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Lethal and sub-lethal selectivity of fenbutatin oxide and sulfur to the predator *Iphiseiodes zuluagai* (Acari: Phytoseiidae) and its prey, *Oligonychus ilicis* (Acari: Tetranychidae), in Brazilian coffee plantations

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Abstract. Lethal concentration (LC) has been widely used to estimate pesticide toxicity. However, it does not consider the sub-lethal effects. Therefore we included the instantaneous rate of increase in association with LC to estimate population-level effects of the acaricides fenbutatin oxide and sulfur on the predator *Iphiseiodes zuluagai* and its prey, the phytophagous southern red mite, *Oligonychus ilicis*. The predator was 32.84*x* and 17.20*x* more tolerant to fenbutatin oxide and sulfur, respectively, than its prey, based on LC_{50} estimates obtained from acute concentration–mortality bioassays. The instantaneous rate of population growth in both mite species decreased with increasing acaricide concentration. Both acaricides provided effective control of *O. ilicis* at their recommended concentrations, but sulfur drastically compromised the predator populations quickly leading them to extinction due to the low reproductive potential of this species compared with its prey.

Introduction

Lethal concentrations (LC) are commonly used to assess pesticide toxicity to arthropods (e.g., Overmeer and van Zon 1981; Stark and Rangus 1994) but does not properly assess the population-level effect of the chemical (Forbes and Calow 1999; Stark and Banken 1999; Stark and Banks 2003). Sub-lethal pesticide effects may range from decrease in life span to decreases in fertility and changes in sex ratio, feeding and oviposition behaviors, etc. (Dempster 1968; Stark and Rangus 1994; Stark and Banks 2003).

Population growth rate, and especially the intrinsic rate of increase (r_m), has been recommended together with LC estimates for toxicity assessments to

provide a more accurate estimate of population-level effects of toxic compounds (Stark et al. 1997; Walthall and Stark 1997a; Forbes and Calow 1999). The r_m is an estimate of population growth expressed as the number of female progeny daily produced by each female (Carey 1993). The calculation of r_m is time-consuming and requires life table studies, but Hall (1964) suggested a simplified population growth estimate – the instantaneous rate of increase (r_i), which has proved very robust (Stark et al. 1997; Walthall and Stark 1997b; Stark and Banken 1999; Stark and Banks 2003). The r_i-values are closely correlated with the intrinsic rate of increase (r_m) and positive r_i-values indicate population increase, while negative r_i-values indicate population decrease and $r_i = 0$ indicates a stable population (Hall 1964; Walthall and Stark 1997b).

The phytophagous mite species *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) is an important coffee pest in Neotropical America (Reis and Alves 1997). Sulfur and fenbutatin oxide, are commonly recommended for its control (Reis et al. 1997; Andrei 1999), but their selectivity to predatory mites, such as *Iphiseiodes zuluagai* Denmark and Muma (Acari: Phytoseiidae) (Reis and Alves 1997; Reis et al. 1997, 1998a), is based only on acute lethal assessments (Reis et al. 1998b; Reis and Souza 2001). The aim of this study is to provide a more robust population-level assessment of the selectivity of these compounds to the Brazilian coffee pest *O. ilicis* and its predator *I. zuluagai*.

Material and methods

Mite rearing

Iphiseiodes zuluagai and *O. ilicis* were collected in a coffee plantation (*Coffea arabica* L. cv. Catuaí) maintained free from pesticide within the campus of the Federal University of Viçosa, Viçosa County, State of Minas Gerais, Brazil. The rearing methods for *I. zuluagai* were adapted from Reis and Alves (1997). The colony of *O. ilicis* was established in 2001 and both mite species were reared indoors, but at natural temperature, humidity and photoperiod. The mites from both species were confined to a black plastic arena (8 cm diameter; Plastigel, São Paulo, Brazil) floating in water within an open polypropylene tray (11 × 11 × 3.5 cm; Plastigel, São Paulo, Brazil). Each arena was centrally perforated by a pin that extended until reaching the bottom of the tray, to where it was attached by a silicon-based glue. This strategy maintained the arena fixed in the tray and floating according with water level preventing the mites from escaping. Two thin microscope slides (18 × 18 mm) were placed over cotton fibers (Johnson & Johnson, São Paulo, Brazil) at the edge of each arena to serve as oviposition site. Pollen of *Ricinus communis* L. from non-sprayed field at the University campus and diluted eucalyptus honey (Associação Apícola, Viçosa, Brazil) were daily provided as food source (Reis and Alves 1997).

Acaricides

Fenbutatin oxide (Torque® 500CS; concentrated suspension; 500 g a.i./l) and sulfur (Kumulus® 800WDG; water dispersible granules; 800 g a.i./l) were obtained from BASF S.A., São Paulo, Brazil.

Concentration–response bioassays

Concentration–response bioassays were carried out for both acaricides using adult females, at the beginning of their reproductive period (Reis et al. 1997, 1998b), of either *I. zuluagai* (8–10 days old) or *O. ilicis* (15–17 days old). The acaricides were sprayed through a Potter tower (Potter 1952) (Burkard, Rickmansworth, UK) in coffee leaf disks (3.5 cm diameter). Acaricide spraying was carried out at 0.34 bar ($=3.44 \times 10^4$ kPa) pressure with a 2.5 ml spray aliquot in accordance with recommendations of the International Organization for Biological Control of Noxious Animals and Plants/West Paleartic Regional Section (IOBC/WPRS) (Hassan et al. 1994).

The sprayed leaf disks (including water sprayed controls) were let to air dry for 1 h before 10 young adult females of either *I. zuluagai* or *O. ilicis* were placed on them. Five replicates were used for each species, acaricide and acaricide concentration. The leaf disks were placed to float in Petri dishes (20 cm diameter) with a pin inserted in its center and connecting it with the bottom of the Petri dish as described for rearing the predator colonies (Reis and Alves 1997). Pollen of *R. communis* was daily provided for *I. zuluagai* (Reis and Alves 1997). Mites were maintained at 25 ± 2 °C, 60 ± 10% R.H. and 14:10 LD photoperiod.

The concentrations used in the bioassays were selected after preliminary tests with broad concentration range allowing selection of lower and upper limits of biological response for each acaricide and mite species. The concentration range for *I. zuluagai* was from 0.10 to 3.85 g a.i./l of fenbutation oxide and from 0.25 to 12.00 g a.i./l of sulfur. The concentration range of fenbutatin oxide used against *O. ilicis* was between 0.060 and 0.125 g a.i./l, and the concentration range of sulfur was between 0.04 and 1.36 g a.i./l for this same species. A control treatment with only distilled water sprayed was also used to correct the data for natural mortality (Abbott 1925). Mite mortality was assessed after 72-h exposure and the mites were recognized as dead if unable to move for a distance at least equal to their body length (Stark et al. 1997). Concentration–mortality curves were estimated by probit analysis (SAS Institute 1997). For each chemical, the index of differential selectivity was obtained by dividing the LC_{50} of the predator by the LC_{50} of its prey and its confidence limits (95%) were calculated following Robertson and Preisler (1992).

Demographic bioassays

The instantaneous rate of increase (r_i) was estimated using the equation $r_i = [\ln (N_f/N_o)]/\Delta T$, where N_f is the final number of living mites, N_o is the initial number of living mites and ΔT is the time interval (days) elapsed between the start and the end of the bioassay (Stark et al. 1997; Walthall and Stark 1997b).

The experimental procedures and acaricides were the same as those used for concentration–mortality bioassays above, but with daily progeny assessments up to 7 days following initial exposure, and five adult females of either *I. zuluagai* or *O. ilicis* were used per replicate. The female mites were as previously described and a male predator was added to each leaf disk of *I. zuluagai*, but not for *O. ilicis* as it was not necessary. The mites were kept in rearing chambers under the same conditions of the concentration–response bioassays. Small amounts of pollen from *R. communis* were daily provided as additional food source for the predatory mites.

The acaricide concentrations for the demographic assays were based on the concentration–mortality curves previously obtained. Control treatments were sprayed only with distilled water. Regression analyses were carried out to assess the effect of acaricide concentrations on the instantaneous rate of increase of each mite species under investigation.

Results

Concentration–mortality bioassays

The predatory mite species *I. zuluagai* was more tolerant to both acaricides than its prey *O. ilicis* (Table 1). The index of differential selectivity at LC_{50} was 32.84x and 17.20x for fenbutatin oxide and sulfur, respectively.

Demographic bioassays

The instantaneous rate of *I. zuluagai* population growth linearly decreased with increasing concentrations of both acaricides (Figure 1a). However, the predator population exposed to concentrations of fenbutatin oxide around its LC_{50} for this species (i.e., ca. 2.66 g a.i./l) showed positive values of r_i (0.037 ± 0.003 day^{-1}) indicating population growth after 7 days of exposure to this acaricide. Predator extinction took place only at the concentration of 51.6 g a.i./l, close to the LC_{90} estimate for this compound and this species. In contrast, sulfur showed a more drastic effect on the predator population growth, which declined at concentrations as low as 0.64 g a.i./l ($r_i = -0.23 \pm 0.00$ day^{-1}) after 7 days exposure. Exposure to the LC_{25} of sulfur to *I. zuluagai* led to predator extinction after 7 days exposure.

Table 1. Toxicity of fenbutatin oxide and sulfur to the predatory mite *Iphiseiodes zuluagai* and its prey *Oligonychus ilicis*.

Acaricide	Mite species	Number of mites tested	Slope ± SE	LC_{50} (95% Fiducial limits) (g a.i./l)	LC_{95} (95% Fiducial limits) (g a.i./l)	χ^2	p	Index of differential selectivity (95% Confidence limits)
Fenbutatin oxide	*I. zuluagai*	290	0.29 ± 0.03	2.66 (1.78–4.83)	157.42 (44.89–184.20)	0.63	0.96	32.84 (20.59–52.73)
	O. ilicis	400	2.79 ± 0.14	0.081 (0.078–0.084)	0.12 (0.11–0.14)	5.05	0.54	–
Sulfur	*I. zuluagai*	550	0.50 ± 0.03	2.58 (2.07–3.12)	25.48 (19.28–36.60)	4.59	0.87	17.20 (2.39–115.58)
	O. ilicis	250	0.71 ± 0.05	0.15 (0.13–0.19)	0.40 (0.30–0.64)	5.28	0.15	–

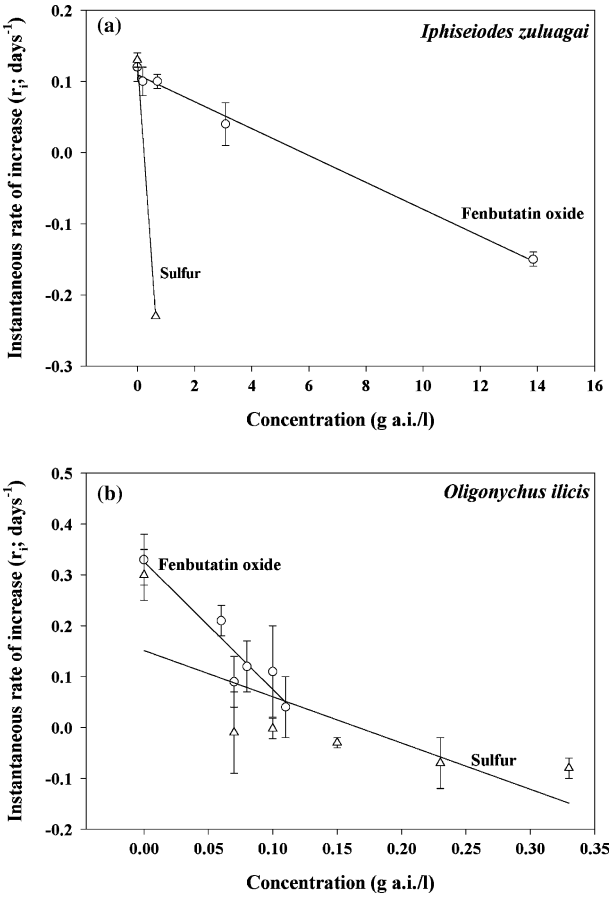

Figure 1. Instantaneous rate of increase (r_i; day^{-1}) of *Iphiseiodes zuluagai* (a) exposed to increased concentrations of fenbutatin oxide (open circle; $y = 0.11 - 0.02x$, $df_{error} = 22$, $F = 80.61$, $p < 0.0001$, $R^2 = 0.79$) and sulfur (open triangle; $y = 0.13 - 0.56x$, $df_{error} = 7$, $F = 520.28$, $p < 0.0001$, $R^2 = 0.99$), and of *Oligonychus ilicis* (b) exposed to fenbutatin oxide (open circle; $y = 0.33 - 2.63x$, $df_{error} = 28$, $F = 60.77$, $p < 0.0001$, $R^2 = 0.68$) and sulfur (open triangle; $y = 0.18 - 1.09x$, $df_{error} = 26$, $F = 18.50$, $p < 0.0001$, $R^2 = 0.41$).

Oligonychus ilicis also showed a linear population decline with increased acaricide concentrations, but the phytophagous species was affected at lower concentrations of fenbutatin oxide than its predator. Fenbutatin oxide showed a stronger effect on this species than sulfur (Figure 1b), but with population growth at concentrations as high as 0.11 g a.i./l (i.e., its LC_{90}) for the first compound ($r_i = 0.037 \pm 0.002$ day^{-1}). Sulfur led to prey population decrease at its estimated LC_{10} (0.072 g a.i./l) ($r_i = -0.012 \pm 0.008$ day^{-1}), but extinction was not reached with concentration as high as the estimated LC_{90} for this acaricide and the phytophagous mite species, unlike what was observed for *I. zuluagai*.

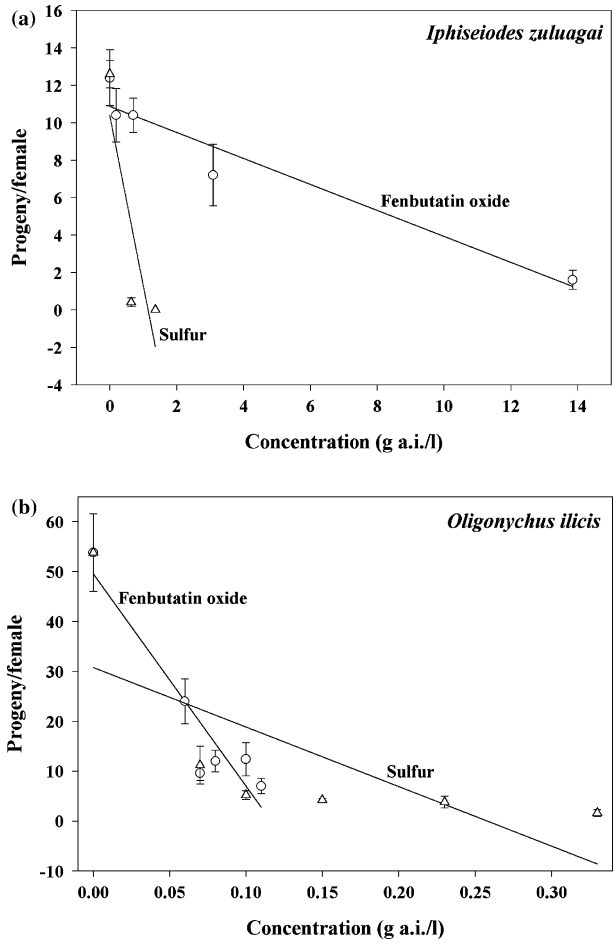

Figure 2. Progeny production per female of *Iphiseiodes zuluagai* (a) exposed to increased concentrations of fenbutatin oxide (open circle; $y = 10.39-0.69x$, $df_{error} = 23$, $F = 41.81$, $p < 0.0001$, $R^2 = 0.64$) and sulfur (open triangle; $y = 10.39-9.08x$, $df_{error} = 13$, $F = 34.17$, $p < 0.0001$, $R^2 = 0.72$) and of *Oligonychus ilicis* (b) also exposed to fenbutatin oxide (open circle; $y = 50.01-436.70x$, $df_{error} = 28$, $F = 69.01$, $p < 0.0001$, $R^2 = 0.71$) and sulfur (open triangle; $y = 31.29-121.48x$, $df_{error} = 28$, $F = 21.94$, $p < 0.0001$, $R^2 = 0.44$).

The progeny produced by each female mite was compromised with increased acaricide concentrations following the same trend reported for population growth of both species and acaricides (Figures 2a and b). Therefore, the patterns of population growth observed were a direct result of reproductive impairment due to the acaricide activity. Again sulfur exhibited stronger reproductive effect than fenbutatin oxide for the mite predator.

Discussion

The predatory mite *I. zuluagai* was more tolerant to both acaricides than its prey species *O. ilicis* based on concentration–mortality bioassays. Such results were expected since it follows previous reports of low impact of fenbutatin oxide at the recommended concentrations on mortality of the phytoseiids *Typhlodromus pyri* Scheuten, *Amblyseius andersoni* (Chant), *A. bibens* (Blommers) and *I. zuluagai* (Overmeer and van Zon 1981; Reis et al. 1998b; Reis and Souza 2001). In contrast, fenbutatin oxide was reported as slightly harmful to *Euseius alatus* (De Leon) (Acari: Phytoseiidae) and sulfur was reported as moderately noxious to *I. zuluagai* based on similar studies (Reis et al. 1998b; Reis and Souza 2001).

The lethal and sub-lethal assessments carried out in the present study indicate that fenbutatin oxide and sulfur used at recommended concentrations for field control of phytophagous mites show distinct impact in populations of predatory and phytophagous mite species. The recommended field concentrations of fenbutatin oxide (i.e., 0.40 g a.i./l) is higher than the estimated LC_{99} for *O. ilicis* (0.15 g a.i./l), what should provide drastic suppression of populations of this pest species. This recommended field concentration though, approaches only the LC_{15} of the predator *I. zuluagai* and caused a small decrease in the population growth rate of this species. In contrast, the recommended field concentration of sulfur (5 g a.i./l) nearly corresponded to the LC_{60} for the predator causing its extinction, while for *O. ilicis* this concentration far surpasses its LC_{99} (0.59 g a.i./l) and will also lead to this species extinction.

The higher reproductive potential of *O. ilicis* compared with its predator *I. zuluagai* is likely to minimize the mortality caused by acaricides in populations of the prey. The greater reproductive output of individuals exposed to sub-lethal doses of noxious agents, such as acaricides, is refereed to as population compensation by Stark et al. (1997). *Oligonychus ilicis* exhibits higher population compensation due to its higher reproductive potential than *I. zuluagai*. Such population compensation may take place because the survivors of acaricide exposure will have greater resource availability and are likely to reproduce at a higher rate mitigating the acaricide effect at the population level. An alternative explanation for population compensation after acaricide exposure is the phenomenon of hormesis, or the improved reproductive output of the population subjected to sub-lethal pesticide concentrations (Calabrese 1999; Forbes 2000; Calabrese and Baldwin 2003; Zanuncio et al. 2003). This is a likely result of the allocation principle put forward by Sibly and Calow (1986) and Calow and Sibly (1990) where the limited energy reserves of the individuals within a population are centered in reproductive output rather than in its maintenance distorting its homeostatic equilibrium, which should prevail in non-stressed individuals, in favor of reproduction.

The predatory mite species *Iphiseius degenerans* (Berlese) (Phytoseiidae) also shows higher population growth rates when exposed to neem and dicofol

compared with the spider mite *Tetranychus urticae* Koch (Tetranychidae) (Stark et al. 1997). The predator in this case also has smaller reproductive potential than its prey resembling what was here reported for *I. zuluagai* and *O. ilicis*. The last species produces four times more progeny than its predator whose population growth was more serious compromised by acaricide exposure due to their heavier impact on its reproductive potential.

In the present case, LC estimates taken alone would not provide evidence for the differential reproductive impairment caused by fenbutatin oxide and sulfur in *I. zuluagai*, turning the last compound particularly undesirable for pest management systems where this predator plays a relevant role, as in the present case.

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