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Toxicity of four pesticides on *Orius insidiosus* under laboratory conditions

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Abstract

The predatory bug *Orius insidiosus* (Say) (Rhynchota Anthocoridae) is marketed in Argentina mainly to control thrips in greenhouse sweet pepper and used along with a recommended pesticide application schedule to simultaneously manage the presence of other pests. The accurate assessment of the compatibility of pesticides with predator activity is key for the success of Integrated Pest Management programs. The susceptibility of *O. insidiosus* to some common pesticides was investigated in the laboratory. Four pesticides (flubendiamide, cyantraniliprole, indoxacarb and azoxystrobin) were assayed for their effects on the predator. These pesticides were tested at a single rate of application, corresponding to their highest label rate. We tested the effect of these compounds on nymphs and adults of *O. insidiosus*, from contact with residuals. Mortality was recorded 24 hours after the treatment. This study assessed the lethal and fecundity effects of the pesticides on *O. insidiosus* which were classified according to the definitions of toxicity given by the International Organization for Biological Control. Flubendiamide and azoxystrobin were categorized as harmless, while cyantraniliprole was slightly harmful and indoxacarb was moderately harmful. Only indoxacarb and cyantraniliprole affected the fecundity of the predatory bug.

Key words: predatory bug, toxic effects, mortality, fecundity, vegetable crops, IPM.

Introduction

The predatory bug *Orius insidiosus* (Say) (Rhynchota Anthocoridae) is an important biological control agent of several insect pests, including thrips, aphids, whiteflies and the eggs and small larvae of Lepidoptera (Silveira *et al*., 2004; Moscardini *et al*., 2013). It is a voracious generalist predator in all its active life stages, which means this species can be used as a biological control agent (Symondson *et al*., 2002).

Predators of the genus *Orius* are mass-produced and released mainly to control thrips pest *Frankliniella occidentalis* (Pergande) (Thysanoptera Thripidae) in various horticultural crops in Europe and North America (Burgio *et al*., 2004; Bosco *et al*., 2008; Weintraub *et al*., 2011). In particular *O. insidiosus* is mass reared and used in commercial biological control in North America since 1985 (van Lenteren, 2012).

The commercialization of biological control agents has grown in Latin America during the last decades. In Brazil for instance this is a viable economic activity (Bueno *et al*., 2014). In particular some studies have demonstrated the effectiveness of *O. insidiosus* against the western flower thrips *F. occidentalis* in greenhouse flowers crops in Brazil (Silveira *et al*., 2004). Nowadays, *O. insidiosus* is also marketed in Argentina for thrips control in greenhouse pepper and used along with a recommended pesticide application schedule to simultaneously manage the presence of other pests (i.e. aphids, mite and fungi diseases) (Cáceres *et al*., 2018).

The evaluation of pesticides for compatibility with Integrated Pest Management (IPM) programs traditionally begins with an assessment of their acute toxicity (lethal effect) that provides important information on the risk they pose to natural enemies (Candolfi *et al*., 2001). The importance of sublethal pesticide effects on development, reproduction and behaviour of predators and parasitoids has also been recognized by many researchers, including Croft (1990), Desneux *et al.* (2007), Biondi *et al*. (2012) and Pekár (2012). Although several authors have worked on the behavioural effects of pesticides on natural enemies recently (Desneux *et al*., 2004; Wrinn *et al*., 2012; Fernandes *et al*., 2016; Herrick and Cloyd, 2017; Cloyd and Herrick, 2018), there are still many open questions about aspects such as the impact on orientation, feeding, reproduction (fecundity and fertility) and learning.

The International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC), the Beneficial Arthropod Regulatory Testing Group (BART) and the European and Mediterranean Plant Protection Organization (EPPO) have elaborated a Joint Initiative program with the aim to develop and validate test methods to assess side effects of plant protection products on non-target arthropods (Candolfi *et al*., 2001; Mead Briggs *et al*., 2010). This initiative also classifies pesticides into four categories depending on the degree of damage they cause to the beneficial (Hassan, 1997; Sterk *et al*., 1999).

We tested three insecticide (cyantraniliprole, flubendiamide, indoxacarb) and one fungicide (azoxystrobin). These pesticides are registered for use in sweet pepper and tomato crops (CIAFA, 2018). Specifically, cyantraniliprole and azoxystrobin are commonly used in sweet pepper greenhouse for the control of whiteflies and powdery mildew, respectively (Molina *et al*., 2018).

Considering the importance of the predator *O. insidious* as a biological control agent, and the need for studies that improve its incorporation in IPM programs, the goal of this work was to assess the lethal and sublethal effects on the reproduction of *O. insidiosus* of some pesticides frequently used in vegetable crops. Furthermore, we aim to classify these pesticides' toxicity according to guidelines given by the International Organization for Biological Control.

Materials and methods

Rearing of the predatory bug *O. insidiosus*

The predatory bug *O. insidiosus* was originally obtained from a commercial orchard of Buenos Aires province, Argentina. They were reared in a climatically controlled room at 24 ± 1 °C temperature, relative humidity of 60 ± 10% and a photoperiod of 14:10 (L:D). Bean seedcases were used as oviposition substrates and *Sitotroga cerealella* (Oliver) (Lepidoptera Gelechiidae) eggs as prey.

Experimental procedure

During these studies, commercial formulations of four pesticides, one fungicide (azoxystrobin, Amistar®, Syngenta) and three insecticides (flubendiamide, Belt®, Bayer CropScience; cyantraniliprole, Benevia®, Dupont; indoxacarb, Avaunt®, Dupont), were assayed for their effects on *O. insidiosus* (table 1). The three insecticides are registered to control *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae), while cyantraniliprole is also effective to control the whitefly *Bemisia tabaci* (Gennadius) (Rhynchota Aleyrodidae). The fungicide azoxystrobin is registered and widely used to control diseases (e.g. *Leveillula taurica*, *Phytophthora capsici*, *Alternaria* spp., *Colletotrichum* sp., *Botrytis* sp.), in pepper, chard, cucurbits, lettuce, tomato and carrots (CIAFA, 2018).

The pesticides were tested for residual contact toxicity at a single rate of application corresponding to their highest label rate since this is the most frequently rate used in vegetable crops in Argentina. For each experiment, all products were diluted in tap water. Experimental units used in this study were a modification of those of Bakker *et al*. (2000). They were glass plates (13 × 13 cm) separated by a PVC cylinder (2 cm high) with 4 holes, 3 of them covered with mesh to ensure ventilation and the other one covered with cotton for the provision of water. In the control treatment, the plates were sprayed only with tap water.

The applications were made in the Instituto de Ingeniería Rural - INTA Castelar, with a sprayer with hollow cone nozzle 8001 Teejet®, at a pressure of 2 bar, from a height of 0.40 m and at a speed of 4.5 km/h.

Experimental units were held in acclimatized room at 24 ± 1 °C temperature, relative humidity of 60 ± 10% and the photoperiod of 14:10 (L:D).

Acute toxicity to nymphs

In this experiment, ten 3th instar nymphs of *O. insidiosus* were placed all together in an experimental unit (10 replicates/treatment) as soon as the sprayed pesticide had dried (approximately 1 hour after the application) and eggs of *S. cerealella* were offered as food. After 24 hours exposure, the number of dead nymphs was recorded. Those nymphs that did not move in response to probing with a camel hair brush were considered dead. Mortality was estimated as the proportion of dead nymphs over the total number of exposed nymphs. To evaluate the effect of the pesticides on the fecundity and fertility, the nymphs that survived after 24 hours were isolated in an untreated plastic container (9 cm diameter, 5 cm high) covered by a gauze, until they reached adulthood (7-8 days approximately). They were fed eggs of *S. cerealella* and water. The females and males coming from each replicate were transferred to a plastic container (8 cm in diameter, 10 cm high) for mating. After 9 days approximately, males were removed and a bean seedcase was introduced into the container as an oviposition substrate for the females. During the following 4 days, the number of eggs inserted into the seedcase was recorded daily and the fecundity was estimated as the total number of eggs/female. Likewise, the eggs were incubated and checked daily until eggs hatched. Fertility was estimated as the proportion of emerged nymphs from the recorded eggs (number of 1st instar nymphs/number of eggs).

Acute toxicity to adults

Five females and five males (24-48 hours old) of *O. insidiosus* were placed together in an experimental unit as soon as the sprayed pesticide has dried, and were fed with eggs of *S. cerealella*. The following experimental steps were the same as in the case of the nymphs described above, and replicated 10 times for each pesticide. To evaluate the effect of the pesticides on the bugs' fecundity and fertility, the surviving females after 24 hours of exposition were placed in an untreated plastic container (8 cm diameter, 10 cm high) covered by gauze. Food, water and the bean seedcases were supplied during 4 days. Fecundity and fertility were estimated as in the previous experiment.

Table 1. Pesticides tested on *O. insidiosus*.

Pesticide group	Common name (active ingredient)	Trade name	Chemical class	Concentration tested*
Insecticide	Cyantraniliprole	Benevia	Anthranilic diamide	0.6 cc/l
Insecticide	Flubendiamide	Belt	Benzenedicarboxamide	0.25 cc/l
Insecticide	Indoxacarb	Avaunt	Oxadiazine	2 g/l
Fungicide	Azoxystrobin	Amistar	Strobilurin	1 cc/l

*The maximum recommended label rate.

Data analyses

Effect of pesticides on nymphs and adults

The effect of pesticides on the nymphs and adults' fecundity was analysed using a one-way ANOVA. The means were separated using the Tukey test at the 0.05 level. Differences due to the treatments on survival (nymph and adult) and fertility were analysed by a Kruskal-Wallis test since the data did not meet the ANOVA assumptions. The means were compared by a post hoc comparison using Dunn's method. Statistical analyses were performed using Statistica for Windows (StatSoft, 2000).

Toxicity classes

The total effect (E) of each pesticide was determined considering the impact on the mortality and the fecundity. We used the formula proposed by Van de Veire *et al.* (1996) to calculate E:

$$E (\%) = 100 - (100 - Ma) \times ER$$

where Ma is the corrected mortality by control using Abbott's formula (Abbott, 1925) and ER is the effect of the product on fecundity:

$$ER = Rt / Rc$$

where Rt is the mean fecundity obtained in the case of pesticide treatment and Rc is the mean fecundity obtained in the case of the control treatment.

The pesticides were assigned to toxicity classes based on the criteria established by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms', and using the E values obtained experimentally. Toxicity classes are defined as follows: class 1 = harmless (E < 30%), class 2 = slightly harmful (30% ≤ E ≤ 79%), class 3 = moderately harmful (80% ≤ E ≤ 99%) and class 4 = harmful (E > 99%) (Hassan, 1997; Sterk *et al.*, 1999).

Results

Acute toxicity to nymphs

Significant differences were observed in the survival of nymphs depending on the treatment, when the nymphs were exposed to dry residues for 24 hours ($H_{4,45} = 21.43$; $P < 0.01$). Indoxacarb was the only pesticide that reduced nymphal survival in comparison to the control case (figure 1), causing 25.77% nymphs' mortality 24 hours after the exposure to insecticide residue (table 2).

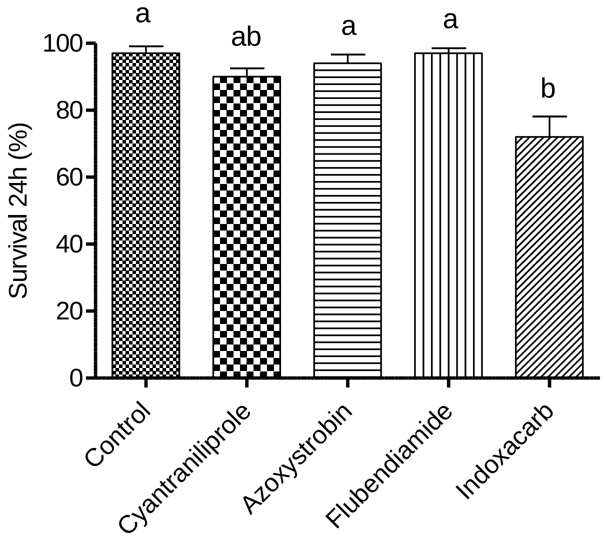

Figure 1. Survival of *O. insidiosus* nymphs after 24 hours exposure to residual pesticides and water control (mean ± SE). Means followed by the same letter are not significantly different (P > 0.05) as determined by Kruskall-Wallis test.

Indoxacarb and cyantraniliprole reduced fecundity, whereas neither flubendiamide nor azoxystrobin affected oviposition ($F_{4,45} = 18.69$; $P < 0.01$) (figure 2). No significant differences were observed in the hatching percentage of the eggs for the different treatments ($H_{3,40} = 0.39$; $P = 0.94$). The average fertility was 79.53 ± 0.69. Indoxacarb treatment was not included in the analysis due to the low number of eggs recorded in this treatment.

Acute toxicity to adults

There were no significant differences in the survival of adults of *O. insidiosus* exposed during 24 hours to dry residues of the different pesticides ($H_{4,50} = 0.68$; $P = 0.95$) (figure 3).

Fecundity of *O. insidiosus* female was reduced significantly when they were treated with indoxacarb ($F_{4,45} = 14.49$; $P < 0.01$) (figure 4). The fertility was on average 80.22 ± 2.52% without significant differences among the treatments ($H_{4,50} = 5.57$; $P = 0.23$).

Table 2. Effect of different pesticides on mortality and fecundity of *O. insidiosus* after 24 hours of exposure of the nymphal stage to the maximum recommended label rates.

Treatment	Ma (%)[1]	Fecundity (eggs female^{-1} 4d^{-1})*	ER[2]	E (%)[3]	Class[4]
Control	-	20.28 ± 1.78 a	-	-	
Cyantraniliprole	7.22	9.16 ± 1.92 b	0.45	58.07	2
Flubendiamide	0.00	19.73 ± 2.32 a	0.97	2.72	1
Indoxacarb	25.77	1.02 ± 1.01 c	0.05	96.28	3
Azoxystrobin	3.09	19.06 ± 2.46 a	0.94	8.91	1

[1]Mortality of nymphs corrected using Abbott's formula; [2]Effect of the pesticide on fecundity (mean fecundity in the pesticide treatment/mean fecundity in the control treatment); [3]Total effect of the pesticide on the predator [E (%) = 100 – (100 – Ma) × ER]; [4]Toxicity class of the compound according to the recommendations of IOBC; *Means (± SE) with the same letter do not differ statistically when compared by Tukey test (P > 0.05).

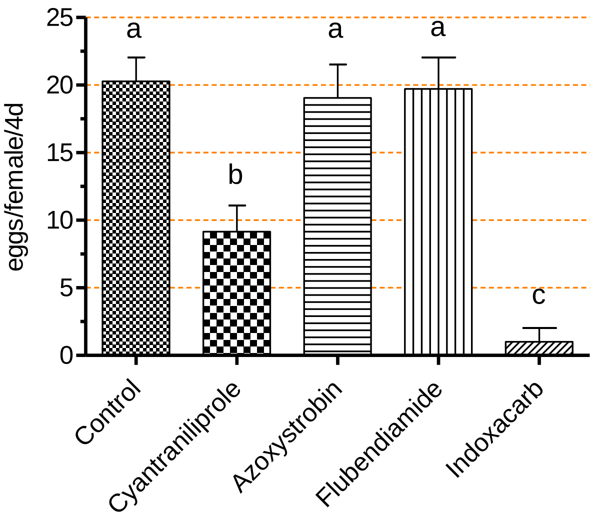

Figure 2. Fecundity of *O. insidiosus* female during 4 days when exposed in nymphal stage to pesticides during 24 hours (mean ± SE). Means followed by the same letter are not significantly different (P > 0.05) as determined by Tukey test.

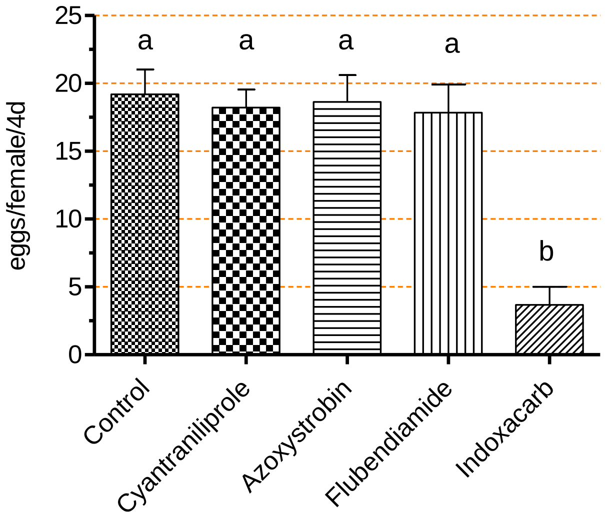

Figure 4. Fecundity of *O. insidiosus* female during 4 days after 24 hours exposure to residual pesticides and water control (mean ± SE). Means followed by the same letter are not significantly different (P > 0.05) as determined by Tukey test.

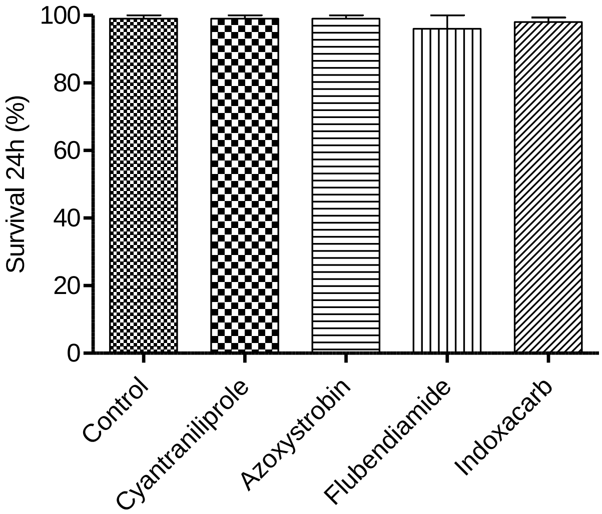

Figure 3. Survival of *O. insidiosus* adults after 24 hours exposure to residual pesticides and water control (mean ± SE).

Toxicity classes

Only flubendiamide and azoxystrobin were classified as harmless (class 1). The residue of cyantraniliprole was harmless to *O. insidiosus* adults, but it was slightly harmful (class 2) to nymphs. The insecticide indoxacarb was moderately harmful (class 3) to both nymphs and adults (tables 2 and 3).

Discussion

O. insidiosus is a polyphagous predator that plays a key role in the management of various pests such as thrips, aphids, mites, whiteflies and moths. Even though this species is effective as a biological control agent, in some cases it might not be able to provide sufficient regulation of pest populations by itself. In those cases, the additional application of pesticides may be required to achieve the suppression of the existing pest populations and prevent plant damage (Van de Veire and Tirry 2003; Stark and Banks 2003; Stark *et al*., 2007). In this

Table 3. Effect of different pesticides on mortality and fecundity of *O. insidiosus* after 24 hours of exposure of the adult stage to the maximum recommended label rates.

Treatment	Ma(%)[1]	Fecundity (eggs female^{-1} 4d^{-1})*	ER[2]	E(%)[3]	Class[4]
Control	-	19.19 ± 1.84 a	-	-	
Cyantraniliprole	0.00	18.22 ± 1.33 a	0.95	5.05	1
Flubendiamide	3.03	17.84 ± 2.09 a	0.93	9.84	1
Indoxacarb	2.00	3.68 ± 1.33 b	0.19	81.19	3
Azoxystrobin	0.00	18.64 ± 1.97 a	0.97	2.84	1

[1]Mortality of nymphs corrected using Abbott's formula; [2]Effect of the pesticide on fecundity (mean fecundity in the pesticide treatment/mean fecundity in the control treatment); [3]Total effect of the pesticide on the predator [E (%) = 100 – (100 – Ma) × ER]; [4]Toxicity class of the compound according to the recommendations of IOBC; *Means (± SE) with the same letter do not differ statistically when compared by Tukey test (P > 0.05).

regard, unfortunately, limited information is available on integrating pesticides and biological control with predators such as *O. insidiosus*.

According to Cloyd (2012), fungicides are commonly applied in greenhouses to deal with fungal diseases and natural enemies may be inadvertently exposed to spray applications. Therefore, it is important to determine the direct and indirect effects of fungicides on *O. insidiosus*. In this work we analysed the effect of the fungicide azoxystrobin which is commonly used for powdery mildew control in horticultural areas of Argentina (Longone and Escoriaza, 2017). We found that this fungicide had no significant effect on the survival, fecundity and fertility of *O. insidiosus* when adults and nymphs were exposed to the fungicide's residues by contact. These results agree with those obtained by Herrick and Cloyd (2017) who found 100% survival when exposing *O. insidiosus* adults to azoxystrobin dried residues in glass Petri dishes for 24, 48, 72 and 96 hours. In our study, the fungicide tested was harmless to nymphs and adults with 97 and 100% survival, respectively, without detrimental effect on reproductive parameters. This fungicide can therefore be recommended to be used in integrated control programs that include *O. insidiosus* to regulate thrips populations.

Flubendiamide belongs to the phthalic acid diamide chemical class and is characterized by its effectiveness in the control of Lepidoptera. This orally ingested toxicant exhibits larvicidal activity by targeting and disrupting the Ca^{2+} balance. This mode of action results in rapid cessation of feeding and the release of calcium causes muscle contraction, resulting in the death of the insect. We found that flubendiamide was harmless when nymphs and adults of *O. insidiosus* were exposed to the dry residue. Kim *et al*. (2018) also showed that flubendiamide, when mixed with other insecticides (bistrifluron) was harmless to *O. laevigatus* exposed to residues 1 day after treatment. On the other hand, Moscardini *et al*. (2013) showed that flubendiamide was harmful to *O. insidiosus* eggs treated with it, causing low egg viability and high emerged nymphs' mortality. These authors attributed their findings to the low molecular weight of the chemical. According to Stock and Holloway (1993) insecticides with this characteristic have better penetration into the corion. Even though our results suggest that this penetration does not occur through the cuticle of nymphs and adults since these stages showed a high survival, the ovicidal effect should be considered when deciding to use this chemical.

Indoxacarb belongs to a new class of insecticides, the oxadiazines. This active ingredient works by inhibiting sodium ion entry into nerve cells, resulting in incoordination, lack of feeding, paralysis and death of the target pests. Indoxacarb is a broad-spectrum Lepidoptera insecticide that also controls selected sucking insect pests including leafhoppers and fleahoppers. The primary route of entry is through ingestion of treated foliage, with some additional absorption through the pest cuticle. Our results showed that nymphs and adults of *O. insidiosus* were susceptible to indoxacarb (E = 96.28% and E = 81.19%, respectively). Similarly, Studebaker and Kring (2003) recorded a high mortality when exposing *O. insidiosus* nymphs and adults to indoxacarb dried residues in a Petri dish for 24 hours. In contrast, Biondi *et al*. (2012) reported that indoxacarb proved to be safe for *O. laevigatus*, although in this study the predator was exposed to dried residues of the pesticide on tomato leaves. Desneux *et al*. (2006) found that pesticides are more toxic on inert material than on plant substrates, mainly because plant enzymes can affect pesticide toxicity. In addition the pesticides may be adsorbed into the waxy layer of the plant's leaf cuticle, making them less available to natural enemies (Desneux *et al*., 2005).

Cyantraniliprole is an anthranilic diamide class insecticide with activity on chewing and sucking insects. It has been reported to be effective on Lepidoptera, tephritid fruit flies, beetles, whiteflies, thrips, aphids, leafhoppers, psyllids and weevils (Zhang *et al*., 2014). In this work we found that this chemical only had affected the reproductive capacity of females that evolved from treated nymphs, although the effects were weak (proportional fecundity = 0.45). On the other hand, it had no lethal or sublethal action on the reproduction on the adult stage of *O. insidiosus*. This result agrees with that of Herrick and Cloyd (2017) who found, under laboratory conditions, that cyantraniliprole was not directly harmful to *O. insidiosus*, with 100% adult survival after 96 hours. Other authors also found that other anthranilic diamide insecticide (chlorantraniliprole) has no or little effects on beneficial invertebrates and their activities (Larson *et al*., 2012) however some detrimental effects on coccinellids were reported (Depalo *et al*., 2017). In addition, several field tests have confirmed minimal or no impact upon beneficial arthropods (Bassi, 2007). In a recent study, Cloyd and Herrick (2018) reported that cyantraniliprole has no sublethal effect on the ability of *O. insidiosus* adults to feed on western flower thrips.

We classified the tested pesticides following the IOBC classification (Hassan *et al*., 1997; Sterk *et al*., 1999) to provide information useful for IPM. Flubendiamide insecticide and azoxystrobin fungicide caused overall effects lower than 30% (class 1), so they are harmless and can be used along with releases of nymphs and adults of *O. insidiosus* without expecting a detrimental action on the seasonal inoculative releases. Indoxacarb was found to be moderately harmful and therefore classified as class 3. Cyantraniliprole was harmless to adults (class 1) and slightly harmful (class 2) when nymphs were exposed to residual pesticide.

The results of this study provide useful insights for IPM programs which incorporate chemical and biological control tactics. The use of selective products allows conservation and enhancement of populations of natural predators in the agroecosystem. According to Van de Veire *et al*. (2002), laboratory tests represent the worst conditions in which an insect can face pesticides. Therefore, pesticides classed as harmless in this 'worst case' conditions, such as azoxystrobin and flubendiamide, do not need further testing and can be used without limit in IPM. However, those products classed from slightly toxic to harmful under rigorous laboratory conditions must be evaluated under field conditions to quantify their persistence and actual effects, since these can be influenced by many factors (such as type of application, mode of action, weather conditions, shelter presence, etc.). Thus, further studies including semi-field and field experiments are necessary in the cases of indoxacarb and cyantraniliprole to evaluate their use in IPM.

With our current knowledge we can only indicate that these last two pesticides be used with caution when *O. insidiosus* has been introduced or is spontaneously present in the crop.

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