

Preliminary Investigation on the Toxicity of Different Formulations on Some Groups of Beneficial Arthropods in Emilia-Romagna Orchards

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Abstract

Control of the most relevant phytophagous of apple and pear trees in Emilia-Romagna (Northern Italy) is achieved by insecticides but it is improved also by defence techniques allowing protection of useful insects. It is therefore relevant to understand in detail the effects of the most common insecticides employed in integrated defence of the two above mentioned cultures on the main auxiliary insects, both predators and parasitoids. With this aim we performed open field tests to evaluate the acute toxicity (48 hours after the treatment), according to the method suggested by IOBC Working Group "Integrated Protection in Orchards" to test three recently developed active ingredient: Spinosad, Indoxacarb and Methoxyfenozide. These three principles were compared to Azinphos methyl, presently one of the most widely employed insecticides with a broad action spectrum. Spinosad is a natural insecticide compound, whose active principle is a toxin produced by *Saccharopolispora spinosa*, Indoxacarb and Methoxyfenozide are synthetic molecules, respectively belonging to the family of oxadiazines and moult accelerators, while Azinphos methyl is an organophosphate compound.

The results show that Azinphos methyl is characterized by a lower selectivity towards generic Coccinellidae, while mortality towards *Antochoris nemoralis* is rather limited for all active principles tested, on the contrary to what observed for parasitoid Hymenoptera.

Key-words: acute toxicity, insecticides, entomophagous insects, pear, apple.

1. Introduction

The research on relationships among useful insects and pesticides is one of the main aspects to consider in the defence of fruit orchards from phytophagous (Croft, 1990). In the last ten years new family of insecticides became available to the market, generally showing a higher selectivity towards useful organisms in comparison to earlier ones have been developed. These new principles can better fulfil the requirements of integrated defence techniques, thus protecting the role of useful insects in natural control of key phytophagous. Consequently, the new active ingredient require a testing of their selectivity degree, to collect information in order to improve their application techniques.

In the Emilia-Romagna region (Northern

Italy) the control of the main phytophagous of apple and pear trees, that is Tortricidae such as *Cydia pomonella* L., *Cydia molesta* (Busck) and *Pandemis cerasana* (Hübner) (Civolani and Pasqualini, 2004; Civolani et al., 2006), was performed until around 1980 by chemical synthesis molecules, often with a broad-spectrum range. In the last years, however, save productions were preferred, based on reduced insecticide use and on principles of integrated agriculture which favour the activity of useful organisms, replacing broad action range insecticides with more selective ones.

The aim of this study is to evaluate in the short period the different selectivity of some insecticides towards auxiliaries found in apple and pear orchards, such as *Anthocoris nemoralis* F. (Heteroptera Anthocoridae), relevant in the

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natural control of *Cacopsylla pyri* L. (Homoptera Psyllidae), and other general predators such as Coleoptera Coccinellidae and Hymenoptera Calcidoidea and Braconidae.

The active ingredients investigated, recently introduced (that is Spinosad, Indoxacarb and Methoxyfenozide) were compared with Azinphos methyl, presently one of the most widely employed insecticides with a broad action range. In more detail, Spinosad is a natural insecticide, whose activity is due to a toxin produced by the bacterium *Saccaropolispora spinosa*; Indoxacarb and Methoxyfenozide are synthetic molecules respectively belonging to the family of oxadiazines and moult accelerators (MAC), while Azinphos methyl is an insecticide belonging to the organophosphate group.

The tests were performed according to the techniques and methods described in the Guidelines IOBC/WPRS (Hassan, 1985), based on previous tests also performed in Emilia-Romagna on other active principles (Civolani and Pasqualini, 1999), only considering short period toxicity, that is the direct (acute) toxicity of the insecticide and excluding its indirect (for example lack of prey) or sublethal effects (for example those concerning reproduction and development). Then the short period mortality was considered in the two days following the treatment, observed on small plots (4-5 plants), excluding in this way immigration or other effects difficult to detect (Jepson, 1989).

2. Materials and methods

Tests were performed in summer 2006 on apple and pear orchards, employing the pesticide compounds listed in Table 1, expressed as either as active ingredient or Commercial Formulation (C.F.) (g or ml hl⁻¹). The compounds were administered in the same periods they are currently used against the phytophagous species, in presence of the entomophagous ones to be investigated.

The standard experimental procedure for the field selectivity tests was the same recommended by IOBC Working Group "Integrated Protection in Orchards" (Hassan, 1985) (Fig. 1). The experimental planning involved randomized blocks (4 blocks) with plots including 4-5 trees (Brown, 1989). The treatments with the active

Table 1. Active ingredient and C.F. doses (g or ml / hl) employed.

Test n°	Active ingredient	Commercial Formulation	C.F. Doses (g or ml / hl)
1	Check (Water)	-	-
2	Azinphos methyl	Gusathion	200
3	Spinosad	Success	120
4	Methoxyfenozide	Prodiigi	40
5	Indoxacarb	Steward	17

ingredient to be tested were all performed the same day (day 1). The insects dead after the treatment were collected by rectangular white cotton sheets, 2 sq. m in size, placed beneath the canopy of two plants at the center of each plot, in the two days following treatment (day 2 and 3). The inventory treatments with deltametrin (Decis, 100 ml C.F. hl⁻¹ in place of DDVP as in the original method), were performed on day 3 on the same plants located in the center of each tested area: as previously mentioned, the rectangular sheets for collecting of dead or dying individuals. In both cases the individuals collected on the sheets were placed in Kartell plastic containers and quickly delivered to the laboratory, where classification at the stereomicroscope was performed, when possible, at the species level.

Tests were performed on areas in the same experimental conditions, in more detail on the same variety and far from the field boundaries. The treatments were performed through a backpack-mounted mist blower, with standard drizzle volumes (15 hl ha⁻¹).

To evaluate selectivity, after laboratory identification and classification of individuals of the useful species collected, we considered only species or groups of entomophagous detected a minimum number of times (more than 5 individuals per sheet). To calculate relative mortal-

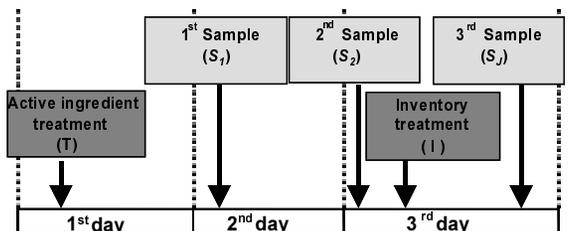


Figure 1. Experimental protocol for field treatments to evaluate short term selectivity.

Table 2. Short term selectivity towards adults of *Anthocoris nemoralis* (pear trial).

Test n	Active ingredient	<i>A. nemoralis</i> (adult)	
		% mortality	% corrected mortality
1	Check (Water)	20 n.s.	-
2	Azinphos methyl	29.92 n.s.	12.41
3	Spinosad	18.33 n.s.	0
4	Methoxyfenozide	27.68 n.s.	9.6
5	Indoxacarb	18.82 n.s.	0

ity (% T_{rel}) of each product we considered the average of four replicates of the initial treatment (S_1, S_2) and the average of the initial treatments and the inventory ones (S_j), using the following formula: $\%T_{rel} = [(S_1 + S_2) / (S_1 + S_2 + S_j)] * 100$. Statistical analyses were performed by analysis of variance (ANOVA). The absolute mortality of each insecticide compound was obtained by the corrected mortality equation by Schneider-Orelli (1947): $\{[\%T_{rel} \text{ (active ingredient)} - \%T_{rel} \text{ (H}_2\text{O)}] / [100 - \%T_{rel} \text{ (H}_2\text{O)}]\} * 100$, taking into account the mechanical effects of water during phytohyatric application (actually the mortality values due to mechanical effect of water change according to the different insect groups here detected).

3. Results and discussion

The results initially showed a different com-

position of insect community on the two tree species examined, probably due to a different presence of preys or hosts (Fig. 2). In the trial on pear trees (Tab. 2), no significant differences were found in the mortality caused by all four active principles on *Anthocoris nemoralis* (found in about 20% of total individual found, both as adults and nymphs). The mortality was generally low also for Azinphos methyl, which is anyway less selective on

Table 3. Short term selectivity towards juvenile stages of *A. nemoralis* (pear trial).

Test n	Active ingredient	<i>A. nemoralis</i> (larvae)	
		% mortality	% corrected mortality
1	Check (Water)	0 n.s.	-
2	Azinphos methyl	15.14 n.s.	15.14
3	Spinosad	16.24 n.s.	16.24
4	Methoxyfenozide	29.52 n.s.	29.52
5	Indoxacarb	19.58 n.s.	19.58

young nymphs and old nymphs in comparison to adults (Tab. 3), supporting previous results (Civolani and Pasqualini, 1999).

Concerning Coccinellidae, they were 73% of total dead insect specimens and mostly represented by *Stethorus punctillum* (Weise) and *Scymnus subvillosus* (Goeze) and by other aphidiphagous species in lower amounts. A significant difference was detected ($p < 0.05$) between Azinphos methyl mortality (over

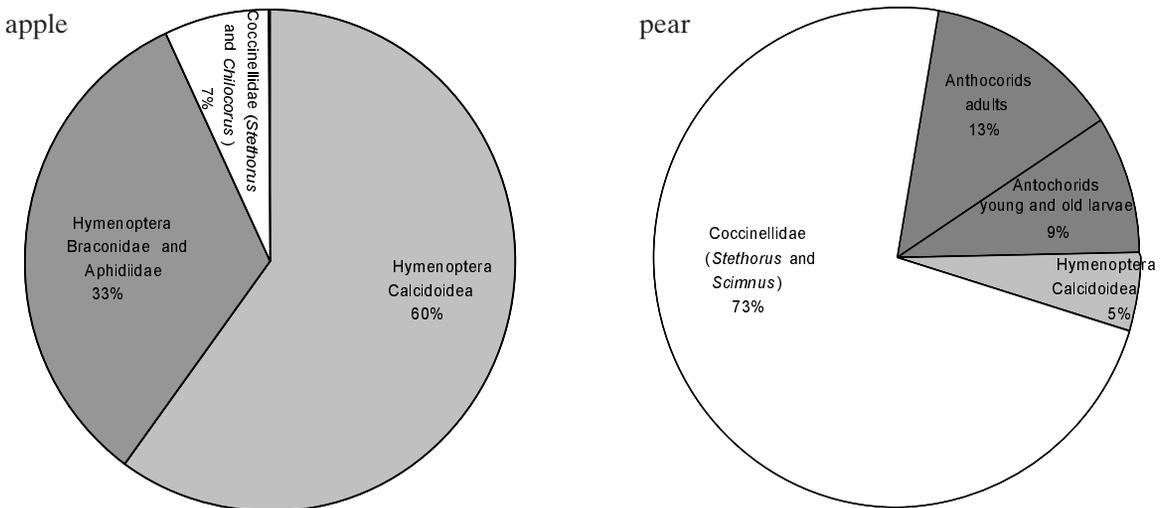


Figure 2. Distribution of entomophagous species on apple and pear trees during tests.

Table 4. Short term selectivity towards Coccinellidae (pear trial).

Test n	Active ingredient	<i>S. punctillum</i> , <i>S. subvillosus</i> , Aphidiphagous Coccinellidae	
		% mortality	% corrected mortality
1	Check (Water)	3.85 a	-
2	Azinphos methyl	43.44 c	41.18
3	Spinosad	5.03 a	1.22
4	Methoxyfenozide	8.43 a	4.76
5	Indoxacarb	9.20 a	5.56

Table 5. Short term selectivity towards Hymenoptera Calcidoidea and Braconidae aphidiidae (pear trial).

Test n	Active ingredient	Hymenoptera Calcidoidea and Braconidae Aphidiidae	
		% mortality	% corrected mortality
1	Check (Water)	0 a	-
2	Azinphos methyl	70.83 b	70.83
3	Spinosad	66.67 b	66.67
4	Methoxyfenozide	66.67 b	66.67
5	Indoxacarb	68.75 b	68.75

Table 6. Short term selectivity towards Hymenoptera Calcidoidea (apple trial).

Test n	Active ingredient	Hymenoptera Calcidoidea	
		% mortality	% corrected mortality
1	Check (Water)	10.34 a	-
2	Azinphos methyl	66.24 b	62.35
3	Spinosad	76.81 b	74.14
4	Methoxyfenozide	60.58 b	56.03
5	Indoxacarb	42.07 ab	35.39

40%) and the other active ingredients (Tab. 4).

Concerning Hymenoptera Calcidoidea and Braconidal Aphidiidae, generally found in lower amounts (about 5% of total), no significant differences were detected among the four principles tested: they all showed a rather high mortality, thus a limited selectivity.

On the contrary of what observed in the trial performed on pear trees, on apple trees (Fig. 2) no anthocorid specimens were detected and the presence of Coccinellidae was also low (7%). On apple trees we noticed a higher mortality with Azinphos methyl in comparison to the other pesticides, even if the low number of individuals collected did not allow to detect significant differences (Tab. 8).

Table 7. Short term selectivity towards Hymenoptera Braconidae aphidiidae (apple trial).

Test n	Active ingredient	Hymenoptera Braconidae aphidiidae	
		% mortality	% corrected mortality
1	Check (Water)	19.44 a	-
2	Azinphos methyl	63.73 b	54.98
3	Spinosad	73.22 b	66.76
4	Methoxyfenozide	66.88 b	58.88
5	Indoxacarb	65.56 b	57.24

Table 8. Short term selectivity towards Coccinellidae (apple trial).

Test n	Active ingredient	<i>S. punctillum</i> , Chilocoridae, aphidiphagous Coccinellidae	
		% mortality	% corrected mortality
1	Check (Water)	25 n.s.	-
2	Azinphos methyl	48.51 n.s.	31.35
3	Spinosad	27.50 n.s.	3.33
4	Methoxyfenozide	17.78 n.s.	0
5	Indoxacarb	34.44 n.s.	12.59

Concerning Hymenoptera Chalcidoidea and Braconidae, all active principles showed similar high mortality values (Tab. 6 and 7).

4. Conclusions

Selectivity field tests performed on the short period, that is two days after treatment with active principles on some entomophagous species in apple and pear orchards in Emilia-Romagna, showed that Azinphos methyl is characterized by a lower selectivity towards predators such as *Stethorus*, *Scymnus* and generally Coccinellidae.

The mortality towards *A. nemoralis* was rather low at all stages and for all active ingredients, including Azinphos methyl, on the contrary to what generally detected for parasitoid Hymenoptera.

On both type of fruit trees the selectivity of Spinosad, Indoxacarb and Methoxyfenozide resulted more or less similar, and sometimes better than that of Azinphos methyl. However, this result must be confirmed in its real "biological effects", especially those concerning the auxiliary species which are truly useful in field to control phytophagous populations.

References

- Brown K.C. 1989. The design of experiment to assay the effects of pesticides on beneficial arthropods in orchards: replication versus plot size. In: Jepson P.C. (ed.): Pesticides and nontarget invertebrates, 77-80. Intercept Ltd., Andover, UK.
- Civolani S., Pasqualini E. 1999. Preliminari indagini sulla tossicità nel breve periodo di diverse formulazioni di insetticidi su alcuni gruppi di entomofagi in pereti dell'Emilia-Romagna. *Inf. Agr.*, 20:1-7.
- Civolani S., Pasqualini E. 2004. Difesa dalla generazione svernante di *Pandemis cerasana* (Hb.) (Lepidoptera Tortricidae) su pero in Emilia-Romagna. *L'inf. Agr.*, 12:89-90.
- Civolani S., Pradolesi G., Melandri M., Pasqualini E. 2006. Impiego di methoxyfenozide nella difesa di melo e pero. *Inf. Agr.*, 6:69-72.
- Croft B.A. 1990. Arthropod biological control agents and pesticides. Wiley Interscience publication, 723 pp.
- Hassan S.A. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites. *Bulletin OEPP-EPPO*, 15:214-255.
- Jepson P.C. 1989. The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. In: Jepson P.C. (ed.): Pesticides and non-target invertebrates, 95-128. Intercept Ltd., Andover, UK.
- Poehling H.M. 1989. Selective application strategies for insecticides in agricultural crops. In: Jepson P.C. (ed.): Pesticides and non-target invertebrates, 151-175. Intercept Ltd., Andover, UK.
- Schneider-Orelli O. 1947. *Entomologisches praktikum*. H.R. Sauerlander and Co. Eds., Aarau (CH).

