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Compatibility of pesticides with the generalist predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae)

Compatibilidad de plaguicidas con el ácaro depredador generalista *Amblyseius largoensis* (Acari: Phytoseiidae)

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Abstract: The aim of this study was to evaluate the compatibility of synthetic and alternative pesticides to an important natural enemy associated with pest mites in citrus orchards, in São Luís Maranhão State, Brazil: the generalist predatory mite *Amblyseius largoensis*. The pesticides tested were abamectin, mineral oil, mancozeb, pyridaben, neem oil (*Azadiractha indica*) and crude cottonseed oil (*Gossypium* spp.). Compatibility of pesticides to *A. largoensis* was assessed as to adverse effects on mortality and fertility as well as to interference with growth rate. Overall, the results of adverse effects and growth rate were corroborative. Mancozeb, mineral oil and neem oil were classified as slightly harmful while the cotton oil and abamectin were classified as moderately harmful and pyridaben was considered harmful. The growth rate of *A. largoensis* females exposed to mancozeb, mineral oil, neem oil and cotton oil was positive indicating population growth. We conclude that neem oil, mineral oil and mancozeb are compatible with *A. largoensis* as they were less harmful to this predator.

Key words: Vegetal oils. Integrated pest management. Physiological selectivity. Toxicity.

Resumen: El objetivo de este estudio fue evaluar la compatibilidad de plaguicidas alternativos y sintéticos con el depredador generalista de ácaros *Amblyseius largoensis,* enemigo natural de plagas de ácaros en cultivos de cítricos en São Luís, Maranhão State, Brazil. Los plaguicidas evaluados fueron la abamectina, el aceite mineral, el mancozeb, el piridaben, y los aceites de neem (*Azadiractha indica*) y de algodón (*Gossypium* spp.). La compatibilidad de los plaguicidas con *A. largoensis* se evaluó mediante la estimación de los efectos adversos en la mortalidad y fecundidad y también por la interferencia con la tasa de crecimiento. En general, los resultados totales fueron complementarios a los efectos adversos de la tasa de crecimiento. El mancozeb, el aceite mineral y el aceite de neem se clasificaron como plaguicidas ligeramente dañinos, el aceite de algodón y la abamectina como moderadamente nocivos y, finalmente, el piridaben se consideró significativamente perjudicial. La tasa de crecimiento de las hembras de *A. largoensis* expuestas a mancozeb, aceite mineral, aceite de neem y aceite de algodón fue positivo, lo que indica crecimiento de la población del depredador. Los resultados en este estudio mostraron que el aceite de neem, el aceite mineral y el mancozeb fueron los plaguicidas más compatibles y que menos efectos dañinos provocaron en el depredador generalista de ácaros *A. largoensis.*

Palabras clave: Aceites vegetales. Manejo integrado de plagas. Selectividad fisiológica. Toxicidad.

Introduction

The use of pesticides that are compatible with natural enemies is pivotal to the success of biological control as selective pesticides have lower toxicity to beneficial organisms and they are therefore prioritized in Integrated Pest Management (IPM) programs (Varenhorst and O'Neal 2012; Abraham *et al*. 2013). Selectivity bioassays in which natural enemies are submitted to pesticides under controlled conditions help to classify such toxicants into innocuous and less harmful to harmful (Hassan *et al*. 1994; Reis *et al.* 2006). The use of selective pesticides is an important strategy of chemical control as such pesticides are less harmful to natural enemies (Reis *et al*. 1998; Abraham *et al*. 2013).

Predatory mites belonging to the family Phytoseiidae are major natural enemies of pest mites (Moraes 1992; McMurtry and Croft 1997; Sarmento *et al*. 2011; Hannef and Sadanandan 2013; Costa *et al*. 2014). The predatory mite *Amblyseius largoensis* (Muma, 1955) (Acari: Phytoseiidae) has been found inhabiting citrus orchards (Childers and Denmark 2011; Silva *et al.* 2013) associated with pests such as the citrus rust mite *Phyllocoptruta oleivora* (Ashmead, 1879) (Acari: Eriophyidae), the Texas citrus mite *Eutetranychus banksi* (McGregor, 1814) (Acari: Tetranychidae) and other tetranychid mites (Tanaka and Kashio 1977; Jamieson *et al.* 2005). This mite has a worldwide distribution and it is also found in South American countries including Brazil and Colombia (Moraes *et al*. 2004). Type III generalist phytoseiid mites like *A. largoensis* feed on pest insects and mites as well as on alternative food like pollen and nectar (McMurtry and Croft 1997; Carrillo *et al.* 2010) which allow them to survive in the field even in periods of low pest populations (Reis and Alves 1997; McMurtry and Croft 1997; Sarmento *et al*. 2011; Gerson and Weintraub 2012).

The control of pests is usually conducted with pesticides which may cause people poisoning and environmental contamination besides pest outbreaks and mortality of natural enemies (Gallo *et al*. 2002; Geiger *et al*. 2011; Abraham *et al*. 2013). Pesticides such as abamectin, mineral oil, mancozeb and pyridaben are registered to control citrus pests in Brazilian citrus orchards (Agrofit 2013). Alternative pesticides such as vegetal oils are usually less toxic to beneficial arthropods

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(Duso *et al*. 2008), however studies to assess their toxicity to natural enemies like the predatory mite *A. largoensis* are needed. Here, we aimed to evaluate the compatibility of synthetic and alternative pesticides to the generalist predatory mite *A. largoensis*.

Material and methods

The predatory mite *A. largoensis* was collected from Orange trees (*Citrus sinensis* L.) located in the city of São Luís (2°35'44.40"S 44°10'30.03"W), Maranhão state, Brazil. Mites were kept in plastic discs (7 cm diameter) floating on Petri dishes (10 cm diameter x 1.5 cm height) without lid filled with water under controlled conditions of temperature (29 ± 5 ºC) and natural relative humidity and photoperiod. Mites were fed with castor bean pollen (*Ricinus communis* L.) and honey every other day (Reis and Alves 1997).

The pesticides abamectin (Abamectina DVA™ 18 EC, 18 g de a.i./L, dosage 0.3 ml/L), mineral oil (Iharol™ CE, 760 g de a.i./L, dosage 20 ml/L), mancozeb (Manzate™ WG, 750 g de a.i./kg, dosage 1.5 g/L) and pyridaben (Sanmite™ CE, 200 g de a.i./L, dosage 0.5 ml/L) were chosen because they are registered in Brazil to control the citrus rust mite *P. oleivora* (Agrofit 2013). The neem oil (*Azadiractha indica* A. Juss) (Sempre Verde Killer™, 3 ml a.i./L, dosage 15 ml/L) and the cotton crude oil (*Gossypium* spp.) (dosage 15 ml of oil + 10 ml of neutral detergent/L of water) (Ferreira and Michereff Filho 2002) were selected because they have been efficiently used to control pests of several crops. Pesticides were sprayed at their highest label dosage recommended to control the citrus rust mite while the neem oil was used in its maximum dosage to control pest mites. The cotton oil was used at the dosage recommended to control the coconut mite *Aceria guerreronis* Keifer 1965 (Acari: Eriophyidae) (Ferreira and Michereff Filho 2002).

Pesticides were sprayed on plastic discs (5 cm diameter) through a Potter tower (Burkard, Rickmansworth, UK) at 5 psi/pol^2 pressure with an 1.3 ml spray aliquot which resulted in an residue of 1.7 ± 0.25 mg/cm^2 (Hassan *et al.* 1994). Control discs were sprayed only with distilled water. Sprayed discs dried up in open air for 1 hour before five adult females of *A. largoensis* in the beginning of their reproductive period (6 days old) and a male were transferred to them. Discs were placed to float on Petri dishes as described above. The experiment consisted of seven treatments (abamectin, mineral oil, mancozeb, pyridaben, neem oil, cotton oil and control) and six replicates in a randomized experimental design. Surviving predatory mites were daily fed with castor bean pollen and honey. The number of living mites and eggs laid were daily recorded over a period of 7 days.

Our approach to determine pesticide compatibility with the predatory mite *A. largoensis* was based on the total adverse effect (E%) and the interference on growth rate. The total adverse effect (E%) was based on mortality and effect on reproduction using the equation: $E\% = 100\% - (100\% - M_c) \times E_r$, where M_c = corrected mortality and E_r = reproduction effect obtained from the quotient between the mean number of viable eggs in the treatment and the mean number of viable eggs in control. E% values were used to rank pesticides into classes from 1 to 4 according to the IOBC/WPRS (Hassan *et al*. 1994), i.e. class 1 = E < 30% (innocuous); class 2 = 30% < E < 79% (slightly harmful); class 3 = 80% < E < 99% (moderately harmful) e class 4 = E > 99% (harmful) (Reis *et al*. 1998).

The instantaneous rate of increase (r_i), a proxy for growth rate, was used to assess the effect of pesticides on growth rate of the predatory mite *A. largoensis* using reproduction and mortality data according to the equation: $r_i = [\ln (N_f / N_0)] / \Delta t$, where N_f is the final number of individuals, N_0 is the initial number of individuals and Δt is the time elapsed between the onset and final of the experiment (7 days) (Stark *et al*. 1997). The r_i is a snapshot of population growth in a given period and positive values mean population increase and negative values indicate population decline (Stark *et al.* 1997). The r_i was calculated based on the number of eggs, immatures and adults daily added to the population over a period of 7 days. Anovas followed by post hoc Fisher tests were used to determine the influence of synthetic and alternative pesticides on the growth rate of and the number of descendants produced by *A. largoensis* using the software Statistica 10 (Statsoft Inc 1984-2011).

Results and discussion

Pyridaben (100%) and abamectin (69.56%) inflicted the highest mortality on females of the predatory mite *A. largoensis* while the mineral oil and the neem oil were less toxic (30.43%) (Table 1). Mancozeb, mineral oil and neem oil were considered slightly harmful (class 2), cotton oil and abamectin were ranked in class 3 as moderately harmful and pyridaben was classified as harmful (class 4) to *A. largoensis*. Although the mineral and the neem oil caused the lowest mortality on the predatory mite they were not considered

Table 1. Toxicity of synthetic and alternative pesticides to the predatory mite *Amblyseius largoensis*.

Pesticides	M_c[1]	E_r[2]	E%[3]	Class[4]
Control	—	—	—	—
Abamectin	69.56	0.112	96.59	3
Mancozeb	47.83	0.436	77.27	2
Pyridaben	100.00	0.065	100.00	4
Mineral oil	30.43	0.319	77.84	2
Cotton oil	47.83	0.298	88.35	3
Neem oil	30.43	0.331	76.99	2

[1] M_c: corrected mortality; [2] E_r: effect on reproduction; [3] E%: total adverse effect. [4] Toxicological classes according to the IOBC WPRS: class 1 = innocuous, class 2 = slightly harmful, class 3 = moderately harmful, class 4 = harmful.

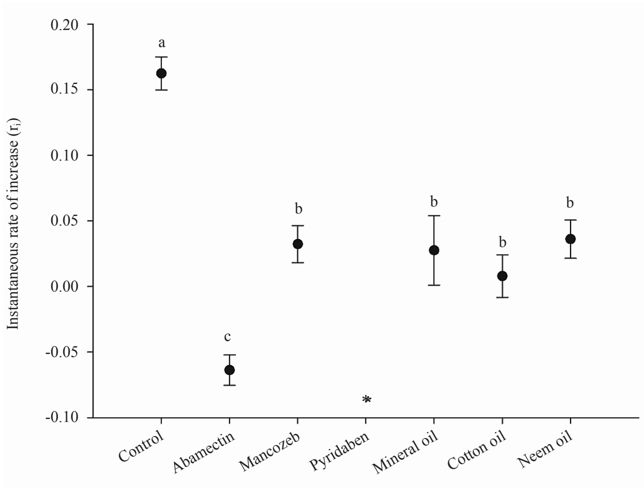

Figure 1. Instantaneous rate of increase (r_i) of the predatory mite *Amblyseius largoensis* submitted to synthetic and alternative pesticides. Means ± SE are presented. Different letters indicate significant differences in growth rate among pesticides based on post hoc Fisher test; * indicates population extinction ($N_f = 0$).

innocuous because they negatively affected reproduction (Table 1).

The growth rates of *A. largoensis* exposed to mancozeb, cotton oil, mineral oil and neem oil were significantly reduced in comparison with control ($F_{5,20} = 19.24$; $P < 0.05$), yet positive r_i values were obtained indicating population growth (Fig. 1). Mites exposed to abamectin had negative r_i values suggesting population decline, while pyridaben eliminated the population of the predatory mite after 7 days ($N_f = 0$) (Fig. 1). The number of descendants per female of *A. largoensis* was affected by the pesticides ($F_{6,35} = 28.20$; $P = 0.00$) (Fig. 2). Abamectin and pyridaben, which were the most toxic pesticides to the predatory mite, also caused the highest reduction in descendants. Mancozeb, cotton oil, mineral oil, and neem oil also reduced the number of descendants (Fig. 2).

Our combined approach of total adverse effect and interference on growth rate allowed us to assess the compatibility of synthetic and alternative pesticides to the predatory mite *A. largoensis*. In general, total adverse effects matched growth rate results. The oils of neem and mineral as well mancozeb were considered slightly harmful (class 2) to the predatory mite *A. largoensis*. In fact, growth rates of *A. largoensis* exposed to these pesticides were positive indicating population increase. Alternative pesticides like the neem oil have additional advantages such as low toxicity to mammals, fast degradation in the environment and relative selectivity to natural enemies (Erler *et al*. 2010; Nicoletti *et al*. 2012). Neem pesticides are efficient against pest mites (Venzon *et al*. 2008) besides being selective to the predatory mites *Neoseiulus californicus* (McGregor, 1954) and *Phytoseiulus macropilis* (Banks, 1904) (Acari: Phytoseiidae) (Bernardi *et al*. 2013). The mineral oil has been used to control insect pests (Najar-Rodriguez *et al*. 2008; Chueca *et al*. 2009), and no resistance has been identified (Najar-Rodriguez *et al*. 2008). Contrasting to our results, Reis *et al.* (1998) ranked mineral oil as harmful (class 4) to another natural enemy found in citrus orchards, the predatory mite *Iphiseiodes zuluagai* Denmark and Muma, 1972 (Acari: Phytoseiidae). The fungicide and acaricide mancozeb exerts it activity through contact and by inhibiting the enzyme acetylcholinesterase in the nervous system (Pang *et al*. 2009). Mancozeb inflicted mortality and reduced oviposition to *A. largoensis* colonies in comparison with control. Adults of the predatory mite *Typhlodromus pyri* Scheuten, 1857 (Acari: Phytoseiidae) treated with mancozeb had low mortality and oviposition reduction (Gadino *et al.* 2011) while this pesticide caused total mortality on *I. zuluagai* populations (Reis *et at.* 1998).

The cotton oil and abamectin were classified as moderately harmful (class 3) and negatively affected the growth rate of *A. largoensis.* Mites exposed to abamectin had negative r_i values suggesting population decline towards extinction. Together with pyridaben, abamectin drastically reduced the fecundity of the predator as shown for Er values (Table 1) and the number of descendants (Fig. 2). The cotton oil is an alternative pesticide which has been used against pests of several crops such as the coconut mite *A. guerreronis* (Ferreira and Michereff Filho 2002), and more studies on this oil are needed to assess its toxicity to pests and natural enemies alike. The abamectin is a broad-spectrum acaricide that have been used against pest mites in citrus orchards (Agrofit 2013). Similarly to pyridaben, abamectin causes mortality and reduces ovipositon of *A. largoensis*. The response to a given pesticide is species-specific. For example, abamectin is considered moderately harmful (class 3) to the predatory mite *Euseius alatus* De Leon, 1966, slightly harmful (class 2) to *Euseius citrifolius* Denmark & Muma, 1970 and innocuous (class 1) to *Amblyseius herbicolus* (Chant, 1959) and *I. zuluagai* (Acari: Phytoseiidae) (Reis *et al*. 2006). Pyridaben was classified as harmful (class 4) and caused the extinction of the population of *A. largoensis* (Nf = 0). Pyridaben is a contact acaricide that inhibits complex I of the mitochondrial respiratory pathway with a fast initial action and has been considered a non selective pesticide model for its high mortality and sharp fecundity reduction to predatory mites (Reis *et al.* 2006; Meyer *et al*. 2009; Park *et al*. 2011).

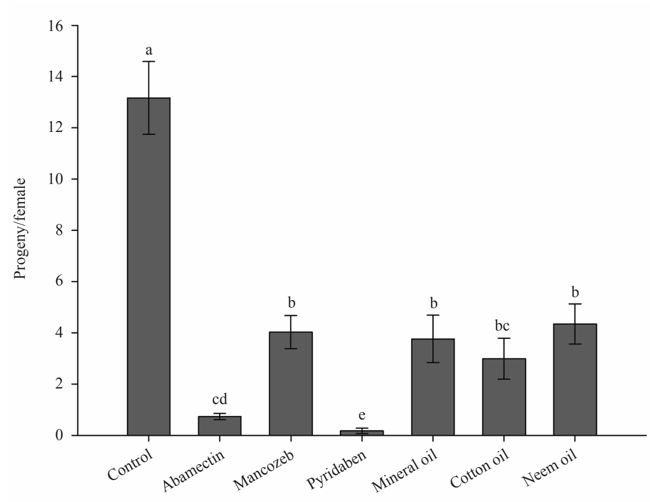

Figure 2. Number of descendants per female of the predatory mite *Amblyseius largoensis* exposed to synthetic and alternative pesticides. Means ± SE are presented. Different letters indicate significant differences in descendant production among pesticides based on post hoc Fisher test.

Conclusions

The oils of neem and mineral as well as mancozeb are compatible with the predatory mite *A. largoensis* as they are less harmful to this predator. The cotton oil had an intermediate selectivity while pyridaben and abamectin were more harmful to *A. largoensis* and therefore should be avoided in IPM programs.

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