Beyond selectivity: Are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*?

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HIGHLIGHTS

- *Amblyseius herbiculus* is more tolerant to deltamethrin than its prey *O. ilicis*.
- The predator *A. herbiculus* do not exhibit avoidance to deltamethrin.
- Low doses of deltamethrin induced hormesis in the red mite *O. ilicis*.
- Deltamethrin-induced hormesis in *O. ilicis* is a likely cause of its outbreaks.

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ABSTRACT

Secondary pest outbreak is a counterintuitive ecological backlash of pesticide use in agriculture that takes place with the increase in abundance of a non-targeted pest species after pesticide application against a targeted pest species. Although the phenomenon was well recognized, its alternative causes are seldom considered. Outbreaks of the southern red mite *Oligonychus ilicis* are frequently reported in Brazilian coffee farms after the application of pyrethroid insecticides against the coffee leaf miner *Leucoptera coffeella*. Selectivity favoring the red mite against its main predatory mites is generally assumed as the outbreak cause, but this theory has never been tested. Here, we assessed the toxicity (and thus the selectivity) of deltamethrin against both mite species: the southern red mite and its phytoseiid predator *Amblyseius herbiculus*. Additionally, behavioral avoidance and deltamethrin-induced hormesis were also tested as potential causes of red mite outbreak using free-choice behavioral walking bioassays with the predatory mite and life-table experiments with both mite species, respectively. Lethal toxicity bioassays indicated that the predatory mite was slightly more susceptible than its prey (1.5<sup>C2</sup>), but in more robust demographic bioassays, the predator was three times more tolerant to deltamethrin than its prey, indicating that predator susceptibility to deltamethrin is not a cause of the reported outbreaks. The predator did not exhibit behavioral avoidance to deltamethrin; however insecticide-induced hormesis in the red mite led to its high population increase under low doses, which was not observed for the predatory mite. Therefore, deltamethrin-induced hormesis is a likely cause of the reported red mite outbreaks.

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1. Introduction

The human health and environmental safety risks imposed by the widespread use of pesticides in agriculture have been broadly recognized since the questions initially raised by Carson’s *Silent Spring* over 50 years ago (Carson, 1962; Metcalf, 1980; Matsumura, 2004; Matthews, 2007). However, pesticides also impose risks to pest management itself, counterintuitively leading to pest outbreaks that were recognized even before the onset of the large-scale use of organo-synthetic pesticides in agriculture (Pickett, 1949; Ripper, 1956). In fact, there is no shortage of reports of pesticide use misfiring and fostering pests instead of controlling them, which is particularly frequent with the use of broad spectrum insecticides, mainly organochlorines and also with organophosphates, carbamates and even pyrethroids (Metcalf, 1980).

Insecticide resistance is the best known and most widely studied ecological backlash resulting from frequent insecticide use, leading to eventual control failures (Georghiou, 1972; Brattsten et al., 1986). In contrast, resurgence and secondary pest outbreaks, although frequent, have received much less attention and studies have not progressed far beyond the reports of occurrence (Ripper, 1956; Hardin et al., 1995). Both the resurgence and secondary pest
outbreaks are short-term (ecological) consequences of pesticide use that are commonly reported in mite and insect pest species, unlike insecticide resistance, which is a long-term (evolutionary) consequence of pesticide use (Ripper, 1956; Georghiou, 1972).

Resurgence is an increase in the abundance of the targeted arthropod pest species to a level exceeding that of uncontrolled populations following an insecticide (or acaricide) application (Ripper, 1956; Hardin et al., 1995). Secondary pest outbreak, or pest replacement (or type II resurgence), is a related but distinct phenomenon of the increase in abundance of a non-target pest species after insecticide (or acaricide) application (Metcalf, 1980; Hardin et al., 1995). Both phenomena have common causes and mechanisms. Three different causes have been associated with resurgence and secondary pest outbreaks since as early as the mid 1950s: the reduction of the natural enemies, an increase of the pests, and the removal of the competitors (Ripper, 1956). Most of the reports of resurgence and secondary pest outbreaks so far implicate the reduction of natural enemies as their main cause (Metcalf, 1980; Hardin et al., 1995).

There is no denying the importance of the elimination of natural enemies for resurgence and secondary pest outbreaks (Metcalf, 1980; Desneux et al., 2007). Nonetheless, it is a mistake to consider it as the sole cause of both phenomena because this precludes the recognition of the underlying mechanisms involved and their consequences in different settings. Although easily perceived as important, the evidence of the involvement of natural enemy reduction in resurgence and secondary outbreaks is usually based on correlational studies and, sometimes, lethal acute selectivity bioassays (Hardin et al., 1995). Not even demographic toxicity bioassays with more robust toxicological endpoints, such as the rate of population growth (e.g., \( r_n \)), are used (Forbes and Calow, 1999; Stark and Banks, 2003). Because of this, the alternative causes of outbreaks, such as pesticide-mediated population increase of the pest species, are not recognized or considered as potential explanations. This is a serious mistake, particularly considering that homesis (i.e., the stimulatory or beneficial effect that is associated with low doses of compounds that are toxic at higher doses) is in the forefront of modern toxicology, widely recognized as a general, real and reproducible biological phenomenon (Calabrese and Baldwin, 2003; Calabrese, 2005, 2008; Cutler, 2013). Additionally, the failure to recognize the potential role of behavior in mediating pest outbreaks should be reviewed based on the growing body of evidence of its effect in insecticide efficacy and pest management (Haynes, 1988; Desneux et al., 2007).

Reports of resurgence and secondary pest outbreaks are frequent regarding tetranychid mite species (Zwick and Fields, 1978; Hall, 1979; Trichilo and Wilson, 1993). A well-recognized example of secondary pest outbreak is the outbreak of the southern red mite \( Oligonychus ilicis \) McGregor (Acari: Tetranychidae) induced by pyrethroid applications against the coffee leaf miner \( Leucomyza coffeella \) (Guérin-Ménéville and Perrottet) (Lepidoptera: Lyonetiidae) in Brazil (Reis and Souza, 1986; Reis et al., 1997). As is frequently the case, the cause of this phenomenon was attributed to the reduction of natural enemies of this mite species in coffee areas, particularly phytoseiid predatory mites such as \( Amblyseius herbicola \) (Chant) (Acari: Phytoseiidae), one of the key players in this scenario (Reis et al., 2007). However, there is an utter lack of evidence to support such assumption, and not even selectivity studies are available to provide support for this contention. Therefore, we performed a series of bioassays to test three potential and non-exclusive hypotheses as underlying causes of pyrethroid-induced outbreaks of the southern red mite: (1) selectivity and (2) pyrethroid-induced homesis in both the prey and the predator, and (3) pyrethroid (behavioral) avoidance in the predatory mite species.

### 2. Materials and methods

#### 2.1. Mites and insecticide

Colonies of the southern red mite \( O. ilicis \) and its predatory mite \( A. herbicola \) were established in the laboratory from coffee field crops in Viçosa County (State of Minas Gerais, Brazil) 1 month before the start of the bioassays and they were periodically subjected to the reintroduction of additional field-collected individuals at each generation in the laboratory. The laboratory colony of the southern red mite was maintained on coffee leaves edged by cotton and placed over wet nylon foam within a tray filled with water to prevent dehydration. The coffee leaves were replaced every 5 d. The laboratory colony of the phytoseiid predator was reared with fresh pollen from castor bean plants \( Ricinus communis \) \( L. \) on a black sheet of polyvinyl chloride (PVC; 15 \( \times \) 20 cm) edged by water-soaked cotton to prevent mite escape (Reis et al., 2007). The mite colonies from both species were maintained under controlled environmental conditions \((27 \pm 2 \degree C\) temperature, \(70 \pm 10\%\) relative humidity, \(12\) h photoperiod), which were also used in the experiments performed.

A commercial formulation of deltamethrin (Decis\textsuperscript{®} 25 CE; 25 g a.i./L, emulsifiable concentrate; Bayer CropScience, São Paulo, Brazil) was used. This formulation is registered for controlling the coffee leaf miner in Brazil (at the label rate of 11.1 mg a.i./L; Ministério da Agricultura, Pecuária e Abastecimento, 2013).

#### 2.2. Concentration–mortality bioassays

Acute concentration–mortality bioassays were performed with three replicates of 15 females each of both mite species \( O. ilicis \) and \( A. herbicola \) exposed to increasing deltamethrin concentrations (in water solution), in addition to an unexposed control (only distilled and deionized water were used), using the 5 s slide immersion methodology \((\text{Entomological Society of America, 1968})\). Briefly, each group of 15 females was dorsally fixed to a piece of double-sided tape (2 cm\(^2\)) on which the other side was fixed to a glass slide \((26 \times 76 \times 1.4 \text{ mm})\). Each slide was immersed for 5 s in the desired insecticide solution under slight agitation, and the mortality of the exposed female mites was recorded after 24 h. After such exposure the mites were considered dead when they could not move their legs when prodded with a fine hair brush. The deltamethrin concentrations used for \( O. ilicis \) were \(0.04, 1.11, 2.22, 3.33\) and \(6.67\) \(\mu\)g a.i./mL, and the concentrations used for \( A. herbicola \) were \(0.11, 1.11, 2.22, 3.33\) and \(4.44\) \(\mu\)g a.i./mL. These concentrations were established based on preliminary bioassays carried out to delimit the concentration range of response for each species.

#### 2.3. Life-table bioassays

The demographic life-table studies were performed to determine not only the chronic toxicity of deltamethrin to both mite species but also to recognize potential homesis. The same basic methodology was used for both mite species. Insecticide (and control) solutions (2.5 mL) were sprayed through a Potter tower \((\text{Standard model, Burkard Scientific, Rickmansworth, UK})\) on coffee leaf disks (3.0 cm diameter) at 0.34 bar \((=3.44 \times 10^4 \text{kPa})\). In addition to the controls, which had no insecticide application, the deltamethrin concentrations used for \( O. ilicis \) were \(0.002, 0.004, 0.005, 0.01, 0.02, 0.025\) and \(0.05\) \(\mu\)g a.i./mL, and the concentrations used for \( A. herbicola \) were \(2.5 \times 10^{-6}, 0.025, 0.10, 0.125, 0.15\) and \(0.245\) \(\mu\)g a.i./mL. These ranges of concentration were established based on the results of the concentration–mortality bioassays and additional preliminary bioassays of demographic response.
The leaf disks were left to dry for 1 h and subsequently placed floating in plastic containers filled with 100 mL distilled water. A pin was silicon-glued to the bottom of the container and attached to the center of the leaf disk allowing the disk to float with the water level but without moving horizontally to prevent mite escape (Reis et al., 1997). Twenty-five females (of either species) were transferred from the laboratory colonies to the untreated leaf disks to lay eggs for 24 h to form cohorts. Three independent cohorts (i.e., replicates) of 15 females (deutochrysalis) each were used and then transferred to the sprayed leaf disks (one female per disk) for each combination of species and deltamethrin concentration (including the control) following a completely randomized design. Because of the arrhenotokous parthenogenesis (i.e., unfertilized eggs develop into males) of the southern red mite, a male was added to each leaf disk when the females reached the adult stage to ensure copulation and the consequent formation of female progeny (Reis et al., 1997). Such addition of the male does not lead to insecticide residue depletion based on preliminary bioassays and therefore does not compromise female exposure to the insecticide. Because the phytoseiid predator A. herbicolus reproduces through thelytokous parthenogenesis (i.e., unfertilized eggs develop into females), male provision was not necessary to ensure female progeny production (Reis et al., 2007). The life-table bioassays were performed with both mite species following Carey (1993), in which cohort age-specific mortality and cohort variation were assessed in each insecticide concentration. The age-specific fertility tables were constructed as described by Carey (1993), and the intrinsic rate of population growth ($r_m$) was calculated for each cohort. This parameter was estimated by successive iterations through the Lotka equation $\Sigma e^{-\lambda x}m_x = 1$, in which $x$ is the pivotal age class, $L_x$ is the number of surviving individuals in the cohort at the interval between ages $x$ and $x+1$, and $m_x$ is the specific fertility and refers to the number of females produced per female at age $x$. The concentration–mortality results were subjected to probit analysis (procedure PROBIT; SAS Institute, 2008). The selectivity ratio for deltamethrin was obtained by dividing the LC$_{50}$ of the southern red mite by the corresponding LC estimate for its predatory phytoseiid A. herbicolus. The 95% confidence limits for this estimate were calculated, and the LC$_{50}$ values were considered to be significantly different ($p < 0.05$) if the confidence limits on the selectivity ratio did not include the value 1 (Robertson et al., 2007). The results of the intrinsic rate of population increase ($r_m$) were subjected to non-linear (log-logistic) regression analysis using the NLIN procedure (SAS SAS Institute, 2008), modified to allow the recognition of hormesis (Brain and Cousen, 1989; Seefeldt et al., 1995; Schabenberger et al., 1999). The significance of the differences observed between the walking behavior parameters that were recorded was tested with Student's $t$-test ($p < 0.05$) using the TTEST protocol from SAS (SAS Institute, 2008). The means of the half-treated portion of the arenas were compared with the untreated portion using a paired $t$-test ($p < 0.05$).

3. Results

3.1. Acute lethal selectivity

The mortality results from the acute lethal bioassays of deltamethrin exposure provided concentration–mortality curves exhibiting low $\chi^2$ values (2.75 and 2.82 for O. ilicis and A. herbicolus, respectively) and high $p$-values (>0.05), indicating their suitability for the probit model. The relative toxicity estimated with these concentration–mortality curves indicated that there was slightly, but significantly higher, deltamethrin susceptibility in the predator A. herbicolus compared with its prey, with a selectivity ratio of 1.5-fold at LC$_{50}$, in which the confidence interval did not include the value 1 and was therefore significant based on Robertson et al. (2007).

3.2. Chronic demographic selectivity

The three-parameter ($\alpha$, $\beta$, and $\delta$) log-logistic model that was used to describe the insecticide toxicity was added by an additional parameter ($\gamma$) to test the hormetic effect in the population growth ($r_m$) data obtained from the life-table (demographic) bioassays (Brain and Cousen, 1989; Seefeldt et al., 1995). The population growth rate of both mite species was significantly compromised by...
Table 1
Relative toxicity and selectivity of deltamethrin to the southern red mite *Oligonychus ilicis* and its phytoseiid predator *Amblyseius herbicolus*.

<table>
<thead>
<tr>
<th>Mite species</th>
<th>Number of mites tested</th>
<th>Slope (±sE)</th>
<th>LC50 (95% FL) (μg/mL)</th>
<th>χ²</th>
<th>p</th>
<th>Selectivity ratio (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oligonychus ilicis</em></td>
<td>225</td>
<td>4.66 (±0.92)</td>
<td>1.37 (1.16–1.67)</td>
<td>2.75</td>
<td>0.25</td>
<td>1.54 (1.41–1.82)</td>
</tr>
<tr>
<td><em>Amblyseius herbicolus</em></td>
<td>225</td>
<td>3.97 (±0.56)</td>
<td>0.89 (0.74–1.03)</td>
<td>2.82</td>
<td>0.42</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2
Summary of the log-logistic (Brain-Cousen) regression analysis of the concentration-response curves of deltamethrin exposure to the southern red mite (*Oligonychus ilicis*) and its phytoseiid predator *Amblyseius herbicolus* exhibited in Fig. 1.

<table>
<thead>
<tr>
<th>Mite species</th>
<th>Model (Brain-Cousen)</th>
<th>Parameters (±sE)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oligonychus ilicis</em></td>
<td>[E[Y</td>
<td>x] = δ + (x - δ)/1 + exp [β Ln(x)]]</td>
<td>99.00 (±5.15)</td>
<td>0.81 (7.46)</td>
</tr>
<tr>
<td><em>Amblyseius herbicolus</em></td>
<td>[E[Y</td>
<td>x] = δ + (x - δ + γ)/1 + exp [β Ln(x)]]</td>
<td>98.00 (±10.34)</td>
<td>0.64 (±0.51)</td>
</tr>
</tbody>
</table>

deltamethrin exposure and even more so for the mite pest, which was three times more susceptible to deltamethrin than its phytoseiid predator at their respective median effective concentrations (EC50) of 0.04 and 0.12 μg a.i./mL for *O. ilicis* and *A. herbicolus*, respectively (Fig. 1). Both the curves were suitably described by the modified log-logistic model to contemplate the possibility of hormesis; however, only the southern red mite exhibited significant hormesis as the hormetic parameter γ estimated for this species did not include the value 0.0 (Table 2) (Schabenberger et al., 1999). Therefore, only the red southern mite exhibited the classical β-curve that is characteristic of the hormesis phenomenon (Stebbing, 2000), indicating the occurrence of deltamethrin-induced hormesis in this mite pest species.

The survival curves of red mite females (Fig. 2A) differed only at deltamethrin concentrations ≥ 0.02 μg a.i./mL, not significantly contributing to the hormesis observed in the population growth rate. By contrast, fertility was enhanced at deltamethrin concentrations lower than 0.002 μg a.i./mL, particularly at 0.004 μg a.i./mL, in which such effects peaked (Fig. 2B and C) and were translated into the population growth profile exhibited in Fig. 1. A reduction in the pre-reproductive period, coupled with an extension of the reproductive period, apparently favored a lower generation time and higher lifetime fertility of the red mite under deltamethrin concentrations between 0.002 and 0.005 μg a.i./mL (Fig. 2).

3.3. Behavioral avoidance
Deltamethrin did not elicit behavioral avoidance in the predatory mite because there was no significant difference in the time spent in each half of the arena (292.89 ± 6.69 s and 280.52 ± 6.31 s for deltamethrin-treated and untreated halves respectively) (paired-*t* = 1.07, *p* = 0.30) in the free-choice test. Additionally, there was no significant difference in walking behavior between the fully treated and fully untreated arenas, in which the pooled mean for the distance walked is 184.27 ± 6.82 cm (*t* = 0.51, *p* = 0.61), for the walking velocity is 0.32 ± 0.01 cm/s (*t* = 0.11, *p* = 0.91), and for the resting time is 33.03 ± 6.28 s (*t* = 1.89, *p* = 0.07). These results suggest a lack of deltamethrin-induced behavioral avoidance in the predatory mite *A. herbicolus*.

3.4. Discussion
Secondary pest outbreaks are an environmental consequence of the use of broad spectrum pesticides, particularly insecticides (and acaricides), with serious consequences to pest management. The general assumption that natural enemies are naturally more susceptible to pesticides is the underlying cause of such mechanisms leading to pest outbreaks (Trichilo and Wilson, 1993; Hardin et al., 1995). However, alternative mechanisms exist as explored in a few different species, including aphids and mites, among others (Kerns and Gaylor, 1993; Szczepaniec et al., 2011). Pyrethroid-induced outbreaks of southern red mites are broadly regarded as a consequence of the elimination of their phytoseiid mite predators by pyrethroid application. However, this hypothesis was never tested, and the entire rationale is based on speculation without any confirmatory data (Reis and Souza, 1986; Reis et al., 1997). Here, we tested this hypothesis and two alternative ones, insecticide avoidance and insecticide-induced hormesis, to explain these reported outbreaks.

Deltamethrin behavioral avoidance was not detected in the predator *A. herbicolus* and, therefore, is not a likely mechanism underlying the outbreaks of the southern red mite. The basic expectation, however, was a higher susceptibility to deltamethrin of the phytoseiid predator *A. herbicolus* than of its prey, the southern red mite. However, the acute lethal toxicity bioassays that were performed indicated that *A. herbicolus* is only slightly more susceptible to deltamethrin than *O. ilicis*. Moreover, demographic bioassays using population growth as a toxicological endpoint are more robust for such predictions, and these bioassays were also performed for the red mite and its predator.

The demographic results are also important because they consider the sublethal range of exposure to the insecticide, which is particularly important in agriculture since the target and non-target species remain exposed to sublethal concentrations of the applied insecticide for longer periods than to their lethal concentration (Guedes et al., 2009; Cordeiro et al., 2010). This takes place for two reasons: (1) because the label rates recommended for field application aim at a particular pest species thus leading to sublethal exposure to a broader range of species, and (2) insecticide degradation rapidly occurs in the field limiting the time of exposure to the lethal concentration initially applied. The demographic results obtained here indicate a threefold higher tolerance, not susceptibility, of the predator to deltamethrin compared with the southern red mite, leading to the rejection of the hypothesis that deltamethrin leads to the reduction of the predator population. The range of population growth of both species falls within the reported range for tetranychids and phytoseiids, indicating the consistency of our results (McMurtry and Croft, 1997; Reis et al., 1997, 2007).

The potential stimulation of the red mite population under sublethal exposure to deltamethrin was the last hypothesis tested to recognize the potential causes of deltamethrin-induced outbreaks of this species. Insecticide-induced hormesis has been recognized not only in insects but also in mites with potentially important consequences for pest management remaining to be addressed (Guedes et al., 2009; Cutler, 2013). The demographic (life-table) studies performed with the red mite and its phytoseiid predator allowed the recognition of significant deltamethrin-induced
hormesis in the former but not in the latter. Such a finding indicates that sublethal deltamethrin exposure leads to higher population growth rates of the red mite, overcoming its higher susceptibility to this insecticide under higher doses, potentially leading to its outbreak.

The delay in the outbreak relative to the initial insecticide spraying, as usually reported for the red mite (Reis and Souza, 1986; Reis et al., 1997), is further suggestive that the role of sublethal insecticide exposure is particularly important in such cases. This is so because it allows the southern red mite to escape the population regulation provided by A. herbicolus, which is not significantly affected by the insecticide. Although deltamethrin-induced hormesis was not detected for the predator A. herbicolus, it may still occur. However, the expression of hormesis may be low for predators, as reported for the spined soldier bugs Podisus distinctus (Stål) and Suppitis cincticeps (Stål) (Heteroptera: Pentatomidae) (Zanuncio et al., 2003; Guedes et al., 2009). Additionally, hormesis is believed less likely to be important among predators than prey because the populations of the former are limited by prey abundance, potentially restricting the expression of hormesis on predators, as suggested by Forbes (2000).

In summary, we tested three alternative hypotheses as potential underlying causes of southern red mite outbreaks in coffee after pyrethroid applications - (1) selectivity, (2) pyrethroid-induced hormesis in both the prey and the predator, and (3) pyrethroid (behavioral) avoidance in the predatory mite species. Among these hypotheses, only the occurrence of pyrethroid-induced hormesis in the southern red mite was verified and provides a consistent (causal) explanation for the outbreaks of this pest species in coffee plantations after pyrethroid sprays.

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