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Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*

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ABSTRACT

The generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is a key natural enemy of various arthropods in agricultural and natural ecosystems. Releases of this predator are frequently carried out, and it is included in the Integrated Pest Management (IPM) programs of several crops. The accurate assessment of the compatibility of various pesticides with predator activity is key for the success of this strategy. We assessed acute and sublethal toxicity of 14 pesticides on *O. laevigatus* adults under laboratory conditions. Pesticides commonly used in either conventional or organic farming were selected for the study, including six biopesticides, three synthetic insecticides, two sulfur compounds and three adjuvants. To assess the pesticides' residual persistence, the predator was exposed for 3 d to pesticide residues on tomato sprouts that had been treated 1 h, 7 d or 14 d prior to the assay. The percentage of mortality and the sublethal effects on predator reproductive capacity were summarized in a reduction coefficient (E_x) and the pesticides were classified according to the IOBC (International Organization for Biological Control) toxicity categories. The results showed that the pesticides greatly differed in their toxicity, both in terms of lethal and sub lethal effects, as well as in their persistence. In particular, abamectin was the most noxious and persistent, and was classified as harmful up to 14 d after the treatment, causing almost 100% mortality. Spinosad, emamectin, metaflumizone were moderately harmful until 7 d after the treatment, while the other pesticides were slightly harmful or harmless. The results, based on the combination of assessment of acute mortality, predator reproductive capacity pesticides residual and pesticides residual persistence, stress the need of using complementary bioassays (e.g. assessment of lethal and sublethal effects) to carefully select the pesticides to be used in IPM programs and appropriately time the pesticides application (as function of natural enemies present in crops) and potential releases of natural enemies like *O. laevigatus*.

1. Introduction

Generalist arthropod predators are worldwide known for their ability to control phytophagous insects and mites in many cultivated crops (Symondson et al., 2002). For example, most Anthocoridae are polyphagous predators which play a key role in management of various pests such as aphids, mites, whiteflies and moths (Chambers et al., 1993; Desneux et al., 2006b; Bosco et al., 2008; Fathi and Nouri-Ganbalani, 2010; Ragsdale et al., 2011; Weintraub et al., 2011) in both greenhouses and fields. These predators are able to build up their populations before pests arrive using alternative prey (Harwood et al., 2007; Desneux and O'Neil, 2008) and host plants as alternative food sources (Lattin, 1999; Lundgren et al., 2009). In addition, predators of the genus *Orius* (Heteroptera: Anthocoridae) are mass-produced and released mainly to control thrips pest *Frankliniella occidentalis* (Pergande) in various horticultural crops in Eurasia and North America (Bosco et al., 2008; Weintraub et al., 2011).

Despite the potential effectiveness of biological control, many crop protection practices are primarily based on broad spectrum chemical pesticides which are noxious to beneficial arthropods (Desneux et al., 2007) and that affect agricultural sustainability (Wilson and Tisdell, 2001). An alternative to conventional pest control is Integrated Pest Management (IPM), an approach that aims to reduce pest status to tolerable levels by using effective, ecologically-sound and economically-sustainable management methods (Van Lenteren and Woets, 1988). IPM involves using

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Table 1
Tested pesticides.[b,c]

Active ingredient (a.i.)	Trade name	Field rate (a.i.%)	Chemical family	Mode of action	Crops	Target
Biopesticides						
Abamectin	Cal-EX EW®	75 mL hL^{-1} (1.8)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, lettuce, cucumber, melon, cabbages, citrus, grape, ornamental plants and flowers, forest trees	Mites. thrips, psyllids, aphids, leafminers, moths
Azadirachtin[a]	Oikos®	150 mL hL^{-1} (3.2)	Botanical	Ingestion. molting disruptor	Tomato, eggplant, sweet pepper, strawberry, carrot, fennel, beans, cabbages, cucurbit crops, garlic, onion, leek, leafy vegetables, celery, stone fruits, pome fruits, actinidia, walnut, chestnut	Thrips, Hemiptera, Lepidoptera
Bacillus thuringiensis var. *kurstaki* strain SA12[a]	Costar® WG	200 g hL^{-1} (90 000 IU μg^{-1})	Cry proteins	Ingestion. Disruptor of insect midgut epithelium	Tomato, eggplant, sweet pepper, strawberry, artichoke, corn, cotton, tobacco, potato, leafy vegetables, cucurbit crops, sugar beet, cabbages, sugar beet, beans, soybean, sunflower, citrus, grape, olive, actinidia, chestnut, ornamental plants, forest trees	Lepidoptera
Emamectin benzoate	Affirm®	150 g hL^{-1} (0.95)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, beans, artichoke, lettuce, stone fruits, pome fruits, grape, cole crops	Lepidoptera
Borax and citrus oil[a]	PreVam®	400 mL hL^{-1} (6)	Borates tetra sodium salts and Oil - essential	Contact. Miscellaneous non-specific inhibitor	Tomato, strawberry, grape	Mites, whiteflies, mealybugs, Tomato borer Fungicide
Spinosad[a]	Laser®	25 mL hL^{-1} 75 mL hL^{-1} (48)	Spinosyn	Ingestion and contact. Nicotinic acetylcholine receptor agonist	Tomato, eggplant, sweet pepper, strawberry, potato, fennel, legumes, garlic, onion, leek, stone fruits, cucurbit crops, artichoke, leafy vegetables, caper, pome fruits, stone fruits, grape, small fruits, tree nuts, ornamental plants, grass	Thrips, Planthoppers, Lepidoptera, Coleoptera, Diptera
Synthetic insecticides						
Chlorantraniliprole	Altacor®	11.5 g hL^{-1} (20)	Anthranilic diamide	Ingestion. Ryanodine receptor modulator	Tomato, eggplant, sweet pepper, cucurbit crops, cole crops, leafy vegetables	Lepidoptera
Indoxacarb	Steward 30 WG®	12.5 g hL^{-1} (30)	Oxadiazine	Ingestion and contact. Voltage-dependent sodium channel blocker	Tomato, eggplant, sweet pepper, cucurbit crops, cole crops, leafy vegetables, artichoke, corn, pome fruits, stone fruits, grape	Lepidoptera
Metaflumizone	Alverde®	100 mL hl^{-1} (24)	Semicarbazone	Ingestion. Sodium channel modulator	Tomato, eggplant, sweet pepper, cabbages, potato, leafy vegetables	Lepidoptera, Colorado potato beetle
Fungicides						
Dust sulfur[a]	Zolfo Ventilato Stella®	30 kg ha^{-1} (94.5)	Inorganic	Contact. Repellent	Tomato, melon, pea, artichoke, leafy vegetables, cucumber, grape, ornamental flowers	Fungal disease
Wettable sulfur[a]	Zolfo Bagnabile Bayer®	200 g hL^{-1} (90)	Inorganic	Contact. Repellent	Tomato, cucumber, melon, peas, apple, peach, grape, ornamental flowers	Fungal disease
Adjuvants						
Parafinic mineral oil[a]	Ufo®	2000 mL hL^{-1} (98.8)	Petroleum derivative	Contact. Asphyxiant	Vegetable crops, citrus, pome fruits, stone fruits, grape, olive, fig, ornamental plants	–
Para-menthene[a]	Nu-film-P®	40 mL hL^{-1} (96)	Unclassified	Contact. Repellent	Vegetable and fruit crops	–
Rapeseed oil[a]	Codacide®	2500 mL hL^{-1} (86.4)	Botanical	Contact. Asphyxiant	Tomato, wheat, rice, corn, sugar beet	–

[a] Indicate pesticides that are authorized for organic farming.
[b] Italian Ministry of Health, phytosanitary products database 2011, http://www.salute.gov.it/fitosanitariwsWeb_new/FitosanitariServlet.
[c] Pesticide Action Network pesticide database 2011.

pesticides when required, though their harmful effects on natural enemies should be mitigated. Accurate assessment of potential side effects of pesticides on natural enemies is critical for developing effective IPM strategies (Desneux et al., 2006a; Stark et al., 2007) and is increasingly important because the recent European Union directive on sustainable use of pesticides, i.e. 2009/128/EC, stated that IPM should be implemented in all member states by January 1st 2014 (EEC/CEE, 2009). Many laboratory assays rely almost exclusively on the assessment of lethal effects. However, pesticides could induce multiple sublethal effects in individuals that survive an exposure to a given pesticide (Desneux et al., 2007), and these effects could have important impact on natural enemies population dynamics (Stark and Banks, 2003). Sublethal effects could impair the physiology (e.g. neurophysiology, development, longevity, fecundity and sex-ratio) and the behavior (e.g. mobility, orientation, feeding, host searching, oviposition and mating) of natural enemies (Desneux et al., 2004a,b; Suma et al., 2009; Evans et al., 2010; Arnó and Gabarra, 2011; Saber, 2011; Stara et al., 2011; He et al., in press and see Desneux et al. (2007) for a thorough review). Studying more subtle endpoints (e.g. behaviors, Desneux et al., 2004c) and using multistep bioassays to evaluate the potential effects of pesticides on natural enemies is therefore required to assess risk in a more complete way (Desneux et al., 2006a, 2006c, 2007; Stark et al., 2007).

Previous laboratory studies on the effects of pesticides on predators have focused mostly on direct contact toxicity by topical application (James, 2004; Mahdian et al., 2007; Rimoldi et al., 2008) or residual toxicity (Giolo et al., 2009; Gradish et al., 2011). By contrast, fewer studies have documented effects of pesticides on predators when exposed through feeding on contaminated prey (Banken and Stark, 1998; Urbaneja et al., 2008; Cabral et al., 2011; He et al., in press) and very few have tested relationship between age of pesticide residues and lethal and sublethal effects on predators and other natural enemies of a given pest (Van de Veire et al., 2002b; Desneux et al., 2005; Gradish et al., 2011).

In this context, the aim of the present work was to provide lethal and sublethal toxicity assessment of various pesticides on the generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae). This species was chosen as predator model because it is widely distributed in the Palaearctic region, it is a key natural enemy of various pests in agricultural ecosystems (Chambers et al., 1993; Weintraub et al., 2011) and had shown some potential for biological control of the invasive South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Desneux et al., 2010; Lins et al., 2011). We evaluated the effects of pesticides that are commonly used in organic and/or conventional cropping systems (see Table 1 for detailed information), including pesticides that have been increasingly used on tomato owing to the recent invasion of Afro-Eurasian countries by *T. absoluta* (Desneux et al., 2010,2011). In laboratory conditions, we assessed acute toxicity on predator adults and sublethal effects on their reproductive capacity (progeny size) when exposed to pesticide residues on tomato leaves. To test for potential relationship between age of pesticide residues and effects in exposed predators, we tested three different ages of pesticides residues: 1-h old, 7-d old and 14-d old.

2. Materials and methods

2.1. Insects

Orius laevigatus individuals used in the trials were provided by Bioplanet (Cesena, Italy) in commercial bottles containing 500 individuals dispersed in inert material (LeviPAK500®). Before being used in experiments, the predators were fed using UV sterilized *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Entofood®, Koppert Biological Systems, The Netherlands) and stored in growth chambers (26 ± 2 °C, 60 ± 10% RH, 14: 10 L.D.). Predator adults belonged to the same cohort and the individuals were 4-d old when used for the experiments.

2.2. Pesticides

The pesticides tested varied in their mode of action, chemical families and pesticide types (detailed information on active ingredients [a.i.] are provided in Table 1). We tested six biopesticides, three synthetic insecticides, two fungicides (sulfur compounds), and three adjuvant compounds. These plant protection products are currently used in various crops (including tomato) and some of them are also authorized for organic farming. The highest recommended rates for tomato crops were used for our experiments, except for spinosad, which was tested at two different recommended rates (both on tomato); one for management of *T. absoluta* and of Thrips (lowest one, hereafter named spinosad 25) and one for management of agromizid (Diptera) leafminers (highest one, hereafter named spinosad 75). All the pesticides were stored and applied following their label guidelines. For a given pesticide, plants were sprayed with the formulated product which was diluted with water (to obtain 0.5 L of solution) and applied at the rate of 1000 L ha^{-1} of solution (3 plants per square meter). The treatment was applied using a 2L power-pack aerosol hand sprayer (Matabi®, Antzuola, Guipuzcoa, Spain) and the nozzle of the sprayer was directed toward the plants from a distance of 0.5 m (this resulted in a complete and uniform wetting of the young tomato plants). An acid fertilizer (Fertacid®, Biointrachem Italia) was added to adjust pH to 4.5 in case of *Bacillus thuringiensis* (*Bt*) and Azadirachtin (neem oil) solutions as recommended by the companies.

2.3. Toxicity trials

2.3.1. Exposure to pesticides and lethal effect

The experiments were conducted at the Department of Agri-food and Environmental Systems Management of the University of Catania (Italy) under controlled environmental conditions in growth chambers (26 ± 2 °C, 60 ± 10% RH, 14:10 L.D.). The trials were performed by exposing *O. laevigatus* adults for 3 d to dried pesticide residues on tomato leaves. Plants were used as a substrate for the pesticides to avoid overestimation of toxicity that usually occurs when using pesticide residues on inert material like glass (Desneux et al., 2005, 2006a). The plants used were 40 cm high, 40-d old tomato plants (cv Missouri), grown from seeds in 2 L pots. The treatments were performed following regular agricultural practices, and control plants (untreated) were sprayed with tap water. Five plants for each trial were sprayed per pesticide tested. The plants were left to allow pesticides to dry for 1 h, 7 d or 14 d (see Section 2.3.2. *Assessment of pesticides persistence* below) and then the upper plant part (about 17 cm) was cut and placed into a bioassay isolator made up of two superposed plastic glasses. The top glass (600 mL, length: 13 cm) had a central hole on its bottom to allow tomato plant stem to reach the water present in a second (bottom) glass (350 mL, length: 11 cm). A fine mesh net was fixed on the upper opening of the largest top glass to allow ventilation (the design is presented in Fig. S1). Five females and five males of *O. laevigatus* were introduced in the described arena. Untreated *E. kuehniella* eggs were provided daily as food at the rate of 50 eggs per predator and a water source was offered *ad libitum* in a 1.5 mL Eppendorf tube sealed with cotton. Five replicates ($5 \times 10 = 50$ predators) were performed per pesticide and per each residual trial (see Section 2.3.2). Mortality (number of dead predators) was recorded daily for 3 d. Predators were considered dead when they remained immobile after being touched with a fine paintbrush.

2.3.2. Assessment of pesticides persistence

Persistence of pesticides toxicity was studied by exposing *O. laevigatus* adults to pesticides applied at three different times prior to the assay: 1 h, 7 d and 14 d (see Section 2.3.1. for description of the bioassay). For a given pesticide, all the plants (for the three different delays after application of pesticide) were sprayed at the same time and they were maintained in insect proof cages in a greenhouse (min < *mean temperature* < max: 19.5 °C < 26.2°C < 38 °C; min < *mean RH* < max, 29% < 61.9% < 92%; natural ambient light: September 2010) to allow aging of pesticides in regular cropping conditions. Plants sprayed with tap water were used as control plants for each of the treatments.

2.3.3. Side effects on offspring production

Various plants are suitable substrate for oviposition of *Orius* spp. and various results on reproductive performances have been reported (Coll, 1996; Lundgren and Fergen, 2006; Butler and O'Neil, 2007; Bonte and De Clercq, 2009; Lundgren et al., 2009). Pilot studies showed that *O. laevigatus* effectively laid eggs and produced offspring on tomato plants in our experimental conditions (Biondi, Desneux and Zappalà, unpublished data). In order to allow the individuals to oviposit from the first day of exposure to the pesticides (most realistic scenario), potential effects of pesticides on reproductive capacity of predator females were assessed using the treated tomato leaves that also served as substrates for exposure to pesticides (described in Sections 2.3.1 and 2.3.2.). After having checked mortality for the lethal assessment assay (3 d), the survivors were removed from the experimental arena. Then the number of emerged progeny (nymphs) was scored daily for 10 d after the first day of pesticide exposure. To avoid cannibalism, the emerged nymphs were removed daily using a mouth aspirator.

2.4. Data analysis

Datasets were first tested for normality and homogeneity of variance using Kolmogorov–Smirnov D test and Cochran's test respectively, and transformed if needed. For the lethal effect and persistence assessment results, we tested the effects of pesticides (factor *pesticide*) and of application timing (factor *application time*) and potential interactions between these two factors on the number of predator found dead after 3 d of exposure to the various pesticides. For this, we used a factorial ANOVA. Subsequently, additional one-way ANOVA followed by LSD post hoc tests for multiple comparisons inside the different application time sub datasets were carried out.

Data recorded on side effects of pesticides and of application time on production of predator nymphs produced were used to calculate two different estimates which provided information on two different effects: (i) *Offspring production*: effect of pesticides on total offspring production. In such case, we analyzed the exact numbers of offspring produced per glass (5 females per each glass were tested per each replicate) and any early death of predator females in a given glass was therefore included in the overall reduction of offspring production estimate (i.e. realistic to general effect of pesticides on predator populations), (ii) *Predator reproductive capacity*: offspring production was corrected by daily survival of predator females in each glass. In this case, the number of nymphs produced was corrected by early death of a predator female per day (if any) and therefore this value provided a more accurate estimate of actual sublethal effects of pesticides on reproductive capacity of predator females. We statistically tested the effect of the two factors; pesticides and application time (and their interaction) on the two estimates, *Offspring production* and *Predator reproductive capacity*, and for this we used a factorial ANOVA. Subsequently, additional one-way ANOVA followed by LSD post hoc tests for multiple comparisons inside pesticide age sub datasets were carried out for each estimate.

Finally, to provide a single value summarizing potential deleterious effects of pesticide tested, the toxic effects (both lethal and sublethal effects) of each pesticide were also expressed as the *Reduction coefficient* E_x for pesticide x (Urbaneja et al., 2008) using the formula:

$$E_x = 100\left\{1 - \left[\left(1 - \frac{E_{mx}}{100}\right)\left(1 - \frac{E_{fx}}{100}\right)\right]\right\}$$

where E_{mx} is the corrected mortality (Abbott, 1925) and E_{fx} is the corrected *Predator reproductive capacity* estimated using the formula:

$$E_{fx} = 100 - \frac{F_x 100}{F_c}$$

where F_x is the mean *Predator reproductive capacity* for pesticide x and F_c is the *Predator reproductive capacity* recorded in the control group (untreated group). The values (E_x) were then classified and interpreted accordingly to the standards of the International Organization for Biological Control (IOBC) which include four categories: (1) harmless: $E_x < 30\%$, (2): slightly harmful: $30\% < E_x < 80\%$, (3): moderately harmful: $80\% < E_x < 99\%$, and (4): harmful: $E_x > 99\%$.

3. Results

3.1. Lethal effect and persistency

The statistical results are summarized in Table 2A. The mortality of the predators during the 3-dexposure period varied significantly among pesticides tested (significant pesticide factor) and delay between exposure to pesticides and application time (significant application time factor). There was a significant interaction between the pesticide and the application time factors, i.e. aging of residues did not affect pesticide toxicity to *O. laevigatus* in the same way among pesticides tested.

Six pesticides significantly increased *O. laevigatus* mortality ($F_{15,68} = 5.67$; $P < 0.001$) with rates of mortality ranging from 75% for spinosad 25 to 98% for abamectin. The other pesticides caused mortality levels always lower than 44% (Fig. 1a). The 7-d-old residues significantly affected *O. laevigatus* survival ($F_{15,69} = 12.14$; $P < 0.001$) in case of abamectin, emamectin benzoate (emamectin), spinosad 75 and metaflumizone, causing almost the same mortality rates (98%, 92%, 85% and 87% respectively) as those observed in case of the 1-h old residues (Fig. 1b). Finally, in case of 14-d old pesticide residues, only abamectin and spinosad 75 still induced

Table 2
Statistics from the factorial ANOVA used to analyze (A) the numbers of predator found dead after 3 d of exposure to pesticides (mortality), (B) the total numbers of offspring produced per replicate, i.e. *offspring production,* and (C) total numbers of emerged nymphs per live *O. laevigatus* females (checked and corrected per day) during 3 d of exposure (i.e. *predator reproductive capacity*) among pesticides tested (pesticide factor) and as function of the age of pesticide residues (application time factor).

Source of variation	Degrees of freedom	*F*	*p*-value
A: Mortality			
Pesticide	15	30.77	<0.001
Application time	2	28.25	<0.001
Pesticide *x* application time	30	4.64	<0.001
B: Offspring production			
Pesticide	15	11.04	<0.001
Application time	2	17.65	<0.001
Pesticide *x* application time	30	2.72	<0.001
C: Predator reproductive capacity			
Pesticide	15	4.85	<0.001
Application time	2	7.41	<0.001
Pesticide *x* application time	30	3.07	<0.001

significant mortality in exposed predators when compared to the untreated control group ($F_{15,63} = 9.09$; $P < 0.001$; Fig. 1c).

3.2. Side effects on O. laevigatus reproduction

The statistical results are summarized in Table 2B and C. Offspring production varied significantly as a function of pesticide tested (significant pesticide factor) and the delay between exposure to pesticides and application time (significant application time factor) (Table 2B). In addition, the persistence of pesticide

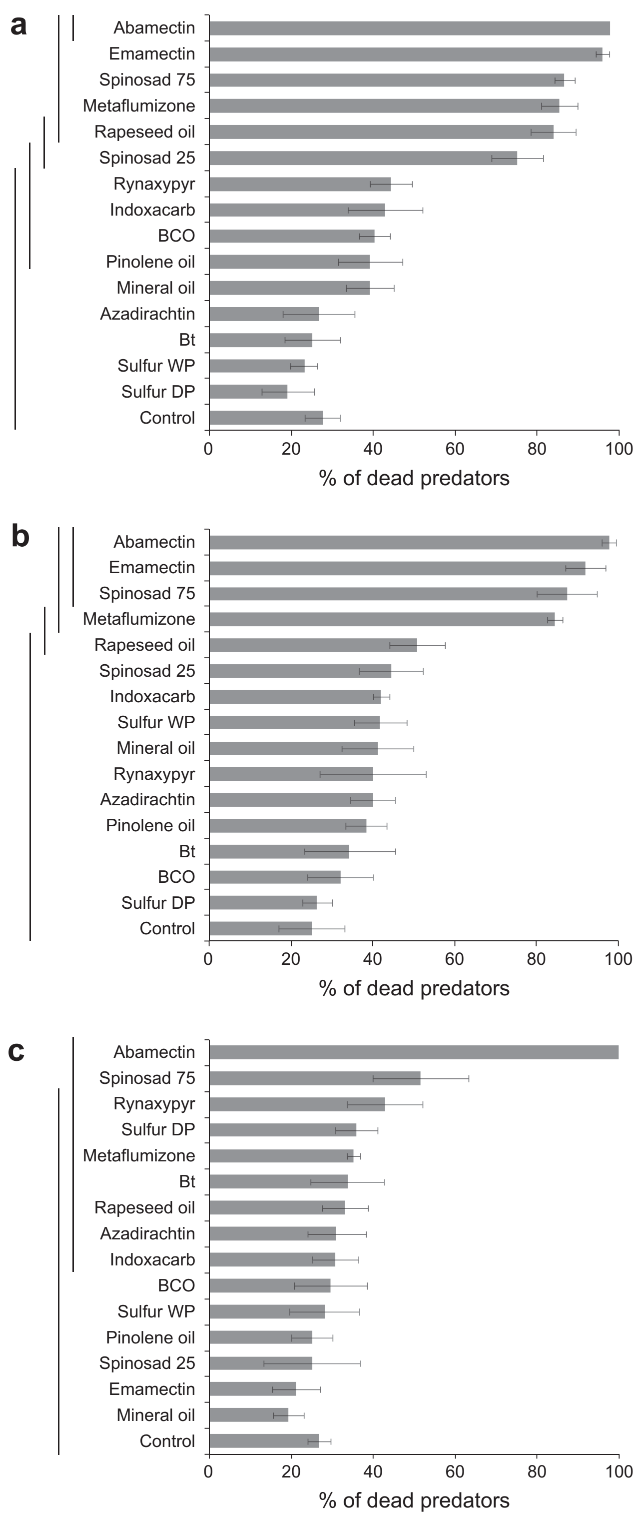

Fig. 1. Lethal effect. Means (±SEM) of mortality percentages of *Orius laevigatus* after 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

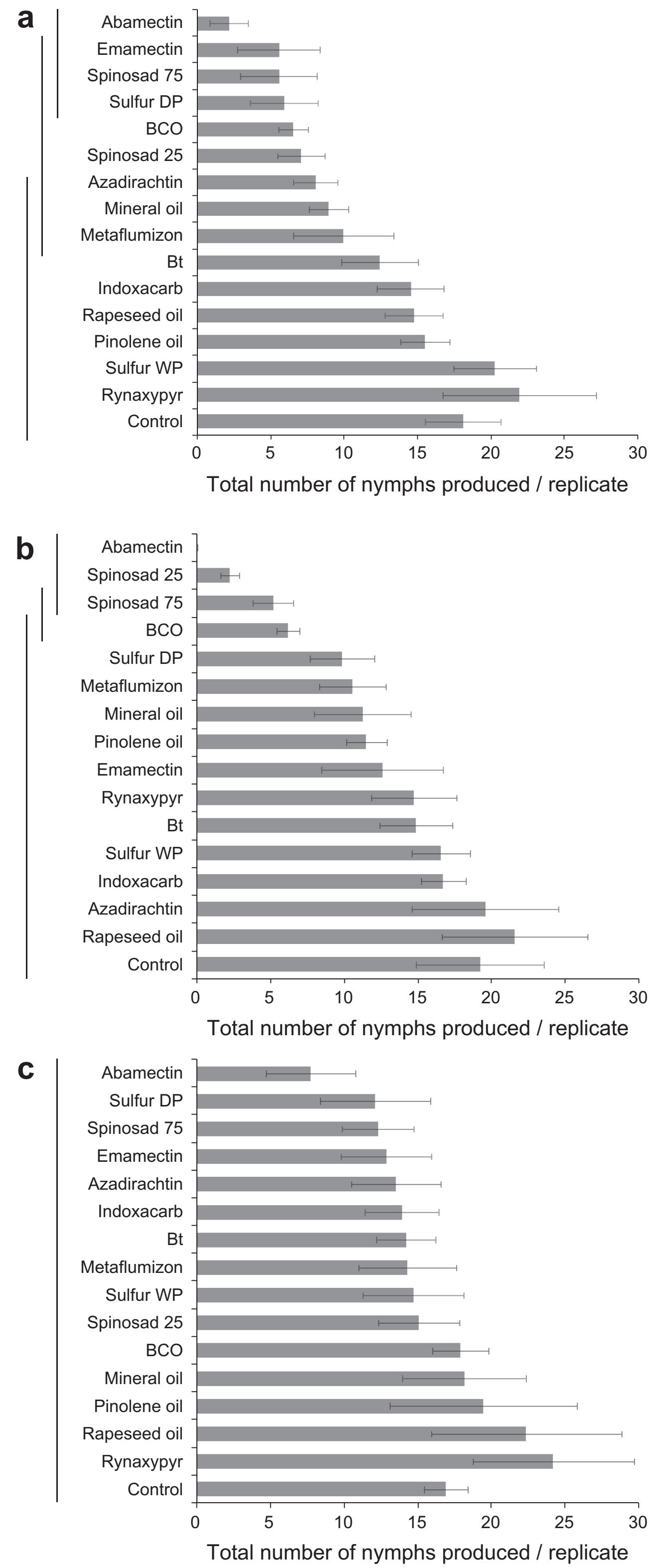

Fig. 2. Side effects of pesticides on *offspring production* by *Orius laevigatus*. Means (±SEM) of total emerged nymphs of *O. laevigatus* in each replicate during 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

toxicity (i.e. effects of pesticide residues when aging) varied among the pesticides (significant interaction between pesticide and application time factors) (Table 2B). Mean number of offspring produced varied significantly among pesticides in case of 1-h old pesticide residues (one-way ANOVA: $F_{15,83} = 5.58$; $P < 0.001$). Abamectin, emamectin, borax plus citrus oil (BCO), dust sulfur (sulfur DP), spinosad 75 and spinosad 25 significantly reduced offspring production when compare to untreated control (Fig. 2a). Offspring production was also significantly reduced when the adults were exposed to the 7-d old pesticide residues ($F_{15,84} = 9.98$; $P < 0.001$) with abamectin, spinosad 75 and 25 causing significant decrease (Fig. 2b). No significant decrease in offspring production were observed when individuals were exposed to 14-d old pesticide residues ($F_{15,78} = 1.49$; $P = 0.135$; Fig. 2c).

Predator reproductive capacity varied also significantly as function of pesticides (significant pesticide factor) and age of pesticide residues (significant application time factor) (Table 2C). As observed for mortality and offspring production, the persistence of pesticide toxicity varied among the pesticides (significant interaction between the two factors) (Table 2C). Predator reproductive capacity varied among pesticides tested in case of exposure to 1-h old residues ($F_{15,83} = 2.85$; $P = 0.002$) with abamectin, sulfur DP, BCO, azadiractin and spinosad 75 significantly decreasing *O. laevigatus* reproductive capacity of predator females when compared to untreated control group (Fig. 3a). In case of exposure to 7-d old residues, abamectin, BCO, Spinosad 25 and Spinosad 75 significantly reduced the number of nymphs produced per *O. laevigatus* female ($F_{15,84} = 7.7$; $P < 0.001$; Fig. 3b). No significant effect was observed when predators were exposed to 14-d old residues ($F_{15,78} = 1.26$; $P = 0.225$; Fig. 3c).

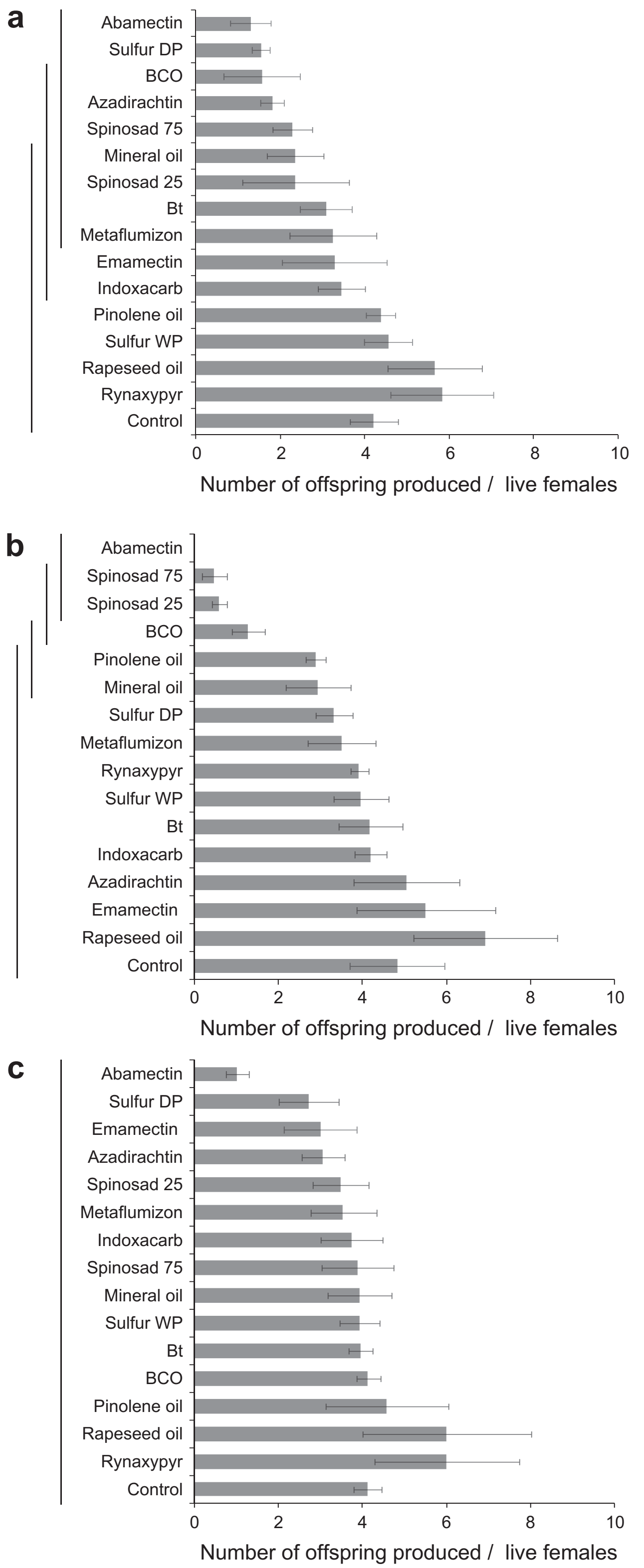

Fig. 3. Sublethal effects of pesticides on *predator reproductive capacity*. Means (±SEM) of total number of emerged nymphs per live *O. laevigatus* females (checked and corrected per day) during 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

3.3. Reduction coefficient (E_x)

Abamectin was the only pesticide with a reduction coefficient $E_x > 99\%$ and it was classified as harmful when using IOBC toxicity categories (class 4; Table 3). Its harmfulness was high for all application time of residues tested (i.e. 1 h, 7-d and 14-d old). For others, four pesticides (emamectin, spinosad 25 and 75, and metaflumizone) were classified as moderately harmful (class 3), i.e. $80\% < E_x < 99\%$, and they remained classified as such when pesticide residues were 7-d old. When 14-d old, pesticide residues were harmless with $E_x < 30\%$, except for spinosad 75 that was categorized as slightly harmful ($E_x = 42.9\%$). Azadiractin, BCO, indoxacarb, sulfur DP, paraffinic mineral oil (mineral oil) and rapeseed oil were classified as slightly harmful (class 2) in the 1-h old residue trial. Then, toxicity of azadiractin, indoxacarb and sulfur DP was reduced when residues were 7-d old but BCO, mineral oil and rapeseed oil. Other pesticides tested had E_x lower than 30% were classified as harmless (class 1; Table 3).

4. Discussion

The present study demonstrated that lethal and sublethal effects of different types of pesticide residues (both in term of chemical and of toxicity persistence) on the generalist predator *O. laevigatus* varied widely, including among pesticides within the same product group (e.g. biopesticides). *Orius laevigatus* was very susceptible to abamectin ($E_x > 99\%$, IOBC class 4) even 14 d after pesticide application, notably because of high mortality induced in adults. Abamectin proved to be not compatible with this predator for IPM. Emamectin, metaflumizone and spinosad were less toxic but still induced high levels of mortality and reduced offspring produced (IOBC class 3), even in case of exposure to 7-d old residues. Finally, chlorantraniliprole (rynaxypyr), *Bt*, indoxacarb, wettable sulfur (sulfur WP), mineral oil and para-menthene (pinolene oil) proved to be harmless ($E_x < 30\%$, IOBC class 1) with mortality and reproductive capacity levels similar to what was recorded in untreated control group. Taken as a whole, the results

Table 3
Reduction coefficient E_x and IOBC toxicity classes. Pesticides, per pesticide type (i.e. biopesticide, synthetic insecticide, fungicide and adjuvant), that are the most harmful to *Orius laevigatus* are indicated in bold.

	Pesticide	1-h old residue		7-d old residue		14-d old residue	
		E_x	IOBC class	E_x	IOBC class	E_x	IOBC class
Biopesticides	**Abamectin**	**99.4**	**4**	**100**	**4**	**100**	**4**
	Azadirachtin	57.1	2	20.9	1	26.4	1
	Bacillus thuringiensis	26.8	1	0	1	0	1
	Emamectin benzoate	95.7	3	88.7	3	27.1	1
	Borax and citrus oil	69.4	2	74.5	2	0	1
	Spinosad 25	82.2	3	90.2	3	15.5	1
	Spinosad 75	91.6	3	97.0	3	42.9	2
Synthetic insecticides							
	Chlorantraniliprole	22.9	1	26.6	1	22.0	1
	Indoxacarb	35.6	2	28.9	1	0	1
	Metaflumizone	**84.8**	**3**	**87.2**	**3**	**24.1**	**1**
Fungicides							
	Dust sulfur	**69.2**	**2**	**17.1**	**1**	**25.4**	**1**
	Wettable sulfur	0	1	20.0	1	0	1
Adjuvants							
	Mineral oil	48.8	2	35.6	2	0	1
	Para-menthene	16.0	1	28.1	1	0	1
	Rapeseed oil	**77.9**	**2**	**39.1**	**2**	**0**	**1**

show that side effects of pesticides can vary largely depending upon various factors studied, like endpoint considered (lethal vs. sublethal), pesticide chemical family and pesticide type. Consequently, comprehensive and specific risk assessment should be undergone before implementing any IPM programs.

4.1. Differential effects and persistence among pesticides

The synthetic insecticides rynaxypyr and indoxacarb proved to be safe for *O. laevigatus*, at least for the traits assessed during our study. These results contrast with those obtained in a study by Studebaker and Kring (2003) in which 100% of mortality was observed when exposing *O. insidiosus* to indoxacarb dried residues on glass plates for 24 h. This is consistent with the finding that pesticides are more toxic on intert material than on plant substrate (Desneux et al., 2005,2006a), mainly because plant enzymes can affect pesticide toxicity (Schuler, 1996) and because pesticides may be adsorbed into the waxy layer of the plant leaf cuticle, making them less available to natural enemies (Desneux et al., 2005). The other synthetic insecticide tested, metaflumizone, proved to be relatively toxic with high acute mortality observed (>80%) and persistence of toxicity for at least 7 d. However, no sublethal effects on reproductive capacity were observed which means that this product might allow a rapid recolonization of treated areas from untreated surroundings or new predators releases by the process of "horizontal recruitment" (Desneux et al., 2006a,2006c), thought additional assessment of potential behavioral sublethal effects (e.g. orientation behavior and repellency) should be assessed to confirm this hypothesis.

In contrast to synthetic pesticides, the biopesticides largely differed in their lethal and sublethal effects on *O. laevigatus*. Azadirachtin was harmless in terms of mortality, most likely because it mainly acts on insects as molting disruptor (Sieber and Rembold, 1983) and therefore could not negatively affect adult insects. However, it showed slight negative effects on overall offspring production in case of exposure to fresh residues (which is consistent with marginally significant reduction of predator reproductive capacity in case of 1-h old residues). The effect probably resulted from lower survival of young nymphs at the time they hatched and got exposed to azadirachtin residues still present on the tomato leaves. In the same way, BCO did not affect predator survival but had negative sublethal effects, reducing both predator reproductive capacity and offspring production, and this occurred even when residues were 7-d old. This is consistent with known properties of this type of botanical insecticide, notably their repellent effect (which obviously reduced time spent by adults on leaves and consequently reduced number of eggs potentially laid) and their negative effect on egg-hatching in insects (Isman, 2000; Cordeiro et al., 2010).

However, several biopesticides, namely abamectin, emamectin and spinosad, proved to be highly toxic to *O. laevigatus*. Emamectin and abamectin showed relatively similar pattern of effects and persistence which is consistent because they are from the same chemical family: Avermectin. Among these two products, and more broadly among all tested pesticides, abamectin was the most noxious and was classified harmful, killing almost all predators, even in case of 14-d old residues, and inducing major sublethal effects on reproduction of rare survivors. These results are in agreement with previous studies which reported negative side effects of abamectin on *Orius* spp. (Van de Veire et al., 2002a,b; Studebaker and Kring, 2003; Bostanian and Akalach, 2004). The high persistence of abamectin in our study may relate to low degradation by UV, low foliar uptake and/or low translaminar activity on/in tomato plants. However, this persistence should be associated with climatic conditions occurring in Mediterranean basin greenhouses and thus abamectin degradation and subsequent effects might be different under different climatic conditions. For example, abamectin is known to be subjected to photolysis when exposed to sunlight Tomlin, (2011) and Gradish et al. (2011) found low persistence of this insecticide when plants were exposed directly to sunlight in Canadian greenhouses. By contrast, plants in Mediterranean basin greenhouses are usually protected from direct sunlight through the use greenhouse shade nets that actually reduce the exposure of plants to UV.

Spinosad was also very toxic with persistence proving to be relatively high; most of effects were still observed when predators were exposed to 7-d old residues. These results matched those of previous studies (Van de Veire et al., 2002a; Studebaker and Kring, 2003) which assessed side effects of spinosad on *O. insidiosus* and *O. laevigatus* when exposed to spinosad residues on glass plates. In our study, in most cases the highest concentration of spinosad tested (75) was twice as toxic and persistent as the lower one (25). Effects may result from side effects of spinosad on reproduction-related behaviors because spinosad is based on spinosyns (produced by the bacteria *Saccharopolyspora spinosa*) which are neurotoxic compounds primarily targeting post-synaptic nicotinic acetylcholine and GABA receptors, i.e. which are likely to impact predator behaviors (Desneux et al., 2007). Williams et al. (2003) reported that predators generally suffered very few sublethal effects following exposure to spinosad, but that parasitoids often showed multiple sublethal effects (notably a decrease in reproductive capacity). It is unclear why the predator *O. laevigatus* was negatively affected by spinosad in our study, but effects may relate to its omnivory behavior (it feeds also on plants and therefore could receive more toxins than if consuming only spinosad-contaminated prey). Finally, *Bt* showed high selectivity (i.e. harmless to *O. laevigatus*) likely owing of the inability to the *Bt* toxins to reach the insect gut in sucking insects (Gill et al., 1992). In addition, results suggest that adjuvant compounds in *Bt* formulations are also harmless to *O. laevigatus*.

During the trials, the adjuvant compounds showed low toxicity to *O. laevigatus*. Only rapeseed oil reduced predator survival in case of exposure to 1-h old residues, but no sublethal effects were observed on predator reproductive capacity and overall offspring production was never reduced. The lack of negative residual effects of adjuvant residues on *O. laevigatus* is likely because adverse effects of adjuvant compounds on insects are thought to occur mostly through suffocation at the time the insects are actually directly sprayed with the product (Acheampong and Stark, 2004; Desneux et al., 2006c). This result supports the idea that adjuvant compounds are not primarily involved in adverse effects that predators (like those from *Orius* genus) can suffer when they are exposed to residues of commercial pesticidal products. It may also mean that potential effects of adjuvant compounds on predators should be considered only when pesticides are going to be used subsequently to releases of predators in crops.

In case of fungicides, both sulfur compounds did not induce mortality in predators, but predator reproductive capacity was reduced by sulfur DP in case of exposure to 1-h old residues. This result may be related to the activity of the compound as an oviposition repellent or as an egg dryer. The establishment of *O. laevigatus* in a treated crop may be possible only if untreated refuge areas (without dust sulfur residues) are available to the predators, as reported for the same fungicides in case of the predator *Nesidiocoris tenuis* on tomato plants (Zappalà et al., in press). However, side effects of sulfur compounds should be also tested in relation to the temperature, because insecticidal activity of these compounds (notably on parasitoids) is positively correlated with temperature (Flanders, 1943).

4.2. Importance of results for IPM and organic farming

Our study provides information that could be useful for IPM programs in identifying (and avoiding) products that may prevent predator population to build up because of side effects on predator reproductive capacity and survival. These products may, in addition to compromising the efficacy of IPM programs, prevent efficient colonization or recolonization of treated crops, particularly when pesticides are highly persistent. Interestingly, Avermectin-based biopesticides, the biopesticides based on Spinosad and BCO fall in this last category. These results indicate that pesticidal products that are classified as biopesticides can easily be of major concerns when they are supposed to be used in combination with natural enemies. Given the results obtained, the use of abamectin together with natural enemies like *O. laevigatus* in IPM programs should not be considered for effective and sustainable pest management programs. Unfortunately, a similar situation applies to pesticides that are recommended for organic cropping systems and therefore that are usually thought to be harmless for non-target arthropods. Spinosad induced both lethal and sublethal effects in *O. laevigatus* and its effects were persistent for at least 7 d after initial application. This biopesticide has been classified as an environmentally-safe product and has been embraced by IPM practitioners as a biorational pesticide. However, present results suggest that other pesticides with higher selectivity should be preferred, at least for management of pests that require usage of the highest application rates authorized (like Diptera leaf miners). For this reason, spinosad application should be avoided for at least one week before predators such as *O. laevigatus* are released in crops (which would prevent sublethal effects on the reproductive capacity of predators). Overall, the safest insecticides for *O. laevigatus* appeared to be azadirachtin, *Bt*, rynaxypyr and indoxacarb. Also, sulfur WP compounds should be preferred over sulfur DP compounds as fungicide. For example, when dealing with Lepidopteran pests, *Bt* should be prioritized because (i) it proved to be safe for *O. laevigatus* (especially true because we tested one of the highest concentrations recommended in crops), (ii) it induces no lethal and sublethal effects on predators that fed on prey which fed *Bt*-treated plants (Angeli et al., 2005; Tian et al., 2010), and (iii) it is highly efficient even against leaf miners (Gonzalez-Cabrera et al., 2011). Finally, in case of azadirachtin, negative effects on the development of predator nymphs (present study and high susceptibility reported in young insect instars; Schmutterer, 1990) could be avoided by delaying predator release after insecticide application.

4.3. Interest of the approach and model for risk assessment

The experimental design used enabled us to demonstrate contrasting effects between lethal and sublethal effects among various pesticides, notably because predators had the possibility to avoid being permanently in contact with pesticide-treated plant by walking on refuge areas (untreated areas like the glass surface). Because individuals also had to oviposit on plant substrate (for laying eggs), it could lead to chronic exposure which is comparable what usually happens in field conditions (Desneux et al., 2007). Alternatively, a strong repellent effect can prevent individuals from being in contact with pesticides (Desneux et al., 2005,2007; Cordeiro et al., 2010), and it could ultimately lead to reduced number of eggs laid and subsequent offspring production (Umoru et al., 1996; Desneux et al., 2005; Urbaneja et al., 2008). For example, sulfur DP and BCO induced only sublethal effects on reproductive capacity and no lethal effect. An alternative hypothesis would be that sublethal effects of products reduced female fecundity and/or impaired female oviposition behaviors (Banken and Stark, 1998; Desneux et al., 2004a,2007) but this should be specifically tested to confirm this hypothesis. The tomato–*O. laevigatus* model proved to be a valid experimental system; we observed normal reproduction levels (i.e. 1.82 ± 0.24 nymphs produced daily per female) and survivorship levels >72% in the control groups. These offspring production and longevity values are in concordance with those recorded on other host plants for various other *Orius* spp. (Lundgren and Fergen, 2006; Butler and O'Neil, 2007) and our results match those from Coll (1996) in which *O. insidiosus* showed preference toward tomato over other plant species as oviposition substrate in laboratory trials.

5. Conclusion

One of the goals of IPM is to preserve and/or increase pests natural mortality factors by combining various compatible control measures. Better knowledge on risks associated with specific pesticides toward natural enemies is of primary importance when incorporating them in IPM programs. The results obtained in the present study could improve IPM programs in which *O. laevigatus* is involved and more broadly may be useful for implementing IPM programs involving the use of *Orius* spp. as natural enemies.

These results were obtained in laboratory conditions, which represent a high exposure scenario, and it would be ideally completed with semi-field and field experiments (Sterk et al., 1999; Desneux et al., 2005; Suma et al., 2009). The results also stress the urgent need to clarify and re-organize how pesticides are labeled and classified, e.g. several biopesticides could be more toxic than synthetic ones, and pesticides that are recommended for organic farming may be more toxic than conventional pesticides.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.12.082.

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