UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA
ÁREA DE CONCENTRAÇÃO: FITOTECNIA

STATUS OF CHEMICAL MANAGEMENT OF THE NEOTROPICAL COFFEE LEAF MINER, *Leucoptera coffeella* (GUÉNRI-MÈNEVILLE & PERROTTET) (LEPIDOPTERA: LYONETIIDAE), IN BAHIA STATE, BRAZIL

SUZANY AGUIAR LEITE

VITÓRIA DA CONQUISTA
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Thesis presented to the State University of Southwest Bahia, as part of the requirements of the Graduate Program in Agronomy, area of concentration in Crop Science, to obtain the title of Doctor.

Advisor: Profa. Dra. Maria Aparecida Castellani

Coadvisor: Prof. Dr. Raul Narciso Carvalho Guedes

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DECLARAÇÃO DE APROVAÇÃO

Título: Status of chemical management of the Neotropical coffee leaf miner, *Leucoptera coffeella* (Guénri-Mèneville & Perrottet) (Lepidoptera: Lyonetiidae), in Bahia State, Brazil

Autor: Suzany Aguiar Leite

Aprovado como parte das exigências para obtenção do Título de DOUTOR EM AGRONOMIA, ÁREA DE CONCENTRAÇÃO EM FITOTECNIA, pela seguinte Banca Examinadora:

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DEDICATION

To my family,
I thank and offer,

To my parents Reinaldo and Dilêne; to my brothers, Suzety and Sudson, for the values transmitted, affection, patience and love. And to all who cheered and believed in me.
With you I learned that each day is a new beginning to face the challenges! Love you so much.

I Dedicate

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“Although no one can go back and make a new start, anyone can start now and make a new end”.

Chico Xavier

GENERAL ABSTRACT

LEITE, S. A. STATUS OF CHEMICAL MANAGEMENT OF THE NEOTROPICAL COFFEE LEAF MINER, *Leucoptera coffeella* (GUÉNRI-MÈNEVILLE & PERROTTET) (LEPIDOPTERA: LYONETIIDAE), IN BAHIA STATE, BRAZIL, Vitória da Conquista – BA, UESB, 2020. 150 p. (Thesis: Doctor Science in Agronomy; Area of Concentration: Crop Science)[*]

The state of Bahia ranks fourth in national coffee production. *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) is a key crop pest, which can cause losses of 50% in production. The use of insecticides has been intensified in recent decades as the only solution for the control of the leaf miner, causing selection pressure for resistant populations in coffee regions of Brazil. In Bahia, the phenomenon for leaf miner resistance has not registered. Thus, the thesis was organized into four articles developed to achieve the following objectives: know aspects of the profile of coffee crops and the management of the leaf miner, including the use of insecticides in the western and highland regions of Bahia; determine the incidence of resistance of the leaf miner to chlorantraniliprole and to evaluate the risk of failure of control with the insecticide and the existence of spatial dependence among populations; detect incidence and determine levels of resistance of the pest insecticides to the chlorantraniliprole, thiametoxam and chlorpyrifos; and evaluate the effect of the insecticide thiamethoxam on the vegetative vigor of *Coffea arabica* seedlings and its effectiveness in the pest control. The survey of the profile of coffee crops and management of the leaf miner showed an increase in the frequency of annual application of systemic insecticides in recent years and standardized use in most farm properties, without adopting the principles of Integrated Pest Management (IPM). Resistance to the insecticide chlorantraniliprole is an eminent problem in the populations of the leaf miner in the western and highland regions of Bahia. The effectiveness of the insecticide is reduced, and the risk of control failure is significant, requiring practices of resistance management. The occurrence of insecticide-resistant populations of the leaf miner in the assessed regions was confirmed at low to moderate levels for chloranthranilprole and thiamethoxam, and low to chlorpyrifos. The western region presented the most concerned scenario of *L. coffeella* resistance to insecticides, though the phenomenon requires attention in both regions. There is an increase in the use of insecticides from the subgroup of neonicotinoids in the control of the leaf miner, mainly the active thiamethoxam. This promotes morphophysiological responses acting as bioactivator, altering the plant's metabolism and morphology, in addition to being effective in controlling the leaf miner in populations from regions where selection pressures by the active are reduced. The continuous use of chlorantraniliprole and thiamethoxam tend to increase the selection pressure over resistant individuals, decreasing these insecticides effectiveness and compromising the management of the leaf miner in the western and highland regions of Bahia.

Keywords: Bioactivador, *Coffea arabica*, Control Failure Likelihood, Integrated Pest Management.

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RESUMO GERAL

LEITE, S.A. STATUS DO MANEJO QUÍMICO DO BICHO-MINEIRO DO CAFÉ, *Leucoptera coffeella* (GUÉNRI-MÈNEVILLE & PERROTTET) (LEPIDOPTERA: LYONETIIDAE), NO ESTADO DA BAHIA, BRASIL, Vitória da Conquista - BA, UESB, 2020. 150p. (Tese: Doutorado em Agronomia; Área de Concentração: Fitotecnia)[*]

O estado da Bahia ocupa o quarto lugar na produção nacional de café. *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) é uma praga-chave da cultura, podendo ocasionar perdas de 50% na produção. O uso de inseticidas tem sido intensificado nas últimas décadas como única solução para o controle do bicho-mineiro, acarretando pressão de seleção para populações resistentes em regiões cafeeiras do Brasil. Na Bahia, o fenômeno da resistência ao bicho-mineiro ainda não foi registrado. Assim, a tese foi organizada em quatro artigos desenvolvidos para atingir os seguintes objetivos: conhecer aspectos do perfil das lavouras cafeeiras e do manejo do bicho-mineiro, englobando o uso de inseticida, para as regiões Oeste e Planalto da Bahia; determinar a incidência da resistência do bicho-mineiro ao clorantraniliprole e avaliar o risco de falha de controle com o inseticida e a existência de dependência espacial entre populações; detectar a incidência e determinar os níveis de resistência da praga aos inseticidas clorantraniliprole, tiametoxam e clorpirifós; e avaliar o efeito do inseticida tiametoxam no vigor vegetativo de mudas de *Coffea arabica* e sua eficácia no controle da praga. O levantamento do perfil das lavouras cafeeiras e manejo do bicho-mineiro evidenciaram aumento na frequência de aplicação anual de inseticidas sistêmicos nos últimos anos e padronização do uso na maioria das propriedades, sem a adoção dos princípios do Manejo Integrado de Pragas (MIP). A resistência ao inseticida clorantraniliprole é um problema eminente nas populações do bicho-mineiro nas regiões Oeste e Planalto da Bahia. A eficácia do inseticida é reduzida e o risco de falha de controle é significativo, exigindo práticas de manejo da resistência. Há ocorrência de populações do bicho-mineiro resistentes a inseticidas nas regiões estudadas em níveis moderado a baixo ao clorantranilprole e ao tiametoxam, e baixo ao clorpirifós. A região oeste apresentou o cenário mais preocupante de resistência de *L. coffeella* a inseticidas, mas o fenômeno requer atenção em ambas as regiões. Há um aumento no uso de inseticidas do subgrupo dos neonicotinoides no controle do bicho-mineiro, principalmente o ingrediente ativo tiametoxam. Este promove respostas morfofisiológicas atuando como bioativador, alterando o metabolismo e a morfologia da planta, além de ser eficaz no controle do bicho-mineiro em populações de regiões onde as pressões de seleção ao ingrediente ativo são baixas. O uso contínuo de clorantraniliprole e tiametoxam tende a aumentar a pressão de seleção para indivíduos resistentes, diminuindo a eficácia desses inseticidas e comprometendo o manejo do bicho-mineiro nas regiões Oeste e Planalto da Bahia.

Palavras-chave: Bioativador, *Coffea arabica*, Risco de Falha de Controle, Manejo Integrado de Pragas.

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[*] **Orientadora**: Profa. Dra. Maria Aparecida Castellani, UESB e **Coorientador**: Prof. Dr. Raul Narciso Carvalho Guedes, UFV.

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LIST OF ABBREVIATIONS, INITIALS AND SYMBOLS

A	Photosynthetic Rate
a.i.	Active ingrediente
C	Partial Sill
Ci	Internal concentration CO_2 in the leaf (µmol CO_2 m^{-1} ar)
Co	Nugget
E	Transpiration Rate
GPS	Global Positioning System
Gs	Stomatal conductance (mol $m^{-2}s^{-1}$)
Hr	Range
IQD	Index of quality of Dickson
IRGA	Infrared Radiation Gas Analyzer
LT_{50}	Lethal time to death of 50 % of the population

SUMMARY

GENERAL INTRODUCTION

Coffee production is an activity of great importance for the Brazilian economy (Righi et al. 2013), with estimated production area of 2.1 million hectares, being the world's largest producer and exporter, with a production of 61.7 million 60 kg sacks of processed coffee. The state of Bahia ranks fourth in Arabica coffee production *Coffea arabica* (western and highland regions) and *Coffea canephora* (coastal region) (Conab, 2020).

Currently for insertion into new markets, coffee farmers must cope with tactics of Integrated Pest Management (IPM) basis and not just chemical control (Świtek and Sawinska, 2017; Sawinska et al., 2020). The adoption of the integrated pest management philosophy in agricultural and forestry crops is consistent with requirements of the new consumer markets and new vision and trends in agriculture that goes far beyond crop productivity (Ha, 2014). The use of scouting techniques and economic injury levels to make decisions-making about the need or not to intervene in the agroecosystem is the basis for applying this management philosophy. However, if the population suppression of an organism that has reached pest status is carried out through chemical control, the choice of an insecticide is of fundamental importance, taking into account not only the effectiveness and price of the product, but mainly the selectivity in favor of natural enemies, toxicity, residual power, preharvest interval, persistence, application method and formulation (Crocomo, 1990).

The use of insecticides in crops was intensified in the period after Second World War, starting to be used indiscriminately as definitive solution for pests, resulting in the selection of insecticide resistant insects along the generations. Several species of insects and mites were reported as resistant, including virtually all insecticide groups (Sparks and Nauen, 2015; Nauen et al., 2019). Insecticide resistance is the development of an ability a lineage of an organism to tolerate doses or concentrations of toxics and / or pathogens that would be lethal to the majority of the normal (susceptible) population of the same species (Who, 1958). It is a microevolutionary phenomenon and an ecological reaction (Guedes et al., 2017), that usually manifests it over the years (Whalon et al., 2008; Sparks and Nauen, 2015). Worldwide, 597 species of arthropods are resistant to one or more pesticides (Nauen et al. 2019). In Brazil, resistance of 33 species of arthropods to pesticides has been detected, these 13 are of medical or veterinary importance and 20 of agricultural importance (Sparks and Nauen, 2015).

The evolution of pest resistance to insecticides and crescent concerns about the impact on the environment and mammals has become a tool to drive the introduction of integrated pest management (IPM), resulting in the search for more selective insecticides which has led the emergence of new insecticidal classes as a tool for implementing strategies for adopting resistance management (Sparks et al., 2019; Sparks et al., 2020).

The application of insecticides to control pest insects has caused biological imbalances in populations and communities of arthropods. Recent studies have indicated the impact of these compounds on the structure agroecosystem populations and communities, in addition to the selection of resistant insects (Guedes et al., 2016). The preservation of susceptible insects with the reduction of applications, rotation of insecticides and use of those with low residual power, as well as the creation of refuge areas are effective measures when applied preventively. (Horowitz and Denholm, 2000). The basic principle of resistance management is based on reducing pressure of selection (Sparks and Nauen, 2015).

Among the insect species that cause economic damage to Brazilian coffee production, the leaf miner *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) stands out as the main coffee pest in the Neotropical America, and especially in conditions cultivation in full sun (Tuelher al., 2003; Pereira et al., 2007a; 2007b; Magalhães et al., 2010; Pantoja-Gomez et al., 2019). The highest incidences of the leaf miner occur in Central America and, especially, in Brazil due edaphoclimatic conditions (Tuelher et al. 2003, Pereira et al. 2007a, 2007b, Magalhães et al. 2010, Pantoja-Gomez et al. 2019). The leaf miner damages result from injuries caused by the insect larvae feeding on the palisade parenchyma, reducing the photosynthesis leading to destruction and falling of leaves, consequently decreasing fruit production. (Parra and Reis, 2013). The biological cycle lasts from 28 to 39 days and may occur from 4 to 5 generations annually (Enríquez et al. 1975) and in dry periods they increase the incidence in coffee plantations (Custódio et al. 2009). The main method employed by coffee farmers to control the coffee leaf miner is the chemical (Fragoso et al. 2002, Ramiro et al. 2004).

Insecticide resistance in populations of the coffee leaf miner in Brazil has been reported in coffee crops regions in the states of Minas Gerais, São Paulo and

Pernambuco (Alves et al., 1992; Fragoso et al., 2002, 2003; Costa et al., 2016). The evolution of resistance in populations of the leaf miner has been occurring due to the intensive use of insecticides in pest control. These insects use different resistance mechanisms adapt to the pressure of selection imposed by the same insecticide, favoring the increase in resistant populations.

As a strategy for monitoring the resistance of the leaf miner in the state of Bahia, new tools with approaches and geographical components should be used to estimate the spatial dimension of the problems resulting from the intensive use of insecticides, which favors the increase the index insects populations resistant and, consequently, in the occurrence of control failure. The mapping of areas allows better decision making for monitoring resistance and setting in measures to be applied in the management program (Guedes, 2017). The spatial distribution of insecticide resistance and possible control failures is a largely neglected topic, but fundamental to the management of the problem, as it allows guiding efforts and resources for integrated management of the coffee leaf miner.

High rates of *L. coffeella* infestation can cause losses in production above 50% (Ramiro et al., 2004). The species finds favorable conditions in the state of Bahia for its development, causing damage to coffee farmers in the main producing regions. In region western the use of insecticides for control leaf miner, reach until 17 applications per year (15 spraying and two systemic insecticide applications) (Castellani et al., 2016).

Survey on the profile of coffee crops, management of the leaf miner and the use of insecticides for control are of great importance, once situation of the management of the leaf miner and the use of insecticides in Bahia coffee culture is not clearly know producing regions of *C. arabica*. The use of the active ingredients chlorantraniliprole, thiamethoxam, abamectin, novaluron and chlorpyrifos, as well as the frequency of application of these, which reach until reach until 17 applications per year (Castellani et al., 2016), and consequently the increase in the frequency of resistant individuals insecticide, due larger pressure of selection.

There is an evidence of increased use of insecticides from the subgroup of neonicotinoids in the control of the leaf miner in Bahia, mainly the active ingredient thiamethoxam, due to its toxic action to insect and it is also believed that its characteristics of promoting changes physiological effects on plants (Pereira et al., 2010). Thiamethoxam is the precursor of clothianidin, another neonicotinoid, a secondary metabolite that can alter function of the thiamentoxam molecule in plants, in addition to causing the death of the target insect (Nauen et al., 2003). Studies that correlate the bioactivating effect and the effective control of the population growth of the coffee leaf miner are necessary. In this context, changes morphological and physiological promoted in *Coffea arabica*, due use of the insecticide thiamethoxam to control leaf miner, and studies on its bioactivator action in the vegetative vigor are still scarce to coffee crops.

Thus, the objectives of the present study were: 1) know aspects of the profile of coffee crops and the management of the leaf miner, including the use of insecticide, for the western and highland regions of Bahia; 2) determine the incidence of resistance of the leaf miner to chlorantraniliprole and to evaluate the risk of failure of control with the insecticide and the existence of spatial dependence between populations; 3) detect incidence and determine levels of resistance of the pest to the insecticides chlorantraniliprole, thiamentoxam and chlorpyrifos; 4) and evaluate the effect of the insecticide thiamethoxam on the vegetative vigor of *Coffea arabica* seedlings and its effectiveness in control of Neotropical leaf miner.

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ARTICLE I

Profile of Coffee Crops and Management of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella*[*]

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Article

Profile of Coffee Crops and Management of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella*

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Abstract: The Neotropical coffee leaf miner is a key coffee pest and in the state of Bahia, one of the major coffee-producing states in the country. The insect finds favorable conditions for its development, causing production losses and intensive use of insecticides. Thus, the objective of the study was to analyze aspects of the profile of coffee crops and the management of the leaf miner, including the use of insecticide for the western and highland regions of Bahia. Data were obtained through questionnaires applied to coffee growers and/or production technicians and included information on the total area, area with coffee, and native vegetation, type of cultivation, cultivars, pest monitoring, methods of control and use, insecticide rotation, and doses used. Descriptive statistical analysis, principal component analysis (PCA), and canonical correlations indicated differences between farm size, and areas with coffee and native vegetation. Chemical pest control prevails as a management strategy in all farms. The results are important for managing the coffee leaf miner while providing an overview and diagnosis of insecticide use in coffee production in the state of Bahia. An increase in the application of systemic insecticides took place in recent years, similarly (same active ingredients) among most coffee growers. This fact increases the risk of selecting populations resistant to insecticides, compromising the management of the leaf miner in the regions.

Keywords: chemical subgroup; control methods; integrated pest management; monitoring; survey

1. Introduction

Coffee production is an activity of great importance for the Brazilian economy [1], with an estimated production area of 2.1 million hectares. The country is the world's largest producer and exporter with an annual production of 61.7 million 60 kg sacks of processed coffee, where few states account for more than 90% of national production [2].

The state of Bahia ranks fourth in Arabica coffee production (*Coffea arabica*, in the western and highland regions) and conilon coffee production (*Coffea canephora*, in the coastal region) with production of 76,135 and 40,930 thousand 60-kg sacks, respectively [2]. Coffee production in Bahia stands out on the national scenario due to the quality of coffee produced in the highland region, mainly in municipalities located in Diamantina highlands (i.e., Chapada Diamantina), that is responsible for the production of specialty coffees due to the particular climatic conditions [3].

Consumer expansion in new markets has led coffee growers to search for systems for sustainable production. The highland region of Bahia, with municipalities located in the Diamantina highlands, has played an important role in the adoption of measures based on agricultural practices that cause lower environmental impact and greater economic value of the product. Currently, for accessing the European and North American markets, farmers must use methods consistent with the Integrated Pest Management (IPM) philosophy, and not just chemical control methods [4,5].

Modern agriculture considers economic, environmental, ecological, and food security aspects taken into account in management decisions. The adoption of the IPM philosophy in agricultural and forestry crops is consistent with the requirements of the new consumer markets and new vision and trends in agriculture that goes far beyond crop productivity [6,7].

Considering the principles of IPM, the use of control tactics must be based on knowledge about the phytophagous species and its natural enemies, and the pest population growth trends. Decision-making regarding adoption of pest control must use control levels and economic thresholds. If the phytophagous species causing the injury reaches the population level of control, assuming the status of pest, the decision is for intervention aimed at suppressing the population [8,9]. If the decision is for chemical control, the choice of the insecticide is of fundamental importance considering not only the effectiveness and price of the product but mainly its selectivity in favor of natural enemies, toxicity, residual power, grace period, persistence, method of application, and formulation [10].

Coffee leaf miner, *Leucoptera coffeella* (Guérin-Méneville and Perrottet, 1842) (Lepidoptera: Lyonetiidae) is a key crop pest, especially of unshaded coffee, which is prevalent in most Neotropical America and particularly in Brazil. The highest incidences of the coffee leaf miner occur in Central America and mainly in Brazil due to the high infestation rates recorded [11–15]. The damage caused by the insect is a result of injuries caused by its larvae that feed on the palisade parenchyma of coffee leaves, reducing the photosynthetic capacity, which leads to destruction and fall of leaves and, consequently, reducing fruit production [16]. The biological cycle lasts from 28 to 39 days, and four to five generations of the leaf miner may occur per year [17]. In dry periods, the leaf miner incidence in coffee crops increases [18].

In Brazil, the main method used by coffee growers to control the coffee leaf miner is chemical [19,20]. Neuroinsecticides are the most widely used, including several organophosphates, carbamates, pyrethroids, and neonicotinoids, some of which are (relatively) persistent in the environment and exhibit low selectivity in favor of natural enemies. The diamide, chlorantraniliprole, is conversely of more recent use against the control of coffee leaf miner and has low impact on non-target insects [21,22].

The management of leaf miner populations is linked to factors such as frequency of insecticide applications and migration of individuals and development of resistant populations, which are of primary importance for the effective control of the species [23]. The neglect of these factors by coffee growers and the frequent use of insecticides in the control of pest species lead to high selection pressure on the pest individuals and the development of resistance to the most frequently used insecticides [24].

The western and highland regions have increased production costs due to the chemical control of the coffee leaf miner. This is the result of the high number of insecticide applications required for the leaf miner control, mainly in the western region, where conditions are more favorable to the pest development [3]. In both of these coffee producing regions of Bahia, insecticide resistance and risk of control failure have already been observed [25]. There are knowledge lacunas related to leaf miner in the main coffee regions of Bahia that can subsidize research and extension actions on IPM in coffee growing. Thus, the objective of the study was to analyze aspects of the profile of coffee crops and the management of leaf miner, including the use of insecticide, for the western and highland regions of Bahia.

2. Materials and Methods

2.1. Study Sites

Sampling took place in farms located in the western (Barreiras, Cocos, Luís Eduardo Magalhães, and São Desiderio) and highland regions of Bahia (Barra do Choça, Barra da Estiva, Encruzilhada, Ibicoara, Mucugê, Piatã, and Vitória da Conquista) (Figure 1) between September 2017 and May 2018, totaling 116 farms surveyed (western region (Farms 1 to 21) and highland region (Farms 22 to 116)).

Information about the profile of coffee crops and management of coffee leaf miner was obtained from coffee growers and/or production technicians. Interviews were carried out in loco, on a voluntary basis, guaranteeing the confidentiality of responses. The questionnaire was designed with structured multiple-choice questions, free and dichotomous responses. Questions involved the following aspects: (1) Farm and cultivation, total area with coffee, area of native vegetation, type of cultivation (irrigated or non-irrigated) and cultivars, (2) history of the area regarding coffee leaf miner monitoring (if so, what level of control is used), types of control adopted, annual frequency of applications, insecticides used, rotation of insecticides, and use of the label rate specified by the manufacturer.

2.2. Data Analysis

Data consistency was measured by Cronbach’s Alpha coefficient test to verify the reliability and consistency of the group of multiple-choice and dichotomous responses. The intensity of the relations was very high ($\alpha = 0.91$) to moderate ($\alpha = 0.65$).

Questionnaire data were tabulated and analyzed in Microsoft Excel, using the Chi-Square test to determine differences between regions (western and highland) related to the distribution of coffee growers within the characteristics addressed, as well as a multivariate analysis with groupings of variables: Total area, area with coffee, area of native vegetation. The adopted technique was the multivariate analysis of PCA (Principal Component Analysis) using the R *FactoMinerR* package software [26] applying the selected variables to transform data from a wide spectrum to low spectrum space. PCA was calculated using the correlation matrix for each variable to deduce the eigenvector and eigenvalue. The eigenvector indicates the direction of the main axis with the greatest variance and the eigenvalue indicates the magnitude of the variability of the secondary axis with the next variance. The Bartlett test was used to verify the measure of the correlation matrix and the identity matrix to indicate the existence of the relationship among variables evaluated and the Kaiser–Meyer–Olkin test (KMO) to measure the adequacy of data for the PCA [27].

For data referring to the number of insecticide applications in the agricultural year, canonical variates analysis (CVA) was performed using the procedure CANDISC on the SAS software Basic Edition, Cary, NC, USA (SAS Institute 2011) to verify possible linear associations of applications among locations in each region under study. Data on the use of chemical subgroups and number of applications were correlated using canonical correlation analysis (partial) in order to test the relationship among these variables using the PROC CANCORR procedure [28].

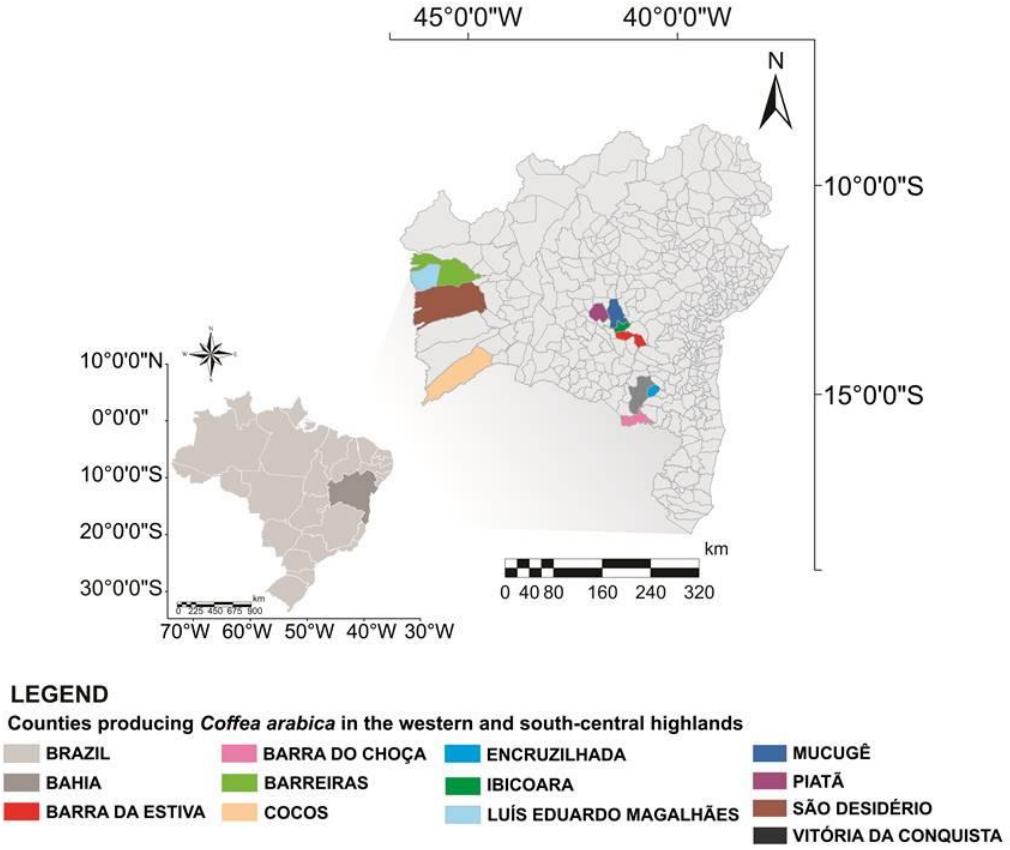

Figure 1. Municipalities producing Arabica coffee (*Coffea arabica*), with sampled farms, belonging in the western and south-central highlands regions of the state Bahia.

3. Results

The size of farms sampled in the survey ranged from 2 to 44 thousand hectares (Figure 2a), the cultivation area ranged from 0.5 to 1800 hectares (Figure 2b), and included farms without area of native vegetation and farms with up to 200 hectares of native vegetation (Figure 2c).

The variables presented in Table 1 and Figure 2 provide total components and the proportion of variance indicating the total variation of the principal component. For the total area component, two distinct axes were obtained, PC1 and PC2, accounting for 56.03% and 43.97% of the total variance observed (Figure 2a). These results indicate the prevalence of small farm size in the highlands (frequently lower than 100 ha), and a broader range of farm size variation in western Bahia with the prevalence of large farm size (i.e., >200 ha) (Figure 2a).

When the area cultivated with coffee was analyzed, linear correlations were also significant, with PC1 and PC2 representing 54.28% and 45.71% of variance, respectively. The profile of farms in their respective coffee cultivated areas closely follows the trend of overall farm size with greater coffee areas prevailing in the western region and small coffee areas prevailing in the highlands (Figure 2b), where more uniform and smaller farm (and coffee field) sizes prevail.

Areas covered with native vegetation and recognized as permanently maintained preservation areas were also surveyed for each farm in each region. The PC1 and PC2 obtained accounted for 62.61 and 37.39% of the observed variance, respectively (Figure 2c). Large areas of native vegetation are frequently associated with larger farm size, while smaller areas of native vegetation are associated with small farm size (Figure 2c).

The results indicated that the occupation of the farms with the coffee crop ($\chi^2 = 42.85$; $p < 0.0001$) is more expressive on smaller properties, on average, 62.4% of the total area is used with coffee in the highlands region, varying from 20% to 100%, in the western, the average occupation with culture vary with an average of 19.3% (Figure 2d). With respect to the native vegetation area ($\chi^2 = 11.55$; $p < 0.0001$), most farms in the highlands have up to 20%, and in the western 11% to 30% of the total area comprehend areas of vegetation reserve (Figure 2e).

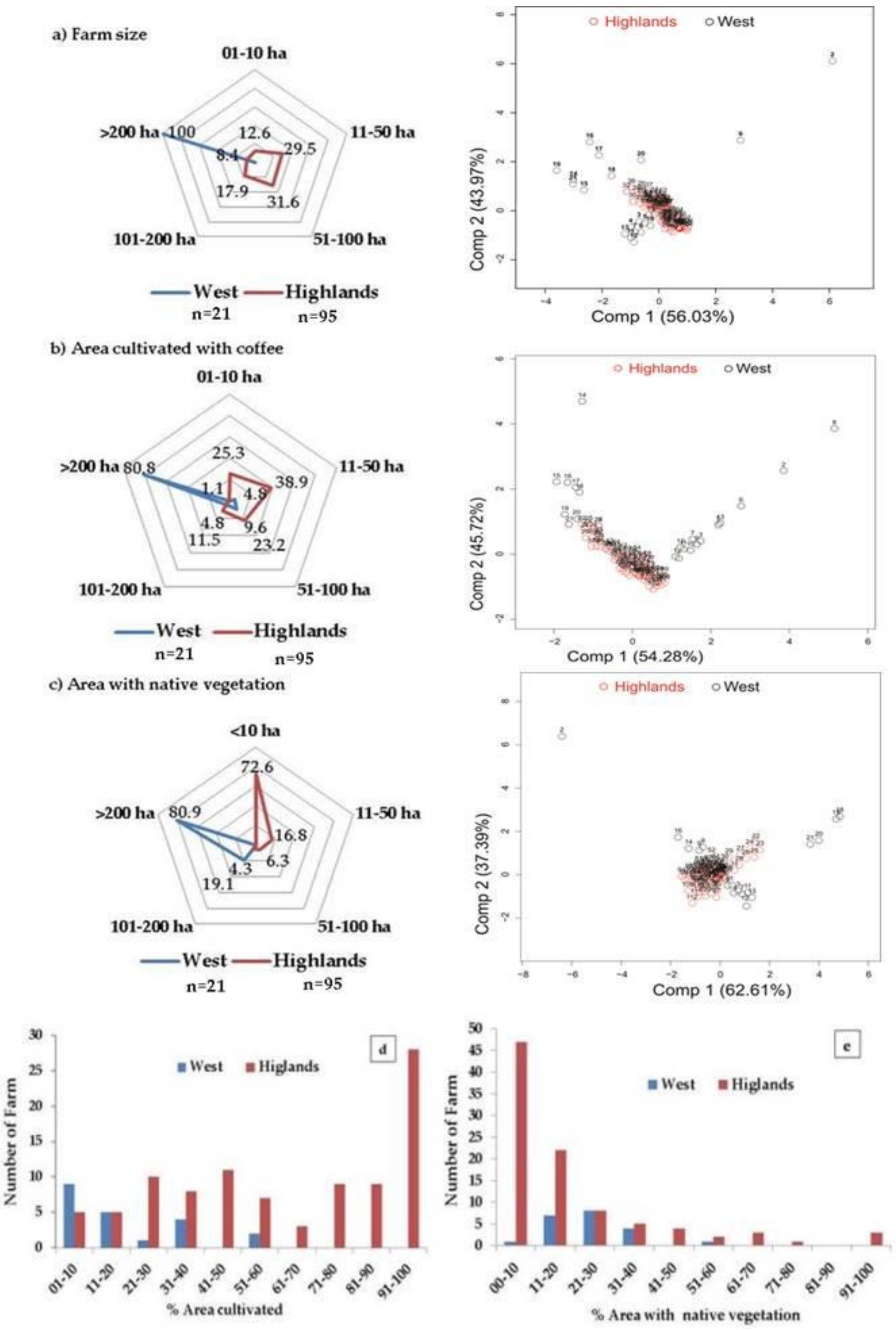

Figure 2. Percentage of coffee farmers' responses and graph of the matrix of 116 sampled farms, belonging to the western and south-central highlands regions of the state Bahia: Total area **a**), area cultivated with Arabica coffee **b**), area of native vegetation **c**), percentage of area cultivated with coffee **d**), and native vegetation **e**) in relation to total area.

Table 1. Principal components, eigenvalues, proportion of explained variance, and proportion accumulated by components for total area, cultivated area and native vegetation area.

Component	Eigenvalues	Proportion	Proportion Accumulated
		Total Area	
PC1	1.12	56.03	56.03
PC2	0.87	43.96	100.00
		Cultivated Area	
PC1	1.08	54.28	54.28
PC2	0.91	45.71	100.00
		Native Vegetation Area	
PC1	1.90	62.61	62.61
PC2	0.89	37.39	100.00

Data related to type of coffee cultivation ($\chi^2 = 6.00$; $p = 0.014$) and cultivars ($\chi^2 = 37.59$; $p < 0.0001$) indicated significant differences between regions. In the western region, 100% of coffee crops are irrigated, while non-irrigated cultivation prevails in the highland region (76.4%) (Figure 3a). The ‘Catuaí’ cultivar is predominant in both regions, reaching 100.0% of the coffee cultivated area in the western region and 90.6% in the highland region (Figure 3b).

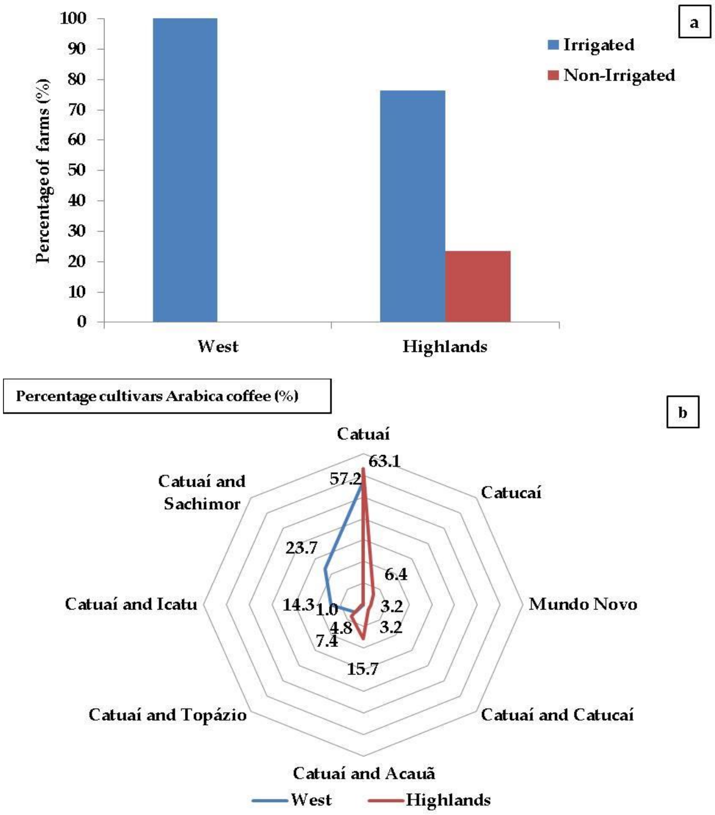

Figure 3. Irrigation prevalence (**a**) and prevailing cultivars of Arabica coffee (**b**) cultivated in the western and south-central highlands regions of the state Bahia.

Regarding the management of the coffee leaf miner, it was observed differences between regions ($\chi^2 = 12.11$; $p = 0.0005$), control tactics used ($\chi^2 = 7.86$; $p = 0.048$), use of insecticide rotation ($\chi^2 = 3.96$; $p = 0.046$), and range of insecticide dose used ($\chi^2 = 33.81$; $p < 0.0001$), without difference in the level of control ($\chi^2 = 2.48$; $p = 0.289$). The infestation level of the coffee leaf miner is monitored by 76.2% (western region) and 34.7% (highland region) of coffee growers (Figure 4a). However, most coffee growers in the western (95.2%) and highland regions (93.7%) do not consider the action (or control) threshold for decision-making, performing only non-quantitative (visual or qualitative) sampling for the adoption of chemical control (Figure 4b).

The prevalent control method is chemical (i.e., by means of insecticide use), mainly in the western region where 100% of farms use insecticides, and only 14.3% associate chemical control with cultural management (e.g., weed management). In the highland region, 57.9% of coffee growers carry out only chemical control. On the other hand, 26.3% of coffee growers in the highland region do not adopt any control method, 14.7% associate chemical and cultural methods, and 1.1% associate chemical and behavioral methods through food bait (based on oleoresins and sugar, Noctovi®) (Figure 4c,d).

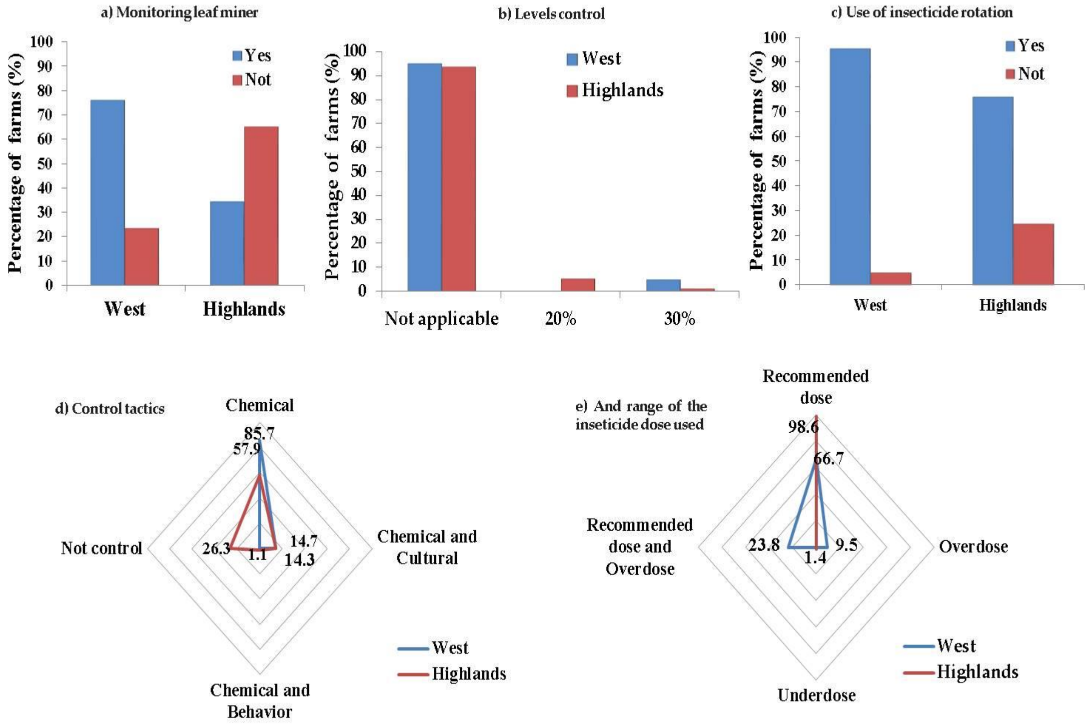

Figure 4. Percentage of responses of coffee farmers to the management of the Neotropical coffee leaf miner (*Leucoptera coffeella*): Monitoring leaf miner **a**), levels control **b**), use of insecticide rotation **c**), control tactics **d**), and range of the insecticide dose used **e**) in Arabica coffee crops in the western and south-central highlands regions of the state Bahia.

The rotation of insecticides is carried out by the majority of coffee growers in the western (95.2%) and highland regions (75.7%). Among the coffee growers, 66.7% use the recommended label rate in western Bahia, and 98.6% use that in the highlands. About a third (33.3%) of the coffee growers in western Bahia overdose the insecticide applications (Figure 4e).

Canonical correlation (partial) in the group of variables was formed by insecticide classes and their frequency of application (Table 2 and Figure 5), which was positive and significant. The main constituents of the canonical pair were based on values of correlations and canonical coefficients with the two canonical axes significant and the first axis explaining 99% of the total data variance for both western and highland regions (Table 2).

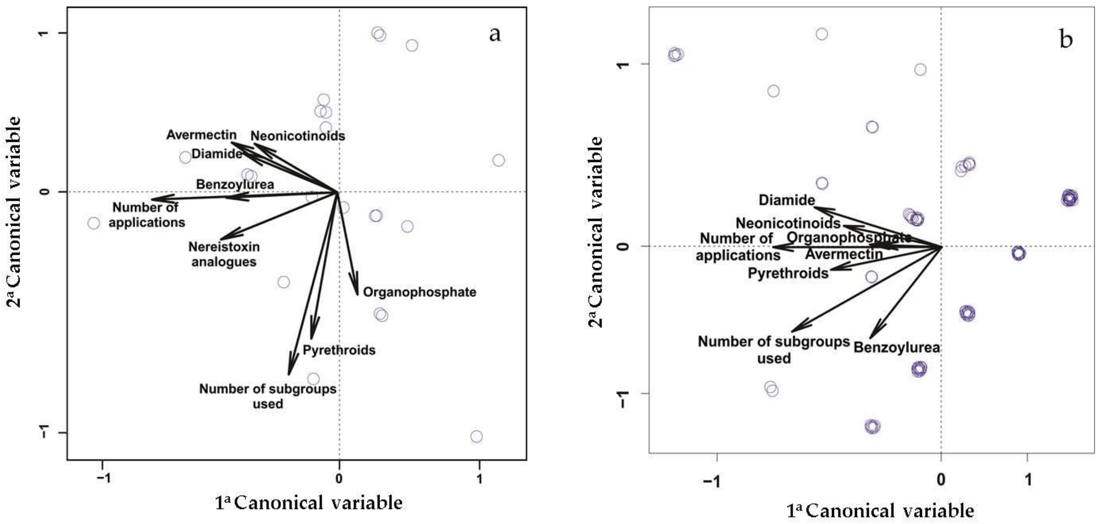

Figure 5. Ordination diagram showing the discrimination between the frequency of application of insecticides for the chemical control of *Leucoptera coffeella* populations in the western (**a**) and highlands (**b**) regions of Bahia. The symbols are centroid of the localities and represent the average of the classes of canonical variables. The vectors indicate groups of farms without significant difference between them (Wilks' Lambda and approximate F, $p < 0.001$).

Absolute values of the highest coefficients were obtained for insecticides diamides, avermectin, nereistoxin analogs, neonicotinoids, and benzoylureas, which contributed to the pattern of divergence between number of applications among the different farms of the western region, in contrast with the diamide, neonicotinoid and benzoylurea more frequent use in the highland region (Figure 5). The first canonical axis of the greatest weight in the analysis indicates frequent use in the western region of all insecticide classes, except organophosphate and pyrethroids (Table 2). The use of diamides prevailed in the highlands and insecticides with more uniform use of insecticides from different classes, and lack of use of nereistoxin analogs (Table 2 and Figure 5). It was observed that in the highland region, the frequency of insecticide applications is lower in comparison to the western region. The range of annual insecticide applications is one to 12 applications in the highlands, and 6 to 20 applications in the western region.

Table 2. Canonical axes and coefficients (grouped in the canonical structure) of the frequency of application of insecticides of the different classes used in the control of *Leucoptera coffeella* in the west and highlands regions of Bahia.

Variable	**Canonical Axes**			
	West		**Highlands**	
	1	**2**	**1**	**2**
Diamide	0.5070	−0.1915	0.7548	−0.2161
Neonicotinoids	0.4467	−0.2930	0.5815	−0.1152
Pyrethroids	0.1424	−0.2875	0.6578	0.1237
Avermectin	0.5694	0.0037	0.3793	−0.0096
Benzoylurea	0.5996	0.3062	0.4246	0.4954
Organophosphate	−0.1109	0.6159	0.4268	−0.0131
Nereistoxin analogs	0.6256	0.7459	-	-
F	140.96	12.43	338.29	8.70
Degrees of Freedom (num.; den)	14; 24	6; 13	12; 174	5; 88
p	<0.0001	<0.0001	<0.0001	<0.0001
Canonical squared correlation	0.99	0.85	0.99	0.33

The application interval for a product is, on average, 20 days. Products without registration with the Ministry of Agriculture and Livestock [29] for coffee crop, such as Ampligo® (lamda-cyhalothrin + chlorantraniliprole), Interprid® (methoxyfenozide), Dimilin® (diflubenzuron), Match® (lufenuron), Oregon® (novaluron), Talisman® (bifenthrin + carbosulfan) are used by some coffee growers in both regions.

4. Discussion

Descriptive analyses, PCA, and the correlations indicated differences between Arabica coffee producing regions of Bahia, as to the size of the farms, occupation of the land with the coffee crops, and native vegetation, adoption of irrigation and, in some aspects, of the management of the leaf miner. In western Bahia, farms are characterized by larger extensions, with areas of up to 44 thousand hectares and areas of coffee reaching 1800 hectares, exhibiting permanent preservation areas in compliance with Brazilian Forest Code (Law 4771/65), a minimum of 20% of the total area. In highlands, most of the farms have smaller extensions, from 2 to 350 hectares (Figure 2a), in some cases, totally occupied with the coffee crop, with a more heterogeneous permanent reserve occupancy rate among the farms.

Coffee production of western Bahia stands out for having 100% of the coffee area completely irrigated (center-pivot and drip irrigation), contrasting with the prevalent non-irrigated coffee of the highland region. The predominant cultivar in these regions is the ‘Catuaí’, which is characterized by being of small size, short internodes, abundant secondary branching, red (cultivar IAC 144), or yellow (cultivar IAC 62) fruits of medium to late maturation, high yield and adaptation to extreme temperatures [30]. However, besides to areas cultivated with Catuaí, other cultivars such as ‘Acauã’, ‘Mundo Novo’ and ‘Catucaí’ are also used only in the highland region, while the ‘Sarchimor’ cultivar is present only in the western region.

These differences reflect in several aspects of the coffee production chain, with mechanized systems and outsourced manpower in the western region, enabling the management of crop in macro scale. Coffee crops in this region is characterized by present productivity above the national Brazilian average and using agricultural inputs, irrigation, appropriate genotypes, and mechanization, among other practices. The climate is favorable to the quality of the coffee, because at the time of harvest, conditions of low relative humidity of the air occur, with the rains concentrated in the summer [31]. In highlands, the use of inputs is less intense and family manpower predominates. The specificities of some microregions in the highlands, such as the Chapada Diamantina, about the climate and the realization of selective and manual harvesting of the fruits, have guaranteed the production of special coffees of excellent quality, with high value aggregated [32].

In the last two years, only one farm in the western region did not show infestation by the coffee leaf miner due to the monitoring used and the application of insecticides in a preventive way in order not to compromise coffee production. However, the other farms have a history of high infestation by the coffee leaf miner resulting in a higher frequency of insecticide applications. In the highland region, some crops fields located in the municipalities of Piatã, Ibicoara, Barra da Estiva, and Vitória da Conquista have low infestation levels, and without a history of insecticide application for over a decade. On the other hand, crops located on municipality Mucugê presented high infestations by the coffee leaf miner in the last five years. This fact caused serious problems for coffee growers, resulting in an increasing number of applications of different insecticide classes.

When asked whether or not to carry out monitoring of the coffee leaf miner, most respondents carried out monitoring, which is the basis for IPM. However, throughout the interview, it was observed that the concept of monitoring is not suitably adopted in practice. This is because, in most cases, the monitoring performed is a visual, non-quantitative, and non-systematic analysis based only on the presence or absence of live larvae detected in quick and casual visual observation. Interestingly, there is no shortage of studies on sampling of the coffee leaf miner, both with conventional [33] and sequential plans [34]. Another problem detected in the survey is that the percentages of predation and natural parasitism are also not quantified by coffee growers [35]. Thus, decision-making about whether or not to use a control method, another IPM support pillar, is not based on the analysis of numerical variations of pest populations and their main natural enemies. The consequence of such neglect of the preventive use of insecticides for leaf miner resulting in insecticide overuse and unnecessary increase in production costs [36].

Quantitative population assessments based on activity levels and economic injury thresholds form the basis of IPM to minimize unnecessary interventions for pest population suppression, especially by the chemical method. When correctly performed and based on a validated sampling plan, monitoring favors decision-making with reduced application of insecticides and reduced production costs. Despite the economic and environmental advantages resulting from the use of the limit established for decision making in agriculture pest management [37,38], their effective use remains as one of the main obstacles to the use of IPM programs in various agricultural crops in Brazil and worldwide [5,37,39,40]. On the other hand, IPM plays a fundamental role in adapting production systems to the trends of modern agriculture for the economic benefit of growers and the reduction of environmental impacts [5,41–43].

Although there are edaphoclimatic differences between regions, the coffee leaf miner occurs throughout the year in Bahia, finding optimal conditions for its development in the western region (low relative humidity and high temperatures) and favorable conditions in the south-central highland region (lower temperatures and high relative humidity at certain times of the year). However, in the highlands at altitudes around 1000 m and average annual temperature of around 20 °C, the coffee leaf miner population remains at the equilibrium level for most of the year, with the presence of at least six species of parasitoids that act in the pest regulation [44]. The same species of parasitoids have also been observed in the western region in coffee crops located in Luis Eduardo Magalhães, which has an average annual temperature of approximately 24.3

°C, but with lower parasitism rates and with variations in the structure of their communities [44].

Most coffee growers adopt only chemical control for coffee leaf miner population suppression, an aspect that, associated with lack of suitable monitoring for control decision-making, impairs the proper use of IPM in coffee farms of the region. With rare exceptions, there are coffee growers in both regions who adopt chemical and cultural controls. On the other, 26.3% of highlands coffee growers do not use any control tactics for the leaf miner, which reveals that the insect meets a “non-pest” situation, probably due to the regulation of its population by factors, such as parasitoid wasps and predators whose survival and permanence in coffee plantations are favored by the absence of insecticides, higher altitude of the region (1000 to 2000 m), and lower average temperatures in relation to the western region. In some cases, coffee growers have not used insecticides for more than 15 years, with the reestablishment of beneficial fauna.

In the case of coffee production, this agro-ecosystem has the capacity of harboring several natural enemies [45]. The harmonization of these practices tends to reduce impacts on the communities of predators and parasitoids, reducing the incidence of the coffee leaf miner. According to Faria and Angelini [46], cultural control aids chemical control by reducing the incidence of the coffee leaf miner in coffee crops. Another important data obtained in the survey was the use of behavioral control using the Noctovi® food-based attractant in a farm located in the municipality of Barra do Choça (highland). Such use allows the recording of the pest population dynamics and can be used simultaneously with insecticides.

Vegetation diversification reduces the pest incidence favoring and providing alternative foods to natural enemies [47]. Natural enemies, such as parasitoids, are efficient when associated with integrated management of the coffee leaf miner [44,48,49], and the use of more selective insecticides favors their prevalence in coffee crops [22,50].

Organophosphate, carbamate, and pyrethroid insecticides were the most used in the control of the coffee leaf miner, but other insecticide classes were more recently introduced and are broadly used against the coffee leaf miner, including neonicotinoids [51], diamides [52], avermectins [53], all of which act on the nervous system, and benzoylureas, which are insect growth disruptors interfering with chitin synthesis, a major component of the insect exoskeleton [54]. Interestingly, rather than rotating the insecticide molecules for controlling the coffee leaf miner, a pivotal recommendation to minimize selection for insecticide resistance in pest species, the growers tend to rotate trade names or formulations, frequently maintaining the use of the same insecticide, but using different commercial products. Thus, no wonder insecticide resistance is a problem in the region against this pest species [25].

The frequent use of insecticides causes selection pressure on pest individuals, favoring the emergence of individuals resistant to products used in their control [24], leading coffee growers to use an overdose of product, greater number of applications, and consequently, replacement of an ineffective insecticide by a new insecticide [55–57]. Fragoso et al. [23] reported the applications of 22 insecticides in a year, 10 of which were organophosphates. Furthermore, many coffee growers make frequent use of insecticides, including relatively more persistent and less selective compounds. Such patterns of insecticide use enhance insecticide resistance risk and environmental problems [22].

A concerning piece of data regarding the use of insecticides in the western region is the residual period indicated by manufacturers, which is not respected by coffee growers. The application interval for a product is, on average, 20 days. In addition to data on the coffee leaf miner management, products without registration with the Ministry of Agriculture and Livestock [29] should not be used according to the regulation of Law No. 7.802 of July 11, 1989, Art.73. Nonetheless, the use of unregistered products is one of the major problems faced when thinking about adapting production to the trends of modern agriculture, as observed for custard apple (*Annona squamosa* L.) crops [58].

There is an increase in the frequency of annual applications of the neonicotinoids thiamethoxam and imidacloprid (two to four) and uniform use of the same compounds in several farms of the region, which increases the risk of selecting for insecticide resistance. Such a trend takes place regardless of the size of cultivated area, climate, temperature, and rainfall.

It is noteworthy that the greater the number of applications and use of insecticides with the same site of action, the greater the likelihood of insecticide resistance. Most coffee growers use an application schedule, not considering parameters population trend coffee leaf miner and natural enemy population densities [32]. Nonetheless, measures for the successful reduction of the pest population must be carried out considering ecological, environmental, and economic and food security aspects for sustainable and high-quality coffee production. Therefore, there is an urgent need for integrative action between research and extension agencies, agricultural companies and coffee growers to expand the use of IPM principles in line with global agriculture megatrends, which are the bases for the sustainability of agriculture production chains [59].

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ARTICLE II

Area-wide survey of chlorantraniliprole resistance and control failure likelihood of the Neotropical coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)[*]

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Area-Wide Survey of Chlorantraniliprole Resistance and Control Failure Likelihood of the Neotropical Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)

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Abstract

The Neotropical coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842), is a key pest species of unshaded coffee plantations in Neotropical America, particularly in Brazil, where pest management involves intensive insecticide use. As a consequence, problems of resistance to conventional insecticides are frequent, and more recently developed insecticide molecules, such as diamide insecticides, are at risk of becoming ineffective. Thus, a survey of resistance to the diamide insecticide chlorantraniliprole was carried out in high-yield coffee-producing areas in the State of Bahia, Brazil. The likelihood of control failure with this insecticide was also assessed. Spatial dependence among the insect sampling sites was assessed and spatial mapping of chlorantraniliprole resistance and risk of control failure was carried out. The frequency of chlorantraniliprole resistant populations was high (34 out of 40 populations, or 85%), particularly in western Bahia, where 94% of the populations were resistant. Resistance levels ranged from low (<10-fold) to moderate (between 10-and 40-fold) with more serious instances occurring in western Bahia. This results in lower chlorantraniliprole efficacy among these populations, with a higher risk of control failure and exhibiting spatial dependence. These findings invite attention to problems with the intensive use of this relatively recent insecticide and demand management attention, but they suggest that local, farm-based management efforts are likely to be the most effective actions against resistance problems in this pest species.

Key words: anthranilic diamides, insecticide control failure, control failure likelihood, insecticide resistance, resistance survey

Life happens; coffee helps! At least that is the belief of a fair share of the human population stressed, blessed, and even obsessed with coffee. The statement is equally valid for coffee producers particularly when facing likely losses due to the Neotropical leaf miner *Leucoptera coffeella* (Guerin-Meneville & Perrottet, 1842). This leaf miner is the key coffee pest species in unshaded coffee plantations, the dominant cultivation system of high-quality coffee (*Coffea arabica* L.) in Neotropical America, particularly Brazil (Tuelher et al. 2003; Pereira et al. 2007a,b; Magalhaes et al. 2010; Pantoja-Gomez et al. 2019), the largest producer and exporter of this prized commodity (MAPA 2018; CONAB 2019). The annual losses by this pest species average about 40% yield but can reach values as high as 80% under high population densities where consumption of palisade parenchyma compromises photosynthetic leaf area leading to early leaf senescence (Tuelher et al. 2003, Pereira et al. 2007a). Thus, the management of this species is of paramount importance in such areas and is achieved mainly with the use of insecticides (Fragoso et al. 2003).

The importance of the leaf miner in coffee production and the (over-)reliance on insecticide use for managing this species naturally raises concern about evolving insecticide resistance in leaf miner populations. Eventual insecticide control failure may result from this, in addition to other hierarchical consequences beyond the population level (Guedes et al. 2016, 2017, 2019). Curiously, studies of insecticide resistance in the coffee leaf miner are rare (Alves et al. 1992; Fragoso et al. 2002, 2003), and the likelihood of insecticide control failure is neglected, as is the potential spatial dependence of both interdependent but distinct phenomena (Guedes 2017).

Insecticide resistance may lead to control failure, but not necessarily since this interaction depends on patterns of cultivation and insecticide use, among other factors, which potentially exhibit spatial dependence (Liebhold et al. 1993; Fragoso et al. 2002; Bacca et al. 2006, 2008; Gontijo et al. 2013; Guedes 2017; Tuelher et al. 2018; Guedes et al. 2019). The possibility of simultaneously surveying both phenomena and geographically mapping their incidenceis seldom attempted despite their strategic relevance for pest management, although some progress has been recently made (Chediak et al. 2016, Guedes 2017, Tuelher et al. 2018).

Insecticide resistance in Neotropical coffee leaf miners was earlier recorded in Brazil against organophosphates, the main insecticide class for management of this species at the time (Alves et al. 1992). However, increases in coffee prices in the international market and consequent concern with leaf miner losses has led to na intensification of insecticide use and magnification of problems with insecticide resistance (Fragoso et al. 2002, 2003). Organophosphate resistance reached very high levels (>1,000-fold) in some of the main producing areas of high-quality coffee in Brazil (Fragoso et al. 2002, 2003). This has led to a diversification of insecticides used against the Neotropical coffee leaf miner, which came to rely on neonicotinoid and diamide use in recent years (MAPA 2019). As a consequence, reports of moderate levels of neonicotinoid resistance have recently emerged (Costa et al. 2016), while diamide use has further intensified.

The diamides are a sound alternative for insect pest control because of their peculiar mode of action distinct from other insecticides available on the market (Lahm et al. 2009). They act as ryanodine receptor activators in the calcium channels regulating muscle cell contractions, through calcium release in the sarcoplasmic reticulum (Lahm et al. 2005, Nauen 2006). The diamide chlorantraniliprole is broadly used against lepidopteran pest species in different crops, including coffee, because of its low nontarget impact and lack of cross-resistance to other insecticides making it a useful pest management tool (Gao et al. 2013). Nonetheless, the growing use of this insecticide is leading to increasing reports of resistance to this molecule in populations of the diamond back moth *Plutella xylostella* (Troczka et al. 2012, Wang and Wu 2012), the Neotropical tomato pinworm *Tuta absoluta* (Roditakis et al. 2015), and the rice stem borer *Chilo suppressalis* (Lu et al. 2017, Wei et al. 2019).

Diamide resistance among coffee leaf miner populations have not yet been a target of attention, and the use of this class of insecticides remains intensive. This scenario has led to the current concern that diamide resistance and particularly chlorantraniliprole resistance may be evolving and may result in future control failures with this insecticide. Therefore, the objectives of the present study were as follows: 1) to survey the incidence of chlorantraniliprole resistance among populations of the Neotropical coffee leaf miner from two important regions of Arabica coffee production in Brazil; 2) to assess the likelihood of control failure with chlorantraniliprole due to the occurrence of resistance to this insecticide in the region; and 3) to preliminarily test whether spatial dependence in chlorantraniliprole resistance exists among sampling sites and to tentatively map such occurrences, if such is the case.

The intensive use of insecticides in the coffee growing regions of the state of Bahia has led us to hypothesize that chlorantraniliprole resistance may already exist in the region, although probably in its initial stages. This suspicion is justified because the use of this compound for coffee protection has only increased recently, but reaching up to

17 annual applications, 2 on soil and 15 spray applications (Castellani et al. 2016). Consequently, resistance to this diamide is likely recent and control failure of chlorantraniliprole was not yet expected since it takes longer to occur as it usually requires incidence of high levels of resistance, a scenario that allows efficient implementation of resistance management practices to minimize such risk. Spatial dependence was not expected because variation in the incidence of insecticide resistance was unlikely to be high (and diverse), compromising the recognition of such a relationship and the spatial mapping of this phenomenon.

Materials and Methods

Insects and Insecticide

Sampling of populations of the Neotropical coffee leaf miner was carried out in 40 sites from two high-quality coffee-producing regions in the state of Bahia (Brazil) – western Bahia (17 sites), and its south-central highlands (23 sites; Table 1; Fig. 1). Leaves containing intact mines were collected from each site, and geo-referenced with a global positioning system (GPS) receiver (Garmin E-Trex Vista HCx, Olathe, KS). The samples were collected between March and December 2018, avoiding leaves with open and/or torn mines indicative of parasitism or predation. The collected leaves were placed in Kraft-type paper bags (17 × 45 cm) and stored in polystyrene boxes for transportation to the laboratory for subsequent bioassays under environmentally controlled conditions.

A commercial formulation of the diamide insecticide chlorantraniliprole was used in the bioassays (350 g a.i. /kg, water dispersible granules, DuPont, Paulinia, SP, Brazil). The insecticide was used at its label rate, as registered at the Brazilian Ministry of Agriculture (MAPA 2019), following the manufacturer’s recommendations. This is the main insecticide currently used in the region against this pest species. The use of a fixed concentration varying exposure allows estimates of both the level of resistance, through time-mortality bioassays, and frequency of resistant individuals, through discriminating time bioassays. This approach parallels others, like Dângelo et al. (2018) with whiteflies, but based on fixed concentration and varying length of exposure and including spatial analyses and spatial mapping of the phenomenon. Furthermore, the discriminating time bioassays also allow estimation of control failure likelihood due to insecticide resistance justifying the presente approach.

Table 1. Identification and geographical coordinates of the sampling sites for populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in our survey of chlorantraniliprole resistance, efficacy and control failure likelihood in the State of Bahia, Brazil.

Meso-region	County	Code	Longitude	Latitude
West	Barreiras	WBAR1	-11° 52' 30.0"	-45° 43' 06.3"
	Barreiras	WBAR2	-12° 16' 30.9"	-45° 30' 35.1"
	Barreiras	WBAR3	-12° 16' 30.9"	-45° 35' 32.6"
	Barreiras	WBAR4	-11° 51' 35.6"	-45° 44' 47.0"
	Cocos	WCOC1	-14° 38' 50.6"	-45° 15' 41.9"
	Cocos	WCOC2	-14° 40' 56.8"	-45° 49' 03.6"
	Luiz Eduardo Magalhães	WLEM1	-11° 57' 43.08"	-45° 44' 01.7"
	Luiz Eduardo Magalhães	WLEM2	-12° 08' 59.1"	-45° 47' 18.1"
	Luiz Eduardo Magalhães	WLEM3	-12° 03' 46.4"	-45° 54' 10.5"
	Luiz Eduardo Magalhães	WLEM4	-12° 16' 19.4"	-45° 56' 02.6"
	Luiz Eduardo Magalhães	WLEM5	-12° 16' 49.0"	-45° 44' 17.6"
	São Desiderio	WSDE1	-12° 08' 06.4"	-45° 53' 20.3"
	São Desiderio	WSDE2	-12° 33' 21.4"	-45° 51' 59.1"
	São Desiderio	WSDE3	-12° 54' 12.7"	-45° 32' 29.4"
	São Desiderio	WSDE4	-12° 33' 06.8"	-45° 47' 23.7"
	São Desiderio	WSDE5	-12° 52' 46.6"	-46° 02' 13.2"
	São Desiderio	WSDE6	-12° 35' 04.0"	-45° 40' 03.4"
Highlands	Barra da Estiva	HBES1	-13° 37' 15.0"	-41° 20' 37.3"
	Barra da Estiva	HBES2	-13° 33' 18.0"	-41° 20' 09.1"
	Barra da Estiva	HBES3	-13° 36' 45.3"	-41° 19' 53.9"
	Barra do Choça	HBCH1	-14° 50' 27.5"	-40° 31' 13.0"
	Barra do Choça	HBCH2	-14° 53' 55.3"	-40° 35' 35.4"
	Barra do Choça	HBCH3	-14° 55' 25.2"	-40° 36' 43.5"
	Barra do Choça	HBCH4	-14° 55" 05.8"	-40° 36" 01.9"
	Barra do Choça	HBCH5	-14° 50' 15.9"	-40° 31' 04.4"
	Barra do Choça	HBCH6	-14° 54' 58.1"	-40° 36' 24.8"
	Barra do Choça	HBCH7	-14° 51' 37.5"	-40° 31' 33.2"
	Barra do Choça	HBCH8	-14° 54' 59.4"	-40° 37' 30.6"
	Encruzilhada	HENC1	-15° 36' 50.1"	-40° 44' 32.3"
	Encruzilhada	HENC2	-15° 37' 14.3"	-40° 45' 59.0"
	Encruzilhada	HENC3	-15° 39' 37.0"	-40° 45' 38.0"
	Mucugê	HMUC1	-13° 02' 38.8"	-41° 26' 02.4"
	Mucugê	HMUC2	-13° 09' 02.9"	-41° 28' 19.8"
	Mucugê	HMUC3	-13° 07' 37.1"	-41° 29' 25.4"
	Mucugê	HMUC4	-13° 05' 57.6"	-41° 26' 38.2"
	Vitória da Conquista	HVDC1	-14° 59' 52.0"	-40° 47' 55.2"
	Vitória da Conquista	HVDC2	-15° 16' 37.5"	-40° 56' 49.2"
	Vitória da Conquista	HVDC3	-15° 14' 39.6"	-40° 59' 11.9"
	Vitória da Conquista	HVDC4	-15° 00' 30.0"	-40° 45' 25.6"
	Vitória da Conquista	HVDC5	-14° 58' 15.3"	-40° 46' 09.6"

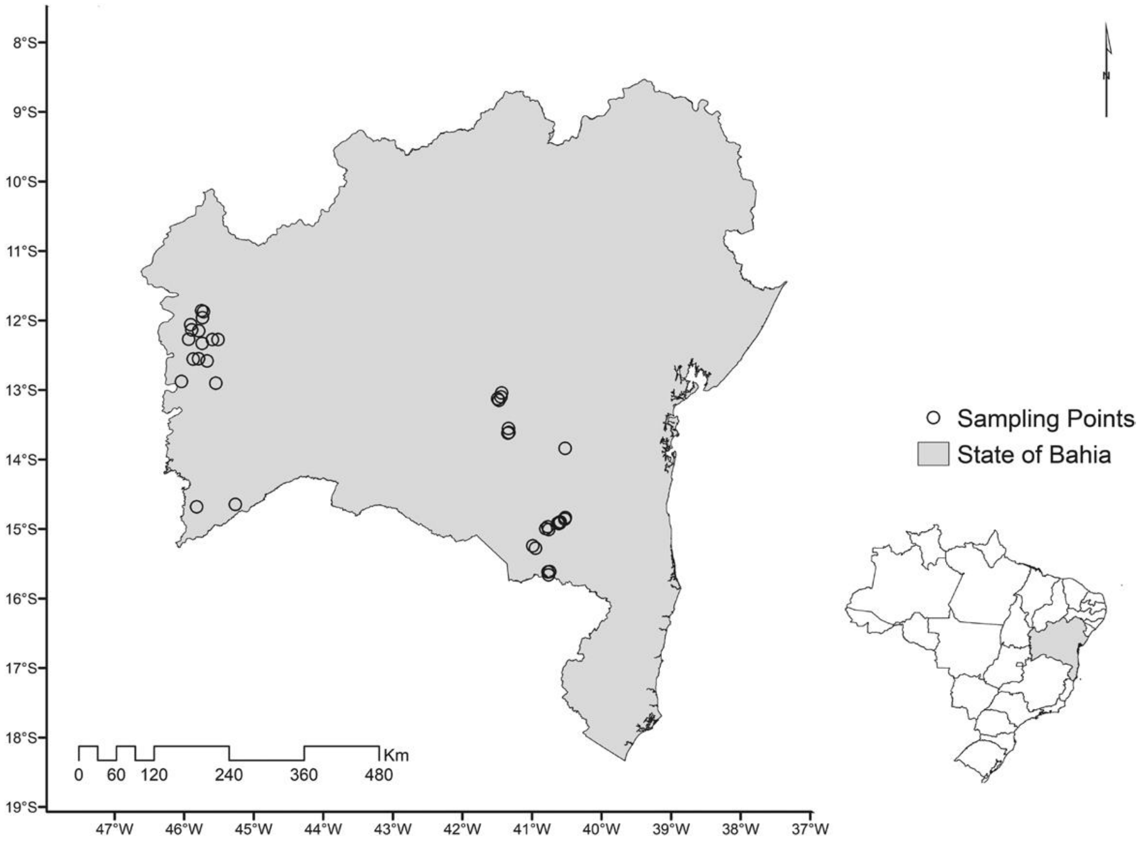

Fig. 1. Distribution of the sampling sites for populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in the spatial survey of chlorantraniliprole resistance in Brazil. Identification for each sampling site and its coordinates are found in Table 1.

Time-Mortality Toxicity Bioassays

Time-mortality insecticide bioassays were carried out following methods adapted from Fragoso et al. (2002), which were derived from earlier work on the tomato pinworm *Tuta absoluta* (Siqueira et al. 2000, 2001). A single chlorantraniliprole concentration was used, the field label rate (90 g a.i./ha), at a rate of 400 liter/ha (=0.78 g a.i./ml), and the exposure times of 2, 4, 6, 12, 18, 24, 36, and 48 h. Filter paper disks (Whatman no. 1; 9.0-cm diameter) were immersed in the insecticide solution for 10 s and allowed to dry for 1 h at ambient temperature, after which they were placed in Petri dishes (9.0-cm diameter × 1.5-cm high). Twenty third instar larvae removed from the field-collected leaves were placed in each Petri dish using a fine hair-brush, and they were subsequently maintained in an environmental chamber under controlled conditions of 25 ± 2°C temperature and 70 ± 5% relative humidity. The experiment was replicated three times for each insect population. Larval mortality was recognized by the inability to move a body length when prodded by a hair-brush. Untreated controls for each insect population were maintained to record natural larval mortality for correction of the chlorantraniliprole-exposed mortality observed (Abbott 1925).

Expected Efficacy and Control Failure Likelihood

The same procedures and experimental units described above were used for a final mortality assessment after 48 h of exposure as a determination of expected chlorantraniliprole efficacy, after proper correction for natural mortality (as indicated above). These data were subsequently used to estimate the control failure likelihood (CFL) of chlorantraniliprole due to insecticide resistance in each of the field-collected insect populations. The control failure likelihood was estimated using 80% mortality as the minimum threshold of efficacy as required by the Brazilian Ministry of Agriculture for conventional insecticides (MAPA 1995), following methods by Guedes (2017) where CFL = 100-[observed mortality (%) × 100]/expected mortality (i.e., 80%). CFL values ≤ 0 indicate a negligible risk of control failure.

Statistical Analyses

Time-mortality data were subjected to probit analysis (PROC PROBIT; SAS Institute, SAS, Cary, NC). The levels of insecticide resistance, or resistance ratios, were estimated by dividing the median lethal time (LT_{50}) of a given population by the LT_{50} of the most susceptible population as recognized through the toxicity bioassays with chlorantraniliprole. Significant chlorantraniliprole resistance was recognized through estimation of the 95% FIs of the resistance ratios, and they were identified as significant if not including the value of 1 (Robertson et al. 2007). The efficacy and control failure results after 48-h exposure were subjected to a one-sided *Z*-test at 95% confidence level with correction for continuity to test their departure from the expected mortality (Roush and Miller 1986). The relationship between levels of chlorantraniliprole resistance and control failure likelihood was tested using regression analysis with the curve-fitting procedure of TableCurve 2D (Systat, San Jose, CA); model selection was based on parsimony, high *F*-values (and reduced error), and R^2 (steep) increase with model complexity.

Spatial analyses were carried out using the distance between pairwise sampling sites obtained from the GPS recorded geographical coordinates and the insect response data (levels of insecticide resistance, efficacy, and control failure likelihood). The relatively low number of sampling sites prevented the use of ordinary kriging methods for the desired estimates, but cokriging circumvented this shortcoming amplifying the data set (i.e., sampling points) used for the estimates. Thus, resistance levels and estimates of control failure likelihood were subjected to cokriging methods with chlorantraniliprole efficacy allowing selection of suitable semivariogram functions for distance interpolation (Isaaks and Srivastava 1989).

The semivariogram functions allow estimation of three parameters: range (*hr*), partial sill (*C*), and nugget (*Co*). The former refers to the distance in which a plateau is reached, thus referring to the maximum distance where spatial dependence exists. The second refers to the mortality-based semivariance value in which the maximum distance of interference (i.e., range) is reached. The latter is the semivariance value where the model intercepts the *y*-axis representing the measurement errors and/or resolution involved. These three parameters were used to obtain three more parameters balancing the mortality semivariance and the measurement error or resolution obtained: sill (*C*o + *C*), proportion [*C*/(*C*o + C)], and randomness (*Co*/*C*) of the data. The semivariogram models were selected based on the best data adjustment (i.e., regression equation with slope closest to one, and intercept and mean error closest to zero) and the highest randomness values. The selected semivariance models were subsequently used to generate spatial maps of chlorantraniliprole resistance levels and control failure likelihood. The spatial analyses were performed using ArcGIS 10.5 (ESRI, Redlands, CA).

Results

Chlorantraniliprole Resistance

The time-mortality results for each leaf miner population with independente time-dependent estimates were subjected to probit analyses and resulted in low χ^{2-} and *P*-values >0.05. These χ^{2-} and *P*-values attest to the suitability of the probit model for the intended analyses and estimation of the desired toxicological endpoints, namely, the median lethal concentrations (LT_{50}'s). The frequency of chlorantraniliprole resistant populations was high (34 out of 40 populations, or 85%), and particularly so in western Bahia, where 94% of the populations were resistant to chlorantraniliprole (Table 2).

The levels of chlorantraniliprole resistance were usually low (<10-fold) in the highlands with four exceptions reaching moderate levels of resistance (between 10- and 100-fold), although distributed in different counties (Table 2). Western Bahia presents a contrasting case, with the prevalence of moderate levels of resistance reaching over 30-fold in two instances, in Cocos and Luis Eduardo Magalhães (Table 2). Low levels of resistance were limited to five sites, and chlorantraniliprole susceptibility was detected in western Bahia at only one site: São Desiderio (WSDE4).

Chlorantraniliprole Efficacy and Control Failure Likelihood

Chlorantraniliprole efficacy remained above the 40% level for all the populations tested, but most did not reach the minimum required threshold of 80% efficacy (Table 3). This is a clear indication that chlorantraniliprole control failure is likely in some populations, which was also estimated (Table 3). The risk or likelihood of control failure was significant in 72.5% of the tested insect populations and sites (29 out of 40 populations). Such risk was usually lower than 30% in the highland populations with just three exceptions: Barra do Choça, Encruzilhada, and Vitória da Conquista. The risk of control failure tended to be higher in western Bahia, reaching over 30% in five instances and up to 50% in one, Cocos (Table 3).

Table 2. Relative toxicity of chlorantranilprole to Brazilian populations of the coffee leaf miner (*Leucoptera coffeella*).

Meso-region	County	Code	No.	Slope ± SE	LT$_{50}$ (95% FI) hours	χ^2	Df	*P*	Resistance ratio at LT$_{50}$ [RR$_{50}$ (95% CI)]
West	Barreiras	WBAR1	480	0.92 ± 0.14	19.96 (14.89-28.95)	0.34	6	0.99	5.72 (2.34-16.86)*
	Barreiras	WBAR2	480	1.33 ± 0.19	65.91 (46.44-115.74)	4.05	6	0.67	18.88 (8.88-48.50)*
	Barreiras	WBAR3	480	1.56 ± 0.19	50.20 (38.46-74.21)	1.19	6	0.98	14.38 (7.70-32.43)*
	Barreiras	WBAR4	480	1.09 ± 0.15	43.03 (31.01-70.78)	3.16	6	0.79	12.33 (5.41-33.96)*
	Cocos	WCOC1	480	0.94 ± 0.17	111.79 (62.93-338.55)	1.17	6	0.98	32.03 (9.66-.128.28)*
	Cocos	WCOC2	480	1.17 ± 0.14	14.96 (11.86-19.17)	7.22	6	0.30	4.29 (2.28-9.74)*
	Luiz Eduardo Magalhães	WLEM1	480	0.82 ± 0.13	9.13 (6.25-12.61)	2.09	6	0.91	2.62 (1.07-7.75)*
	Luiz Eduardo Magalhães	WLEM2	480	0.61 ± 0.14	113.31 (53.11-700.41)	2.36	6	0.88	32.47 (3.95-322.72)*
	Luiz Eduardo Magalhães	WLEM3	480	1.28 ± 0.17	46.47 (34.51-72.35)	2.22	6	0.89	13.32 (2.95-72.57)*
	Luiz Eduardo Magalhães	WLEM4	480	1.01 ± 0.16	70.06 (45.25-148.93)	1.70	6	0.95	20.08 (7.43-65.54)*
	Luiz Eduardo Magalhães	WLEM5	480	0.97 ± 0.14	35.50 (25.48-58.79)	6.69	6	0.35	10.17 (4.04-30.95)*
	São Desiderio	WSDE1	480	1.53± 0.16	23.13 (19.11-28.99)	10.07	6	0.12	6.63 (3.86-13.75)*
	São Desiderio	WSDE2	480	0.92 ± 0.14	38.61 (29.94-68.28)	2.69	6	0.84	11.06 (4.03-36.68)*
	São Desiderio	WSDE3	480	1.12 ± 0.14	24.71 (19.13-34.45)	1.81	6	0.94	7.08 (3.46-17.51)*
	São Desiderio	WSDE4	480	1.00 ± 0.13	5.72 (3.87-7.64)	2.60	6	0.86	1.64 (0.87-3.71)
	São Desiderio	WSDE5	480	1.28 ± 0.14	8.34 (6.50-10.40)	0.45	6	0.99	2.39 (1.39-4.98)*
	São Desiderio	WSDE6	480	1.12 ± 0.15	38.19 (28.26-59.52)	3.92	6	0.69	10.94 (5.00-28.93)*
Highlands	Barra da Estiva	HBES1	480	1.17 ± 0.15	3.18 (2.02-4.35)	0.54	6	0.99	1.00 (0.49-2.03)
	Barra da Estiva	HBES2	480	1.39 ± 0.15	3.58 (2.53-4.63)	1.74	6	0.94	1.03 (0.60-2.12)
	Barra da Estiva	HBES3	480	1.07 ± 0.14	4.06 (2.63-5.52)	2.77	6	0.84	1.16 (0.65-2.52)
	Barra do Choça	HBCH1	480	0.76 ± 0.13	30.70 (20.85-57.26)	2.65	6	0.85	8.80 (2.224-42.10)*
	Barra do Choça	HBCH2	480	0.97 ± 0.13	24.81 (18.49-37.05)	1.73	6	0.94	7.11 (2.98-20.47)*
	Barra do Choça	HBCH3	480	0.80 ± 0.13	21.26 (15.18-33.65)	0.55	6	0.99	6.09 (2.00-22.44)*
	Barra do Choça	HBCH4	480	1.01 ± 0.15	48.63 (33.66-87.69)	2.65	6	0.85	13.93 (5.53-42.36)*
	Barra do Choça	HBCH5	480	1.04 ± 0.16	64.75 (42.96-128.84)	0.81	6	0.99	18.55 (7.25-57.31)*
	Barra do Choça	HBCH6	480	1.03 ± 0.14	26.70 (20.13-39.27)	1.75	6	0.94	7.65 (3.42-20.69)*
	Barra do Choça	HBCH7	480	1.24 ± 0.14	17.47 (14.02-22.40)	1.42	6	0.96	5.01 (2.72-11.12)*
	Barra do Choça	HBCH8	480	1.07 ± 0.15	34.66 (25.72-53.40)	1.05	6	0.98	9.93 (4.48-26.60)*
	Encruzilhada	HENC1	480	1.57 ± 0.17	34.34 (27.68-45.68)	1.16	6	0.98	9.84 (5.57-37.74)*
	Encruzilhada	HENC2	480	1.28 ± 0.17	52.40 (38.20-85.05)	0.99	6	0.99	15.01 (7.21-8.71)*
	Encruzilhada	HENC3	480	1.34 ± 0.14	14.41 (11.71-17.87)	1.66	6	0.95	4.13 (2.36-8.71)*
	Mucugê	HMUC1	480	1.72 ± 0.17	24.71 (20.71-30.47)	3.56	6	0.73	7.78 (4.25-14.26)*
	Mucugê	HMUC2	480	0.74 ± 0.13	5.18 (2.84-7.60)	0.88	6	0.98	1.63 (0.61-4.39)
	Mucugê	HMUC3	480	0.65 ± 0.13	41.06 (25.25-104.01)	0.85	6	0.17	12.93 (2.16-77.21)*
	Mucugê	HMUC4	480	0.99 ± 0.14	31.08 (22.79-48.75)	3.26	6	0.77	8.78 (1.08-88.79)*
	Vitória da Conquista	HVDC1	480	0.98 ± 0.14	3.49 (2.04-4.95)	9.48	6	0.15	1.00 (0.53-2.28)
	Vitória da Conquista	HVDC2	480	0.56 ± 0.13	45.68 (25.68-166.03)	2.79	6	0.83	13.09 (1.85-111.94)*
	Vitória da Conquista	HVDC3	480	0.90 ± 0.13	11.59 (8.43-16.33)	3.38	6	0.76	3.32 (1.38-10.85)*
	Vitória da Conquista	HVDC4	480	0.90 ± 0.13	8.57 (6.00-11.52)	2.17	6	0.90	2.45 (1.13-6.46)*
	Vitória da Conquista	HVDC5	480	0.51 ± 0.05	23.89 (15.30-49.73)	3.35	6	0.76	6.84 (1.10-51.51)*

The asterisk in the resistance ratio indicate a significant difference from the standard susceptible population based on Robertson et al. (2007).

Table 3. Estimated chlorantraniliprole mortality (%) and control failure likelihood (%) of populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*) using Brazilian recommended label rates.

Meso-region	County	Code	No.	Mortality [control failure likelihood] (%)
West	Barreiras	WBAR1	60	69.0 [13.7]*
	Barreiras	WBAR2	60	52.8 [34.0]*
	Barreiras	WBAR3	60	56.4 [29.5]*
	Barreiras	WBAR4	60	61.8 [22.7]*
	Cocos	WCOC1	60	40.0 [50.0]*
	Cocos	WCOC2	60	76.3 [4.6]
	Luiz Eduardo Magalhães	WLEM1	60	78.3 [2.1]
	Luiz Eduardo Magalhães	WLEM2	60	52.8 [34.0]*
	Luiz Eduardo Magalhães	WLEM3	60	61.8 [22.8]*
	Luiz Eduardo Magalhães	WLEM4	60	52.8 [34.0]*
	Luiz Eduardo Magalhães	WLEM5	60	60.0 [25.0]*
	São Desiderio	WSDE1	60	67.3 [15.9]*
	São Desiderio	WSDE2	60	54.5 [31.9]*
	São Desiderio	WSDE3	60	69.0 [13.7]*
	São Desiderio	WSDE4	60	85.4 [0.0]
	São Desiderio	WSDE5	60	90.8 [0.0]
	São Desiderio	WSDE6	60	52.7 [34.1]*
Highlands	Barra da Estiva	HBES1	60	91.7 [0.0]
	Barra da Estiva	HBES2	60	100.0 [0.0]
	Barra da Estiva	HBES3	60	96.3 [0.0]
	Barra do Choça	HBCH1	60	63.6 [20.5]*
	Barra do Choça	HBCH2	60	70.1 [12.4]*
	Barra do Choça	HBCH3	60	63.6 [20.5]*
	Barra do Choça	HBCH4	60	58.1 [27.4]*
	Barra do Choça	HBCH5	60	49.1 [38.6]*
	Barra do Choça	HBCH6	60	61.8 [22.8]*
	Barra do Choça	HBCH7	60	76.3 [4.6]
	Barra do Choça	HBCH8	60	60.0 [25.0]*
	Encruzilhada	HENC1	60	65.4 [18.3]*
	Encruzilhada	HENC2	60	52.7 [34.1]*
	Encruzilhada	HENC3	60	78.3 [2.1]
	Mucugê	HMUC1	60	78.3 [2.13]*
	Mucugê	HMUC2	60	87.2 [0.0]
	Mucugê	HMUC3	60	58.1 [27.37]*
	Mucugê	HMUC4	60	65.4 [18.3]*
	Vitória da Conquista	HVDC1	60	91.7 [0.0]
	Vitória da Conquista	HVDC2	60	54.5 [31.9]*
	Vitória da Conquista	HVDC3	60	74.5 [6.9]*
	Vitória da Conquista	HVDC4	60	81.8 [0.0]*
	Vitória da Conquista	HVDC5	60	67.3 [15.9]*

Mortalities followed by an asterisk are significantly lower than the minimum efficacy threshold of 80% (one-sided Z-test at 95% confidence level with correction for continuity and Bonferroni correction; n = 120), as required by Brazilian legislation (MAPA 1995).

Relationship Between Resistance and the Likelihood of Control Failure

The relationship between chlorantraniliprole resistance and control failure likelihood was tested using regression analysis with the former trait as the independent variable determining the latter. The relationship was significant, with the level of chlorantraniliprole resistance largely determining the likelihood of control failure with this insecticide (Fig. 2). The likelihood of control failure with chlorantraniliprole increases with the level of resistance to this insecticide (Fig. 2).

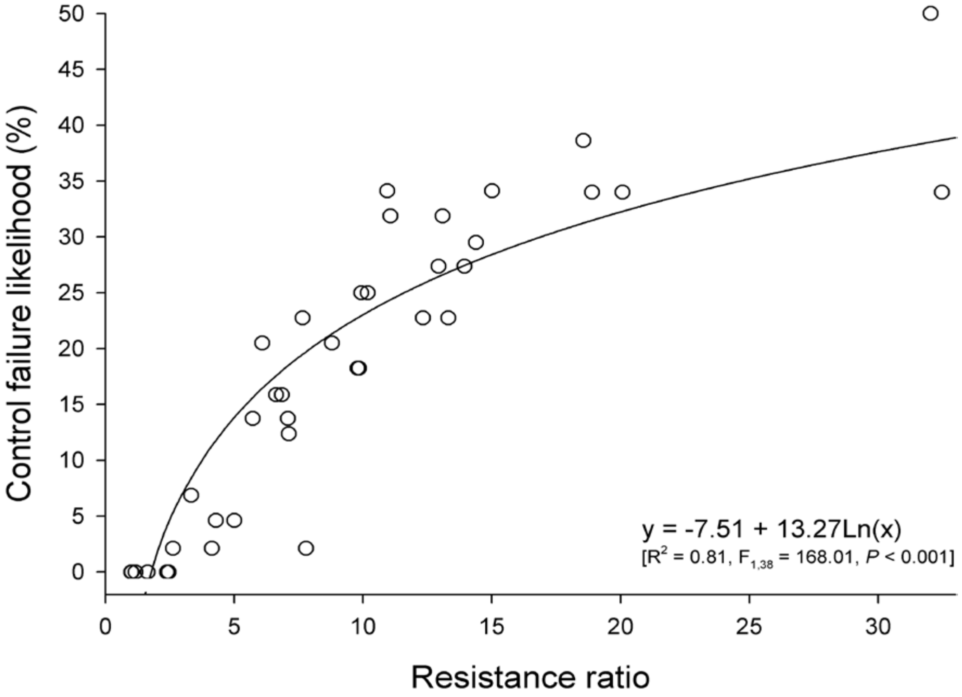

Fig. 2. The relationship between chlorantraniliprole resistance and control failure likelihood. The symbols indicate the observed data.

Spatial Dependence

The relatively large variation in chlorantraniliprole resistance, efficacy, and control failure likelihood is suggestive of county-wide variation in these traits; thus, spatial dependence is a potential characteristic that allows geographical mapping of the phenomenon if significant and suitable models are identified for extrapolation. The number of sampling sites from each region was limited and required the use of cokriging for meaningful estimates. This was carried out in two separate regions-one encompassing the sampling sites of the northern counties of western Bahia (except Cocos), and another encompassing the highland sampling sites.

The best semivariogram models obtained from the results of chlorantraniliprole resistance and control failure likelihood are exhibited in Table 4 together with their respective parameters for model selection. The nugget (*Co*) values of zero and partial sill (*C*) around the value of one allowed robust estimates with spatial dependence reaching distances <500 m (Table 4). The model parameters and mean errors obtained allowed distance interpolation and subsequent mapping of chlorantraniliprole resistance ratio and control failure likelihood.

The mapping of chlorantraniliprole resistance indicates a scenario provoking more concern in western Bahia than in the highlands with higher within-county variability (Fig. 3), although the later exhibited lower distance of interference between sampling sites (Table 4). This was translated into the likelihood of control failure with this insecticide (Fig. 4). The range of variability was smaller when control failure was considered, but western Bahia exhibited higher variation and higher risks of control failure; however, the risks were localized (Fig. 4).

Discussion

Insecticide resistance is a genetic change in response to selection that may compromise insecticide efficacy leading to control failure (Guedes 2017). The concepts of insecticide resistance, efficacy and control failure are interdependent although distinct, since the former is not always the underlying cause of the latter two (Tabashnik et al. 2014, Guedes 2017). Such distinction is seldom recognized, and control failure is usually assumed when insecticide resistance is detected. However, a recent shift in this trend seems to be taking place based on recent studies with the tomato leaf miner *Tuta absoluta*, the putative whitefly species MEAM1, and the Neotropical brown stink bug *Euchistus heros* (Gontijo et al. 2013, Roditakis et al. 2013, Silva et al. 2015, Dangelo et al. 2018, Tuelher et al. 2018, Guedes et al. 2019). Such studies were able to recognize insecticide resistance as the determinant cause of insecticide control failures of these pest species (Gontijo et al. 2013, Roditakis et al. 2013, Silva et al. 2015, Dangelo et al. 2018, Tuelher et al. 2018).

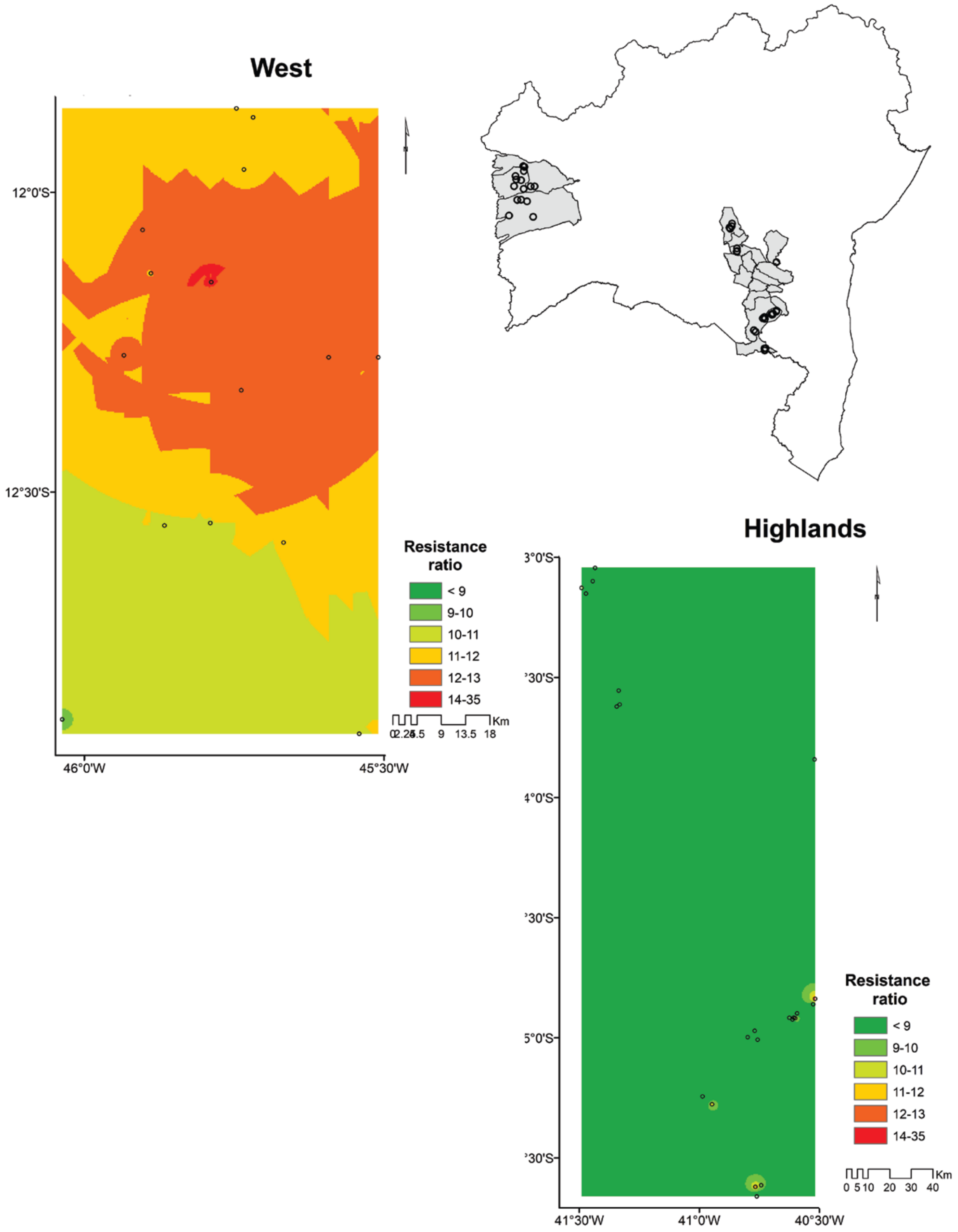

Fig. 3. Contour maps of the levels of chlorantraniliprole resistance in populations of the Neotropical coffee leaf miner *Leucoptera coffeella*. The maps were generated using spatial interpolation. The color legend indicates the represented range of resistance ratios of the coffee leaf miner.

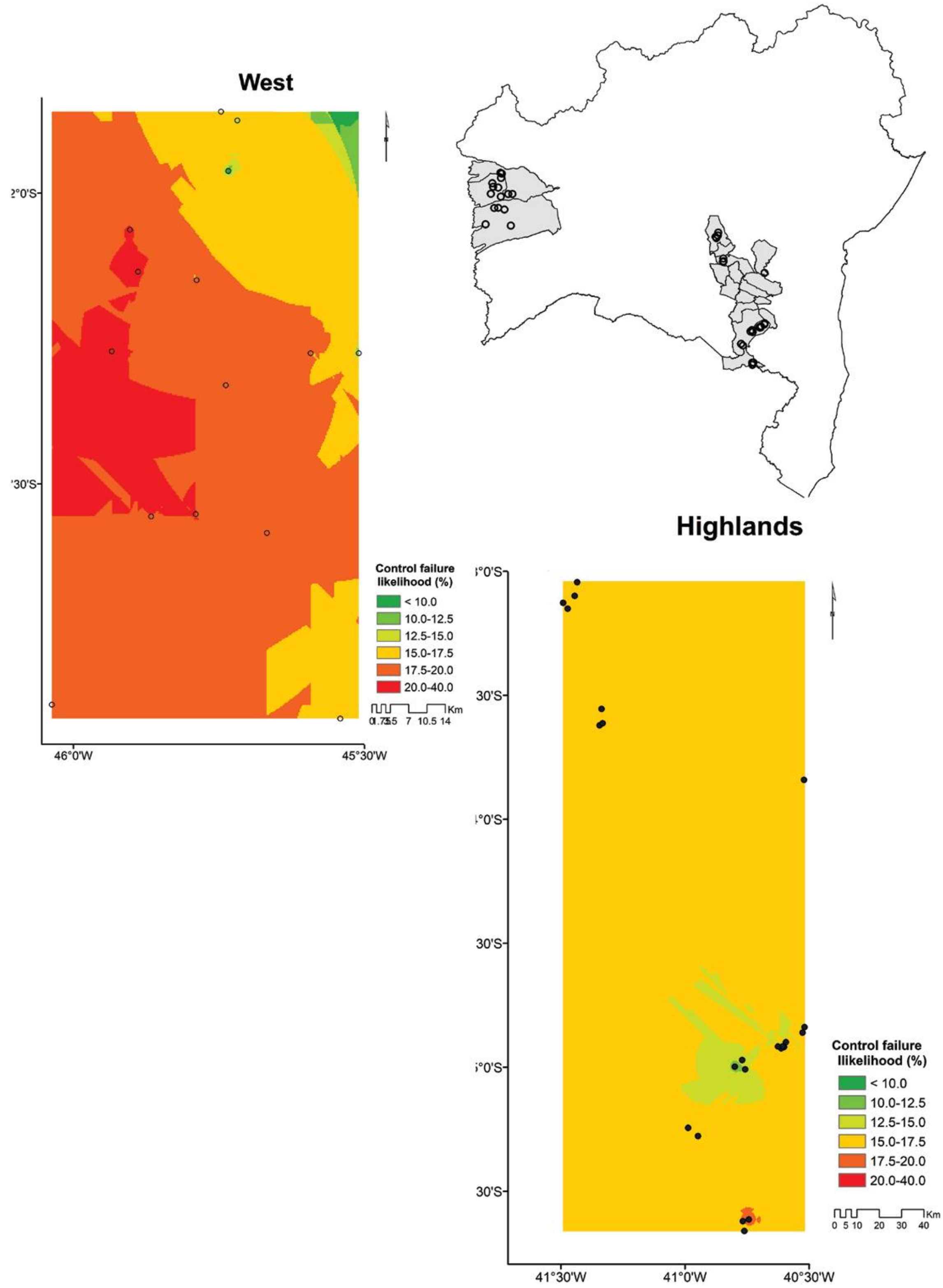

Fig. 4. Contour maps of the control failure likelihood of chlorantraniliprole used against populations of the Neotropical coffee leaf miner *Leucoptera coffeella*. The maps were generated using spatial interpolation. The color legend indicates the represented range of resistance ratios of the coffee leaf miner.

Table 4. Semivariogram models and parameters of chlorantraniliprole resistance and control failure likelihood in populations of the Neotropical coffee leaf miner *Leucoptera coffeella*.

Response	Region	Cokriging	Model	Nugget (C_0)	Partial sill (C)	Sill ($C + C_0$)	Proportion ($C/C+C_0$)	Range (h_r, m)	Randomness (C_0/C)	Mean errors	Slope	Intercept
Resistance ratio	West	Simple	K-Bessel	0	1.1868	1.19	1	0.40	0	0.056	0.81	2.21
	Highlands	Simple	K-Bessel	0	1.0415	1.04	1	0.20	0	-0.013	0.94	0.50
Control failure Likelihood	West	Simple	K-Bessel	0	1.1681	1.17	1	0.40	0	0.073	0.72	4.76
	Highlands	Simple	K-Bessel	0	1.3705	1.37	1	0.06	0	-0.530	1.02	0.81

Insecticide resistance in the Neotropical coffee leaf miner *L. coffeella* has been hardly studied, which is limited to three surveys (Alves et al. 1992, Fragoso et al. 2003, Costa et al. 2016). These surveys indicate that the phenomenon may be frequent in this species and is likely to result in control failures, as particularly indicated by the high levels of organophosphate resistance (>1,000-fold) among leaf miner populations (Fragoso et al. 2003). The relatively recent increase and spread in the use of the diamide insecticide chlorantraniliprole against the coffee leaf miner suggests the potential emergence of resistant populations, which are targeted in the present study. We aimed 1) to survey the incidence of chlorantraniliprole resistance; 2) to assess the control failure likelihood of chlorantraniliprole due to this phenomenon; and 3) to test if spatial dependence exists for these traits. All these objectives were achieved, although some contrasted with our earlier expectations of a limited occurrence of chlorantraniliprole resistance, a low expectation of control failure and a lack of spatial dependence.

Incidence of insecticide resistance is usually low for recently used insecticides because the results of selection for the phenomenon usually takes a few years to manifest (Roush and McKenzie 1987, McKenzie 1996, Whalon et al. 2008, Sparks and Nauen 2015), but exceptions do exist including for diamide resistance (e.g., Troczka et al. 2017). Despite reported exceptions in different species, the more general expectation of a longer period for insecticide resistance to evolve prevails. Therefore, chlorantraniliprole resistance in the Neotropical coffee leaf miner was expected to be limited and in its initial stages. Nonetheless, the incidence of this phenomenon was high in the region under investigation, with 85% of the insect populations exhibiting chlorantraniliprole resistance. Curiously, the levels of resistance were low to moderate, although reaching levels over 30-fold in some instances, particularly in western Bahia. The widespread and intensive use of chlorantraniliprole in the region is the likely reason for the high incidence of resistance to this compound among the insect populations sampled and tested. However, the evolution of this phenomenon is still in its early stages at most sites, as the levels of resistance detected did not reach high levels (>100-fold), but remained below the 40-fold threshold.

The levels of chlorantraniliprole resistance detected in the coffee leaf miner may not be high enough to compromise this insecticide's efficacy but that requires the testing and proper estimation provided by the present study. Efficacy was indeed compromised considering the levels of chlorantraniliprole resistance observed and the risk of control failure does already exist in the region. Nonetheless, the risk is significant although reduced in most of the tested populations. Instances of 30–50% risk of control failure exist and are distributed through most of the counties sampled. They are frequently located side-by-side with sites exhibiting negligible risk of control failure, limiting the range of spatial dependence for the recorded traits. The situation appears to be more serious in western Bahia, but both regions exhibit the reported pattern and control concern. The recognition of the potential spatial dependence of insecticide resistance and control failure likelihood is important for scaling up the required resistance management effort, sustaining the potential use of chlorantraniliprole as a management tool against the coffee leaf miner.

The notion that spatial proximity favors resemblance is rather intuitive and widespread. Surveys of insecticide resistance assume this relationship, which is usually not tested despite its importance in determining the scale and scope of resistance management programs. Thus, the scale of management programs, whether local, microregional, meso-regional, or even country-wide, is not recognized as a factor compromising their potential efficacy (Guedes 2017). The number of sampling sites and populations tested in our survey of the coffee leaf miner may potentially allow recognition and possibly mapping of chlorantraniliprole resistance and control failure likelihood. However, the samples were not established a priori for such a purpose, imposing limitations on the effort. Cokringing with a secondary trait (i.e., chlorantraniliprole efficacy) allowed suficiente resolution to recognize that spatial dependence does exist for the traits assessed, considering the scale of our study, encompassing a few counties in western Bahia and the south-central highlands. The scale of spatial dependence is restricted, not spanning more than half a kilometer. Variation is smaller for the control failure likelihood, a consequence of the relatively low ranges involved, except for two instances in western Bahia.

These findings are important for managing the coffee leaf miner. Despite its relative recent use, chlorantraniliprole already exhibits significant and widespread problems of resistance in both regions, especially in western Bahia. However, the levels of resistance detected are low to moderate, reaching 30-fold in few instances. The problem is still recent and allows for proper resistance management to slow or even prevent further exacerbation. The levels of resistance detected are already in a range that compromises chlorantraniliprole, with estimated risks of control failure <30% in most instances, but reaching the 50% threshold at a site in Cocos County in western Bahia. Nonetheless, spatial dependence is limited to a small scale, allowing the design of resistance management practices at a local (farm) level (Guedes 2017). Despite previous problems with resistance to organophosphates and emerging problems with neonicotinoids, alternative insecticides with distinct modes of action and prevailing detoxification mechanisms are still available, among which azadirachtin, pyrethroids, spynosins, and growth regulators are promising alternatives for rotation at the farm level (Spark and Nauen 2015, MAPA 2019).

In summary, chlorantraniliprole resistance is already widespread among the Neotropical coffee leaf miners in western and southcentral Bahia (Brazil). The resistance levels are low to moderate but are already leading to reduced efficacy and significant risk of control failure, demanding resistance management practices. Among these, replacement and rotation of alternative insecticides with distinct underlying mechanisms of resistance are sound practices for use at the local scale, and they are likely to extend the potential use of diamides against this species not only in this region, but also elsewhere as well.

Acknowledgments

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Periodíco Científico: Agricultural and Forest Entomology – A2

ARTICLE III

Time-concentration interplay in insecticide resistance among populations of the Neotropical coffee leaf miner, *Leucoptera coffeella*[*]

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[*] **Situation:** In press.

Agricultural and Forest Entomology
Original Research Paper

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Time-concentration interplay in insecticide resistance among populations of the Neotropical coffee leaf miner, *Leucoptera coffeella*

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Short title: Insecticide resistance of *L. coffeella*

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ABSTRACT

1 *Leucoptera coffeella* is one of the main coffee pests in the Neotropical region and particularly important in crops cultivated under full sun, as in Brazil. The intensive use of insecticides in the last two decades, including molecules of recent use such as chlorantranilprole, has not been successful in suppressing this pest species.

2 Thus, study aimed to detect insecticide resistance and determine the levels of resistance to commercial formulations of the insecticides chlorantraniliprole, chlorpyrifos and thiamethoxam in populations of *L. coffeella* from Arabica coffee fields in western and the highlands of the state Bahia (Brazil). Furthermore, as the expression of insecticide toxicity varies with the length of exposure and the compound concentration, what is frequently neglected, these two variables were considered while determining the levels of resistance in nine populations.

3 Moderate levels of resistance were detected to chlorantranilprole in two populations, while the others exhibited low levels of resistance. All populations were resistant to thiamethoxam ranging from low to moderate levels. Low levels of resistance to chlorpyrifos were detected in all populations from western, but none from highlands region.

4 The western presents a more concerning scenario of insecticide resistance the *L. coffeella*, but the phenomenon requires attention in both regions.

Key words: chemical control, chlorantraniliprole, chlorpyrifos, *Coffea arabica*, resistance ratio, thiamethoxam

1. Introduction

Pesticides, particularly insecticides to reduce populations of pest species that reach high infestation levels, are tools of great importance for agriculture. Despite this, or even for this reason, the application of insecticides to control pest insects has caused biological imbalances in populations and communities of arthropods, mainly related to ecological and physiological changes in their behavior, triggered by the use of pesticides (Guedes *et al*., 2016, 2017).

In the context of globalized agriculture of highly productive crops, Brazil stands out in the production of commodities of great importance, such as coffee, topping as the world's largest producer and exporter (Mapa, 2019). Among insect species that cause economic damage to coffee production, the Neotropical coffee leaf miner [*Leucoptera coffeella* (Guérin-Méneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae)] stands out as one of the main coffee pest species in the Neotropical region, especially under conditions of cultivation in full sun, as in Brazil (Tuelher *et al.*, 2003; Pereira *et al.*, 2007a,b; Magalhães *et al*., 2010; Pantoja-Gomez *et al*., 2019).

High infestation rates of *L. coffeella* can cause production losses above 50% in Brazil (Ramiro *et al*., 2004). The increase in the infestation levels of this pest has caused significant production losses, resulting in the intensive use of pesticides reaching up to up to 20 applications per year (Leite *et al*., 2020a). Neurotoxic insecticides are the most used by coffee growers, mainly organophosphates, carbamates, pyrethroids and neonicotinoids, which are more persistent in the environment, in addition to diamides, the most recent group of insecticides used against *L. coffeella* (Costa *et al*., 2016).

Resistance to organophosphates in *L. coffeella* populations in Brazil was reported by early the 1990s and more recent surveys (Alves *et al*., 1992; Fragoso *et al*., 2002, 2003; Ribeiro *et al*., 2003; Costa *et al*., 2016). Reports of resistance to neonicotinoids (thiamethoxam) and diamides (chlorantraniliprole) are recent in *L. coffeella* populations, but detected in different coffee-producing states (Costa *et al*., 2016; Leite *et al*., 2020b). However, there was diversification in the use of insecticides in the last decade to control the coffee leaf miner, mainly with increased frequency of the use of diamides and neonicotinoids. Regarding chlorantraniliprole, leaf miner resistance and the risk of control failure have already been confirmed in recent studies with the recommended label rate in the western and south-central regions of Bahia in Brazil (Leite *et al*., 2020b). However, the situation for other commonly-used insecticides remains unknown.

Recent studies indicate the impact of these compounds on the structure of agroecosystem populations and communities, in addition to the selection of resistant insects (Guedes *et al*., 2016). This is the case for the chlorantraniliprole diamide and its effects on populations of *Plutella xylostella* (Troczka *et al*., 2012; Wang & Wu 2012), *Tuta absoluta* (Roditakis *et al*., 2015; Grant *et al*., 2019; Silva *et al*., 2019), *Chilo suppressalis* (Lu *et al*., 2017; Wei *et al*., 2019), *Bemisia tabaci* (Dângelo *et al*., 2018) and *Spodoptera frugipera* (Boaventura *et al*., 2019; Bolzan *et al*., 2019).

For the coffee leaf miner, the existence of populations resistant to chlorantraniliprole was recently detected in the state of Bahia in Brazil with 84% incidence of resistance among the populations surveyed (Leite *et al*., 2020b). The intensive use of insecticides leads to concerns about the development of insecticide resistance in populations of this pest species, in addition to favoring the reproduction and survival of individuals in the field through the use of sublethal concentrations, influencing the selection of individuals through additional phenomena such as induced hormesis and cross-induction of enzymes by metabolic detoxification (Guedes *et al*., 2017).

In this context, the aims of the present study were: 1) to detect resistance and to determine the levels of resistance to the diamide chlorantraniliprole, the organophosphate chlorpyrifos and the neonicotinoid thiamethoxam using time-mortality bioassays in field-collected populations of *Leucoptera coffeella* from western and south-central Bahia; 2) to assess the variation in resistance levels with the concentration of the insecticide, what is seldom considered, but it is relevant in the expression of the phenomenon as insecticdes degrade with time under field conditions, but retain enough selection pressure for reistance even with the its reduction (Gressel, 2011; Guedes *et al*., 2017).

2. Material and Methods

2.1 *Insects and insecticides*

Leucoptera coffeella populations were collected from properties located in the western (Barreiras and Luís Eduardo Magalhães) and south-central highland regions of Bahia (Barra Choça, Mucugê and Vitória da Conquista), which are important and representative coffee-producing regions of Brazil. Nine field-populations from intensive coffee-producing field with frequent insecticide application were used in addition to a susceptible standard population from a property not subjected to insecticide use for at least 10 years (Table 1, Figure 1).

Leaves with intact mines (without signs of predation or parasitism) containing live larvae and chrysalis were collected and stored in Kraft-type paper bags (2kg) (17cm x 45cm), and transported in polystyrene box to the laboratory. Leaves were placed in plastic cages (2 L capacity) until the emergence of adults, which were released in wooden cages (2x2x2 m) covered by organza fabric and containing coffee seedlings (cv. 'Catuaí 144').

The insecticides and respective registered commercial formulations used were, as follows: chlorantraniliprole (350 g i.a. kg^{-1}, water-dispersible granules, DuPont, Paulínia, SP, Brazil), chlorpyrifos (480 g i.a. L^{-1}, emulsifiable concentrate, Nufarm, Maracanaú, CE, Brazil) and thiamethoxam (250 g i.a. kg^{-1}, water-dispersible granules, Syngenta, São Paulo, SP, Brazil) (Mapa 2020).

2.2 *Time-mortality bioassays under increasing insecticide concentrations*

Time-mortality bioassays were performed using methods adapted from Fragoso *et al*. (2002) and Leite *et al*. (2020) with the exposure of larva to filter paper impregnated with dry insecticide residues, as earlier developed for the tomato leaf miner *Tuta absoluta* (Siqueira *et al*., 2000, 2001). Five concentration ranges were used for commercial formulations of thiamethoxam (0.67; 1.34; 2.00; 2.67 and 3.34 mg a.i. mL^{-1}), chlorantraniliprole (0.026; 0.052; 0.078; 0.105 and 0.131 mg a.i. mL^{-1}) and chlorpyrifos (2.4; 4.8; 7.2; 9.6 and 12.0 mg a.i. mL^{-1}); the exposure times tested were 2, 4, 6, 12, 18, 24, 36 and 48 hours.

Filter paper discs (9.0 cm in diameter) were immersed in insecticide solution for 10 s and allowed to dry for 1 h at room temperature, after which they were subsequently placed in Petri dishes (9.0 cm in diameter x 1.5 cm in height). Twenty 3rd instar larvae were removed from leaves and placed in each Petri dish using a fine-tipped brush and kept in ambient chamber under controlled conditions of 25 ± 2°C of temperature and 70 ± 5% of relative humidity. The experiment was replicated three times for each exposure time, insecticide concentration and insect population. Larval mortality was recognized by the insect's inability to move its body length when prodded with a fine-tipped brush. Time-response curves were estimated for each concentration

2.3 *Statistical analysis*

Time-morality data was subjected to probit analyses (PROC PROBIT; SAS Institute, SAS, 2011). The levels of resistance to insecticides, or resistance ratios, were estimated by dividing the median lethal time (LT_{50}) of a given population by the LT_{50} of the susceptible population. Significant resistance to chlorantraniliprole, thiamethoxam and chlorpyrifos was recognized by estimating 95% confidence intervals of resistance ratios, which were identified as significant if they did not include the value of 1 (Robertson *et al*., 2007).

The resistance ratio estimates at LT_{50} were subjected to regression analysis using insecticide concentration as independent variable. Model selection was based on parsimony, high F values (reduced error) and R^2 (accentuated error), increasing complexity using the software TableCurve 2D (Systat, San Jose, CA, USA 2002).

3. Results

3.1 *Insecticide resistance*

The time-mortality data for each leaf miner population were subjected to probit analyses. The low $\chi 2$ and *P* values (>0.05) obtained indicate the suitability of the adjusted probit model, allowing the estimates of the desired toxicological endpoints, and particularly the median lethal time (LT_{50}).

The frequency of resistance in populations of the coffee leaf miner to chlorpyrifos (3 in 10 populations), chlorantraniliprole (8 in 10 populations) and thiamethoxam (9 in 10 populations) diverged between insecticides, concentration, lethal time and region. All populations in western Bahia were resistant to chlorantranilprole, thiamethoxam and chlorpyrifos. In the south-central highlands, all populations were resistant to thiamethoxam, and all were susceptible to chlorpyrifos, except one from Barra do Choça (BCH2) that was also susceptible to chlorantraniliprole (Tables 2, 3 and 4).

Chlorpyrifos resistance was low in three populations (BAR1, LEM1 and LEM2) from western Bahia with the LEM2 population exhibiting resistance at four concentrations, but no resistance was detected at the recommended label rate (7.2 mg a.i. mL^{-1}). All populations from the south-central highlands were susceptible to chlorpyrifos (Table 2).

Moderate levels of resistance to chlorantraliniprole (> 10x and < 100x) were detected in the MUC1 (Mucugê; south-central highlands) and in the LEM1 population (Luís Eduardo Magalhães). Low levels of resistance (<10 times) were observed in six populations, including BAR1, which exhibited resistant in all tested concentrations (Table 3). Susceptibility was detected only in the BCH2 population from the south-central highlands.

Moderate levels of resistance to thiamethoxam were detected in LEM1 (Luís Eduardo Magalhães) at three concentrations (0.67, 1.34 and 2.0 mg a.i. mL^{-1}), and particularly at 1.34 mg a.i. mL^{-1} (75.20 times RR). Low levels of resistance were observed in seven populations (70%) at concentrations of 0.67 and 1.34 mg a.i. mL^{-1}. However, for BAR1 levels of resistance were low at all concentrations except 3.34 mg a.i. mL^{-1}, which exhibited moderate level of resistance (Table 4).

3.2 *Relationship between insecticide concentrations and resistance ratios*

The relationship between concentration of chlorantraniliprole, thiamethoxam and chlorpyrifos and resistance ratios (LT_{50}) was tested using regression analyses with insecticide concentrations as independent variable. The range of variation in chlorpyrifos resistance was smaller than that of thiamethoxam, with intermediate values for chlorantraniliprole.

Relationships were significant ($P < 0.05$) in four populations for chlorpyrifos (Figure 2), in five populations for chlorantraniliprole (Figure 3); and in two populations for thiamethoxam (Figure 4). The resistance ratio tended to decrease with increasing concentrations in most cases. The trend was more homogeneous for chlorpyrifos (Figure 2). The increase in the concentration of insecticides determines a lower likelihood of higher levels of resistance to insecticides.

4. Discussion

The aim of this study was to quantify the levels of resistance to chlorantraniliprole, thiamethoxam and chlorpyrifos insecticides considering both exposure time and insecticide concentrations to which the insects were exposed. The aims were achieved indicating to existing resistance chlorantraniliprole, thiamethoxam and chlorpyrifos among coffee leaf miner populations from western and south-central highlands of Bahia.

The emergence of resistant populations in coffee-producing regions of the state of Bahia is due to the intensive use of insecticides against the coffee leaf miner, with annual applications frequency varying between 17 and 20 (Castellani *et al*., 2016; Leite *et al*., 2020a). As a likely consequence, low and moderate levels of resistance, reduced efficacy, and risk of control failure of *L. coffeella* with chlorantraniliprole were already detected in Bahia (Leite *et al*., 2020). Our results reinforce this notion.

There are few studies of insecticide resistance in the Neotropical coffee leaf miner (Alves *et al*., 1992; Fragoso *et al*., 2002, 2003; Costa *et al*., 2016). The chemical group most used against this pest species is organophosphates, consequently this was the first group to exhibit resistance in populations of the coffee leaf miner (Alves *et. al*, 1992). High levels of resistance to organophosphates have been detected subsequently in one of the main coffee producing areas in the country, the savannah-like region of Minas Gerais (Fragoso *et al*., 2002; Costa *et al*., 2016). However, the intensive use of insecticidal molecules such as the neonicotinoid thiamethoxam and diamide chlorantraniliprole of more recent use against lepidopteran pest species (Lahm *et al*., 2005; Mapa, 2020), increased the potential for emergence resistance to these insecticides.

The detection of resistant populations is not strictly related to the use of organophosphates, insecticides commonly used for *L. coffeella* control. Only populations located in the western region of Bahia were resistant to chlorpyrifos. Therefore, the phenomenon of resistance of *L. coffeella* is not limited to a particular insecticide and region. The incidence is high for other insecticides with 80% of the insect populations exhibiting resistance to chlorantraniliprole and 90% to thiamethoxam. Exceptionally, a population of Barra do Choça (BCH2) located in the south-central highlands of Bahia was susceptible to chlorantraniliprole. The widespread and intensive use of the most recent insecticides in coffee fields, namely chlorantraniliprole and thiamethoxam, is the likely reason for the resistance profile observed for *L. coffeella* populations in our study.

Recently, moderate levels of resistance to thiamethoxam and low levels to chlorantraniliprole were detected (Costa *et al.*, 2016). Moderate levels of chlorantraniliprole resistance was detected in Luís Eduardo Magalhães (LEM1) in western Bahia, reinforcing the perception that the high use of this compound led to the current situation in recent years evoking concern and changes for in this species management (Leite *et al.*, 2020b).

Low levels of resistance prevail in most populations of the coffee leaf miner. Yet, the phenomenon was significant and relatively frequent among the leaf miner populations surveyed, particularly in western Bahia. Furthermore, the expression of resistance was relatively consistent at different concentration ranges and particularly so for chlorpyrifos. Such variation was steeper for chlorantraniliprole, which sharply droped with increased concentrations suggesting a lower risk of field control failure, as the higher field-concentrations used remain effective for longer. Thiamethoxam provides a contrasting scenario where increased concentration did not affect the levels of resistance to this compound in most instances, what is an added concern for the likelihood of field control failure with this compound.

The phenomenon of insect resistance to thiamethoxam and chlorantraniliprole insecticides is relatively recent, occurring in several pest species including *Plutella xylostella;* (Troczka *et al*. 2012; Wang & Wu, 2012; Ribeiro *et al.*, 2014); *Tuta absoluta* (Roditakis *et al.*, 2015; Grant *et al.*, 2019); *Chilo suppressalis* (Lu *et al.*, 2017; Wei *et al.*, 2019); *Bemisia tabaci* (Feng *et al.*, 2009; Dângelo *et al.*, 2018); *Spodoptera frugipera* (Boaventura *et al.*, 2019; Bolzan *et al.*, 2019). Furthermore, insecticide resistance compromises the insecticide effectiveness resulting in control failure, as already recognized for the tomato pinworm *Tuta absoluta* (Gontijo *et al.*, 2013; Roditakis *et al.*, 2018), the white fly *Bemisia tabaci* (MEAM1; B biotype) (Dângelo *et al.*, 2018), the Neotropical brown stink bug *Euschistus heros* (Tuelher *et al*., 2018), and more recently for the Neotropical coffee leaf miner *L. coffeella* (Leite *et al*., 2020b).

Understanding and determining the levels of resistance in field populations is essential to designing resistance management strategies (Roush & McKenzie, 1987). Thus, the information here gathered allowed recognition of insecticide resistance as a major problem in the coffee leaf miner and will direct future insecticide use against this species in the studied regions. Populations *L. coffeella* are resistant to chlorantraniliprole (western and south-central highlands), thiamethoxam (western and south-central highlands) and chlorpyrifos (western). The problem is recent and the levels of resistance detected range from low (<10x), whose incendence was more frequent, to moderate (< 10x, and <100x) to chlorantraniliprole and thiamethoxam insecticides, and low to chloripyrifos in western Bahia (Brazil).

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1 Identification and geographical coordinates of the sampling sites of the populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in our survey of chlorantraniliprole, thiamethoxam and chlorpyrifos resistance, in the state of Bahia, Brazil.

County	Code	Longitude	Latitude
Barra do Choça	BCH1	-14º50'27.5"	-40º31'13.0"
Barra do Choça	BCH2	-14º54'58.1"	-40º36'24.8"
Barra do Choça	BCH3	-14º50'15.9"	-40º31'04.4"
Barreiras	BAR1	-12°16'30.9"	-45º30'35.1"
Luís Eduardo Magalhães	LEM1	-12°16'19.4"	-45º56'02.6"
Luís Eduardo Magalhães	LEM2	-12°03'46.4"	-45º54'10.5"
Mucugê	MUC1	-13°02'38.8"	-41º26'02.4"
Mucugê	MUC2	-13°07'37.1"	-41º29'25.4"
Mucugê	MUC3	-13°07'53.0"	-41º28'16.1"
Vitória da Conquista (Susceptible)	VDC	-14º59'52.0"	-40º47'55.2"

Table 2 Relative toxicity of chlorpyrifos to populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*); the asterisk in the resistance ratio indicate significant difference from the standard susceptible population based on Robertson et al. (2007).

Chlorpyrifos concentration (mg a.i. mL^{-1})	County	Code	Slope ± SE	LT_{50} (95% FI) h	χ^2	df	*P*	Resistance ratio at LT_{50} [RR_{50} (95% CI)]
2.4	Luís Eduardo Magalhães	LEM2	1.52 ± 0.21	25.73 (20.64-33.78)	5.26	6	0.51	4.55 (2.63-7.89)*
	Barreiras	BAR1	1.66 ± 0.19	12.99 (10.54-15.76)	10.36	6	0.11	2.30 (1.54-3.44)*
	Luís Eduardo Magalhães	LEM1	1.78 ± 0.22	8.73 (6.67-10.93)	1.28	6	0.97	1.55 (1.04-2.30)*
	Mucugê	MUC2	1.99 ± 0.19	7.56 (6.16-9.00)	3.29	6	0.77	1.34 (0.97-1.85)
	Barra do Choça	BCH3	1.65 ± 0.30	6.94 (3.57-10.51)	11.61	6	0.07	1.23 (0.72-2.11)
	Mucugê	MUC3	2.37 ± 0.22	6.24 (5.15-7.34)	7.03	6	0.32	1.10 (0.81-1.51)
	Mucugê	MUC1	2.11 ± 0.20	5.78 (4.69-6.92)	4.20	6	0.65	1.03 (0.74-1.42)
	Barra do Choça	BCH1	1.67 ± 0.35	5.59 (2.23-9.06)	23.00	6	0.01	0.99 (0.54-1.82)
	Barra do Choça	BCH2	1.02 ± 0.21	4.37 (1.34-7.58)	12.00	6	0.06	0.77 (0.36-1.65)
	Vitória da Conquista	VDC	2.14 ± 0.20	5.65 (4.59-6.72)	6.84	6	0.34	1.00 (0.72-1.38)
4.8	Luís Eduardo Magalhães	LEM2	1.42 ± 0.18	15.19 (12.10-19.06)	4.10	6	0.66	2.69 (1.63-4.43)*
	Barreiras	BAR1	2.67 ± 0.35	11.92 (8.84-15.07)	11.11	6	0.09	2.11 (1.52-2.93)*
	Luís Eduardo Magalhães	LEM1	2.56 ± 0.22	10.70 (9.15-12.32)	10.16	6	0.11	1.89 (1.42-2.53)*
	Barra do Choça	BCH2	2.18 ± 0.36	7.85 (4.75-10.94)	10.80	6	0.09	1.39 (0.91-2.11)
	Barra do Choça	BCH1	1.36 ± 0.16	6.68 (4.95-8.47)	6.98	6	0.32	1.18 (0.77-1.82)
	Barra do Choça	BCH3	1.84 ± 0.30	5.54 (3.08-8.04)	15.27	6	0.02	0.98 (0.61-1.58)
	Mucugê	MUC3	1.62 ± 0.17	5.53 (4.21-8.25)	4.63	6	0.59	0.98 (0.67-1.42)
	Mucugê	MUC1	1.99 ± 0.19	5.42 (4.31-6.54)	6.02	6	0.42	0.96 (0.68-1.36)
	Mucugê	MUC2	1.78 ± 0.28	4.22 (2.26-6.17)	13.34	6	0.04	0.75 (0.45-1.25)
	Vitória da Conquista	VDC	2.14 ± 0.21	3.55 (2.76-4.33)	9.37	6	0.15	1.00 (0.63-1.58)
	Barreiras	BAR1	1.54 ± 0.18	12.25 (9.84-15.01)	7.54	6	0.27	2.17 (1.42-3.31)*
	Luís Eduardo Magalhães	LEM1	1.65 ± 0.17	11.41 (9.26-13.81)	6.23	6	0.40	2.02 (1.37-2.97)*
	Barra do Choça	BCH3	1.62 ± 0.17	5.28 (4.00-6.58)	6.62	6	0.36	0.94 (0.64-1.36)
	Mucugê	MUC2	1.58 ± 0.17	5.27 (3.97-6.59)	3.18	6	0.79	0.93 (0.64-1.37)

7.2	Mucugê	MUC3	1.47 ± 0.17	4.51 (3.25-5.79)	2.99	6	0.81	0.80 (0.53-1.21)
	Barra do Choça	BCH1	1.74 ± 0.18	4.32 (3.25-5.39)	3.64	6	0.73	0.77 (0.52-1.12)
	Mucugê	MUC1	1.71 ± 0.18	4.12 (3.07-5.17)	4.10	6	0.66	0.73 (0.49-1.09)
	Luís Eduardo Magalhães	LEM2	1.05 ± 0.15	4.05 (2.43-5.70)	0.68	6	0.99	0.72 (0.41-1.25)
	Barra do Choça	BCH2	1.37 ± 0.17	3.61 (2.43-4.80)	3.46	6	0.75	0.64 (0.40-1.03)
	Vitória da Conquista	VDC	2.97 ± 0.31	3.79 (3.15-4.42)	1.36	6	0.97	1.00 (0.70-1.43)
9.6	Luís Eduardo Magalhães	LEM2	1.77 ± 0.22	17.80 (14.68-21.58)	4.34	6	0.63	3.15 (2.08-4.78)*
	Barreiras	BAR1	2.47 ± 0.23	12.18 (10.38-14.07)	9.17	6	0.27	2.16 (1.60-2.90)*
	Luís Eduardo Magalhães	LEM1	1.57 ± 0.24	6.85 (4.04-9.88)	12.63	6	0.05	1.21 (0.74-1.98)
	Barra do Choça	BCH1	1.58 ± 0.27	5.20 (2.61-7.86)	14.78	6	0.02	0.92 (0.53-1.61)
	Mucugê	MUC2	1.26 ± 0.20	4.59 (3.54-5.64)	5.36	6	0.50	0.81 (0.56-1.17)
	Mucugê	MUC1	1.62 ± 0.17	4.15 (3.05-5.25)	0.90	6	0.99	0.73 (0.49-1.10)
	Barra do Choça	BCH3	1.73 ± 0.26	3.94 (2.09-5.79)	12.39	6	0.05	0.70 (0.41-1.19)
	Barra do Choça	BCH2	2.66 ± 0.35	3.43 (2.61-4.20)	6.34	6	0.38	0.61 (0.38-0.96)
	Mucugê	MUC3	1.43 ± 0.17	3.03 (1.99-4.06)	6.83	6	0.34	0.54 (0.31-0.93)
	Vitória da Conquista	VDC	3.15 ± 0.36	3.30 (2.73-3.84)	1.68	6	0.95	1.00 (0.67-1.48)
12.0	Luís Eduardo Magalhães	LEM2	2.04 ± 0.23	15.78 (13.22-18.63)	6.20	6	0.40	2.79 (1.96-3.97)*
	Barreiras	BAR1	1.98 ± 0.40	7.71 (3.55-12.19)	18.59	6	0.01	1.36 (0.78-2.38)
	Luís Eduardo Magalhães	LEM1	2.06 ± 0.19	7.34 (6.00-8.72)	4.49	6	0.61	1.30 (0.84-1.79)
	Mucugê	MUC2	1.12 ± 0.20	2.43 (1.07-3.86)	1.25	6	0.97	0.86 (0.59-1.24)
	Barra do Choça	BCH1	1.38 ± 0.34	4.57 (1.03-8.27)	16.62	6	0.02	0.81 (0.53-1.23)
	Barra do Choça	BCH3	1.59 ± 0.17	4.09 (2.98-5.21)	5.32	6	0.50	0.72 (0.48-1.09)
	Mucugê	MUC1	1.41 ± 0.17	3.61 (2.46-4.76)	3.43	6	0.75	0.64 (0.40-1.02)
	Mucugê	MUC3	1.35 ± 0.17	3.23 (2.10-4.36)	1.87	6	0.93	0.57 (0.34-0.97)
	Barra do Choça	BCH2	1.80 ± 0.40	2.10 (0.50-3.62)	12.18	6	0.05	0.37 (0.08-1.64)
	Vitória da Conquista	VDC	2.61 ± 0.30	2.80 (2.19-3.38)	1.53	6	0.96	1.00 (0.42-2.35)

Table 3 Relative toxicity of chlorantraniliprole to populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*); the asterisk in the resistance ratio indicate significant difference from the standard susceptible population based on Robertson et al. (2007).

Chlorantraniliprole concentration (mg a.i. mL^{-1})	County	Code	Slope ± SE	LT$_{50}$ (95% FI) h	χ^2	Df	*P*	Resistance ratio at LT$_{50}$ [RR$_{50}$ (95% CI)]
0.026	Luís Eduardo Magalhães	LEM1	1.54 ± 0.34	184.08 (96.02-903.29)	0.83	6	0.99	12.95 (4.09-41.06)*
	Barreiras	BAR1	0.86 ± 0.16	135.16 (70.17-512.99)	4.72	6	0.58	9.51 (2.22-40.74)*
	Luís Eduardo Magalhães	LEM2	1.99 ± 0.55	106.38 (65.09-480.37)	8.99	6	0.17	7.49 (2.54-22.09)*
	Mucugê	MUC1	6.55 ± 21.67	81.45 (58.06-199.66)	21.36	6	0.01	5.73 (1.09-369.75)*
	Barra do Choça	BCH1	1.87 ± 0.64	52.77 (33.79-182.36)	11.51	6	0.05	3.71 (1.44-9.55)*
	Mucugê	MUC2	0.89 ± 0.18	51.21 (32.37-126.99)	2.70	6	0.85	3.60 (1.07-15.05)*
	Barra do Choça	BCH3	0.94 ± 0.15	48.88 (33.00-94.07)	6.44	6	0.37	2.94 (1.12-10.60)*
	Mucugê	MUC3	1.40 ± 0.22	34.50 (26.57-50.27)	2.48	6	0.87	2.43 (1.10-5.38)*
	Barra do Choça	BCH2	1.11 ± 0.15	32.82 (24.63-49.30)	1.33	6	0.97	2.31 (0.98-5.46)
	Vitória da Conquista	VDC	1.30 ± 0.17	14.21 (11.12-18.13)	0.87	6	0.99	1.00 (0.50-2.00)
0.052	Luís Eduardo Magalhães	LEM1	1.23 ± 0.27	241.92 (111.14-1607.00)	3.49	6	0.74	17.02 (4.70-61.73)*
	Barreiras	BAR1	1.03 ± 0.17	103.12 (61.26-270.36)	0.55	6	0.99	7.26 (2.29-22.96)*
	Mucugê	MUC1	4.76 ± 4.99	79.86 (38.35-182.96)	21.20	6	0.01	5.62 (1.05-31.57)*
	Barra do Choça	BCH1	0.76 ± 0.13	37.03 (28.50-54.96)	3.30	6	0.77	2.61 (1.16-5.84)*
	Mucugê	MUC3	1.77 ± 0.26	34.87 (28.10-46.92)	4.32	6	0.63	2.45 (1.24-4.85)*
	Barra do Choça	BCH3	1.09 ± 0.15	34.24 (25.51-52.40)	5.25	6	0.51	2.41 (1.02-5.83)*
	Mucugê	MUC2	1.39 ± 0.28	34.08 (26.21-49.90)	1.44	6	0.96	2.40 (1.07-5.39)*
	Luís Eduardo Magalhães	LEM2	0.75 ± 0.16	44.22 (27.08-119.33)	1.17	6	0.98	3.11 (0.56-17.22)
	Barra do Choça	BCH2	1.25 ± 0.15	27.04 (21.29-36.87)	1.80	6	0.94	1.90 (0.91-3.99)
	Vitória da Conquista	VDC	1.86 ± 0.18	4.61 (3.57-5.65)	1.40	6	0.97	1.00 (0.67-1.49)
	Mucugê	MUC1	1.62 ± 0.73	242.69 (88.08-1064.00)	4.11	6	0.66	17.08 (2.16-135.22)*
	Luís Eduardo Magalhães	LEM1	1.53 ± 0.38	225.53 (106.55-1787.00)	1.51	6	0.96	15.87 (4.42-56.99)*
	Barreiras	BAR1	1.55 ± 0.25	90.85 (61.29-182.65)	4.43	6	0.62	6.39 (2.72-15.02)*
	Barra do Choça	BCH3	0.83 ± 0.14	38.91 (26.36-73.80)	1.83	6	0.93	2.73 (0.79-9.43)

0.078	Barra do Choça	BCH1	1.32 ± 0.19	34.53 (26.21-51.13)	9.05	6	0.17	2.12 (0.99-4.52)
	Barra do Choça	BCH2	1.45 ± 0.16	24.04 (19.62-30.74)	5.00	6	0.54	1.69 (0.88-3.26)
	Mucugê	MUC3	1.18 ± 0.17	21.93 (16.84-30.36)	0.97	6	0.99	1.54 (0.68-3.52)
	Mucugê	MUC2	1.20 ± 0.17	19.47 (15.03-26.28)	1.05	6	0.98	1.37 (0.62-3.04)
	Luís Eduardo Magalhães	LEM2	0.88 ± 0.15	14.62 (10.34 -21.15)	0.21	6	0.99	1.03 (0.35-3.02)
	Vitória da Conquista	VDC	1.79 ± 0.18	4.79 (3.68-5.89)	1.81	6	0.93	1.00 (0.63-1.53)
0.105	Luís Eduardo Magalhães	LEM1	1.34 ± 0.40	172.58 (80.79-2662.00)	7.07	6	0.31	12.14 (2.48-59.42)*
	Mucugê	MUC1	3.98 ± 6.38	110.77 (36.70-261.31)	26.47	6	0.01	7.80 (2.29-212.75)*
	Barreiras	BAR1	2.52 ± 0.67	77.18 (54.99-201.16)	9.71	6	0.14	5.43 (2.28-12.94)*
	Mucugê	MUC3	0.90 ± 0.19	69.08 (40.83-214.37)	1.03	6	0.98	4.86 (1.08-21.87)*
	Mucugê	MUC2	1.82 ± 0.25	30.19 (24.77-38.84)	3.63	6	0.73	2.12 (1.11-4.06)*
	Barra do Choça	BCH1	1.45 ± 0.29	30.08 (23.65-41.67)	1.38	6	0.97	2.12 (0.35-12.97)
	Barra do Choça	BCH2	2.36 ± 0.30	24.66 (21.10-29.10)	8.08	6	0.23	1.74 (0.30-9.90)
	Luís Eduardo Magalhães	LEM2	1.34 ± 0.19	23.39 (18.39-31.41)	2.91	6	0.82	1.65 (0.77-3.50)
	Barra do Choça	BCH3	0.83 ± 0.13	11.98 (8.52-16.72)	4.99	6	0.54	0.84 (0.30-2.38)
	Vitória da Conquista	VDC	1.41 ± 0.39	3.90 (2.53-6.32)	9.05	6	0.05	1.00 (0.09-13.35)
0.131	Mucugê	MUC1	1.61 ± 0.41	106.45 (63.43-434.03)	4.47	6	0.61	7.49 (2.34-23.98)*
	Barreiras	BAR1	1.11 ± 0.17	72.58 (47.84-146.95)	0.77	6	0.99	5.11 (1.88-13.85)*
	Luís Eduardo Magalhães	LEM1	0.83 ± 0.14	51.01 (32.91-109.42)	4.60	6	0.59	3.59 (1.04-13.01)*
	Mucugê	MUC2	2.88 ± 0.67	32.95 (24.21-53.72)	13.04	6	0.05	2.32 (1.21-4.46)*
	Luís Eduardo Magalhães	LEM2	0.76 ± 0.19	70.23 (38.93-271.48)	8.23	6	0.22	4.94 (0.81-30.07)
	Barra do Choça	BCH1	0.80 ± 0.15	27.14 (18.40-50.17)	2.59	6	0.86	1.91 (0.17-0.83)
	Barra do Choça	BCH3	1.37 ± 0.15	17.69 (14.45-22.16)	3.27	6	0.77	1.40 (0.65-2.39)
	Barra do Choça	BCH2	1.60 ± 0.16	16.19 (13.57-19.52)	4.16	6	0.65	1.14 (0.63-2.06)
	Mucugê	MUC3	1.42 ± 0.18	15.87 (12.66-19.96)	4.84	6	0.56	1.12 (0.57-2.17)
	Vitória da Conquista	VDC	1.79 ± 0.19	3.79 (2.84-4.76)	1.22	6	0.97	1.00 (0.62-1.61)

Table 4 Relative toxicity of thiamethoxam to populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*); the asterisk in the resistance ratio indicate significant difference from the standard susceptible population based on Robertson et al. (2007).

Thiamethoxam concentration (mg a.i. mL^{-1})	County	Code	Slope ± SE	LT$_{50}$ (95% FI) h	χ^2	df	*P*	Resistance ratio at LT$_{50}$ [RR$_{50}$ (95% CI)]
0.67	Luís Eduardo Magalhães	LEM1	2.24 ± 1.17	116.68 (44.16-204.99)	9.19	6	0.16	11.02 (1.90-63.77)*
	Mucugê	MUC1	0.63 ± 0.17	102.84 (46.71-1002.00)	1.23	6	0.98	9.71 (1.24-110.42)*
	Barra do Choça	BCH3	1.61 ± 0.31	72.60 (49.93-150.61)	6.57	6	0.36	6.86 (2.72-17.25)*
	Luís Eduardo Magalhães	LEM2	0.96 ± 0.20	62.77(38.91-167.50)	1.44	6	0.96	5.93 (1.50-23.45)*
	Mucugê	MUC3	1.04 ± 0.20	56.62 (36.90-128.10)	2.28	6	0.89	5.35 (1.58-18.13)*
	Barreiras	BAR1	1.78 ± 0.33	43.20 (32.38-69.72)	4.27	6	0.64	4.08 (1.82-9.16)*
	Barra do Choça	BCH1	1.39 ± 0.22	39.03 (29.50-59.87)	6.19	6	0.40	3.69 (1.59-8.56)*
	Mucugê	MUC2	2.14 ± 0.27	26.26 (22.22-31.76)	3.06	6	0.80	2.48 (1.36-4.53)*
	Barra do Choça	BCH2	1.77 ± 0.30	12.85 (8.12-19.38)	12.22	6	0.06	1.25 (0.75-1.97)
	Vitória da Conquista	VDC	1.48 ± 0.24	10.59 (6.73-15.29)	11.50	6	0.08	1.00 (0.49-2.05)
1.34	Luís Eduardo Magalhães	LEM1	1.25 ± 0.86	796.23 (298.65-2123.00)	4.66	6	0.59	75.20 (1.35-4195.71)*
	Mucugê	MUC3	1.03 ± 0.23	92.50 (52.45-349.99)	1.40	6	0.97	8.74 (2.04-37.39)*
	Barreiras	BAR1	1.72 ± 0.34	56.17 (39.68-107.36)	8.68	6	0.19	5.30 (2.19-12.83)*
	Barra do Choça	BCH3	1.38 ± 0.23	47.89 (34.95-80.84)	2.34	6	0.89	4.52 (1.85-11.08)*
	Barra do Choça	BCH1	1.66 ± 0.25	40.67 (31.81-58.48)	7.68	6	0.26	3.84 (1.83-8.05)*
	Mucugê	MUC2	1.11 ± 0.18	28.21 (20.96-42-87)	1.77	6	0.94	2.66 (1.04-6.84)*
	Barra do Choça	BCH2	1.89 ± 0.26	21.30 (17.10-27.12)	9.92	6	0.13	2.01 (1.06-3.82)*
	Mucugê	MUC1	1.13 ± 0.16	16.60 (12.69-22.25)	5.94	6	0.43	1.57 (0.70-3.53)
	Luís Eduardo Magalhães	LEM2	1.14 ± 0.16	14.37 (10.94-18.97)	1.29	6	0.97	1.36 (0.61-3.00)
	Vitória da Conquista	VDC	1.87 ± 0.19	4.05 (3.09-5.00)	1.37	6	0.97	1.00 (0.65-1.55)
	Luís Eduardo Magalhães	LEM1	1.47 ± 0.51	183.61 (82.11-8744.00)	4.43	6	0.62	17.34 (3.20-94-02)*
	Barreiras	BAR1	1.85 ± 0.43	70.14 (47.27-171.65)	8.01	6	0.24	6.62 (2.53-17.35)*
	Mucugê	MUC3	1.26 ± 0.27	66.99 (44.04-165.76)	5.69	6	0.46	6.33 (1.98-20.20)*
	Mucugê	MUC1	1.11 ± 0.20	45.42 (31.62-85.59)	0.33	6	0.99	4.29 (1.45-12.79)*

2.00	Barra do Choça	BCH1	1.54 ± 0.23	39.18 (30.30-57.07)	7.35	6	0.29	3.70 (1.72-7.94)*
	Barra do Choça	BCH3	1.74 ± 0.21	26.85 (22.02-34.07)	9.09	6	0.17	2.54 (1.33-4.83)*
	Barra do Choça	BCH2	1.79 ± 0.18	13.89 (11.50-16.64)	8.39	6	0.21	1.31 (0.73-2.35)
	Luís Eduardo Magalhães	LEM2	1.11 ± 0.16	12.60 (9.47-16.57)	1.90	6	0.93	1.19 (0.67-2.11)
	Mucugê	MUC2	1.60 ± 0.18	11.92 (9.59-14.54)	8.36	6	0.21	1.13 (0.61-2.08)
	Vitória da Conquista	VDC	1.72 ± 0.24	5.37 (3.20-7.56)	11.44	6	0.08	1.00 (0.58-1.73)
2.67	Luís Eduardo Magalhães	LEM1	2.19 ± 0.69	101.50 (63.15-631.89)	9.91	6	0.13	9.59 (3.13-29.38)*
	Mucugê	MUC3	2.47 ± 0.54	51.11 (41.00-80.48)	3.64	6	0.72	4.83 (2.30-10.14)*
	Mucugê	MUC1	1.32 ± 0.23	45.03 (32.87-76.13)	2.37	6	0.88	4.25 (1.68-10.74)*
	Barreiras	BAR1	1.44 ± 0.21	29.63 (23.31-40.82)	1.17	6	0.98	2.80 (1.31-5.98)*
	Barra do Choça	BCH3	1.62 ± 0.20	25.32 (20.57-32.45)	9.66	6	0.14	2.39 (1.23-4.64)*
	Luís Eduardo Magalhães	LEM2	0.72 ± 0.16	39.33 (24.25-102.63)	1.55	6	0.96	3.71(0.63-21.96)
	Barra do Choça	BCH1	2.18 ± 0.39	30.71 (22.67-17.84)	12.55	6	0.05	2.90 (0.90-9.35)
	Mucugê	MUC2	1.67 ± 0.19	13.24 (10.77-16.05)	8.32	6	0.22	1.25 (0.68-2.29)
	Barra do Choça	BCH2	1.62 ± 0.17	12.32 (10.03-14.96)	9.56	6	0.14	1.16 (0.38-3.57)
	Vitória da Conquista	VDC	1.32 ± 0.18	2.06 (1.16-2.98)	4.67	6	0.59	1.00 (0.22-4.56)
3.34	Barreiras	BAR1	1.21 ± 0.37	234.22 (94.49-6670.00)	5.58	6	0.47	22.12 (3.75-130.55)*
	Luís Eduardo Magalhães	LEM1	3.78 ± 4.62	129.68 (28.30-594.28)	6.42	6	0.38	12.25 (1.55-96.84)*
	Luís Eduardo Magalhães	LEM2	0.98 ± 0.21	70.91 (42.82-210.80)	1.63	6	0.95	6.70 (1.64-27.40)*
	Mucugê	MUC3	1.82 ± 0.36	43.95 (34.47-67.61)	2.03	6	0.92	4.15 (1.85-9.31)*
	Mucugê	MUC1	1.83 ± 0.33	24.42 (17.34-38.19)	12.83	6	0.05	2.31 (1.10-4.81)*
	Barra do Choça	BCH3	1.97 ± 0.39	18.93 (12.05-31.32)	15.61	6	0.02	1.79 (0.85-3.77)
	Mucugê	MUC2	1.79 ± 0.25	17.48 (13.87-22.07)	7.33	6	0.29	1.65 (0.87-3.14)
	Barra do Choça	BCH1	2.71 ± 0.42	16.35 (11.89-21.71)	11.18	6	0.08	1.54 (0.86-2.78)
	Barra do Choça	BCH2	1.63 ± 0.29	9.77 (5.66-14.85)	11.88	6	0.06	0.92 (0.45-1.91)
	Vitória da Conquista	VDC	1.29 ± 0.18	1.64 (0.83-2.49)	2.48	6	0.87	1.00 (0.06-16.50)

Figure captions

Figure 1 Distribution of the sampling sites for populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in our survey of chlorantraniliprole, thiamethoxam and chlorpyrifos resistance in the state of Bahia, Brazil. Identification for each sampling site and its coordinates are found in Table 1.

Figure 2 The relationship between the concentrations of the insecticide chlorpyrifos and the resistance ratio (at LT_{50}) in populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*). The symbols indicate the observed data.

Figure 3 The relationship between the concentrations of the insecticide chlorantraniliprole and the resistance ratio (at LT_{50}) in populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*). The symbols indicate the observed data.

Figure 4 The relationship between the concentrations of the insecticide thiamethoxam and the resistance ratio (at LT_{50}) in populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*). The symbols indicate the observed data.

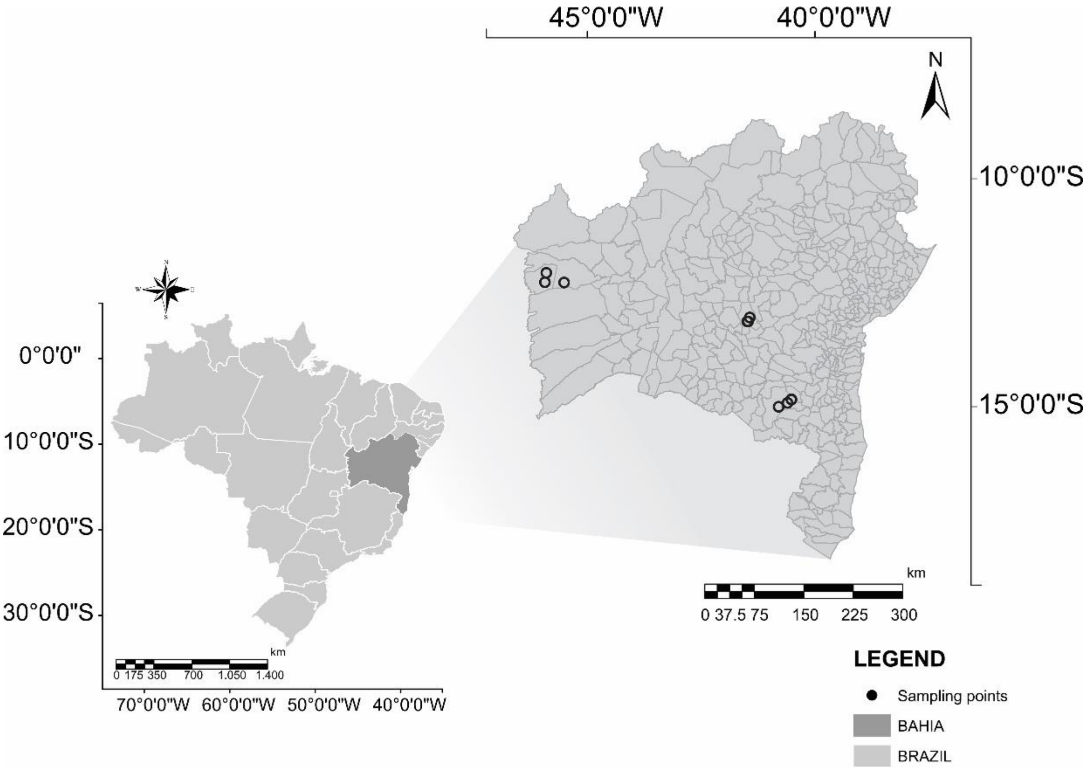
45°0'0"W
40°0'0"W
N
10°0'0"S
15°0'0"S
0°0'0"
10°0'0"S
20°0'0"S
30°0'0"S
km
0 175 350 700 1.050 1.400
70°0'0"W 60°0'0"W 50°0'0"W 40°0'0"W
km
0 37.5 75 150 225 300
LEGEND
● Sampling points
BAHIA
BRAZIL

Fig 1.

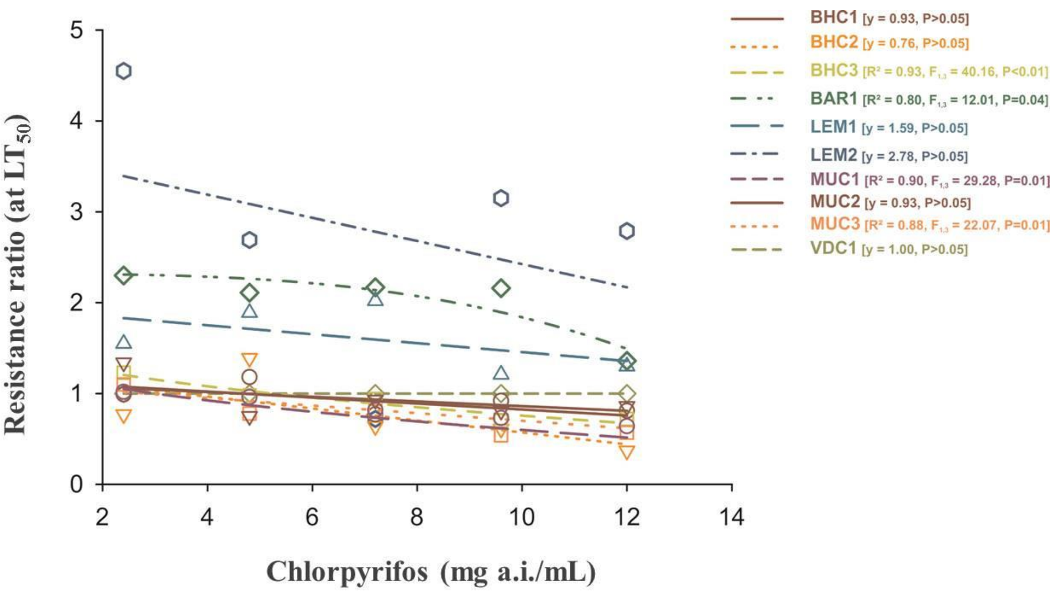
Resistance ratio (at LT$_{50}$)
0
1
2
3
4
5
2
4
6
8
10
12
14
Chlorpyrifos (mg a.i./mL)
BHC1 [y = 0.93, P>0.05]
BHC2 [y = 0.76, P>0.05]
BHC3 [R² = 0.93, F$_{1,3}$ = 40.16, P<0.01]
BAR1 [R² = 0.80, F$_{1,3}$ = 12.01, P=0.04]
LEM1 [y = 1.59, P>0.05]
LEM2 [y = 2.78, P>0.05]
MUC1 [R² = 0.90, F$_{1,3}$ = 29.28, P=0.01]
MUC2 [y = 0.93, P>0.05]
MUC3 [R² = 0.88, F$_{1,3}$ = 22.07, P=0.01]
VDC1 [y = 1.00, P>0.05]

Fig. 2

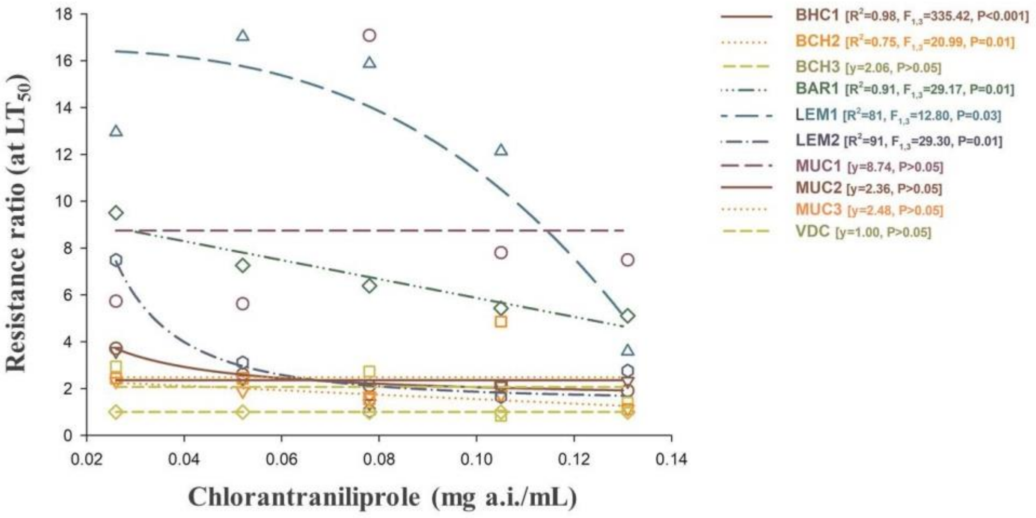
Resistance ratio (at LT_{50})
0
2
4
6
8
10
12
14
16
18
0.02
0.04
0.06
0.08
0.10
0.12
0.14
Chlorantraniliprole (mg a.i./mL)
BHC1 [R^2=0.98, $F_{1,3}$=335.42, P<0.001]
BCH2 [R^2=0.75, $F_{1,3}$=20.99, P=0.01]
BCH3 [y=2.06, P>0.05]
BAR1 [R^2=0.91, $F_{1,3}$=29.17, P=0.01]
LEM1 [R^2=81, $F_{1,3}$=12.80, P=0.03]
LEM2 [R^2=91, $F_{1,3}$=29.30, P=0.01]
MUC1 [y=8.74, P>0.05]
MUC2 [y=2.36, P>0.05]
MUC3 [y=2.48, P>0.05]
VDC [y=1.00, P>0.05]

Fig. 3

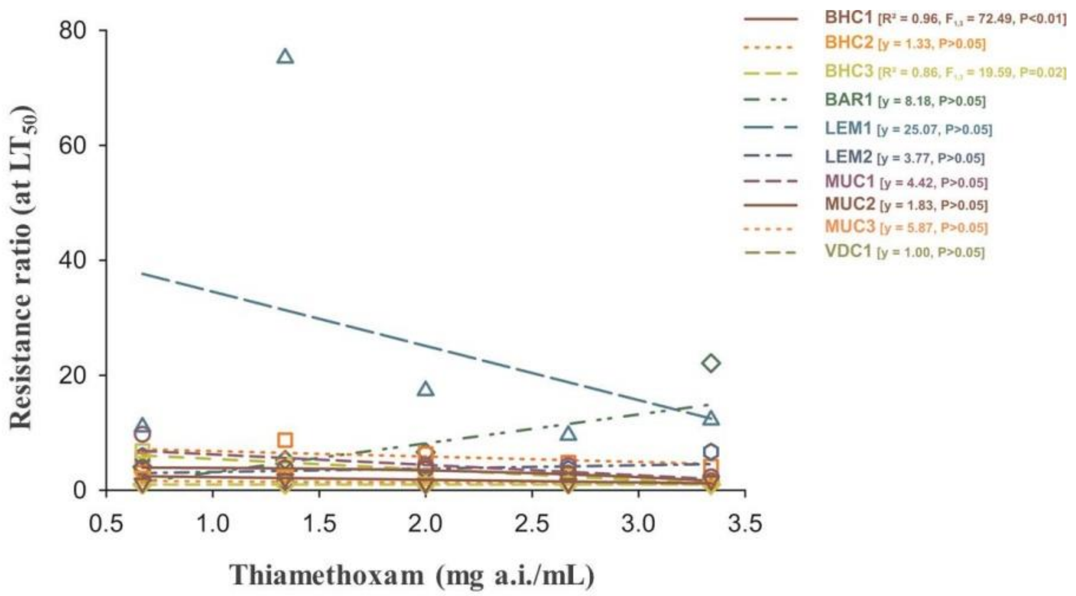
Resistance ratio (at LT$_{50}$)
80
60
40
20
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Thiamethoxam (mg a.i./mL)
BHC1 [$R^2 = 0.96$, $F_{1,3} = 72.49$, $P<0.01$]
BHC2 [$y = 1.33$, $P>0.05$]
BHC3 [$R^2 = 0.86$, $F_{1,3} = 19.59$, $P=0.02$]
BAR1 [$y = 8.18$, $P>0.05$]
LEM1 [$y = 25.07$, $P>0.05$]
LEM2 [$y = 3.77$, $P>0.05$]
MUC1 [$y = 4.42$, $P>0.05$]
MUC2 [$y = 1.83$, $P>0.05$]
MUC3 [$y = 5.87$, $P>0.05$]
VDC1 [$y = 1.00$, $P>0.05$]

Fig. 4

Periódico Científico: Chemosphere – A1

ARTICLE IV

Thiamethoxam on the morphophysiology of coffee seedlings and infestation Neotropical leaf miner, *Leucoptera coffeella*[*]

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[*] **Situation:** Not Submitted

Submitted to: *Chemosphere*
Original Research Paper

Thiamethoxam on the morphophysiology of coffee seedlings and infestation of the Neotropical leaf miner (*Leucoptera coffeella*)

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Abstract

Coffee is one of the main commodities in the Brazilian economy. The use of insecticides prevails for the suppression of pest populations, particularly the Neotropical leaf miner *Leucoptera coffeella*, which is based on the neonicotinoid thiamethoxam as one of the main insecticides used and considered effective against arthropod pest species. One of the reasons for that, besides is pest control efficacy, is its allegeded bioactivation of the plant metabolism. Thus, the aims of the present study were: 1) to evaluate the effect of thiamethoxam on the vegetative vigor of coffee seedlings (*Coffea arabica* 'Catuaí 144' cultivar) at different thiametoxan concentrations (2, 20, 40, 80 and 200 mg i.a. mL^{-1}) applied via drench; and 2) to evaluate the effectiveness of thiamethoxan in controlling the leaf miner. Morphophysiological variables of the coffee seedlings were evaluated at 20, 40, 60, 80 and 100 days after application, and leaf miner infestations were recorded at 20, 40, 60 and 80 days after the release of adults. The results obtained indicated that thiamethoxam indeed interferes with morphysiology of coffee seedlings, besides of providing effective leaf miner control even at the smaller concentration evaluated. Nonethless the continuous use of thiamethoxam in coffee crops for leaf miner control and plant bioactivator may further encourage the unnecessary use of the compound potentially favoring selection for insecticide resistance and eventual decline in its effectiveness against the coffee leaf miner.

Keywords: Bioactivator; biomass; *Coffea arabica*; neonicotinoids, vegetative vigor

1. Introduction

Neonicotinoids are one of the chemical classes of synthetic insecticides on the market most used in agriculture. One of the main neonicotinoid insecticides used for the pest population suppression is thiametoxam. In addition, neonicotinoids can directly modify the metabolism of plant species mainly in the growth and vigor of plants. The use of the neonicotinoids imidacloprid and thiamethoxam favor the expression of genes that induce plant defense and stress tolerance (Theilert et al., 2009; Stamm et al., 2014), regardless of their effectiveness in control pest species.

Thiamethoxam has been used to protect crops against pests, and also as a promoter of changes in plant metabolism and morphology, promoting greater plant vigor and development, in addition to increased productivity, reason of its suspected activity as bioactivator (Castro, 2006). Physiological effects of thiamethoxam have already been described in some crops and instances such as increase in growth rate (Tavares et al., 2007; Pereira et al., 2010; Macedo and Castro, 2011), and greater seed physiological in rice (Almeida et al., 2014) and pumpkin (Lemes et al., 2015). In coffee, changes in morphological characteristics were observed (Costa et al., 2010), an effect similar to that observed when plant regulators are used at the initial plant growth (Santos et al., 2015; d'Arêde et al., 2017; Ribeiro et al., 2017). Nonetheless, studies on the bioactivating effect of thiamethoxam in plants and its relationship with insects in general are scarce (Pereira et al., 2010), as most emphasize only the plant morphophysiological changes (Tavares et al., 2007; Castro and Pereira, 2008; Almeida et al., 2011; Martins et al., 2012).

Coffee is one of the main commodities of the Brazilian economy, occupying a cultivated area of 1,185.5 thousand hectares with estimated production of 62 million bags for 2020, of which 45 million bags of Arabica coffee (*Coffea arabica* L.), which makes the country the main world coffee producer (Conab, 2020). Phytosanitary factors are the main cause for reduction in crop productivity, mainly infestations by the *Leucoptera coffeella* leaf miner (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae), which occur in Neottropical America, reaching the status of key crop pest unshaded coffee, production system that prevails in Brazil (Tuelher et al., 2003; Pereira et al., 2007a; 2007b; Magalhães et al., 2010; Pantoja-Gomez et al., 2019). It is a microlepidoptera, whose caterpillars feed on the palisade parenchym, causing leafmining and, consequently, leaf fall reaching high levels of defoliation and production losses above 50% (Ramiro et al., 2004).

Chemical control is the most used method for leafminer population suppression, with tendency of increasing the frequency of application and doses of insecticides in regions with higher pest pressure (Leite et al., 2020a). The intensive application of broad-spectrum insecticides has caused several impacts on the leaf miner control, especially the development of insecticide resistance (Alves et al., 1992; Fragoso et al., 2002; 2003; Ribeiro et al., 2003; Costa et al., 2016; Leite et al., 2020b).

Insecticides from the neonicotinoid subgroup are prevalent in several crops, such as the continued use in soybean against stinkbugs, whiteflies and aphids (Castellanos et al., 2019), which are important for crop protection (Elbert et al., 2008). Neonicotinoids comprise systemic insecticides acting on the insect nervous system, specifically as agonists of nicotinic acetylcholine receptors (Nauen et al., 2003; Jeschke and Nauen, 2008). Thiamethoxam is considered to be the first of the second generation of neonicotinoids (Maiensfich et al., 2001a; 2001b), and one of the main insecticides used in coffee for leaf miner management (Miranda et al., 2016).

The increasing of thiamethoxan application frequency in coffee in some regions of Bahia, Brazil, does not seem to be related only to leafminer population suppression, but also to its effect of promoting vegetative vigor. However, the intensive use of the insecticide can lead to the selection of resistant populations, compromising the effectiveness of the insecticide molecule in the future. As consequence, there isincreasing the risk of control failure, as already detected for the insecticide chlorantraniliprole in leaf miner populations (Leite et al., 2020). Moderate levels of thiamethoxan resistance in the leaf miner were also recently detected in the Brazilian states of São Paulo, Minas Gerais and Pernambuco (Costa et al., 2016).

In Arabica coffee production regions of the Brazilian state of Bahia, the intensive use of insecticides for leafminer control in recent years has reached 20 annual applications (Leite et al., 2020a). More particularly, an increase in soil applications has been observed in these regions reaching an average of four applications per year (Leite et al., 2020a), which may be related not only to its effect on the pest, but also on the plant. Studies correlating the bioactivating effect and the effective control of coffee leaf miner are again scarce (Costa et al., 2010).

Neonicotinoids can potentially alter morphological and physiological characteristics of plants. Thus, the suspicion that this systemic insecticide acts as a bioactivator of the plant metabolism. In this context, studies on these changes in *Coffea arabica* are essential to assess the influence of thiamethoxam and its efects with the control of *L. coffeella*. Thus, the aim of the present study was to evaluate the effect of different concentrations thiamethoxam on the vegetative vigor of coffee seedlings (*Coffea arabica* L.) and their afficay against the coffee leaf miner.

2. Material and Methods

2.1 Plants, insecticides and insects

Coffea arabica "Catuaí 144" cultivar (n = 108), with four pairs of expanded leaves obtained from certified nursery, were transplanted and conducted in plastic pots containing 15 L of substrate (red-yellow latosol type soil, medium texture with good drainage and tanned manure) The experiment was conducted in a completely randomized design with six treatments (2, 20, 40, 80 and 200 mg i.a. mL^{-1}), an untreated control, and with three replicates, totaling 18 plots consisting of three coffee seedlings kept in field cages (2x2x2 m) covered with voile fabric, totaling 108 seedlings.

A commercial formulation of the insecticide thiamethoxam (250 g i.a. kg^{-1}, water-dispersible granules, Syngenta, São Paulo, SP, Brazil) was used at five concentrations (2, 20, 40, 80 and 200 mg i.a. mL^{-1}), and an untreated control (i.e., without insecticide application). Thirteen days after transplanting, insecticide was applied through soil drench as a single volume of 50 mL of insecticide suspension per plant for all treatments. Of the 108 plants, 90 plants received insecticide application. Twenty days after application, 54 plants were infested with the leaf miner, 45 of which also received insecticide application. The other 54 plants were used for morphological evaluations performed 20 days after insecticide application.

Twenty days after application, the plots were infested with three *L. coffeella* adults, totaling 27 adults per treatment obtained from a population considered susceptible to insecticides. This insect population was obtained from a commercial coffee plantation without insecticide application for 15 years located in the District of Capinal, county of Vitória da Conquista (state of Bahia, Brazil). Leaves infested with the leaf miner were collected and stored in 2 m x 2 m x 2 m cages containing coffee seedlings to obtain adults.

2.2 Morphological plant analyses

2.2.1 Destructive assessment

Evaluations were performed at 20 and 100 days after application. At 20 days, the following morphological parameters were obtained: number of leaves, plant height (cm; considering substrate level up to the apex of the plant), stem diameter (mm; at 5 cm from the base of the plant), leaf area (cm²; measured with a non-destructive leaf area meter model LA = 0.8147 $(L.L)^{0.9543}$ described by Schmildt et al. (2014)), and fresh and dry shoot, root and total weight, besides shoot/root weight relationships.. Fresh weight of seedlings was obtained by separating shoots and roots, and subsequently weighing each of these parts. To obtain dry weight, the material was placed in Kraft-type paper bags (17 x 45 cm) and dried in oven with forced air circulation at the temperature of 65°C for 48 hours until reaching constant weight and then weighed on scale (tipo, fabricante, local de fabricação?) with accuracy of 0.01 grams. Total weight values were obtained by summing up the total fresh and dry shoot and root weight. Shoot / root relationship was calculated using their dry weight relationship.

2.2.2 Non-destructive assessment

At 40, 60 and 80 days after application, number of leaves, height plant, stem diameter and total leaf area were determined following the same methodology described for 20 day after application. At 100 day after application, the last evaluation of morphological parameters was carried out using all 54 seedlings infested with the leaf miner, totaling 54 plants referring to the three seedlings of each plot. Assessment procedures were the same as those adopted at 20 day after application.

2.3 Physiological plant analysis

At 20, 40, 60, 80 and 100 day after application of insecticide thiamethoxam, relative chlorophyll content and gas exchanges were evaluated. The estimate of chloroplast pigments was obtained using the SPAD index (Soil Plant Analysis Development), determined with the aid of portable chlorophyll meter model SPAD 502 (MINOLTA, Japan). Readings were performed at three points on the second pair of leaves from the apex of the plant.

Leaf gas exchanges were determined using infrared radiation gas analyzer (LCPro, ADC, UK), coupled to a 46-actinic light source, and with 1000 μmol photons m^{-2} s^{-2} of photosynthetically active radiation on the same leaf used to determine the SPAD index. CO_2 assimilation rate (Aμmol CO_2 m^{-2} s^{-2}), stomatal conductance (*gs* mol m^{-2} s^{-2}), transpiration rate (Emmol water vapor m^{-2} s^{-2}) and internal CO_2 concentration (Ciμmol CO_2 mol^{-1} air) were also determined.

2.4 Evaluation of leaf miner infestation index and intensity

Infestation was initiated with the release of three adult *L. coffeella* insects per plot at 20 day after application. To monitor the population development, assessments were performed at 20, 40, 60 and 80 days after release, by counting the number of leaves and of mined leaves. Infestation index (number of leaves with mines / total number of leaves x 100) and infestation intensity (number of mines / number of mined leaves) were also estimated.

2.5 Statistical analyses

The means of plant morphological and physiological characteristics were subjected to regression analyses, as was insect infestation. The models were s based on significance (*probability* < 0.05), parsimony, high F values (reduced error) and R^2 with model complexity, and biological explanation of each variable. The statistical analyses were performed using the Analysis System Statistics and Genetics (SAEG), version 9.1, and the graphs plotted using the software SigmaPlot 13.0.

Ordination was used to group treatments according to the fallowing variables: index and infestation intensity with number of leaves, leaf area, SPAD index, transpiration and CO_2 assimilation rate, stomatal conductance and internal CO_2 concentration. PCA (Principal Component Analysis) was used for this purpose and carried out using the R *FactoMinerR* package software (Lê et al., 2008) applying the selected variables to transform data from a wide spectrum to low spectrum space. PCA was calculated using the correlation matrix for each variable to deduce the eigenvector and eigenvalue. The eigenvector indicates the direction of the main axis with the greatest variance and the eigenvalue indicates the magnitude of the variability of the secondary axis with the next variance. The Bartlett test was used to verify the measure of the correlation matrix and the identity matrix to indicate the existence of the relationship among variables evaluated and the Kaiser–Meyer–Olkin test (KMO) to measure the adequacy of data for the PCA.

3. Results

3.1 Effect of thiamethoxam on morphological characteristics

3.1.1 Destructive assessment

To morphological characteristics number of leaves, the cubic-root model was designed, observing a greater increase in the lower concentration (2 mg a.i. mL^{-1}) of the insecticide thiametoxam at 100 days after application (Figure 1A). For plant height, at

100 days after application, the concentration of 2 mg a.i mL^{-1} favored the greatest development of coffee seedlings, verifying the linear effect (Figure 1B); for the stem diameter, the linear model was outlined for 20 and 100 days after application, at 20 days the increase in stem diameter was greater at the highest concentration (200 mg a.i mL^{-1}) and reduction of the increment at 100 days, being observed major diameter development in the lowest concentration (2 mg a.i mL^{-1}) at 100 days after application (Figure 1C). For characteristic number of leaves and leaf area at 20 and 100 days after application, and plant height at 20 days after application, the effect of the insecticide thiametoxam was not significant.

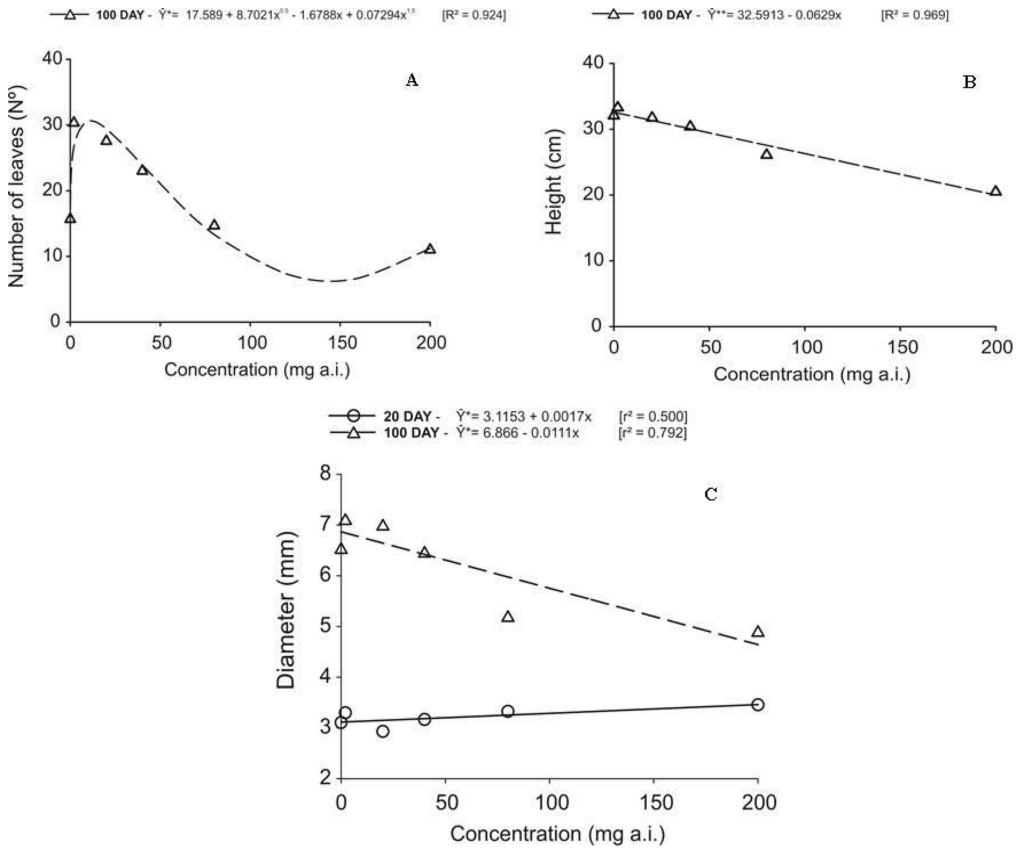

Figure 1. Morphological characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to different concentrations of thiamethoxam, at 20 and 100 days after application of the insecticide. Number of leaves 100 days after application (A); plant height 100 days after application (B); stem diameter 20 and 100 days after application (C). By regression analysis, at 10%, 5% and 1% probability.

To effect of thiamethoxam on the coffee seedling morphological characteristics fresh and dry shoot weight followed the linear model for 20 days after application (Figure 2A), and the cubic-root model at 100 days after application (Figure 2B), with lower values of weight obtained in the highest concentrations.

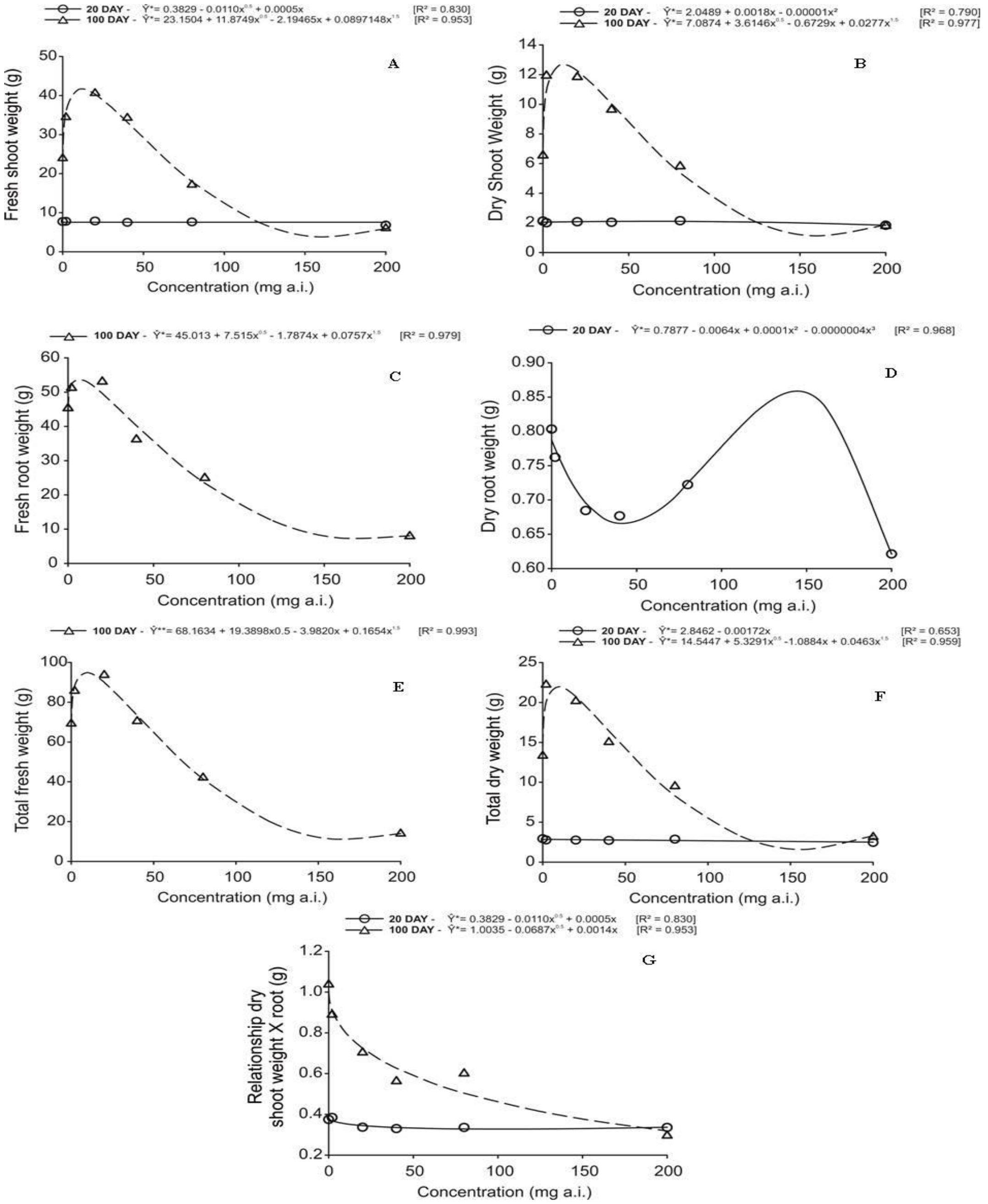

Figure 2. Morphological characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to different concentrations of thiamethoxam, at 20 and 100 days after application of the insecticide. Fresh and dry shoot weight (A and B); fresh and dry root weight (C and D); total fresh and dry weight (E and F); relationship dry shoot weight and dry root weight (G). By regression analysis, at 10%, 5% and 1% probability.

Concerning the root weight, the cubic-root model was designed for fresh weight at 20 and 100 days after application (Figure 2C and 2D). For total fresh weight, the effect was verified at 100 days and explained by the cubic-root model (Figure 2E), while for total dry weight the effect was linear at 20 days and cubic-root for 100 days after application (Figures 2E and 2F). The dry weight relathioship of area and root at 20 and 100 days after application was outlined by the cubic-root model as a function of thiametoxam concentrations (Figure G).

3.1.2 Non-destructive assessment

Decrease was observed in the number of leaves as the concentration of thiametoxam increased at 40, 60 and 80 days after application, however a small increase in the number of leaves at 40 days was observed using the concentration of 200 mg ai mL-1 (Figure 3A).

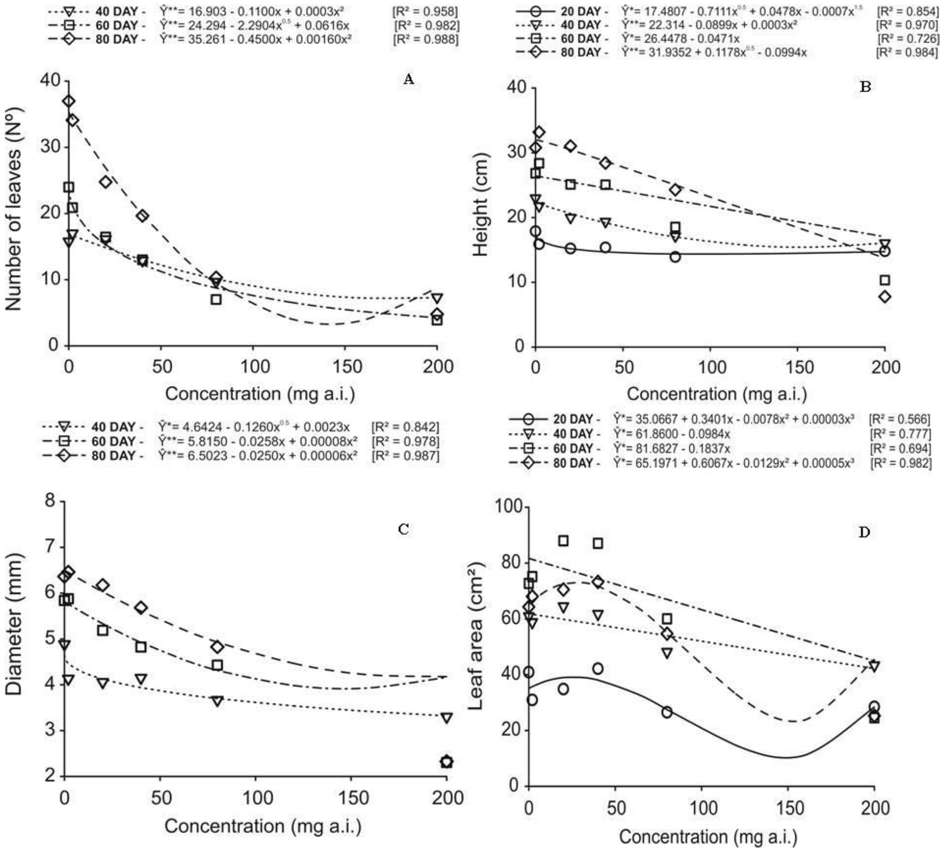

Figure 3. Morphological characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to different concentrations of thiamethoxam, at 20, 40, 60 and 80 days after application of the insecticide. Number of leaves (A); plant height (B); stem diameter (C); total leaf area (D). By regression analysis, at 10%, 5% and 1% probability.

For plant height, the increase was greater in the concentration of 2 mg a.i. mL^{-1} in relation to the control, and a significant reduction in the height of the seedlings with an increase in the concentrations of the insecticide (Figure 3B). Although the application of thiametoxam in the highest concentrations resulted in a larger diameter at 20 days after application, at 40, 60 and 80 days the insecticide reduced the diameter of the plant (Figure 3C). For total leaf area of the coffee seedlings, there was an effect of thiamethoxam concentrations with a decrease in values as the concentration increased, and slight increase at 20 and 40 days after application at a concentration of 200 mg a.i mL^{-1} (Figure 3D).

3.2 Effect of thiamethoxam on physiological characteristics

3.2.1 Destructive assessment

For physiological characteristics, liquid photosynthesis rate (Aμmol CO_2 $m^{-2}s^{-1}$), transpiration rate (Emmol water vapor $m^{-2}s^{-1}$), and stomatal conductance (*gs* mol $m^{-2}s^{-1}$) after application of thiametoxam a decrease in values was observed as the concentration increased, and a slight increase at 20 and 100 days after the application of thiametoxam to a concentration of 200 mg a.i mL^{-1} (Figure 4A and 4B). For internal concentration of CO_2 in the leaf (Ciμmol CO_2 mol^{-1} air) there was no effect of the insecticide thiametoxam at 20 and 100 days after application (Figure 4C).

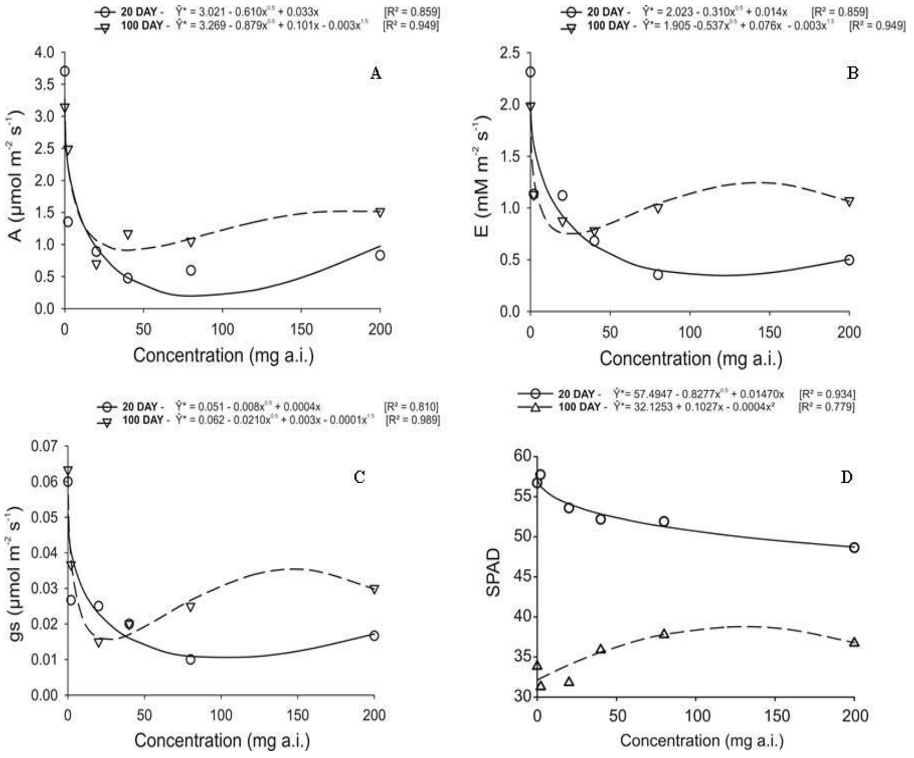

Figure 4. Physiological characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to different concentrations of thiamethoxam, at 20 and 100 days after

application of the insecticide. Photosynthetic Rate of CO_2 (A µmol CO_2 $m^{-2}s^{-1}$) (A); transpiration Rate (E mmol vapor d´água $m^{-2}s^{-1}$) (B); stomatal conductance (*gs* mol $m^{-2}s^{-1}$) (C) and index SPAD (D). By regression analysis, at 10%, 5% and 1% probability.

Concerning the total chlorophyll content, the SPAD index at 20 days after application was designed as the square root model and at 100 days after application, the quadratic effect was verified with an increase in the values of the SPAD index as the concentrations increased (Figure 4D).

3.2.2 Non-destructive assessment

The rate of liquid photosynthesis (Aµmol CO_2 $m^{-2}s^{-1}$) was altered by the action of the insecticide at 20, 40 and 80 days after application, the models outlined were the root cubic, quadratic and linear, respectively (Figure 5A); smaller rates of liquid photosynthesis were observed at 20 days after application and elevation at 40 days in the highest concentrations; for the transpiration rate (Emmol water vapor $m^{-2}s^{-1}$) verified was linear effect at 20, 40 and 80 days after application with increased leaf transpiration at higher concentrations (Figure 5B), and a cubic effect 60 days after application.

To the internal concentration of CO_2 in the leaf (Ciµmol CO_2 mol^{-1} air), verified was cubic-root effect at 20 days after thiametoxam application and a cubic effect at 40 and 60 days, with a different behavior from the levels of CO_2 concentration in the coffee leaves mainly at 40 days after application with the major increase in the concentration of 200 mg a.i mL^{-1} (Figure 5C). Verified was a linear effect at 20 and 40 days after application and a cubic root effect at 80 days after application of thiametoxam with increase of the stomatal conductance (*gs* mol $m^{-2}s^{-1}$) the measure what the concentration increased (Figure 5D).

The total chlorophyll content, the SPAD index was outlined the linear model for the 20 and 40 days after application with the highest SPAD index at 40 days for the concentration of 2 mg a.i mL^{-1} (Figure 5E), and decrease for the other concentrations and a quadratic model for 80 days after application with a reduction in the SPAD index for the lowest concentration of the insecticide (Figure 5E).

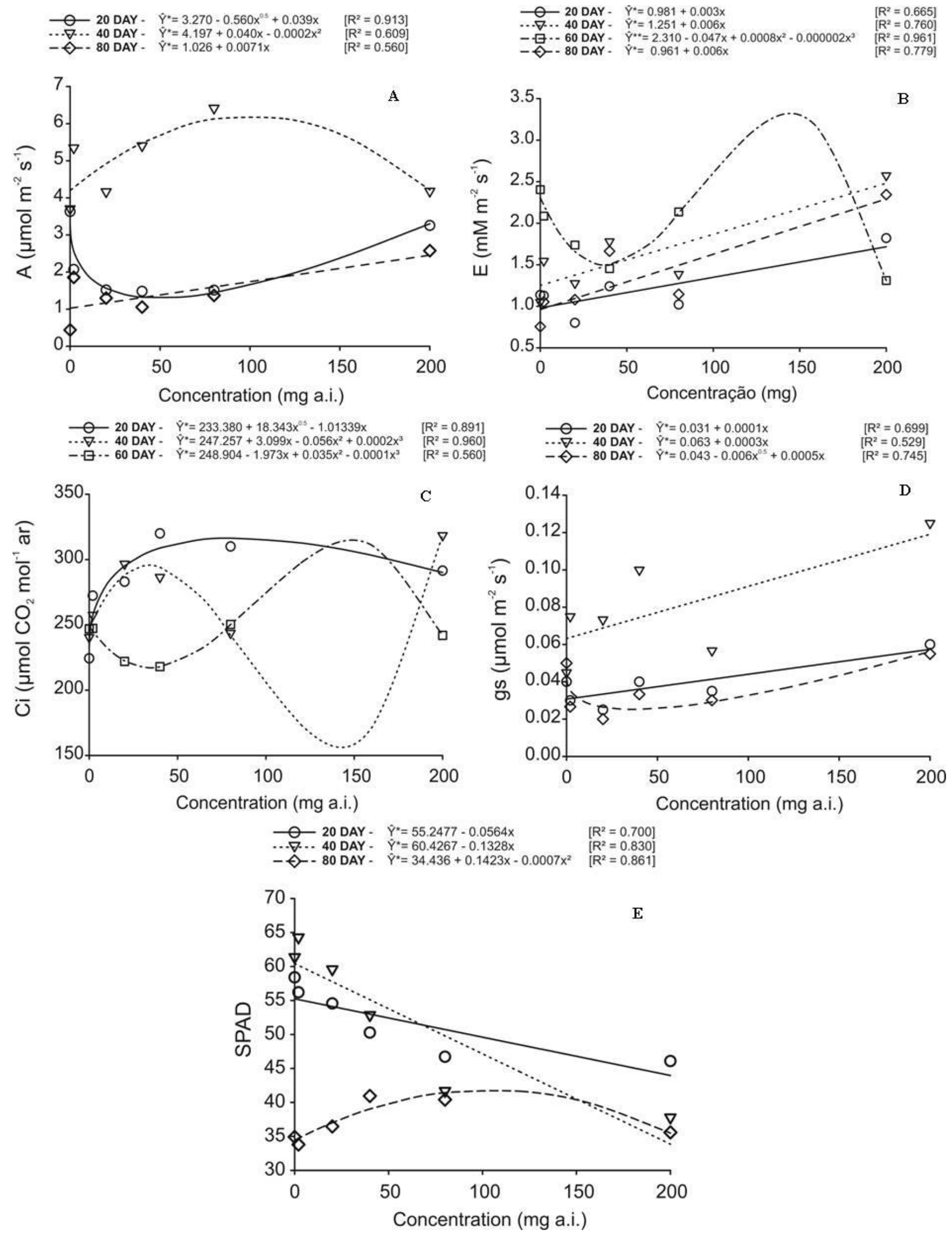

Figure 5. Physiological characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to different concentrations of thiamethoxam, at 20, 40, 60 and 80 days after application of the insecticide. Photosynthetic Rate of CO_2 (A µmol CO_2 $m^{-2}s^{-1}$) (A); transpiration Rate (E mmol vapor d´água $m^{-2}s^{-1}$) (B); internal concentration CO_2 in the leaf (Ci µmol CO_2 mol^{-1} ar) (C); stomatal conductance (*gs* mol $m^{-2}s^{-1}$) (D) and index SPAD (E). By regression analysis, at 10%, 5% and 1% probability.

3.3 Leaf miner infestation

There was no leaf miner population growth in treatments submitted to the application of thiamethoxam, and infestation rate was evaluated only in the control treatment. The infestation of the leaf miner in coffee seedlings was explained by the cubic effect in the number of leaves (Figure 6A) and leaves with mines (Figure 6B), and number mines (Figure 6C) days after release the adults of the leaf miner. At 60 days, seedlings had highest number of leaves (Figure 6A), as well as highest damage caused to coffee leaves (Figure 6C), and highest number leaves with mines (Figure 6B).

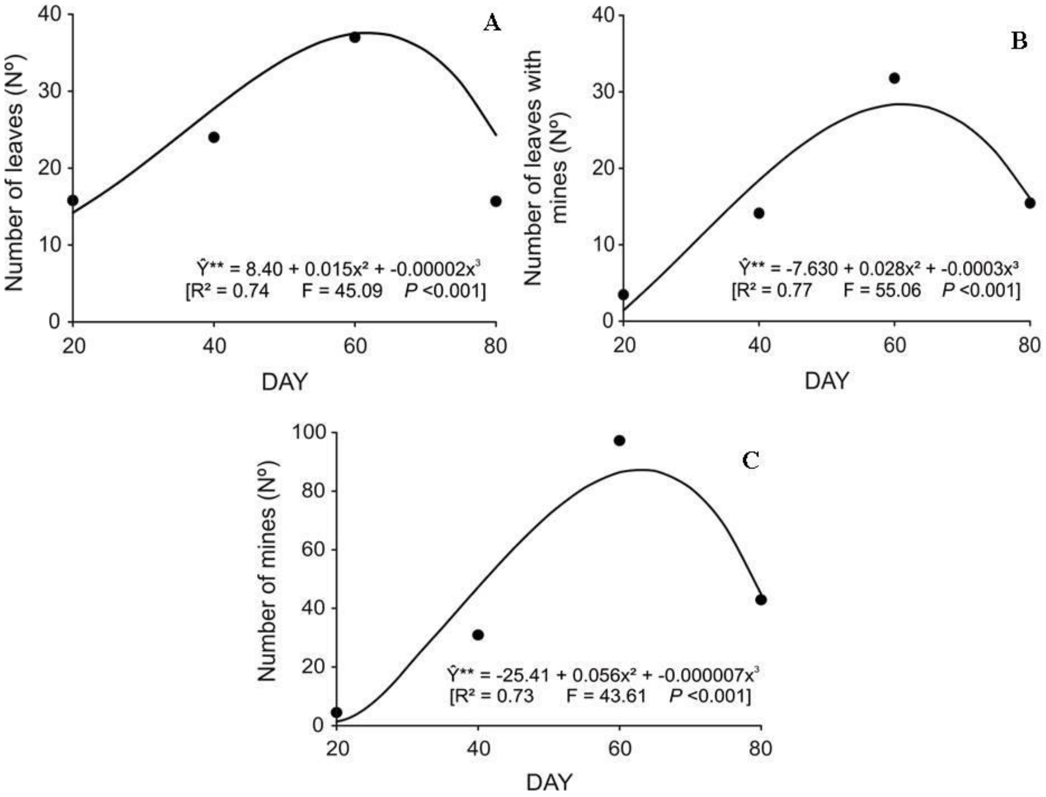

Figure 6. Characteristics of coffee trees (*C. arabica* cv. Catuaí IAC 144) in response to Neotropical leaf miner infestation days after release: number of leaves (A), number of leaves with mines (B) and number mines (C).

The variables presented in Figure 7 and Table 1 provide total components and the proportion of variance indicating the total variation of the principal component. The results indicated that correlations were linear and significant between index and intensity infestation leaf miner, number leaves, and SPAD index (Table 1). For these components four distinct axes were obtained. PC1 accounting for 62.7% of the total variance observed (Figure 7).

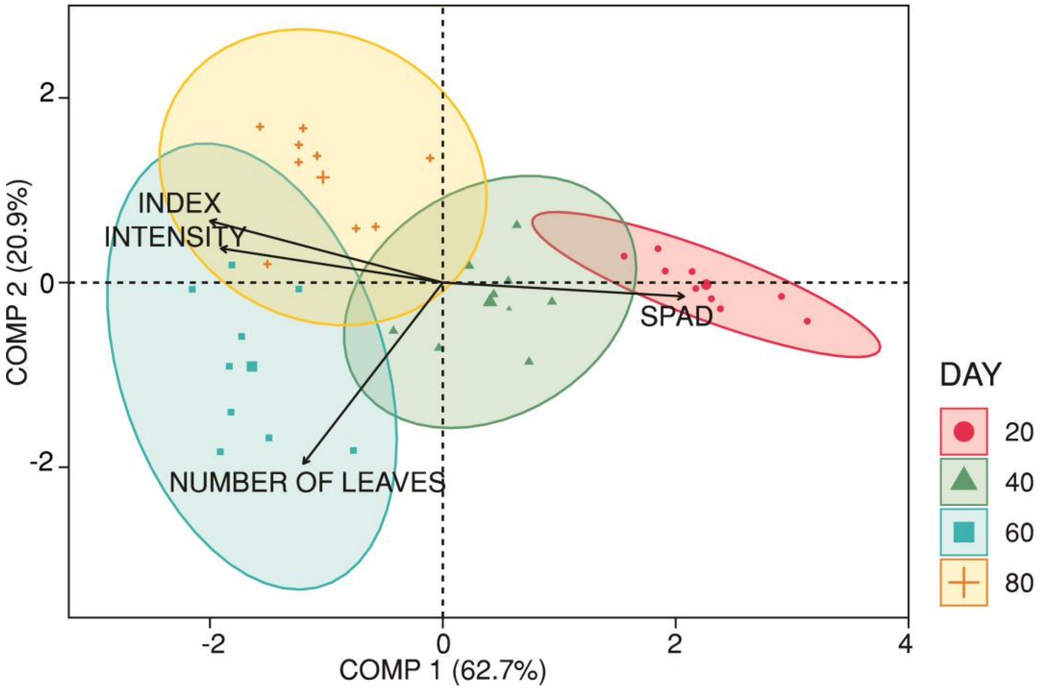

Figure 7. Graph of the matrix of correlations between variables: index and intensity infestation of *Leucoptera coffeella*, number of leaves, and index SPAD in seedlings *C. arabica* cv. Catuaí IAC 144.

Table 1. Principal components, eigenvalues, proportion of explained variance and proportion accumulated components for correlations between variables: index and intensity infestation of *Leucoptera coffeella*, number of leaves, and index SPAD in seedlings *C. arabica* cv. Catuaí IAC 144.

Component	Eigenvalues	Proportion	Proportion Accumulated
PC1	2.51	62.73	62.73
PC2	0.84	20.94	83.67
PC3	0.42	10.48	94.15
PC4	0.23	5.85	100.00

In Figure 8 and Table 2 presented variables provide total components and the proportion of variance indicating the total variation of the principal component, indicating that results of the correlations were significant between index and intensity infestation leaf miner, photosynthesis rate (A), transpiration rate (E), internal leaf CO_2 concentration (Ci), and stomatal conductance (*gs*). For these components six distinct axes were obtained. The total variance observed was better represented by PC1 45.5% (Figure 8).

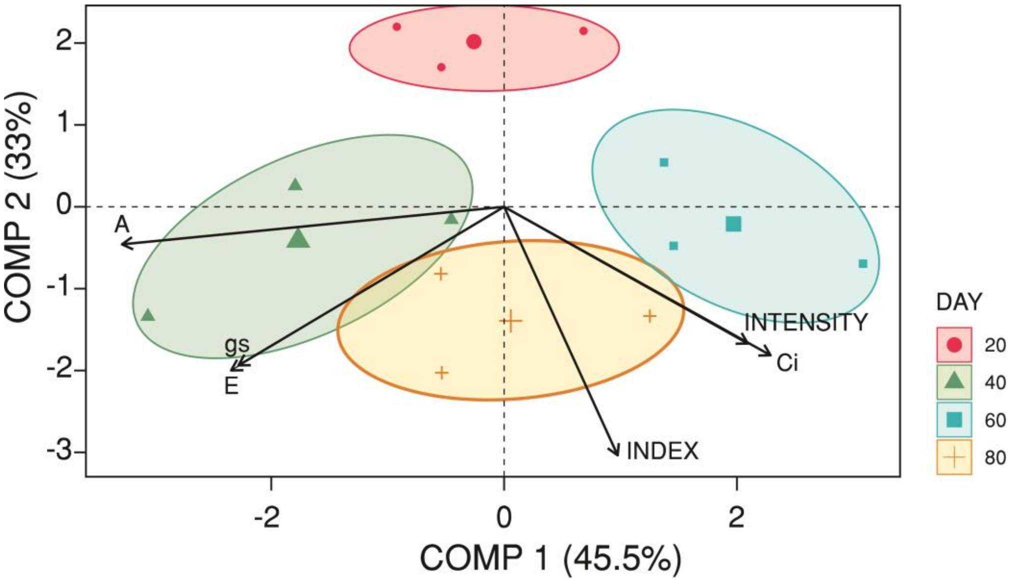

Figure 8. Graph of the matrix of correlations between variables: index and intensity infestation of *Leucoptera coffeella*, photosynthetic rate of CO_2 (A µmol CO_2 $m^{-2}s^{-1}$) (A); transpiration rate (E mmol vapor d´água $m^{-2}s^{-1}$) (B); internal concentration CO_2 in the leaf (Ci µmol CO_2 mol^{-1} ar) (C); stomatal conductance (*gs* mol $m^{-2}s^{-1}$) in seedlings *C. arabica* cv. Catuaí IAC 144.

Table 2. Principal components, eigenvalues, proportion of explained variance and proportion accumulated components for correlations between variables: index and intensity infestation of *Leucoptera coffeella*, photosynthetic rate of CO_2 (A µmol CO_2 $m^{-2}s^{-1}$) (A); transpiration rate (E mmol vapor d´água $m^{-2}s^{-1}$) (B); internal concentration CO_2 in the leaf (Ci µmol CO_2 mol^{-1} ar) (C); stomatal conductance (*gs* mol $m^{-2}s^{-1}$) in seedlings *C. arabica* cv. Catuaí IAC 144.

Component	**Eigenvalues**	**Proportion**	**Proportion Accumulated**
PC1	2.73	45.55	45.55
PC2	1.98	33.05	78.60
PC3	0.71	11.88	90.48
PC4	0.42	6.91	97.39
PC5	0.15	2.46	99.85
PC6	0.01	0.15	100.00

4. Discussion

Applications of systemic insecticides in coffee crops are not only related to the effectiveness of pest control, but also to the effects on the plant. Results indicate that insecticide thiamethoxam alters the vegetative vigor of *Coffea arabica* ‘Catuaí 144’ cultivar and is effective in controlling the leaf miner at recommended concentration. Thiamethoxam has excellent absorption and translocation by the plant due to its bioactivating action that promotes changes in plant physiology and morphology (Maienfisch et al. 2001b) and greater residual power in plants (Maienfisch et al., 2001a), in addition to being one of the insecticides most effective in the control of phytosuccivorous pests, microlepidopterans and various coleopterans (Elbert et al., 2008).

The use of neonicotinoids in cultivated plants promotes metabolic changes that cause several morphological processes in plants due to the bioactivation capacity of products (Almeida et al., 2014). The active ingredient thiamethoxam is the precursor of clothianidin, another neonicotinoid, a secondary metabolite that can alter the function of the thiamentoxam molecule in plants (Nauen et al., 2003), often with independent enhancement of plant vigor and stress tolerance (Ford et al., 2010).

According to Castro (2006), thiamethoxam is not part of the group of plant growth regulators. The increase in initial plant development it related to its bioactivating activity that alters primary and secondary metabolism, capable of altering plant growth, acting on DNA transcription, gene expression, membrane proteins, metabolic enzymes and mineral nutrition (Castro and Pereira, 2008; Macedo et al., 2013a; 2013b). Such changes providing greater root development and shoot vigor, in addition to increasing crop productivity (Pereira et al., 2010). The thiamethoxam is translocated through the roots to the rest of the plant, and metabolized slowly (Castro, 2005), due to greater enzymatic activation what favors root development and vegetative vigor, greater stomatal resistance (Gazzoni et al., 2008), and stomatal conductance as observed in the present study.

The hormonal effect on plant cells promoted by thiamethoxam favors shoot and root growth (Venâncio et al., 2003; Pereira et al., 2010; Macedo and Castro, 2011; Macedo et al., 2013a; 2013b; Annamalai et al., 2018). In soybean and rice, thiamethoxam promotes positive effects such as high photosynthesis rate, increased vigor expression, biomass accumulation and root length (Castro et al., 2007; Almeida et al., 2011).

In coffee seedlings, thiamethoxam promoted increase in leaf area; however, it reduced the fresh shoot weight with increased concentration. Melo and Maciel (2014) did not observe effect of the insecticide on the biomass growth of coffee seedlings using the recommended dose for the crop. However, Durante et al. (2015) found that the use of thiamethoxam at recommended dose after the formation of the second pair of true leaves has positive effect on the development of coffee seedlings.

The active ingredient molecule thiamethoxam has direct action on the enzymatic activities that alter plant hormones in cells, increasing the development potential, which may reflect in the later stages of plant development, such as increased crop productivity (Pereira et al., 2010; Macedo and Castro, 2011). The correlation between vegetative vigor and crop productivity is proportionally linked to the reduction of stresses resulting from the initial development stage, as observed in coffee seedlings with the use of plant growth regulators (d'Aredê et al., 2017, Ribeiro et al., 2017).

Frequently, pesticides act as components responsible for the increase in vegetative vigor of plants and protection against species that cause damage to the crop. In coffee ‘Mundo Novo’ cultivar, thiamethoxam at doses smaller than recommendations, altered the morphological development (diameter, higher percentage of dry shoot and root weight) and protected the plant against the incidence of leaf miner up to 150 days after application (Costa et al., 2010), aspects also verified in the present study.

In coffee crop, this effect on leaves has to be taken into account, mainly in relation to number of leaves. The correlation between infestation index and number of leaves was positive, indicating that the increase in the number of leaves increases infestation, and consequently, the number of leaves with lesions (mines), reducing chlorophyll content and stomatal conductance. The infestation intensity increases over time, reducing the chlorophyll content, photosynthesis and transpiration rate of plants. However, in the present study using susceptible individuals, population growth was observed only at zero concentration, since in the other concentrations, the insecticide was effective in controlling the pest, and it is not possible to infer about the effect of thiamethoxam on the relationship between morphophysiological characteristics and leaf miner infestation.

The damage caused to coffee leaves by the leaf miner reduces the leaf area, causes leaf fall and, consequently, decreases photosynthetic activity and productivity (Reis and Souza, 1998). For Blanco and Folegatti (2005), leaf area is the key variable in studies on the physiological effects on plant growth, photosynthetic efficiency, evapotranspiration and on the responses of fertilizers and pesticides. Larger leaf area, number and size of leaves reduce the leaf miner infestation (Caixeta et al., 2004), and the photosynthetically active area decreases with pest infestation intensity (Caixeta et al., 2004), that is, greater infestation intensity reflects in reduction in the net photosynthesis rate (Neves et al., 2006). The incidence of leaf miner is also correlated with changes in the amount of nitrogen absorbed by plants (Caixeta et al., 2004) and in the total sugar content in coffee leaves (Theodoro et al., 2014).

Studies in some cultures with thiamethoxam reveal gains in crop production due to the use of insecticide in the treatment of seeds and seedlings, favoring plant vigor and growth, root development and alteration in the distribution of photoassimilates (Castro et al., 2007; Macedo and Castro, 2011; Almeida et al., 2014; Durante et al., 2015; Annamalai et al., 2018). However, for coffee plants, when application is carried out in the seedling phase, shoot height, stem diameter and dry shoot and root weight must be taken into account (Binotto et al., 2010). However, it is necessary to add the reading of other essential components such as chlorophyll index and the essential photosynthetic processes when using the application of products to overcome stress and protect crop in the initial growth stage. Regardless of dose used, thiamethoxam promotes less impact on gas exchanges in coffee seedlings and reduces stress, overcoming stresses occurring in the early stages of coffee development, as already observed when using plant growth regulators (Ribeiro et al., 2017).

The liquid photosynthesis rate was not initially changed by the product; however, at 40 days after application, an increase in liquid photosynthesis rate was observed and at 100 days after application, a decrease in the photosynthesis rate associated with increased insecticide dose. Increase in plant biomass is promoted by increase in the photosynthesis rate, and in general, it occurs in coffee plants when CO_2 availability is greater (DaMatta et al., 2016). According to Beasley and Banham (2007) and d'Aredê et al. (2017), plant growth regulators do not impact the distribution of photoassimilates and weight accumulation. For DaMatta et al. (2008) and Martins et al. (2014), the reduction of photosynthesis rate in coffee plants is not directly related to lower CO_2 availability.

The changes in the secondary metabolism of plants promoted by thiamethoxam favor the induction of resistance, mainly by potentiating the expression of salicylic acid. Salicylic acid increases the chlorophyll content of cultivated plants, and the accumulation of chlorophyll effectively favors the photochemical reactions of photosynthesis (Thornber, 1975). Thus, the expression of salicylic acid by the use of neonicotinoids increases resistance to the disease in *Arabidopsis thaliana* (Ford et al., 2010). Thus, changes in chlorophyll content may be associated with physiological processes influenced by the possible hormonal effect that insecticide thiamethoxam causes to plants. In coffee, systemic fungicides cause this effect, which is called the tonic effect, characterized by greater vigor, leafing and darker green color of leaves (Carvalho et al., 1997; Venâncio et al., 2003). Growth regulators favor the increase of chlorophyll content in coffee (d'Aredê et al., 2017; Ribeiro et al., 2017).

The study demonstrat that high doses of thiametoxam used in the study caused phytotoxic effects on coffee seedlings. The direct effects of thiamethoxam on the morphophysiology of C. arabica seedlings (number of leaves, diameter, height, SPAD index, sweating and photosynthesis rates and stomatal conductance) were observed in the lowest concentration studied and recommended for coffee culture to control *L. coffeella*. In soybean, thiamethoxam promotes changes in oxidative stress and cell wall metabolism (Stamm et al., 2014). According Wulff et al. (2019) the metabolic processes (cell wall formation, lignin and defense response) that regulate essential functions in soybean plants without the presence of herbivores were modified by neonicotinoids and, consequently, affected the plant-herbivore interaction.

In summary, this study provides insight into the effect thiamethoxam applied via drench in seedlings *C. arabica*. Thus, insecticide thiamethoxam in high concentrations non stimulat the development morphophysiological reported a high toxicity of thiamethoxam seedlings coffee. The insecticide thiamethoxam in coffee crops changes plant growth, and provides evidence that the neonicotinoids alter metabolic process plants, beyond reduced leaf miner infestations. The continuous use of thiamethoxam in coffee crops over the agricultural years as bioactivator per promoting greater plant development and vigor may further encourage the unnecessary use of the compound potentially favoring selection for insecticide resistance and eventual decline in its effectiveness against the coffee leaf miner.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FINAL CONSIDERATIONS

The results obtained in the present study are of great importance for coffee production, especially for the Arabica coffee producing regions of Bahia, and for the expansion of subárea of the research area in Integrated Pest Management of the Graduate Program in Agronomy, as insetcs resistante to insecticides.

From the point of view of integration with society, the work enabled direct contact with managers or coffee growers of 116 properties, who were visited to survey data on crops and the management of the leaf miner, with the important verifing that the Integrated Management of Pests in coffee farming is very far from what the IMP philosophy advocates. With rare exceptions, the management is chemical, practically using the same insecticides and with increasing frequency of application in majority of the properties, with great selection pressure for resistant populations.

From a scientific point of view, it can be highlighted that unprecedented aspects were achieved for the conditions of Bahia. This is the first work with mapping the occurrence of insect populations resistant to the insecticide chlorantraniliprole and spatial panorama of risk of control failure with this insecticide, as well as determination of resistance levels of the most used insecticides (chlorantraniliprole, thiamethoxam and chlorpyrifos) and effects of thiamethoxam on coffee seedlings and its relationship with leaf miner. Considering if that the execution of the work contributes to advances in knowledge about insect resistance to insecticides.

Although the costs of the detected resistance levels and the environmental impact, due to the increase in the number of insecticide applications, not been estimated certainly the resistance management should be emphasized and practiced in the regions with moderate risks, especially in the western, for reestablishment of susceptible populations. Resistance management measures, if adopted, certainly reduction in economic and environmental impacts by reducing number of insecticide applications.

However, for recommendations of management tactics of insect’s resistance to insecticides in the state of Bahia, there are knowledge lacuna that the research needs act, verifying the existence of cross-selection, which would lead existence of cross or multiple resistance, to determine the existence the resistance mechanism (s) involved and the insects ability to disperse to the edaphoclimatic environment. conditions of coffee regions.

The use of insecticides in majority of the farmlands provided an increase in the selection pressure against populations leading to the resistance, in addition to the imminent risk of failure of control that compromises the management of the pest. The aspects addressed in this study will allow coffee growers to trace strategies to seek minimize environmental, economic and food security impacts and mainly not to compromise the management of *Leucoptera coffeella*.

ANNEX I

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Article

Profile of Coffee Crops and Management of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella*

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Abstract: The Neotropical coffee leaf miner is a key coffee pest and in the state of Bahia, one of the major coffee-producing states in the country. The insect finds favorable conditions for its development, causing production losses and intensive use of insecticides. Thus, the objective of the study was to analyze aspects of the profile of coffee crops and the management of the leaf miner, including the use of insecticide for the western and highland regions of Bahia. Data were obtained through questionnaires applied to coffee growers and/or production technicians and included information on the total area, area with coffee, and native vegetation, type of cultivation, cultivars, pest monitoring, methods of control and use, insecticide rotation, and doses used. Descriptive statistical analysis, principal component analysis (PCA), and canonical correlations indicated differences between farm size, and areas with coffee and native vegetation. Chemical pest control prevails as a management strategy in all farms. The results are important for managing the coffee leaf miner while providing an overview and diagnosis of insecticide use in coffee production in the state of Bahia. An increase in the application of systemic insecticides took place in recent years, similarly (same active ingredients) among most coffee growers. This fact increases the risk of selecting populations resistant to insecticides, compromising the management of the leaf miner in the regions.

Keywords: chemical subgroup; control methods; integrated pest management; monitoring; survey

1. Introduction

Coffee production is an activity of great importance for the Brazilian economy [1], with an estimated production area of 2.1 million hectares. The country is the world's largest producer and exporter with an annual production of 61.7 million 60 kg sacks of processed coffee, where few states account for more than 90% of national production [2].

The state of Bahia ranks fourth in Arabica coffee production (*Coffea arabica,* in the western and highland regions) and conilon coffee production (*Coffea canephora,* in the coastal region) with production of 76,135 and 40,930 thousand 60-kg sacks, respectively [2]. Coffee production in Bahia stands out on the national scenario due to the quality of coffee produced in the highland region, mainly in

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municipalities located in Diamantina highlands (i.e., Chapada Diamantina), that is responsible for the production of specialty coffees due to the particular climatic conditions [3].

Consumer expansion in new markets has led coffee growers to search for systems for sustainable production. The highland region of Bahia, with municipalities located in the Diamantina highlands, has played an important role in the adoption of measures based on agricultural practices that cause lower environmental impact and greater economic value of the product. Currently, for accessing the European and North American markets, farmers must use methods consistent with the Integrated Pest Management (IPM) philosophy, and not just chemical control methods [4,5].

Modern agriculture considers economic, environmental, ecological, and food security aspects taken into account in management decisions. The adoption of the IPM philosophy in agricultural and forestry crops is consistent with the requirements of the new consumer markets and new vision and trends in agriculture that goes far beyond crop productivity [6,7].

Considering the principles of IPM, the use of control tactics must be based on knowledge about the phytophagous species and its natural enemies, and the pest population growth trends. Decision-making regarding adoption of pest control must use control levels and economic thresholds. If the phytophagous species causing the injury reaches the population level of control, assuming the status of pest, the decision is for intervention aimed at suppressing the population [8,9]. If the decision is for chemical control, the choice of the insecticide is of fundamental importance considering not only the effectiveness and price of the product but mainly its selectivity in favor of natural enemies, toxicity, residual power, grace period, persistence, method of application, and formulation [10].

Coffee leaf miner, *Leucoptera coffeella* (Guérin-Méneville and Perrottet, 1842) (Lepidoptera: Lyonetiidae) is a key crop pest, especially of unshaded coffee, which is prevalent in most Neotropical America and particularly in Brazil. The highest incidences of the coffee leaf miner occur in Central America and mainly in Brazil due to the high infestation rates recorded [11–15]. The damage caused by the insect is a result of injuries caused by its larvae that feed on the palisade parenchyma of coffee leaves, reducing the photosynthetic capacity, which leads to destruction and fall of leaves and, consequently, reducing fruit production [16]. The biological cycle lasts from 28 to 39 days, and four to five generations of the leaf miner may occur per year [17]. In dry periods, the leaf miner incidence in coffee crops increases [18].

In Brazil, the main method used by coffee growers to control the coffee leaf miner is chemical [19,20]. Neuroinsecticides are the most widely used, including several organophosphates, carbamates, pyrethroids, and neonicotinoids, some of which are (relatively) persistent in the environment and exhibit low selectivity in favor of natural enemies. The diamide, chlorantraniliprole, is conversely of more recent use against the control of coffee leaf miner and has low impact on non-target insects [21,22].

The management of leaf miner populations is linked to factors such as frequency of insecticide applications and migration of individuals and development of resistant populations, which are of primary importance for the effective control of the species [23]. The neglect of these factors by coffee growers and the frequent use of insecticides in the control of pest species lead to high selection pressure on the pest individuals and the development of resistance to the most frequently used insecticides [24].

The western and highland regions have increased production costs due to the chemical control of the coffee leaf miner. This is the result of the high number of insecticide applications required for the leaf miner control, mainly in the western region, where conditions are more favorable to the pest development [3]. In both of these coffee producing regions of Bahia, insecticide resistance and risk of control failure have already been observed [25]. There are knowledge lacunas related to leaf miner in the main coffee regions of Bahia that can subsidize research and extension actions on IPM in coffee growing. Thus, the objective of the study was to analyze aspects of the profile of coffee crops and the management of leaf miner, including the use of insecticide, for the western and highland regions of Bahia.

2. Materials and Methods

2.1. Study Sites

Sampling took place in farms located in the western (Barreiras, Cocos, Luís Eduardo Magalhães, and São Desiderio) and highland regions of Bahia (Barra do Choça, Barra da Estiva, Encruzilhada, Ibicoara, Mucugê, Piatã, and Vitória da Conquista) (Figure 1) between September 2017 and May 2018, totaling 116 farms surveyed (western region (Farms 1 to 21) and highland region (Farms 22 to 116)).

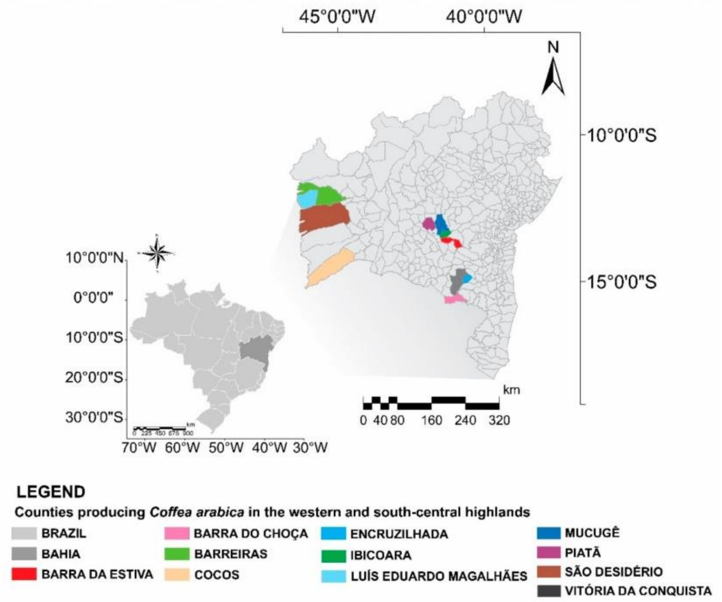

Figure 1. Municipalities producing Arabica coffee (*Coffea arabica*), with sampled farms, belonging in the western and south-central highlands regions of the state Bahia.

Information about the profile of coffee crops and management of coffee leaf miner was obtained from coffee growers and/or production technicians. Interviews were carried out in loco, on a voluntary basis, guaranteeing the confidentiality of responses. The questionnaire was designed with structured multiple-choice questions, free and dichotomous responses. Questions involved the following aspects: (1) Farm and cultivation, total area with coffee, area of native vegetation, type of cultivation (irrigated or non-irrigated) and cultivars, (2) history of the area regarding coffee leaf miner monitoring (if so, what level of control is used), types of control adopted, annual frequency of applications, insecticides used, rotation of insecticides, and use of the label rate specified by the manufacturer.

2.2. Data Analysis

Data consistency was measured by Cronbach's Alpha coefficient test to verify the reliability and consistency of the group of multiple-choice and dichotomous responses. The intensity of the relations was very high ($\alpha = 0.91$) to moderate ($\alpha = 0.65$).

Questionnaire data were tabulated and analyzed in Microsoft Excel, using the Chi-Square test to determine differences between regions (western and highland) related to the distribution of coffee growers within the characteristics addressed, as well as a multivariate analysis with groupings of variables: Total area, area with coffee, area of native vegetation. The adopted technique was the multivariate analysis of PCA (Principal Component Analysis) using the R *FactoMinerR* package software [26] applying the selected variables to transform data from a wide spectrum to low spectrum space. PCA was calculated using the correlation matrix for each variable to deduce the eigenvector and eigenvalue. The eigenvector indicates the direction of the main axis with the greatest variance and the eigenvalue indicates the magnitude of the variability of the secondary axis with the next variance. The Bartlett test was used to verify the measure of the correlation matrix and the identity matrix to indicate the existence of the relationship among variables evaluated and the Kaiser–Meyer–Olkin test (KMO) to measure the adequacy of data for the PCA [27].

For data referring to the number of insecticide applications in the agricultural year, canonical variates analysis (CVA) was performed using the procedure CANDISC on the SAS software Basic Edition, Cary, NC, USA (SAS Institute 2011) to verify possible linear associations of applications among locations in each region under study. Data on the use of chemical subgroups and number of applications were correlated using canonical correlation analysis (partial) in order to test the relationship among these variables using the PROC CANCORR procedure [28].

3. Results

The size of farms sampled in the survey ranged from 2 to 44 thousand hectares (Figure 2a), the cultivation area ranged from 0.5 to 1800 hectares (Figure 2b), and included farms without area of native vegetation and farms with up to 200 hectares of native vegetation (Figure 2c).

The variables presented in Table 1 and Figure 2 provide total components and the proportion of variance indicating the total variation of the principal component. For the total area component, two distinct axes were obtained, PC1 and PC2, accounting for 56.03% and 43.97% of the total variance observed (Figure 2a). These results indicate the prevalence of small farm size in the highlands (frequently lower than 100 ha), and a broader range of farm size variation in western Bahia with the prevalence of large farm size (i.e., >200 ha) (Figure 2a).

When the area cultivated with coffee was analyzed, linear correlations were also significant, with PC1 and PC2 representing 54.28% and 45.71% of variance, respectively. The profile of farms in their respective coffee cultivated areas closely follows the trend of overall farm size with greater coffee areas prevailing in the western region and small coffee areas prevailing in the highlands (Figure 2b), where more uniform and smaller farm (and coffee field) sizes prevail.

Areas covered with native vegetation and recognized as permanently maintained preservation areas were also surveyed for each farm in each region. The PC1 and PC2 obtained accounted for 62.61 and 37.39% of the observed variance, respectively (Figure 2c). Large areas of native vegetation are frequently associated with larger farm size, while smaller areas of native vegetation are associated with small farm size (Figure 2c).

The results indicated that the occupation of the farms with the coffee crop ($\chi^2 = 42.85$; $p < 0.0001$) is more expressive on smaller properties, on average, 62.4% of the total area is used with coffee in the highlands region, varying from 20% to 100%, in the western, the average occupation with culture vary with an average of 19.3% (Figure 2d). With respect to the native vegetation area ($\chi^2 = 11.55$; $p < 0.0001$), most farms in the highlands have up to 20%, and in the western 11% to 30% of the total area comprehend areas of vegetation reserve (Figure 2e).

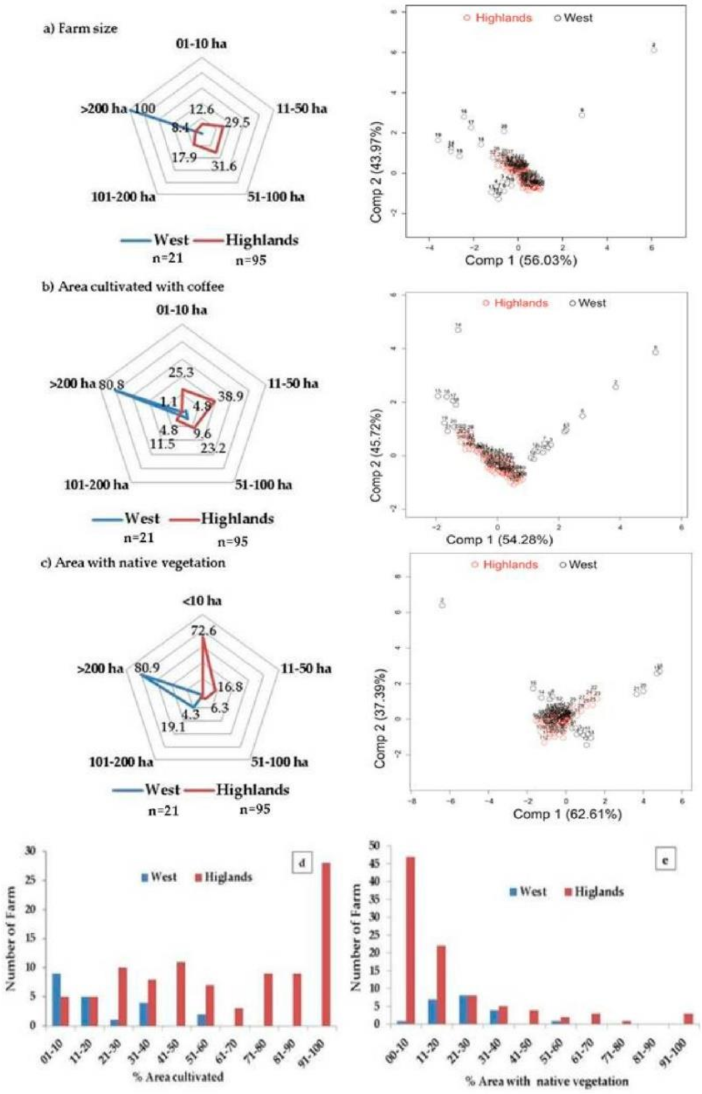

Figure 2. Percentage of coffee farmers' responses and graph of the matrix of 116 sampled farms, belonging to the western and south-central highlands regions of the state Bahia: Total area (**a**), area cultivated with Arabica coffee (**b**), area of native vegetation (**c**), percentage of area cultivated with coffee (**d**), and native vegetation (**e**) in relation to total area.

Table 1. Principal components, eigenvalues, proportion of explained variance, and proportion accumulated by components for total area, cultivated area and native vegetation area.

		Total Area	
Component	**Eigenvalues**	**Proportion**	**Proportion Accumulated**
PC1	1.12	56.03	56.03
PC2	0.87	43.96	100.00
		Cultivated Area	
PC1	1.08	54.28	54.28
PC2	0.91	45.71	100.00
		Native Vegetation Area	
PC1	1.90	62.61	62.61
PC2	0.89	37.39	100.00

Data related to type of coffee cultivation ($\chi^2 = 6.00$; $p = 0.014$) and cultivars ($\chi^2 = 37.59$; $p < 0.0001$) indicated significant differences between regions. In the western region, 100% of coffee crops are irrigated, while non-irrigated cultivation prevails in the highland region (76.4%) (Figure 3a). The 'Catuaí' cultivar is predominant in both regions, reaching 100.0% of the coffee cultivated area in the western region and 90.6% in the highland region (Figure 3b).

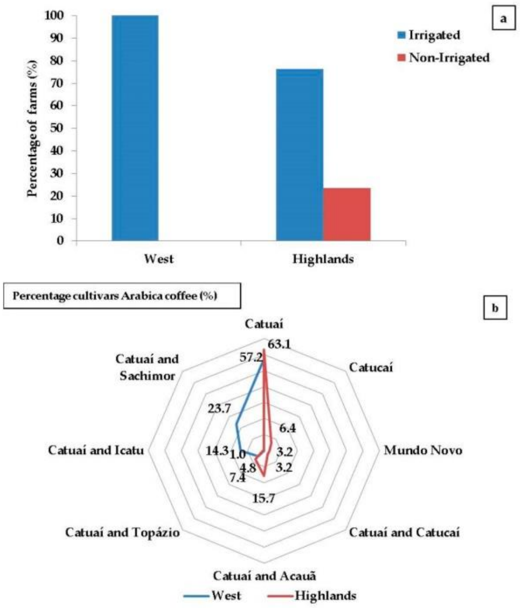

Figure 3. Irrigation prevalence (**a**) and prevailing cultivars of Arabica coffee (**b**) cultivated in the western and south-central highlands regions of the state Bahia.

Regarding the management of the coffee leaf miner, it was observed differences between regions ($\chi^2 = 12.11$; $p = 0.0005$), control tactics used ($\chi^2 = 7.86$; $p = 0.048$), use of insecticide rotation ($\chi^2 = 3.96$; $p = 0.046$), and range of insecticide dose used ($\chi^2 = 33.81$; $p < 0.0001$), without difference in the level of control ($\chi^2 = 2.48$; $p = 0.289$). The infestation level of the coffee leaf miner is monitored by 76.2% (western region) and 34.7% (highland region) of coffee growers (Figure 4a). However, most coffee growers in the western (95.2%) and highland regions (93.7%) do not consider the action (or control) threshold for decision-making, performing only non-quantitative (visual or qualitative) sampling for the adoption of chemical control (Figure 4b).

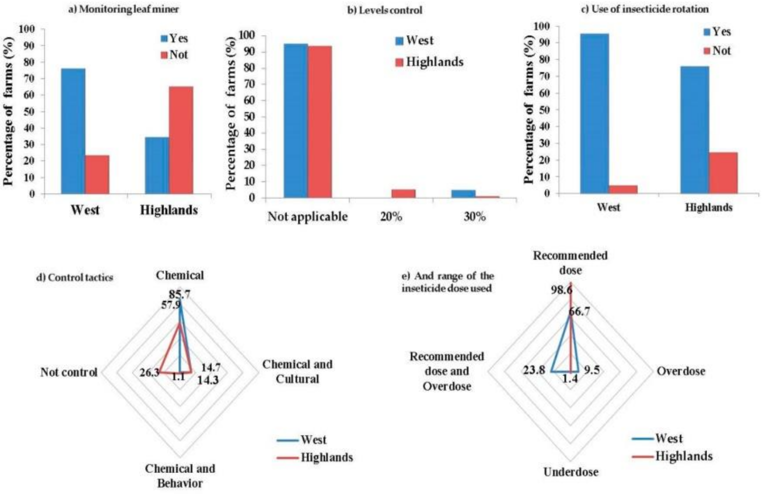

Figure 4. Percentage of responses of coffee farmers to the management of the Neotropical coffee leaf miner (*Leucoptera coffeella*): Monitoring leaf miner (**a**), levels control (**b**), use of insecticide rotation (**c**), control tactics (**d**), and range of the insecticide dose used (**e**) in Arabica coffee crops in the western and south-central highlands regions of the state Bahia.

The prevalent control method is chemical (i.e., by means of insecticide use), mainly in the western region where 100% of farms use insecticides, and only 14.3% associate chemical control with cultural management (e.g., weed management). In the highland region, 57.9% of coffee growers carry out only chemical control. On the other hand, 26.3% of coffee growers in the highland region do not adopt any control method, 14.7% associate chemical and cultural methods, and 1.1% associate chemical and behavioral methods through food bait (based on oleoresins and sugar, Noctovi®) (Figure 4c,d).

The rotation of insecticides is carried out by the majority of coffee growers in the western (95.2%) and highland regions (75.7%). Among the coffee growers, 66.7% use the recommended label rate in western Bahia, and 98.6% use that in the highlands. About a third (33.3%) of the coffee growers in western Bahia overdose the insecticide applications (Figure 4e).

Canonical correlation (partial) in the group of variables was formed by insecticide classes and their frequency of application (Table 2 and Figure 5), which was positive and significant. The main constituents of the canonical pair were based on values of correlations and canonical coefficients with the two canonical axes significant and the first axis explaining 99% of the total data variance for both western and highland regions (Table 2).

Absolute values of the highest coefficients were obtained for insecticides diamides, avermectin, nereistoxin analogs, neonicotinoids, and benzoylureas, which contributed to the pattern of divergence between number of applications among the different farms of the western region, in contrast with the diamide, neonicotinoid and benzoylurea more frequent use in the highland region (Figure 5). The first canonical axis of the greatest weight in the analysis indicates frequent use in the western region of all insecticide classes, except organophosphate and pyrethroids (Table 2). The use of diamides prevailed in the highlands and insecticides with more uniform use of insecticides from different classes, and lack of use of nereistoxin analogs (Table 2 and Figure 5). It was observed that in the highland region, the frequency of insecticide applications is lower in comparison to the western region. The range of annual insecticide applications is one to 12 applications in the highlands, and 6 to 20 applications in the western region.

Table 2. Canonical axes and coefficients (grouped in the canonical structure) of the frequency of application of insecticides of the different classes used in the control of *Leucoptera coffeella* in the west and highlands regions of Bahia.

Variable	**Canonical Axes**			
	West		**Highlands**	
	1	**2**	**1**	**2**
Diamide	0.5070	−0.1915	0.7548	−0.2161
Neonicotinoids	0.4467	−0.2930	0.5815	−0.1152
Pyrethroids	0.1424	−0.2875	0.6578	0.1237
Avermectin	0.5694	0.0037	0.3793	−0.0096
Benzoylurea	0.5996	0.3062	0.4246	0.4954
Organophosphate	−0.1109	0.6159	0.4268	−0.0131
Nereistoxin analogs	0.6256	0.7459	-	-
F	140.96	12.43	338.29	8.70
Degrees of Freedom (num.; den)	14; 24	6; 13	12; 174	5; 88
p	<0.0001	<0.0001	<0.0001	<0.0001
Canonical squared correlation	0.99	0.85	0.99	0.33

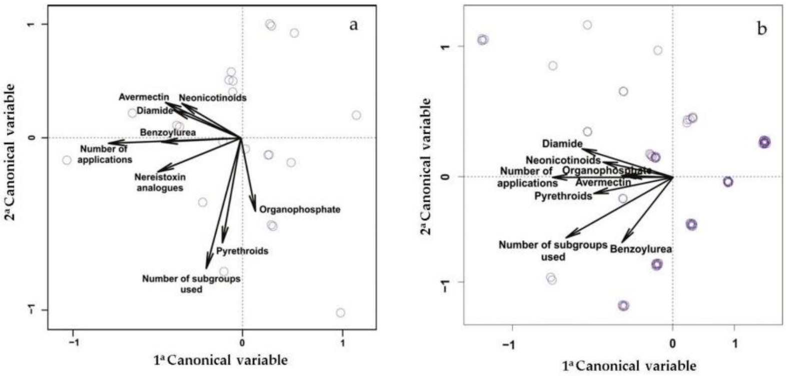

Figure 5. Ordination diagram showing the discrimination between the frequency of application of insecticides for the chemical control of *Leucoptera coffeella* populations in the western (**a**) and highlands (**b**) regions of Bahia. The symbols are centroid of the localities and represent the average of the classes of canonical variables. The vectors indicate groups of farms without significant difference between them (Wilks' Lambda and approximate F, $p < 0.001$).

The application interval for a product is, on average, 20 days. Products without registration with the Ministry of Agriculture and Livestock [29] for coffee crop, such as Ampligo® (lamda-cyhalothrin + chlorantraniliprole), Interprid® (methoxyfenozide), Dimilin® (diflubenzuron), Match® (lufenuron), Oregon® (novaluron), Talisman® (bifenthrin + carbosulfan) are used by some coffee growers in both regions.

4. Discussion

Descriptive analyses, PCA, and the correlations indicated differences between Arabica coffee producing regions of Bahia, as to the size of the farms, occupation of the land with the coffee crops, and native vegetation, adoption of irrigation and, in some aspects, of the management of the leaf miner. In western Bahia, farms are characterized by larger extensions, with areas of up to 44 thousand hectares and areas of coffee reaching 1800 hectares, exhibiting permanent preservation areas in compliance with Brazilian Forest Code (Law 4771/65), a minimum of 20% of the total area. In highlands, most of the farms have smaller extensions, from 2 to 350 hectares (Figure 2a), in some cases, totally occupied with the coffee crop, with a more heterogeneous permanent reserve occupancy rate among the farms.

Coffee production of western Bahia stands out for having 100% of the coffee area completely irrigated (center-pivot and drip irrigation), contrasting with the prevalent non-irrigated coffee of the highland region. The predominant cultivar in these regions is the 'Catuaí', which is characterized by being of small size, short internodes, abundant secondary branching, red (cultivar IAC 144), or yellow (cultivar IAC 62) fruits of medium to late maturation, high yield and adaptation to extreme temperatures [30]. However, besides to areas cultivated with Catuaí, other cultivars such as 'Acauã', 'Mundo Novo' and 'Catucaí' are also used only in the highland region, while the 'Sarchimor' cultivar is present only in the western region.

These differences reflect in several aspects of the coffee production chain, with mechanized systems and outsourced manpower in the western region, enabling the management of crop in macro scale. Coffee crops in this region is characterized by present productivity above the national Brazilian average and using agricultural inputs, irrigation, appropriate genotypes, and mechanization, among other practices. The climate is favorable to the quality of the coffee, because at the time of harvest, conditions of low relative humidity of the air occur, with the rains concentrated in the summer [31]. In highlands, the use of inputs is less intense and family manpower predominates. The specificities of some microregions in the highlands, such as the Chapada Diamantina, about the climate and the realization of selective and manual harvesting of the fruits, have guaranteed the production of special coffees of excellent quality, with high value aggregated [32].

In the last two years, only one farm in the western region did not show infestation by the coffee leaf miner due to the monitoring used and the application of insecticides in a preventive way in order not to compromise coffee production. However, the other farms have a history of high infestation by the coffee leaf miner resulting in a higher frequency of insecticide applications. In the highland region, some crops fields located in the municipalities of Piatã, Ibicoara, Barra da Estiva, and Vitória da Conquista have low infestation levels, and without a history of insecticide application for over a decade. On the other hand, crops located on municipality Mucugê presented high infestations by the coffee leaf miner in the last five years. This fact caused serious problems for coffee growers, resulting in an increasing number of applications of different insecticide classes.

When asked whether or not to carry out monitoring of the coffee leaf miner, most respondents carried out monitoring, which is the basis for IPM. However, throughout the interview, it was observed that the concept of monitoring is not suitably adopted in practice. This is because, in most cases, the monitoring performed is a visual, non-quantitative, and non-systematic analysis based only on the presence or absence of live larvae detected in quick and casual visual observation. Interestingly, there is no shortage of studies on sampling of the coffee leaf miner, both with conventional [33] and sequential plans [34]. Another problem detected in the survey is that the percentages of predation and natural parasitism are also not quantified by coffee growers [35]. Thus, decision-making about whether or not to use a control method, another IPM support pillar, is not based on the analysis of numerical variations of pest populations and their main natural enemies. The consequence of such neglect of the preventive use of insecticides for leaf miner resulting in insecticide overuse and unnecessary increase in production costs [36].

Quantitative population assessments based on activity levels and economic injury thresholds form the basis of IPM to minimize unnecessary interventions for pest population suppression, especially by the chemical method. When correctly performed and based on a validated sampling plan, monitoring favors decision-making with reduced application of insecticides and reduced production costs. Despite the economic and environmental advantages resulting from the use of the limit established for decision making in agriculture pest management [37,38], their effective use remains as one of the main obstacles to the use of IPM programs in various agricultural crops in Brazil and worldwide [5,37,39,40]. On the other hand, IPM plays a fundamental role in adapting production systems to the trends of modern agriculture for the economic benefit of growers and the reduction of environmental impacts [5,41–43].

Although there are edaphoclimatic differences between regions, the coffee leaf miner occurs throughout the year in Bahia, finding optimal conditions for its development in the western region (low relative humidity and high temperatures) and favorable conditions in the south-central highland region (lower temperatures and high relative humidity at certain times of the year). However, in the highlands at altitudes around 1000 m and average annual temperature of around 20 °C, the coffee leaf miner population remains at the equilibrium level for most of the year, with the presence of at least six species of parasitoids that act in the pest regulation [44]. The same species of parasitoids have also been observed in the western region in coffee crops located in Luis Eduardo Magalhães, which has an average annual temperature of approximately 24.3 °C, but with lower parasitism rates and with variations in the structure of their communities [44].

Most coffee growers adopt only chemical control for coffee leaf miner population suppression, an aspect that, associated with lack of suitable monitoring for control decision-making, impairs the proper use of IPM in coffee farms of the region. With rare exceptions, there are coffee growers in both regions who adopt chemical and cultural controls. On the other, 26.3% of highlands coffee growers do not use any control tactics for the leaf miner, which reveals that the insect meets a "non-pest" situation, probably due to the regulation of its population by factors, such as parasitoid wasps and predators whose survival and permanence in coffee plantations are favored by the absence of insecticides, higher altitude of the region (1000 to 2000 m), and lower average temperatures in relation to the western region. In some cases, coffee growers have not used insecticides for more than 15 years, with the reestablishment of beneficial fauna.

In the case of coffee production, this agro-ecosystem has the capacity of harboring several natural enemies [45]. The harmonization of these practices tends to reduce impacts on the communities of predators and parasitoids, reducing the incidence of the coffee leaf miner. According to Faria and Angelini [46], cultural control aids chemical control by reducing the incidence of the coffee leaf miner in coffee crops. Another important data obtained in the survey was the use of behavioral control using the Noctovi® food-based attractant in a farm located in the municipality of Barra do Choça (highland). Such use allows the recording of the pest population dynamics and can be used simultaneously with insecticides.

Vegetation diversification reduces the pest incidence favoring and providing alternative foods to natural enemies [47]. Natural enemies, such as parasitoids, are efficient when associated with integrated management of the coffee leaf miner [44,48,49], and the use of more selective insecticides favors their prevalence in coffee crops [22,50].

Organophosphate, carbamate, and pyrethroid insecticides were the most used in the control of the coffee leaf miner, but other insecticide classes were more recently introduced and are broadly used against the coffee leaf miner, including neonicotinoids [51], diamides [52], avermectins [53], all of which act on the nervous system, and benzoylureas, which are insect growth disruptors interfering with chitin synthesis, a major component of the insect exoskeleton [54]. Interestingly, rather than rotating the insecticide molecules for controlling the coffee leaf miner, a pivotal recommendation to minimize selection for insecticide resistance in pest species, the growers tend to rotate trade names or formulations, frequently maintaining the use of the same insecticide, but using different commercial products. Thus, no wonder insecticide resistance is a problem in the region against this pest species [25].

The frequent use of insecticides causes selection pressure on pest individuals, favoring the emergence of individuals resistant to products used in their control [24], leading coffee growers to use an overdose of product, greater number of applications, and consequently, replacement of an ineffective insecticide by a new insecticide [55–57]. Fragoso et al. [23] reported the applications of 22 insecticides in a year, 10 of which were organophosphates. Furthermore, many coffee growers make frequent use of insecticides, including relatively more persistent and less selective compounds. Such patterns of insecticide use enhance insecticide resistance risk and environmental problems [22].

A concerning piece of data regarding the use of insecticides in the western region is the residual period indicated by manufacturers, which is not respected by coffee growers. The application interval for a product is, on average, 20 days. In addition to data on the coffee leaf miner management, products without registration with the Ministry of Agriculture and Livestock [29] should not be used according to the regulation of Law No. 7.802 of July 11, 1989, Art.73. Nonetheless, the use of unregistered products is one of the major problems faced when thinking about adapting production to the trends of modern agriculture, as observed for custard apple (*Annona squamosa* L.) crops [58].

There is an increase in the frequency of annual applications of the neonicotinoids thiamethoxam and imidacloprid (two to four) and uniform use of the same compounds in several farms of the region, which increases the risk of selecting for insecticide resistance. Such a trend takes place regardless of the size of cultivated area, climate, temperature, and rainfall.

It is noteworthy that the greater the number of applications and use of insecticides with the same site of action, the greater the likelihood of insecticide resistance. Most coffee growers use an application schedule, not considering parameters population trend coffee leaf miner and natural enemy population densities [32]. Nonetheless, measures for the successful reduction of the pest population must be carried out considering ecological, environmental, and economic and food security aspects for sustainable and high-quality coffee production. Therefore, there is an urgent need for integrative action between research and extension agencies, agricultural companies and coffee growers to expand the use of IPM principles in line with global agriculture megatrends, which are the bases for the sustainability of agriculture production chains [59].

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ANNEX II

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Research

Area-Wide Survey of Chlorantraniliprole Resistance and Control Failure Likelihood of the Neotropical Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)

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Abstract

The Neotropical coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842), is a key pest species of unshaded coffee plantations in Neotropical America, particularly in Brazil, where pest management involves intensive insecticide use. As a consequence, problems of resistance to conventional insecticides are frequent, and more recently developed insecticide molecules, such as diamide insecticides, are at risk of becoming ineffective. Thus, a survey of resistance to the diamide insecticide chlorantraniliprole was carried out in high-yield coffee-producing areas in the State of Bahia, Brazil. The likelihood of control failure with this insecticide was also assessed. Spatial dependence among the insect sampling sites was assessed and spatial mapping of chlorantraniliprole resistance and risk of control failure was carried out. The frequency of chlorantraniliprole resistant populations was high (34 out of 40 populations, or 85%), particularly in western Bahia, where 94% of the populations were resistant. Resistance levels ranged from low (<10-fold) to moderate (between 10- and 40-fold) with more serious instances occurring in western Bahia. This results in lower chlorantraniliprole efficacy among these populations, with a higher risk of control failure and exhibiting spatial dependence. These findings invite attention to problems with the intensive use of this relatively recent insecticide and demand management attention, but they suggest that local, farm-based management efforts are likely to be the most effective actions against resistance problems in this pest species.

Key words: anthranilic diamides, insecticide control failure, control failure likelihood, insecticide resistance, resistance survey

Life happens; coffee helps! At least that is the belief of a fair share of the human population stressed, blessed, and even obsessed with coffee. The statement is equally valid for coffee producers particularly when facing likely losses due to the Neotropical leaf miner *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842). This leaf miner is the key coffee pest species in unshaded coffee plantations, the dominant cultivation system of high-quality coffee (*Coffea arabica* L.) in Neotropical America, particularly Brazil (Tuelher et al. 2003; Pereira et al. 2007a,b; Magalhães et al. 2010; Pantoja-Gomez et al. 2019), the largest producer and exporter of this prized commodity (MAPA 2018; CONAB 2019). The annual losses by this pest species average about 40% yield but can reach values as high as 80% under high population densities where consumption of palisade parenchyma compromises photosynthetic leaf area leading to early leaf senescence (Tuelher et al. 2003, Pereira et al. 2007a). Thus, the management of this species is of paramount importance in such areas and is achieved mainly with the use of insecticides (Fragoso et al. 2003).

The importance of the leaf miner in coffee production and the (over-)reliance on insecticide use for managing this species naturally raises concern about evolving insecticide resistance in leaf miner populations. Eventual insecticide control failure may result from this, in addition to other hierarchical consequences beyond the population level (Guedes et al. 2016, 2017, 2019). Curiously, studies of insecticide resistance in the coffee leaf miner are rare (Alves et al. 1992; Fragoso et al. 2002, 2003), and the likelihood of insecticide control failure is neglected, as is the potential spatial dependence of both interdependent but distinct phenomena (Guedes 2017).

Insecticide resistance may lead to control failure, but not necessarily since this interaction depends on patterns of cultivation and insecticide use, among other factors, which potentially exhibit spatial dependence (Liebhold et al. 1993; Fragoso et al. 2002; Bacca et al. 2006, 2008; Gontijo et al. 2013; Guedes 2017; Tuelher et al. 2018; Guedes et al. 2019). The possibility of simultaneously surveying both phenomena and geographically mapping their incidence is seldom attempted despite their strategic relevance for pest management, although some progress has been recently made (Chediak et al. 2016, Guedes 2017, Tuelher et al. 2018).

Insecticide resistance in Neotropical coffee leaf miners was earlier recorded in Brazil against organophosphates, the main insecticide class for management of this species at the time (Alves et al. 1992). However, increases in coffee prices in the international market and consequent concern with leaf miner losses has led to an intensification of insecticide use and magnification of problems with insecticide resistance (Fragoso et al. 2002, 2003). Organophosphate resistance reached very high levels (>1,000-fold) in some of the main producing areas of high-quality coffee in Brazil (Fragoso et al. 2002, 2003). This has led to a diversification of insecticides used against the Neotropical coffee leaf miner, which came to rely on neonicotinoid and diamide use in recent years (MAPA 2019). As a consequence, reports of moderate levels of neonicotinoid resistance have recently emerged (Costa et al. 2016), while diamide use has further intensified.

The diamides are a sound alternative for insect pest control because of their peculiar mode of action distinct from other insecticides available on the market (Lahm et al. 2009). They act as ryanodine receptor activators in the calcium channels regulating muscle cell contractions, through calcium release in the sarcoplasmic reticulum (Lahm et al. 2005, Nauen 2006). The diamide chlorantraniliprole is broadly used against lepidopteran pest species in different crops, including coffee, because of its low nontarget impact and lack of cross-resistance to other insecticides making it a useful pest management tool (Gao et al. 2013). Nonetheless, the growing use of this insecticide is leading to increasing reports of resistance to this molecule in populations of the diamond backmoth *Plutella xylostella* (Troczka et al. 2012, Wang and Wu 2012), the Neotropical tomato pinworm *Tuta absoluta* (Roditakis et al. 2015), and the rice stem borer *Chilo suppressalis* (Lu et al. 2017, Wei et al. 2019).

Table 1. Identification and geographical coordinates of the sampling sites for populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in our survey of chlorantraniliprole resistance, efficacy, and control failure likelihood in the State of Bahia, Brazil

Meso-region	County	Code	Longitude	Latitude
West	Barreiras	WBAR1	–11° 52′ 30.0″	–45° 43′ 06.3″
	Barreiras	WBAR2	–12° 16′ 30.9″	–45° 30′ 35.1″
	Barreiras	WBAR3	–12° 16′ 30.9″	–45° 35′ 32.6″
	Barreiras	WBAR4	–11° 51′ 35.6″	–45° 44′ 47.0″
	Cocos	WCOC1	–14° 38′ 50.6″	–45° 15′ 41.9″
	Cocos	WCOC2	–14° 40′ 56.8″	–45° 49′ 03.6″
	Luiz Eduardo Magalhães	WLEM1	–11° 57′ 43.08″	–45° 44′ 01.7″
	Luiz Eduardo Magalhães	WLEM2	–12° 08′ 59.1″	–45° 47′ 18.1″
	Luiz Eduardo Magalhães	WLEM3	–12° 03′ 46.4″″	–45° 54′ 10.5″
	Luiz Eduardo Magalhães	WLEM4	–12° 16′ 19.4″	–45° 56′ 02.6″
	Luiz Eduardo Magalhães	WLEM5	–12° 16′ 49.0″	–45° 44′ 17.6″
	São Desiderio	WSDE1	–12° 08′ 06.4″	–45° 53′ 20.3″
	São Desiderio	WSDE2	–12° 33′ 21.4″	–45° 51′ 59.1″
	São Desiderio	WSDE3	–12° 54′ 12.7″	–45° 32′ 29.4″
	São Desiderio	WSDE4	–12° 33′ 06.8″	–45° 47′ 23.7″
	São Desiderio	WSDE5	–12° 52′ 46.6″	–46° 02′ 13.2″
	São Desiderio	WSDE6	–12° 35′ 04.0″	–45° 40′ 03.4″
Highlands	Barra da Estiva	HBES1	–13° 37′ 15.0″	–41° 20′ 37.3″
	Barra da Estiva	HBES2	–13° 33′ 18.0″	–41° 20′ 09.1″
	Barra da Estiva	HBES3	–13° 36′ 45.3″	–41° 19′ 53.9″
	Barra do Choça	HBCH1	–14° 50′ 27.5″	–40° 31′ 13.0″
	Barra do Choça	HBCH2	–14° 53′ 55.3″	–40° 35′ 35.4″
	Barra do Choça	HBCH3	–14° 55′ 25.2″	–40° 36′ 43.5″
	Barra do Choça	HBCH4	–14° 55′ 05.8″	–40° 36′ 01.9″
	Barra do Choça	HBCH5	–14° 50′ 15.9″	–40° 31′ 04.4″
	Barra do Choça	HBCH6	–14° 54′ 58.1″	–40° 36′ 24.8″
	Barra do Choça	HBCH7	–14° 51′ 37.5″	–40° 31′ 33.2″
	Barra do Choça	HBCH8	–14° 54′ 59.4″	–40° 37′ 30.6″
	Encruzilhada	HENC1	-15° 36′ 50.1″	–40° 44′ 32.3″
	Encruzilhada	HENC2	–15° 37′ 14.3″	–40° 45′ 59.0″
	Encruzilhada	HENC3	–15° 39′ 37.0″	–40° 45′ 38.0″
	Mucugê	HMUC1	–13° 02′ 38.8″	–41° 26′ 02.4″
	Mucugê	HMUC2	–13° 09′ 02.9″	–41° 28′ 19.8″
	Mucugê	HMUC3	–13° 07′ 37.1″	–41° 29′ 25.4″
	Mucugê	HMUC4	–13° 05′ 57.6″	–41° 26′ 38.2″″
	Vitória da Conquista	HVDC1	–14° 59′ 52.0″	–40° 47′ 55.2″
	Vitória da Conquista	HVDC2	–15° 16′ 37.5″	–40° 56′ 49.2″
	Vitória da Conquista	HVDC3	–15° 14′ 39.6″	–40° 59′ 11.9″
	Vitória da Conquista	HVDC4	–15° 00′ 30.0″	–40° 45′ 25.6″
	Vitória da Conquista	HVDC5	–14° 58′ 15.3″	–40° 46′ 09.6″

Diamide resistance among coffee leaf miner populations have not yet been a target of attention, and the use of this class of insecticides remains intensive. This scenario has led to the current concern that diamide resistance and particularly chlorantraniliprole resistance may be evolving and may result in future control failures with this insecticide. Therefore, the objectives of the present study were as follows: 1) to survey the incidence of chlorantraniliprole resistance among populations of the Neotropical coffee leaf miner from two important regions of Arabica coffee production in Brazil; 2) to assess the likelihood of control failure with chlorantraniliprole due to the occurrence of resistance to this insecticide in the region; and 3) to preliminarily test whether spatial dependence in chlorantraniliprole resistance exists among sampling sites and to tentatively map such occurrences, if such is the case.

The intensive use of insecticides in the coffee growing regions of the state of Bahia has led us to hypothesize that chlorantraniliprole resistance may already exist in the region, although probably in its initial stages. This suspicion is justified because the use of this compound for coffee protection has only increased recently, but reaching up to 17 annual applications, 2 on soil and 15 spray applications (Castellani et al. 2016). Consequently, resistance to this diamide is likely recent and control failure of chlorantraniliprole was not yet expected since it takes longer to occur as it usually requires incidence of high levels of resistance, a scenario that allows efficient implementation of resistance management practices to minimize such risk. Spatial dependence was not expected because variation in the incidence of insecticide resistance was unlikely to be high (and diverse), compromising the recognition of such a relationship and the spatial mapping of this phenomenon.

Materials and Methods

Insects and Insecticide

Sampling of populations of the Neotropical coffee leaf miner was carried out in 40 sites from two high-quality coffee-producing regions in the state of Bahia (Brazil) – western Bahia (17 sites), and its south-central highlands (23 sites; Table 1; Fig. 1). Leaves containing intact mines were collected from each site, and geo-referenced with a global positioning system (GPS) receiver (Garmin E-Trex Vista HCx, Olathe, KS). The samples were collected between March and December 2018, avoiding leaves with open and/or torn mines indicative of parasitism or predation. The collected leaves were placed in Kraft-type paper bags (17 × 45 cm) and stored in polystyrene boxes for transportation to the laboratory for subsequent bioassays under environmentally controlled conditions.

A commercial formulation of the diamide insecticide chlorantraniliprole was used in the bioassays (350 g a.i./kg, water dispersible granules, DuPont, Paulínia, SP, Brazil). The insecticide was used at its label rate, as registered at the Brazilian Ministry of

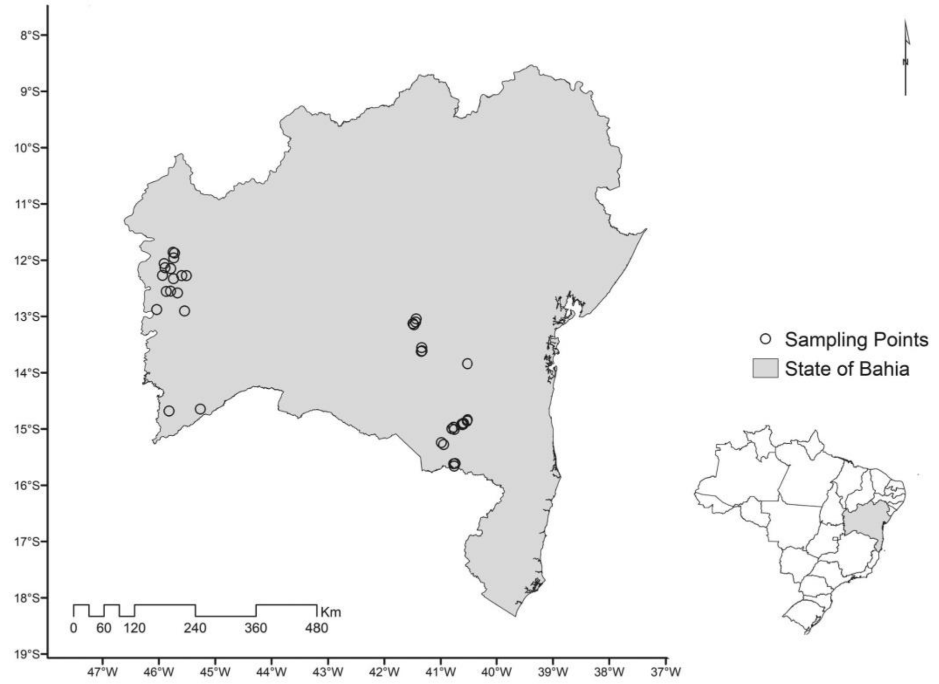

Fig. 1. Distribution of the sampling sites for populations of the Neotropical coffee leaf miner *Leucoptera coffeella* used in the spatial survey of chlorantraniliprole resistance in Brazil. Identification for each sampling site and its coordinates are found in Table 1.

Agriculture (MAPA 2019), following the manufacturer's recommendations. This is the main insecticide currently used in the region against this pest species. The use of a fixed concentration varying exposure allows estimates of both the level of resistance, through time-mortality bioassays, and frequency of resistant individuals, through discriminating time bioassays. This approach parallels others, like Dângelo et al. (2018) with whiteflies, but based on fixed concentration and varying length of exposure and including spatial analyses and spatial mapping of the phenomenon. Furthermore, the discriminating time bioassays also allow estimation of control failure likelihood due to insecticide resistance justifying the present approach.

Time-Mortality Toxicity Bioassays

Time-mortality insecticide bioassays were carried out following methods adapted from Fragoso et al. (2002), which were derived from earlier work on the tomato pinworm *Tuta absoluta* (Siqueira et al. 2000, 2001). A single chlorantraniliprole concentration was used, the field label rate (90 g a.i./ha), at a rate of 400 liter/ha (= 0.23 g a.i./ml), and the exposure times of 2, 4, 6, 12, 18, 24, 36, and 48 h. Filter paper disks (Whatman no. 1; 9.0-cm diameter) were immersed in the insecticide solution for 10 s and allowed to dry for 1 h at ambient temperature, after which they were placed in Petri dishes (9.0-cm diameter × 1.5-cm high). Twenty third-instar larvae removed from the field-collected leaves were placed in each Petri dish using a fine hair-brush, and they were subsequently maintained in an environmental chamber under controlled conditions of 25 ± 2°C temperature and 70 ± 5% relative humidity. The experiment was replicated three times for each insect population. Larval mortality was recognized by the inability to move a body length when prodded by a hair-brush. Untreated controls for each insect population were maintained to record natural larval mortality for correction of the chlorantraniliprole-exposed mortality observed (Abbott 1925).

Table 2. Relative toxicity of chlorantranilprole to Brazilian populations of the coffee leaf miner (*Leucoptera coffeella*)

Meso-region	County	Code	No.	Slope ± SE	LT_{50} (95% FI) hours	χ^2	df	*P*	Resistance ratio at LT_{50} [RR_{50} (95% CI)]
West	Barreiras	WBAR1	480	0.92 ± 0.14	19.96 (14.89–28.95)	0.34	6	0.99	5.72 (2.34–16.86)*
	Barreiras	WBAR2	480	1.33 ± 0.19	65.91 (46.44–115.74)	4.05	6	0.67	18.88 (8.88–48.50)*
	Barreiras	WBAR3	480	1.56 ± 0.19	50.20 (38.46–74.21)	1.19	6	0.98	14.38 (7.70–32.43)*
	Barreiras	WBAR4	480	1.09 ± 0.15	43.03 (31.01–70.78)	3.16	6	0.79	12.33 (5.41–33.96)*
	Cocos	WCOC1	480	0.94 ± 0.17	111.79 (62.93–338.55)	1.17	6	0.98	32.03 (9.66-.128.28)*
	Cocos	WCOC2	480	1.17 ± 0.14	14.96 (11.86–19.17)	7.22	6	0.30	4.29 (2.28–9.74)*
	Luiz Eduardo Magalhães	WLEM1	480	0.82 ± 0.13	9.13 (6.25–12.61)	2.09	6	0.91	2.62 (1.07–7.75)*
	Luiz Eduardo Magalhães	WLEM2	480	0.61 ± 0.14	113.31 (53.11–700.41)	2.36	6	0.88	32.47 (3.95–322.72)*
	Luiz Eduardo Magalhães	WLEM3	480	1.28 ± 0.17	46.47 (34.51–72.35)	2.22	6	0.89	13.32 (2.95–72.57)*
	Luiz Eduardo Magalhães	WLEM4	480	1.01 ± 0.16	70.06 (45.25–148.93)	1.70	6	0.95	20.08 (7.43–65.54)*
	Luiz Eduardo Magalhães	WLEM5	480	0.97 ± 0.14	35.50 (25.48–58.79)	6.69	6	0.35	10.17 (4.04–30.95)*
	São Desiderio	WSDE1	480	1.53 ± 0.16	23.13 (19.11–28.99)	10.07	6	0.12	6.63 (3.86–13.75)*
	São Desiderio	WSDE2	480	0.92 ± 0.14	38.61 (29.94–68.28)	2.69	6	0.84	11.06 (4.03–36.68)*
	São Desiderio	WSDE3	480	1.12 ± 0.14	24.71 (19.13–34.45)	1.81	6	0.94	7.08 (3.46–17.51)*
	São Desiderio	WSDE4	480	1.00 ± 0.13	5.72 (3.87–7.64)	2.60	6	0.86	1.64 (0.87–3.71)
	São Desiderio	WSDE5	480	1.28 ± 0.14	8.34 (6.50–10.40)	0.45	6	0.99	2.39 (1.39–4.98)*
	São Desiderio	WSDE6	480	1.12 ± 0.15	38.19 (28.26–59.52)	3.92	6	0.69	10.94 (5.00–28.93)*
Highlands	Barra da Estiva	HBES1	480	1.17 ± 0.15	3.18 (2.02–4.35)	0.54	6	0.99	1.00 (0.49–2.03)
	Barra da Estiva	HBES2	480	1.39 ± 0.15	3.58 (2.53–4.63)	1.74	6	0.94	1.03 (0.60–2.12)
	Barra da Estiva	HBES3	480	1.07 ± 0.14	4.06 (2.63–5.52)	2.77	6	0.84	1.16 (0.65–2.52)
	Barra do Choça	HBCH1	480	0.76 ± 0.13	30.70 (20.85–57.26)	2.65	6	0.85	8.80 (2.224–42.10)*
	Barra do Choça	HBCH2	480	0.97 ± 0.13	24.81 (18.49–37.05)	1.73	6	0.94	7.11 (2.98–20.47)*
	Barra do Choça	HBCH3	480	0.80 ± 0.13	21.26 (15.18–33.65)	0.55	6	0.99	6.09 (2.00–22.44)*
	Barra do Choça	HBCH4	480	1.01 ± 0.15	48.63 (33.66–87.69)	2.65	6	0.85	13.93 (5.53–42.36)*
	Barra do Choça	HBCH5	480	1.04 ± 0.16	64.75 (42.96–128.84)	0.81	6	0.99	18.55 (7.25–57.31)*
	Barra do Choça	HBCH6	480	1.03 ± 0.14	26.70 (20.13–39.27)	1.75	6	0.94	7.65 (3.42–20.69)*
	Barra do Choça	HBCH7	480	1.24 ± 0.14	17.47 (14.02–22.40)	1.42	6	0.96	5.01 (2.72–11.12)*
	Barra do Choça	HBCH8	480	1.07 ± 0.15	34.66 (25.72–53.40)	1.05	6	0.98	9.93 (4.48–26.60)*
	Encruzilhada	HENC1	480	1.57 ± 0.17	34.34 (27.68–45.68)	1.16	6	0.98	9.84 (5.57–37.74)*
	Encruzilhada	HENC2	480	1.28 ± 0.17	52.40 (38.20–85.05)	0.99	6	0.99	15.01 (7.21–8.71)*
	Encruzilhada	HENC3	480	1.34 ± 0.14	14.41 (11.71–17.87)	1.66	6	0.95	4.13 (2.36–8.71)*
	Mucugê	HMUC1	480	1.72 ± 0.17	24.71 (20.71–30.47)	3.56	6	0.73	7.78 (4.25–14.26)*
	Mucugê	HMUC2	480	0.74 ± 0.13	5.18 (2.84–7.60)	0.88	6	0.98	1.63 (0.61–4.39)
	Mucugê	HMUC3	480	0.65 ± 0.13	41.06 (25.25–104.01)	0.85	6	0.17	12.93 (2.16–77.21)*
	Mucugê	HMUC4	480	0.99 ± 0.14	31.08 (22.79–48.75)	3.26	6	0.77	8.78 (1.08–88.79)*
	Vitória da Conquista	HVDC1	480	0.98 ± 0.14	3.49 (2.04–4.95)	9.48	6	0.15	1.00 (0.53–2.28)
	Vitória da Conquista	HVDC2	480	0.56 ± 0.13	45.68 (25.68–166.03)	2.79	6	0.83	13.09 (1.85–111.94)*
	Vitória da Conquista	HVDC3	480	0.90 ± 0.13	11.59 (8.43–16.33)	3.38	6	0.76	3.32 (1.38–10.85)*
	Vitória da Conquista	HVDC4	480	0.90 ± 0.13	8.57 (6.00–11.52)	2.17	6	0.90	2.45 (1.13–6.46)*
	Vitória da Conquista	HVDC5	480	0.51 ± 0.05	23.89 (15.30–49.73)	3.35	6	0.76	6.84 (1.10–51.51)*

The asterisk in the resistance ratio indicate a significant difference from the standard susceptible population based on Robertson et al. (2007).

Expected Efficacy and Control Failure Likelihood

The same procedures and experimental units described above were used for a final mortality assessment after 48 h of exposure as a determination of expected chlorantraniliprole efficacy, after proper correction for natural mortality (as indicated above). These data were subsequently used to estimate the control failure likelihood (CFL) of chlorantraniliprole due to insecticide resistance in each of the field-collected insect populations. The control failure likelihood was estimated using 80% mortality as the minimum threshold of efficacy as required by the Brazilian Ministry of Agriculture for conventional insecticides (MAPA 1995), following methods by Guedes (2017) where CFL = 100-[observed mortality (%) × 100]/expected mortality (i.e., 80%). CFL values ≤ 0 indicate a negligible risk of control failure.

Statistical Analyses

Time-mortality data were subjected to probit analyses (PROC PROBIT; SAS Institute, SAS, Cary, NC). The levels of insecticide resistance, or resistance ratios, were estimated by dividing the median lethal time (LT_{50}) of a given population by the LT_{50} of the most susceptible population as recognized through the toxicity bioassays with chlorantraniliprole. Significant chlorantraniliprole resistance was recognized through estimation of the 95% FIs of the resistance ratios, and they were identified as significant if not including the value of 1 (Robertson et al. 2007). The efficacy and control failure results after 48-h exposure were subjected to a one-sided *Z*-test at 95% confidence level with correction for continuity to test their departure from the expected mortality (Roush and Miller 1986). The relationship between levels of chlorantraniliprole resistance and control failure likelihood was

Table 3. Estimated chlorantraniliprole mortality (%) and control failure likelihood (%) of populations of the Neotropical coffee leaf miner (*Leucoptera coffeella*) using Brazilian recommended label rates

Meso-region	County	Code	No.	Mortality [control failure likelihood] (%)
West	Barreiras	WBAR1	60	69.0 [13.7]*
	Barreiras	WBAR2	60	52.8 [34.0]*
	Barreiras	WBAR3	60	56.4 [29.5]*
	Barreiras	WBAR4	60	61.8 [22.7]*
	Cocos	WCOC1	60	40.0 [50.0]*
	Cocos	WCOC2	60	76.3 [4.6]
	Luiz Eduardo Magalhães	WLEM1	60	78.3 [2.1]
	Luiz Eduardo Magalhães	WLEM2	60	52.8 [34.0]*
	Luiz Eduardo Magalhães	WLEM3	60	61.8 [22.8]*
	Luiz Eduardo Magalhães	WLEM4	60	52.8 [34.0]*
	Luiz Eduardo Magalhães	WLEM5	60	60.0 [25.0]*
	São Desiderio	WSDE1	60	67.3 [15.9]*
	São Desiderio	WSDE2	60	54.5 [31.9]*
	São Desiderio	WSDE3	60	69.0 [13.7]*
	São Desiderio	WSDE4	60	85.4 [0.0]
	São Desiderio	WSDE5	60	90.8 [0.0]
	São Desiderio	WSDE6	60	52.7 [34.1]*
Highlands	Barra da Estiva	HBES1	60	91.7 [0.0]
	Barra da Estiva	HBES2	60	100.0 [0.0]
	Barra da Estiva	HBES3	60	96.3 [0.0]
	Barra do Choça	HBCH1	60	63.6 [20.5]*
	Barra do Choça	HBCH2	60	70.1 [12.4]*
	Barra do Choça	HBCH3	60	63.6 [20.5]*
	Barra do Choça	HBCH4	60	58.1 [27.4]*
	Barra do Choça	HBCH5	60	49.1 [38.6]*
	Barra do Choça	HBCH6	60	61.8 [22.8]*
	Barra do Choça	HBCH7	60	76.3 [4.6]
	Barra do Choça	HBCH8	60	60.0 [25.0]*
	Encruzilhada	HENC1	60	65.4 [18.3]*
	Encruzilhada	HENC2	60	52.7 [34.1]*
	Encruzilhada	HENC3	60	78.3 [2.1]
	Mucugê	HMUC1	60	78.3 [2.13]*
	Mucugê	HMUC2	60	87.2 [0.0]
	Mucugê	HMUC3	60	58.1 [27.37]*
	Mucugê	HMUC4	60	65.4 [18.3]*
	Vitória da Conquista	HVDC1	60	91.7 [0.0]
	Vitória da Conquista	HVDC2	60	54.5 [31.9]*
	Vitória da Conquista	HVDC3	60	74.5 [6.9]*
	Vitória da Conquista	HVDC4	60	81.8 [0.0]*
	Vitória da Conquista	HVDC5	60	67.3 [15.9]*

Mortalities followed by an asterisk are significantly lower than the minimum efficacy threshold of 80% (one-sided *Z*-test at 95% confidence level with correction for continuity and Bonferroni correction; *n* = 120), as required by Brazilian legislation (MAPA 1995).

tested using regression analysis with the curve-fitting procedure of TableCurve 2D (Systat, San Jose, CA); model selection was based on parsimony, high *F*-values (and reduced error), and R^2 (steep) increase with model complexity.

Spatial analyses were carried out using the distance between pairwise sampling sites obtained from the GPS recorded geographical coordinates and the insect response data (levels of insecticide resistance, efficacy, and control failure likelihood). The relatively low number of sampling sites prevented the use of ordinary kriging methods for the desired estimates, but cokriging circumvented this shortcoming amplifying the data set (i.e., sampling points) used for the estimates. Thus, resistance levels and estimates of control failure likelihood were subjected to cokriging methods with chlorantraniliprole efficacy allowing selection of suitable semivariogram functions for distance interpolation (Isaaks and Srivastava 1989).

The semivariogram functions allow estimation of three parameters: range (*hr*), partial sill (*C*), and nugget (C_o). The former refers to the distance in which a plateau is reached, thus referring to the maximum distance where spatial dependence exists. The second refers to the mortality-based semivariance value in which the maximum distance of interference (i.e., range) is reached. The latter is the semivariance value where the model intercepts the *y*-axis representing the measurement errors and/or resolution involved. These three parameters were used to obtain three more parameters balancing the mortality semivariance and the measurement error or resolution obtained: sill (C_o + C), proportion [$C/(C_o + C)$], and randomness (C_o/C) of the data. The semivariogram models were selected based on the best data adjustment (i.e., regression equation with slope closest to one, and intercept and mean error closest to zero) and the highest randomness values. The selected semivariance models were subsequently used to generate spatial maps of chlorantraniliprole resistance levels and control failure likelihood. The spatial analyses were performed using ArcGIS 10.5 (ESRI, Redlands, CA).

Results

Chlorantraniliprole Resistance

The time-mortality results for each leaf miner population with independent time-dependent estimates were subjected to probit analyses and resulted in low χ^2- and *P*-values >0.05. These χ^2- and *P*-values attest to the suitability of the probit model for the intended analyses and estimation of the desired toxicological endpoints, namely, the median lethal concentrations (LT_{50}'s). The frequency of chlorantraniliprole resistant populations was high (34 out of 40 populations, or 85%), and particularly so in western Bahia, where 94% of the populations were resistant to chlorantraniliprole (Table 2).

The levels of chlorantraniliprole resistance were usually low (<10-fold) in the highlands with four exceptions reaching moderate levels of resistance (between 10- and 100-fold), although distributed in different counties (Table 2). Western Bahia presents a contrasting case, with the prevalence of moderate levels of resistance reaching over 30-fold in two instances, in Cocos and Luís Eduardo Magalhães (Table 2). Low levels of resistance were limited to five sites, and chlorantraniliprole susceptibility was detected in western Bahia at only one site: São Desidério (WSDE4).

Chlorantraniliprole Efficacy and Control Failure Likelihood

Chlorantraniliprole efficacy remained above the 40% level for all the populations tested, but most did not reach the minimum required threshold of 80% efficacy (Table 3). This is a clear indication that chlorantraniliprole control failure is likely in some populations, which was also estimated (Table 3). The risk or likelihood of control failure was significant in 72.5% of the tested insect populations and sites (29 out of 40 populations). Such risk was usually lower than 30% in the highland populations with

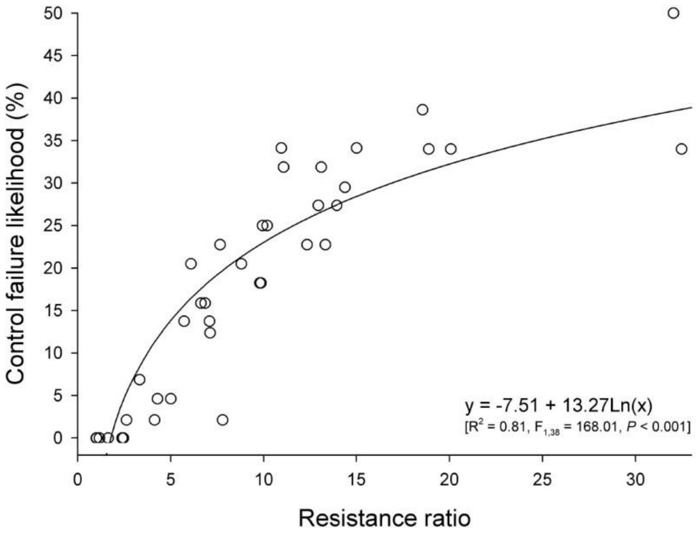

Fig. 2. The relationship between chlorantraniliprole resistance and control failure likelihood. The symbols indicate the observed data.

just three exceptions: Barra do Choça, Encruzilhada, and Vitória da Conquista. The risk of control failure tended to be higher in western Bahia, reaching over 30% in five instances and up to 50% in one, Cocos (Table 3).

Relationship Between Resistance and the Likelihood of Control Failure

The relationship between chlorantraniliprole resistance and control failure likelihood was tested using regression analysis with the former trait as the independent variable determining the latter. The relationship was significant, with the level of chlorantraniliprole resistance largely determining the likelihood of control failure with this insecticide (Fig. 2). The likelihood of control failure with chlorantraniliprole increases with the level of resistance to this insecticide (Fig. 2).

Spatial Dependence

The relatively large variation in chlorantraniliprole resistance, efficacy, and control failure likelihood is suggestive of county-wide variation in these traits; thus, spatial dependence is a potential characteristic that allows geographical mapping of the phenomenon if significant and suitable models are identified for extrapolation. The number of sampling sites from each region was limited and required the use of cokriging for meaningful estimates. This was carried out in two separate regions—one encompassing the sampling sites of the northern counties of western Bahia (except Cocos), and another encompassing the highland sampling sites.

The best semivariogram models obtained from the results of chlorantraniliprole resistance and control failure likelihood are exhibited in Table 4 together with their respective parameters for model selection. The nugget (C_o) values of zero and partial sill (C) around the value of one allowed robust estimates with spatial dependence reaching distances <500 m (Table 4). The model parameters and mean errors obtained allowed distance interpolation and subsequent mapping of chlorantraniliprole resistance ratio and control failure likelihood.

The mapping of chlorantraniliprole resistance indicates a scenario provoking more concern in western Bahia than in the highlands with higher within-county variability (Fig. 3), although the latter exhibited lower distance of interference between sampling sites (Table 4). This was translated into the likelihood of control failure with this insecticide (Fig. 4). The range of variability was smaller when control failure was considered, but western Bahia exhibited higher variation and higher risks of control failure; however, the risks were localized (Fig. 4).

Discussion

Insecticide resistance is a genetic change in response to selection that may compromise insecticide efficacy leading to control failure (Guedes 2017). The concepts of insecticide resistance, efficacy and control failure are interdependent although distinct, since the former is not always the underlying cause of the latter two (Tabashnik et al. 2014, Guedes 2017). Such distinction is seldom recognized, and control failure is usually assumed when insecticide resistance is detected. However, a recent shift in this trend seems to be taking place based on recent studies with the tomato leaf miner *Tuta absoluta*, the putative whitefly species MEAM1, and the Neotropical brown stink bug *Euchistus heros* (Gontijo et al. 2013, Roditakis et al. 2013, Silva et al. 2015, Dângelo et al. 2018, Tuelher et al. 2018, Guedes et al.

Table 4. Semivariogram models and parameters of chlorantraniliprole resistance and control failure likelihood in populations of the Neotropical coffee leaf miner *Leucoptera coffeella*

Response	Region	Cokriging	Model	Nugget (C_0)	Partial sill (C)	Sill ($C + C_0$)	Proportion ($C/C+C_0$)	Range (b_r, m)	Randomness (C_0/C)	Mean errors	Slope	Intercept
Resistance ratio	West	Simple	K-Bessel	0	1.1868	1.19	1	0.40	0	0.056	0.81	2.21
	Highlands	Simple	K-Bessel	0	1.0415	1.04	1	0.20	0	-0.013	0.94	0.50
Control failure likelihood	West	Simple	K-Bessel	0	1.1681	1.17	1	0.40	0	0.073	0.72	4.76
	Highlands	Simple	K-Bessel	0	1.3705	1.37	1	0.06	0	-0.530	1.02	0.81

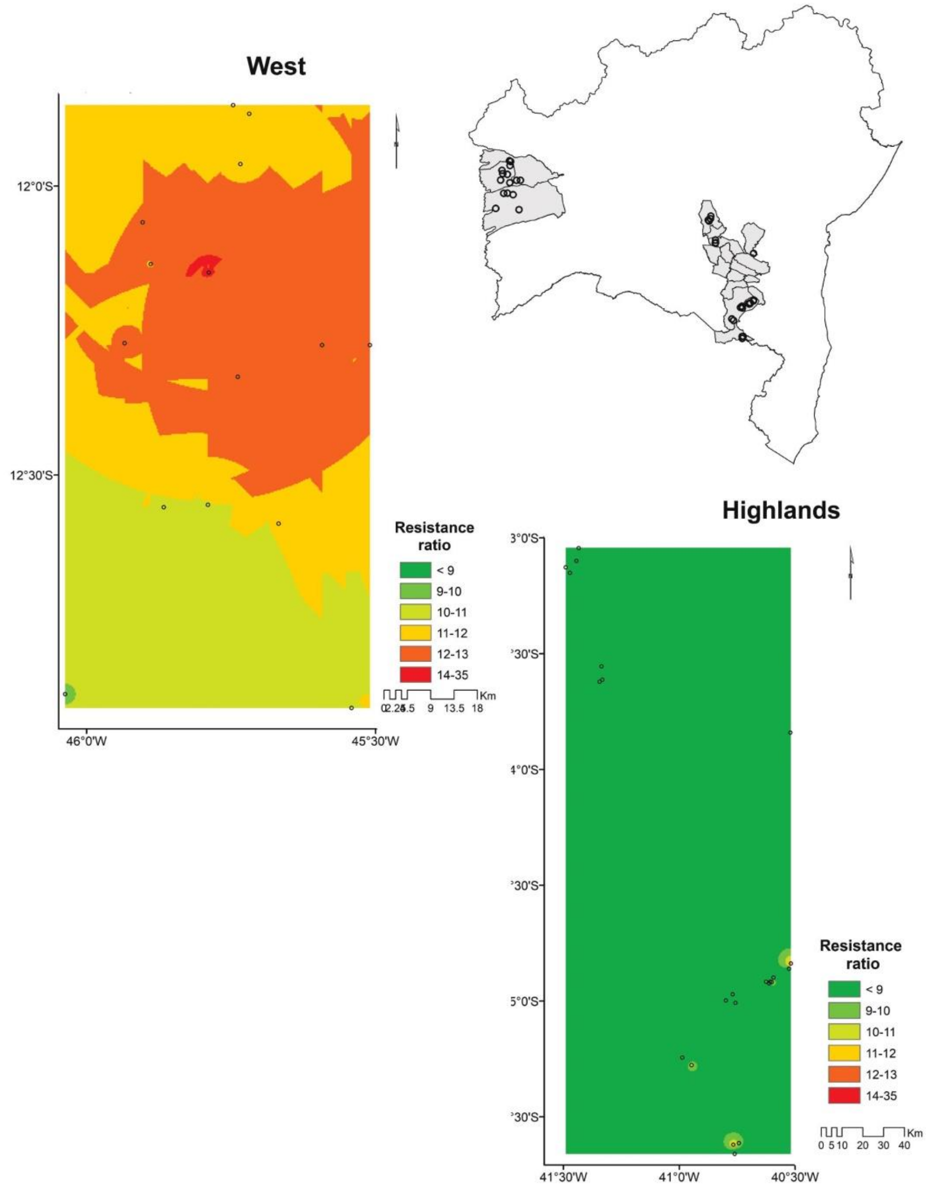

Fig. 3. Contour maps of the levels of chlorantraniliprole resistance in populations of the Neotropical coffee leaf miner *Leucoptera coffeella.* The maps were generated using spatial interpolation. The color legend indicates the represented range of resistance ratios of the coffee leaf miner.

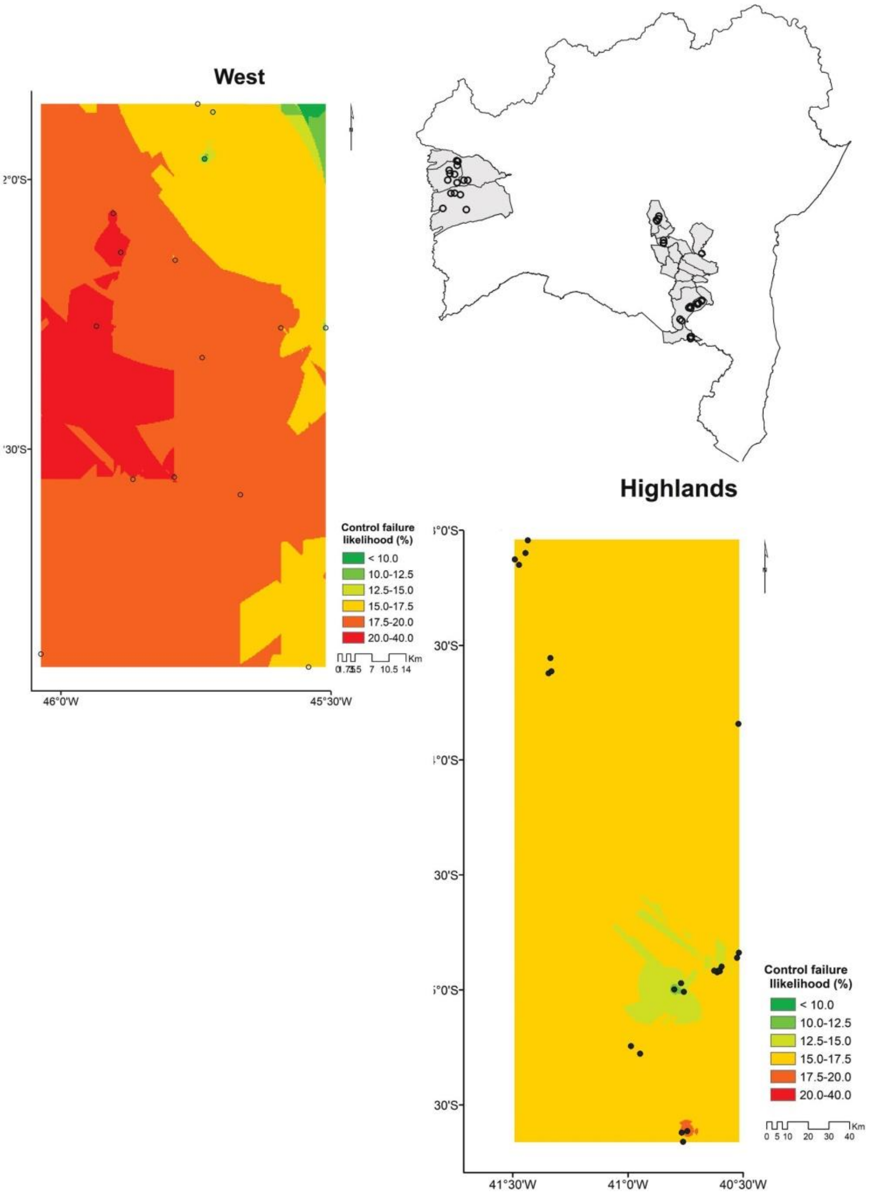

Fig. 4. Contour maps of the control failure likelihood of chlorantraniliprole used against populations of the Neotropical coffee leaf miner *Leucoptera coffeella*. The maps were generated using spatial interpolation. The color legend indicates the represented range of resistance ratios of the coffee leaf miner.

2019). Such studies were able to recognize insecticide resistance as the determinant cause of insecticide control failures of these pest species (Gontijo et al. 2013, Roditakis et al. 2013, Silva et al. 2015, Dângelo et al. 2018, Tuelher et al. 2018).

Insecticide resistance in the Neotropical coffee leaf miner *L. coffeella* has been hardly studied, which is limited to three surveys (Alves et al. 1992, Fragoso et al. 2003, Costa et al. 2016). These surveys indicate that the phenomenon may be frequent in this species and is likely to result in control failures, as particularly indicated by the high levels of organophosphate resistance (>1,000-fold) among leaf miner populations (Fragoso et al. 2003). The relatively recent increase and spread in the use of the diamide insecticide chlorantraniliprole against the coffee leaf miner suggests the potential emergence of resistant populations, which are targeted in the present study. We aimed 1) to survey the incidence of chlorantraniliprole resistance; 2) to assess the control failure likelihood of chlorantraniliprole due to this phenomenon; and 3) to test if spatial dependence exists for these traits. All these objectives were achieved, although some contrasted with our earlier expectations of a limited occurrence of chlorantraniliprole resistance, a low expectation of control failure and a lack of spatial dependence.

Incidence of insecticide resistance is usually low for recently used insecticides because the results of selection for the phenomenon usually takes a few years to manifest (Roush and McKenzie 1987, McKenzie 1996, Whalon et al. 2008, Sparks and Nauen 2015), but exceptions do exist including for diamide resistance (e.g., Troczka et al. 2017). Despite reported exceptions in different species, the more general expectation of a longer period for insecticide resistance to evolve prevails. Therefore, chlorantraniliprole resistance in the Neotropical coffee leaf miner was expected to be limited and in its initial stages. Nonetheless, the incidence of this phenomenon was high in the region under investigation, with 85% of the insect populations exhibiting chlorantraniliprole resistance. Curiously, the levels of resistance were low to moderate, although reaching levels over 30-fold in some instances, particularly in western Bahia. The widespread and intensive use of chlorantraniliprole in the region is the likely reason for the high incidence of resistance to this compound among the insect populations sampled and tested. However, the evolution of this phenomenon is still in its early stages at most sites, as the levels of resistance detected did not reach high levels (>100-fold), but remained below the 40-fold threshold.

The levels of chlorantraniliprole resistance detected in the coffee leaf miner may not be high enough to compromise this insecticide's efficacy but that requires the testing and proper estimation provided by the present study. Efficacy was indeed compromised considering the levels of chlorantraniliprole resistance observed and the risk of control failure does already exist in the region. Nonetheless, the risk is significant although reduced in most of the tested populations. Instances of 30–50% risk of control failure exist and are distributed through most of the counties sampled. They are frequently located side-by-side with sites exhibiting negligible risk of control failure, limiting the range of spatial dependence for the recorded traits. The situation appears to be more serious in western Bahia, but both regions exhibit the reported pattern and control concern. The recognition of the potential spatial dependence of insecticide resistance and control failure likelihood is important for scaling up the required resistance management effort, sustaining the potential use of chlorantraniliprole as a management tool against the coffee leaf miner.

The notion that spatial proximity favors resemblance is rather intuitive and widespread. Surveys of insecticide resistance assume this relationship, which is usually not tested despite its importance in determining the scale and scope of resistance management programs. Thus, the scale of management programs, whether local, micro-regional, meso-regional, or even country-wide, is not recognized as a factor compromising their potential efficacy (Guedes 2017). The number of sampling sites and populations tested in our survey of the coffee leaf miner may potentially allow recognition and possibly mapping of chlorantraniliprole resistance and control failure likelihood. However, the samples were not established a priori for such a purpose, imposing limitations on the effort. Cokringing with a secondary trait (i.e., chlorantraniliprole efficacy) allowed sufficient resolution to recognize that spatial dependence does exist for the traits assessed, considering the scale of our study, encompassing a few counties in western Bahia and the south-central highlands. The scale of spatial dependence is restricted, not spanning more than half a kilometer. Variation is smaller for the control failure likelihood, a consequence of the relatively low ranges involved, except for two instances in western Bahia.

These findings are important for managing the coffee leaf miner. Despite its relative recent use, chlorantraniliprole already exhibits significant and widespread problems of resistance in both regions, especially in western Bahia. However, the levels of resistance detected are low to moderate, reaching 30-fold in few instances. The problem is still recent and allows for proper resistance management to slow or even prevent further exacerbation. The levels of resistance detected are already in a range that compromises chlorantraniliprole, with estimated risks of control failure <30% in most instances, but reaching the 50% threshold at a site in Cocos County in western Bahia. Nonetheless, spatial dependence is limited to a small scale, allowing the design of resistance management practices at a local (farm) level (Guedes 2017). Despite previous problems with resistance to organophosphates and emerging problems with neonicotinoids, alternative insecticides with distinct modes of action and prevailing detoxification mechanisms are still available, among which azadirachtin, pyrethroids, spynosins, and growth regulators are promising alternatives for rotation at the farm level (Spark and Nauen 2015, MAPA 2019).

In summary, chlorantraniliprole resistance is already widespread among the Neotropical coffee leaf miners in western and south-central Bahia (Brazil). The resistance levels are low to moderate but are already leading to reduced efficacy and significant risk of control failure, demanding resistance management practices. Among these, replacement and rotation of alternative insecticides with distinct underlying mechanisms of resistance are sound practices for use at the local scale, and they are likely to extend the potential use of diamides against this species not only in this region, but also elsewhere as well.

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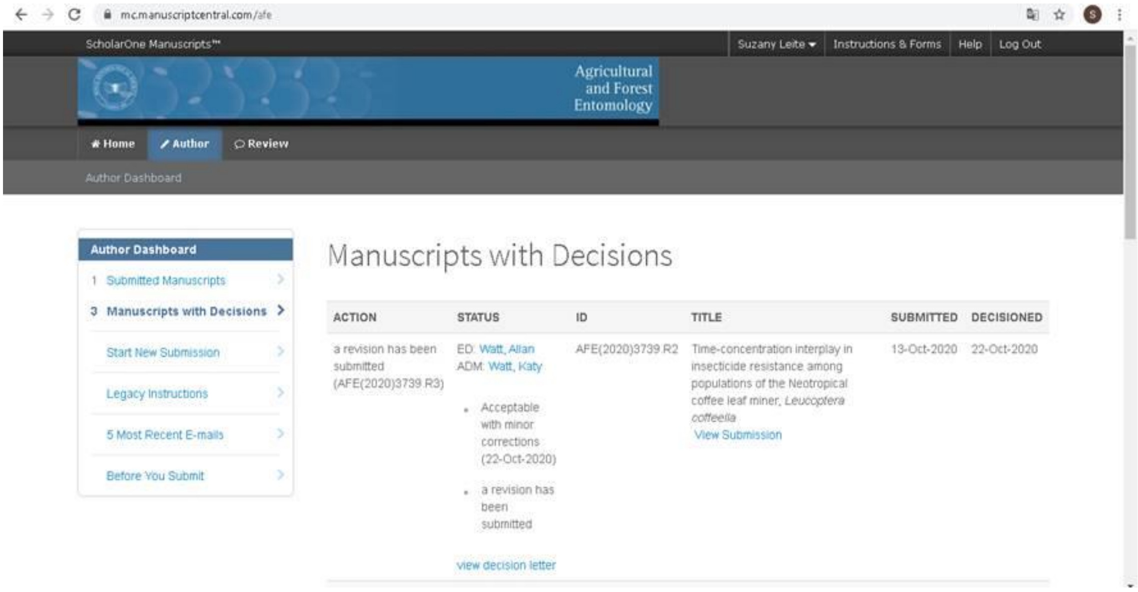
ANNEX III



Manuscripts with Decisions

ACTION	STATUS	ID	TITLE	SUBMITTED	DECISIONED
a revision has been submitted (AFE(2020)3739.R3)	ED: Watt, Allan ADM: Watt, Katy • Acceptable with minor corrections (22-Oct-2020) • a revision has been submitted view decision letter	AFE(2020)3739.R2	Time-concentration interplay in insecticide resistance among populations of the Neotropical coffee leaf miner, *Leucoptera coffeella* View Submission	13-Oct-2020	22-Oct-2020