

## Insecticide Resistance and Resistance Management

# 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* (Trocza 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.

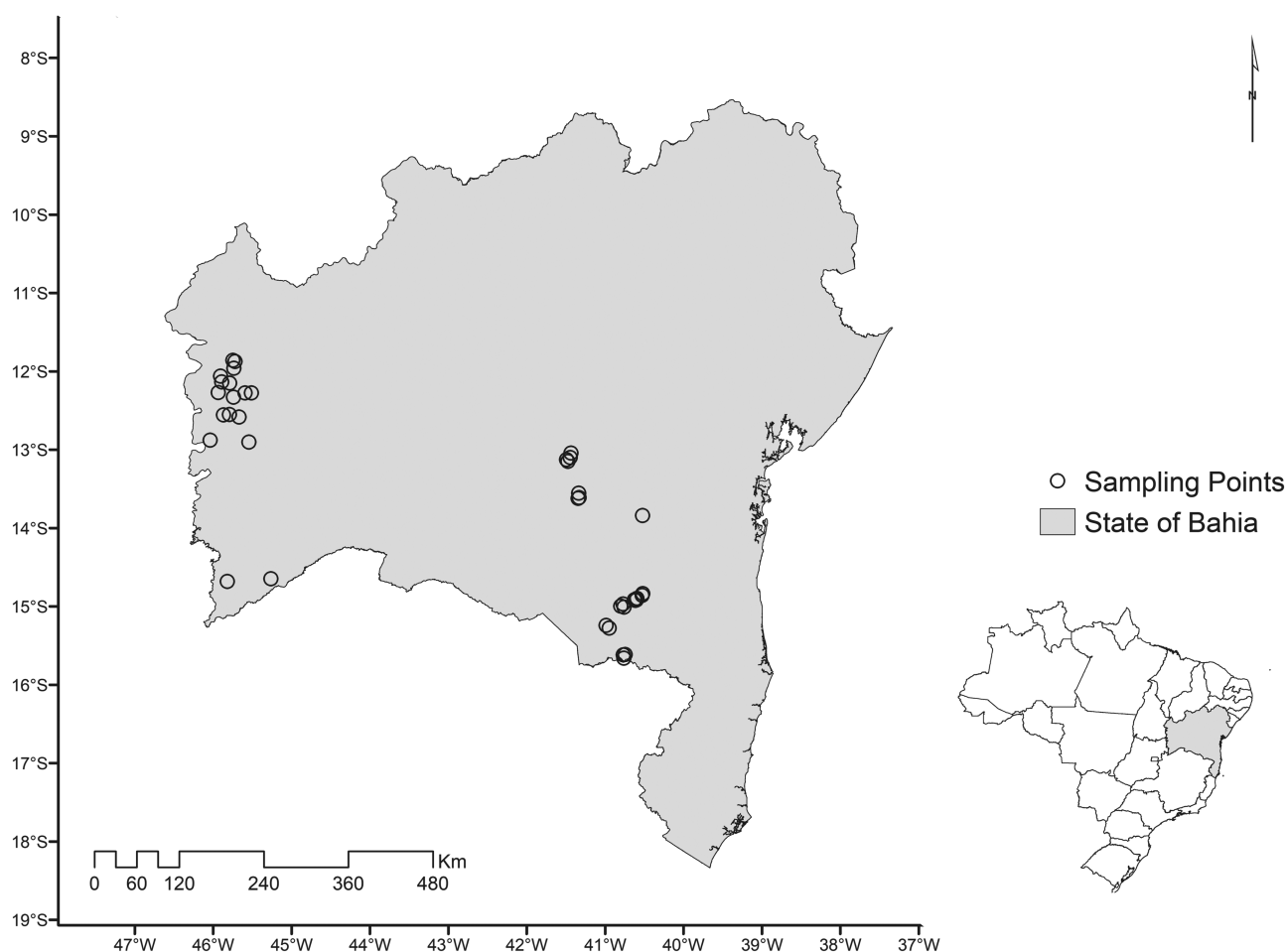
Spatial dependence was not expected because variation in the incidence of insecticide resistance was unlikely to be high (and diverse), compromising the recognition of such a relationship and the spatial mapping of this phenomenon.

## 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  $25 \pm 2^\circ\text{C}$  temperature and  $70 \pm 5\%$  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 chlorantraniliprole to Brazilian populations of the coffee leaf miner (*Leucoptera coffeella*)

Meso-region	County	Code	No.	Slope $\pm$ SE	LT <sub>50</sub> (95% FI) hours	$\chi^2$	df	P	Resistance ratio at LT <sub>50</sub> [RR <sub>50</sub> (95% CI)]
West	Barreiras	WBAR1	480	0.92 $\pm$ 0.14	19.96 (14.89–28.95)	0.34	6	0.99	5.72 (2.34–16.86)*
	Barreiras	WBAR2	480	1.33 $\pm$ 0.19	65.91 (46.44–115.74)	4.05	6	0.67	18.88 (8.88–48.50)*
	Barreiras	WBAR3	480	1.56 $\pm$ 0.19	50.20 (38.46–74.21)	1.19	6	0.98	14.38 (7.70–32.43)*
	Barreiras	WBAR4	480	1.09 $\pm$ 0.15	43.03 (31.01–70.78)	3.16	6	0.79	12.33 (5.41–33.96)*
	Cocos	WCOC1	480	0.94 $\pm$ 0.17	111.79 (62.93–338.55)	1.17	6	0.98	32.03 (9.66–128.28)*
	Cocos	WCOC2	480	1.17 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 0.13	30.70 (20.85–57.26)	2.65	6	0.85	8.80 (2.24–42.10)*
	Barra do Choça	HBCH2	480	0.97 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 0.15	34.66 (25.72–53.40)	1.05	6	0.98	9.93 (4.48–26.60)*
	Encruzilhada	HENC1	480	1.57 $\pm$ 0.17	34.34 (27.68–45.68)	1.16	6	0.98	9.84 (5.57–37.74)*
	Encruzilhada	HENC2	480	1.28 $\pm$ 0.17	52.40 (38.20–85.05)	0.99	6	0.99	15.01 (7.21–8.71)*
	Encruzilhada	HENC3	480	1.34 $\pm$ 0.14	14.41 (11.71–17.87)	1.66	6	0.95	4.13 (2.36–8.71)*
	Mucugê	HMUC1	480	1.72 $\pm$ 0.17	24.71 (20.71–30.47)	3.56	6	0.73	7.78 (4.25–14.26)*
	Mucugê	HMUC2	480	0.74 $\pm$ 0.13	5.18 (2.84–7.60)	0.88	6	0.98	1.63 (0.61–4.39)
	Mucugê	HMUC3	480	0.65 $\pm$ 0.13	41.06 (25.25–104.01)	0.85	6	0.17	12.93 (2.16–77.21)*
	Mucugê	HMUC4	480	0.99 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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  $CFL = 100 - [\text{observed mortality (\%)} \times 100] / \text{expected mortality (i.e., 80\%)}$ . CFL values  $\leq 0$  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 ( $br$ ), partial sill ( $C$ ), and nugget ( $C_0$ ). 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 ( $C_0 + C$ ), proportion [ $C/(C_0 + C)$ ], and randomness ( $C_0/C$ ) 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  $\chi^2$ - and  $P$ -values  $>0.05$ . These  $\chi^2$ - 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

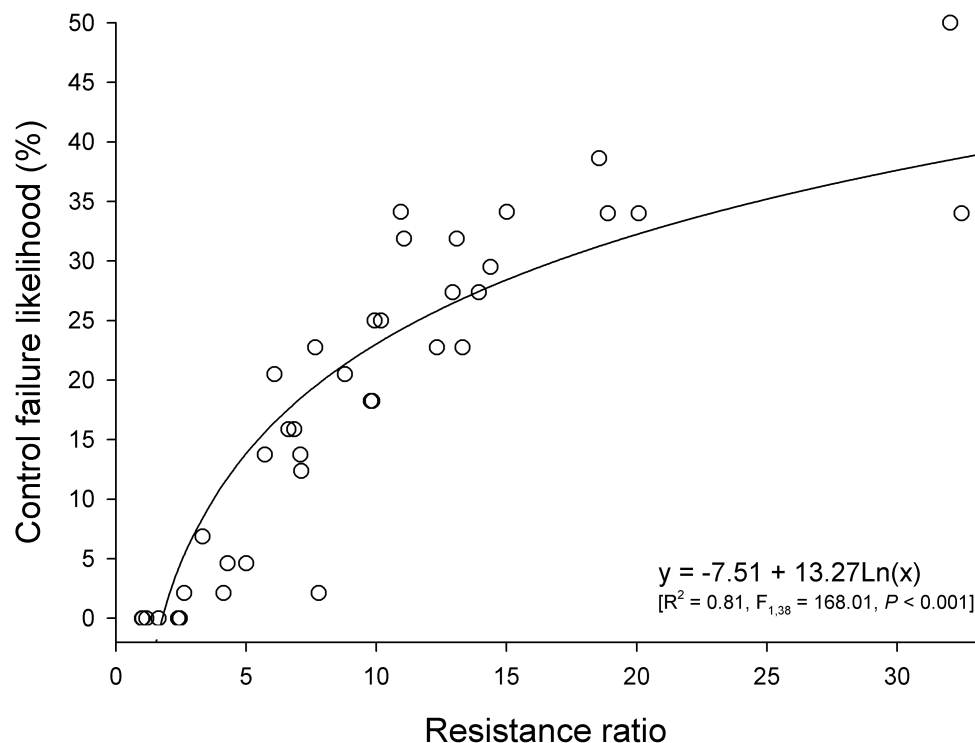


Fig. 2. The relationship between chlorantraniliprole resistance and control failure likelihood. The symbols indicate the observed data.

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 ( $C_0$ ) 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.

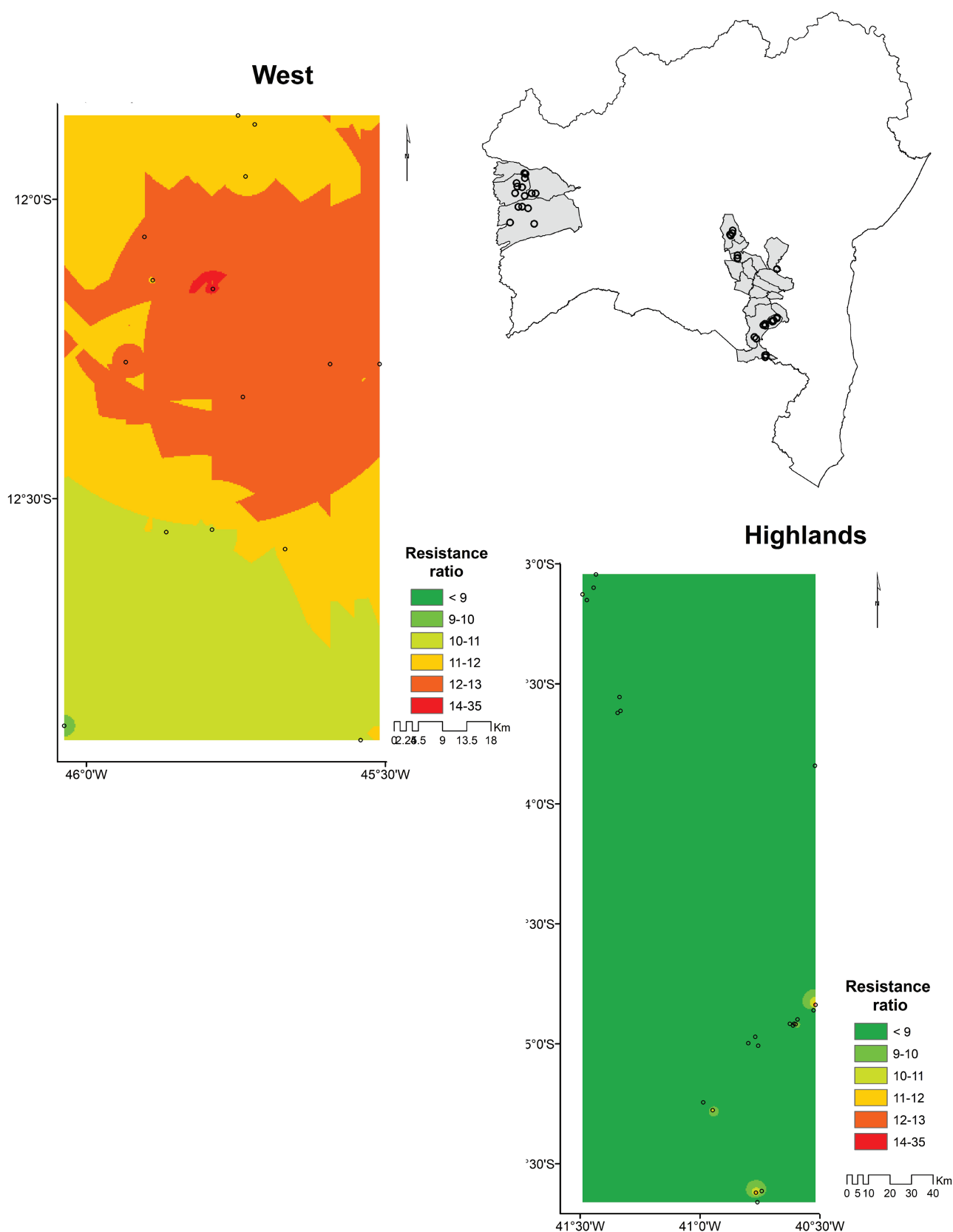
The mapping of chlorantraniliprole resistance indicates a scenario provoking more concern in western Bahia than in the highlands with higher within-county variability (Fig. 3), although the latter exhibited lower distance of interference between sampling sites (Table 4). This was translated into the 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.

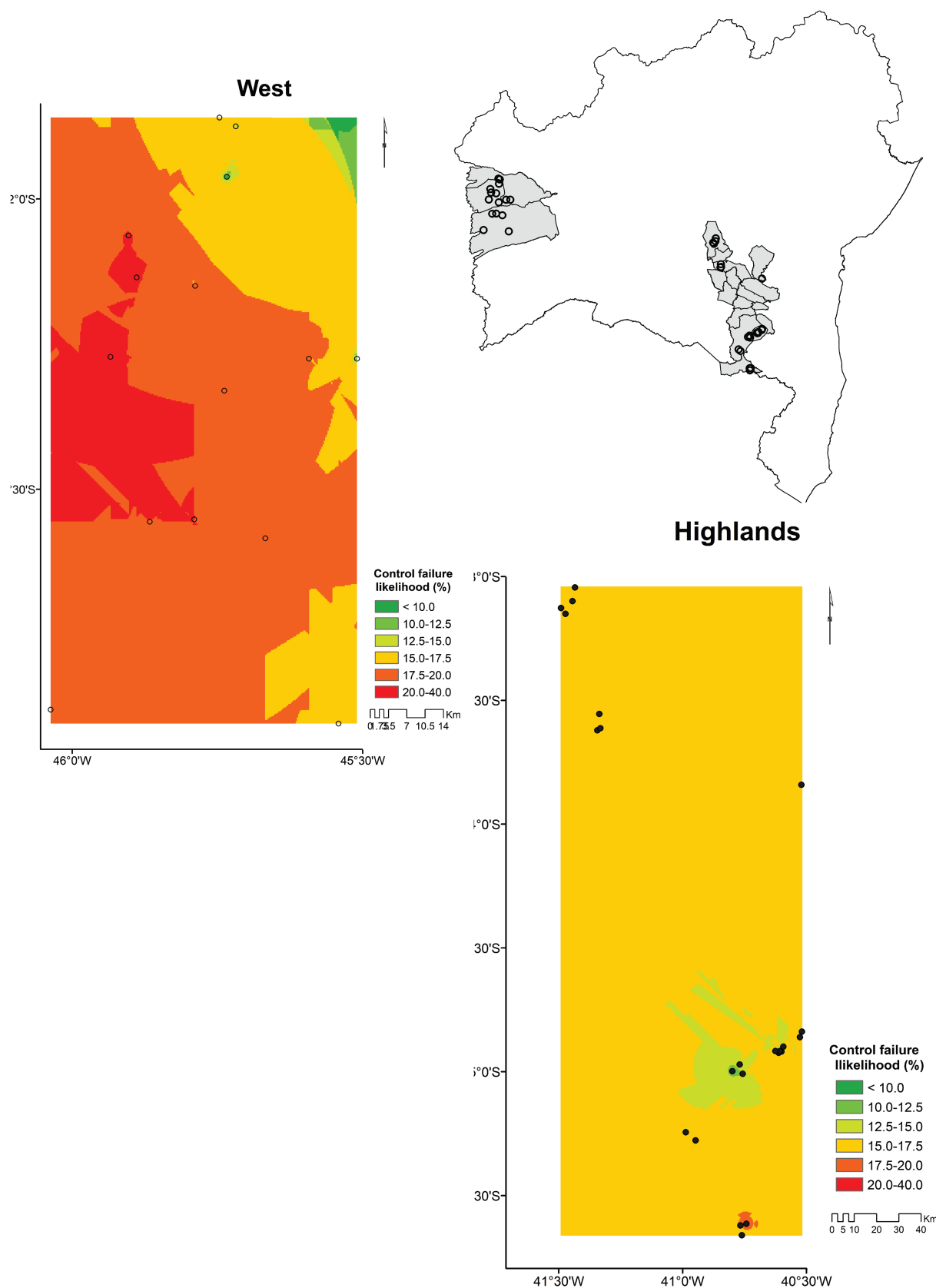
**Table 4.** Semivariogram models and parameters of chlorantraniliprole resistance and control failure likelihood in populations of the Neotropical coffee leaf miner *Leucoptera coffeella*

Response	Region	Cokriging	Model	Nugget ( $C_0$ )	Partial sill ( $C$ )	Sill ( $C + C_0$ )	Proportion ( $C/(C+C_0)$ )	Range ( $h_s$ , 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, spinosins, 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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