

SENSITIVITY OF NON-TARGET ARTHROPODS AND BENEFICIAL FUNGAL SPECIES TO CHEMICAL AND BIOLOGICAL PLANT PROTECTION PRODUCTS: RESULTS OF LABORATORY AND SEMI-FIELD TRIALS

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INTRODUCTION

Testing side-effects on beneficial organisms is increasingly important in the development of new pesticides and the reregistration of old active ingredients in Europe. This testing is partly done for regulatory assessment of potential risks to terrestrial, non-target arthropods, which are an important part of the environment, both in and out of crop fields. Tests are designed to determine the effects of pesticides on beneficial insects, mites or nematodes, used in Integrated Pest Management in horticulture. Direct acute toxicity is the most important parameter measured in such tests.

Microbial agents such as entomopathogenic fungi or fungi active against soil-borne diseases are a special case. For these agents, tests measure both the effects of pesticides (mainly fungicides) on the agents and the effect of microbial agents on other beneficial organisms. In addition, we tested the effects of several old and new pesticides (both microbial and botanical) under semi-field conditions on the most sensitive stages of one pollinator (*Bombus terrestris* [L.]), one predatory mite (*Phytoseiulus persimilis* Athias-Henriot), two predatory bugs (*Orius laevigatus* [Fieber] and *Macrolophus caliginosus* Wagner), one parasitoid (*Encarsia formosa* Gahan), and three species of entomopathogenic fungi (*Paecilomyces fumosoroseus* [Wize] Brown and Smith strain Apopka 97, *Verticillium lecanii* [Zimmerman] Viegas, and *Beauveria bassiana* [Balsamo] Vuillmin). Also under laboratory conditions, one strain of a fungus antagonistic to leaf pathogens (*Trichoderma harzianum* Tul. strain T-39) was tested.

New active ingredients tested included spinosad, fipronil, thiamethoxam and chlorfenapyr, in addition to insecticides, acaricides and fungicides already on the European market.

Testing for Registration

The testing of the effects of pesticides on non-target arthropods in Europe is necessary, both to meet regulatory requirements and to find compounds suitable for use in IPM programs. Registration testing is currently conducted according to the Council Directive of July 15, 1991 (91/414/EEC), updated by the Commission Directive 96/12EC. The directive refers to the first ESCORT guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al.*, 1994) and to the EPPO scheme for risk assessment on natural enemies. The first was revised, simplified and harmonized at the ESCORT 2 workshop (Candolfi *et al.*, 2000). This workshop dealt with the discrimination between in-field, off-field (e.g., borders) and IPM situations. A testing procedure for use with non-target terrestrial arthropods and a risk assessment scheme applicable to pesticides for regulatory purposes was developed, in accordance with the previous guidelines. It provides a tiered testing and assessment procedure and gives decision criteria and trigger values for both in-field and off-field risk assessment, together with options for risk mitigation measures (Candolfi *et al.*, 2000).

Testing for IPM: Bumblebees (*Bombus terrestris* [L.])

The use of bumblebees in greenhouse crops, introduced worldwide for the first time by Biobest in 1987, results in great labor savings, improved crop quality and increased yield, especially for tomatoes. Because many pesticides are harmful to bumblebees, the introduction of bumblebee pollination stimulated the use of biological pest control (Sterk *et al.*, 1995). However, quite often biological control is not able to keep pest populations at acceptable levels. Therefore, selective chemical or biological compounds, are used as corrective agents. Some of these compounds are toxic to bumblebee larvae; others harm adult bees or influence their behavior. Extrapolation from the known side-effects of pesticides on honeybees, *Aphis mellifera* L., is not always possible. Tests on bumblebees are required for product registration. Fenoxycarb, for instance, is very toxic to larvae of honeybees, but has hardly any effect on bumblebee larvae. Teflubenzuron, on the other hand, is almost harmless on larvae of honeybees, but is used as a toxic standard in feeding trials on larvae of bumblebees.

Testing for IPM: Beneficial Arthropods

Pest insects and mites in protected culture have been controlled successfully with beneficial arthropods for several decades. Nevertheless, at times, due to exceptional climatic conditions or the introduction of new exotic insects, beneficial arthropods are sometimes not able to keep pests below the economic damage thresholds. Selective biological or chemical compounds are needed in such cases. With the withdrawal of EC-registrations for several older pesticides, several new products are now being developed. Side-effect trials with these new compounds were carried out to see if they could be used safely in IPM programs. All trials (both old and new products) were done under semi-field conditions, following protocols developed by the IOBC group 'Side-effects of Pesticides on Beneficial Organisms' (Sterk *et al.* 1999). The effect of old and new compounds were tested on first and second nymphal instars of *Orius laevigatus* (Fieber) (Anthocoridae) and *Macrolophus caliginosus* Wagner (Miridae), adult females of *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae), and pupae and adults of *Encarsia formosa* Gahan (Aphelinidae).

Testing for IPM: Fungi

Four entomopathogenic fungi used in biological control were tested for their sensitivity to fungicides. These species were three entomopathogenic fungi—*Paecilomyces fumosoroseus* (Wize) Brown and Smith strain Apopka 97, *Verticillium lecanii* (Zimmerman) Viegas, *Beauveria bassiana* (Balsamo) Vuillemin—and one biological fungicide used against leaf diseases—*Trichoderma harzianum* Tul. strain T39. These microbial pesticides are often used in combination or in rotation with chemical fungicides, so the effects of such fungicides must be considered.

METHOD AND MATERIALS

Test Methods for Bumblebee Trials

The toxicity of several chemical or biological pesticides was tested on bumblebees. Three kinds of tests were used: (1) individual contact on five workers in each hive, four hives per test compound, (2) *ad libitum* feeding of treated pollen and (3) *ad libitum* feeding of a solution of sugar water and the test compound. Both feeding tests were made on 10 worker bees in each hive, five hives per compound. Tests followed methods of Sterk *et al.* (1995) and Merck (2002). In the individual contact test 50 µl of the test compound were put by pipette on each worker bee after cooling the bees for about 15 to 20 minutes in a freezer to make them immobile. Microbial products were only tested by individual contact with the test compound. Products and rates tested are given in Table 1. For the direct toxicity trials, the mortality was expressed as the percentage of surviving adult worker bees compared with the

control. In both oral tests it was calculated as the number of male offspring. The pesticides were classified into the toxicity categories proposed by the IOBC working group for semi-field trials on beneficial arthropods: Class 1: harmless (<25% mortality or effect), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%) and Class 4: harmful (>75%).

Table 1. Compounds and rates tested on bumblebees.

Product Class	Active Ingredient	Product	Concentration gram a.i./l
Microbial (fungi)	<i>Paecilomyces fumosoroseus</i>	PreFeRal 20 WDG (2x10 ⁹ CFU/g)	0.2
	<i>Trichoderma harzianum</i>	Trichodex 25 WP (1010 CFU/g)	1.0
Microbial (bacteria)	<i>Bacillus thuringiensis</i> var. <i>aizawai</i>	Xentari 10 WG (3500 DMU/g)	0.1
	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	Scutello 6.4 WP (20000 UAAK/g)	0.064
	<i>Bacillus thuringiensis</i> var. <i>israelensis</i>	Vectobac 12 AS (1200 ITU/mg)	0.06
Microbial (viruses)	<i>Adoxophyes orana</i> Granulose Virus	Capex 1% SC (5 x 10 ¹³ gv/l)	0.001
	<i>Cydia pomonella</i> Granulose Virus	Granupom 1% EC (2.2 x 10 ¹³ gv/l)	0.003
Insecticides	Imidacloprid	Confidor 200 SL	0.2
	Thiamethoxam	Actara 25 WG	0.1
	Fipronil	Regent 80 WG	0.024
	Spinosad	Tracer 480 SC	0.096
	Dimethoate	Perfekthion 500 EC	0.5
	Teflubenzuron	Nomolt 150 SC	0.15

Test Methods for Effects on Biological Control Agents

The trials were run using published methods for semi-field trials: *O. laevigatus* (Van de Veire et al., 2002), *M. caliginosus* (Van der Linden, 2000), *P. persimilis* (Sterk and Van Wetswinkel, 1988), and *E. formosa* (Jaco, 2001). In all trials, biphenthrin was used as a positive (toxic) standard. In the side-effect trials on predators, five larvae L1-L2 (for *O. laevigatus* and *M. caliginosus*) or 10 female adults (for *P. persimilis*) were put on the dry residue of the test compounds on each plant. The trials on *O. laevigatus* were done on sweet pepper; *M. caliginosus* was tested on tomato plants; and *P. persimilis* was tested on beans. In each trial, there were five replicates. In the tests on predators, the mortality of the surviving mobile stages was corrected according to Abbott (1925).

Tests on *E. formosa* were done on cucumber. There were 10 replicates for each compound. Test plants were heavily infested with greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) a few weeks before the start of trials. When sufficient number of nymphs were found on the plants, the infested leaves were marked. To test effects on adult parasitoids, plants were treated first and about 20 to 50 adults were released on each treated plant. To measure the influence of the pesticides on larvae of *E. formosa*, whitefly-infested plants were first exposed to parasitoids for oviposition. About one week later, the plants with the then-parasitized whitefly pupae were sprayed. Effects of compounds on adult females of *E. formosa* were recorded by counting the number of resulting parasitized pupae. To measure the effects of the test products on immature stages of *E. formosa*, the number of emerged parasitoids was determined. The mortality percentages were also corrected in both cases using Abbott's formula (Abbott, 1925). Pesticides were classified into the toxicity categories proposed by the IOBC working group for semi-field trials: Class 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%) and Class 4: harmful (>75%). Products and rates tested are given in Table 2.

Table 2. Compounds and rates tested against biological control agents.

Product Class	Active Ingredient	Product	Concentration gram a.i./l
Microbial (fungi)	<i>Paecilomyces fumosoroseus</i>	PreFeRal 20WDG (2x10 ⁹ CFU/g)	0.2
	<i>Trichoderma harzianum</i>	Trichodex 25WP (1010 CFU/g)	1.0
Insecticide/Acaricide	chlorfenapyr	Intrepid 240 SC	0.24
	biphentrine	Talstar 100 EC	0.03
Insecticides	imidacloprid	Confidor 200 SL	0.2
	thiamethoxam	Actara 25 WG	0.1
	fipronil	Regent 80 WG	0.024
	spinosad	Tracer 480 SC	0.096
	azadirachtin	Nemix 4.5 EC	0.0225
	pyriproxifen	Admiral 100 EC	0.025

Methods to Assess Effects of Fungicides on Beneficial Fungi

These tests, done by mixing the normal dose rate of the fungicides with the agar, were carried out with mycelium of all four fungi under laboratory conditions. The test method was partly based on the guideline for testing side-effects of pesticides on *B. bassiana* (Coremans-Pelseneer, 1994) and *P. fumosoroseus* (Aerts *et al.*, 1997), but instead of spores, small plugs of mycelium were placed in the treated agar (Bock, 2002). The assessment was based on the mean diameter of the fungal colonies, corrected according to Abbott's formula. The same IOBC classes as in the previous trials were used to categorize the degree of harm of each fungicide.

Table 3. Compounds and rates of fungicides used in tests with beneficial fungi.

Product Class	Active Ingredient	Product	Concentration gram a.i./l
Fungicides	<i>captan</i>	Captan 80 WP	1.2
	<i>azoxystrobin</i>	Amistar 240 SC	0.24
	<i>kresoxim-methyl</i>	Candit 50 WG	0.1
	<i>trifloxystrobin</i>	Flint 50 WG	0.75
	mepanipyrim	Frupica 50 WP	0.3
	procymidone	Sumisclex 50 WP	0.75
	sulfur	Hermovit 80 WP	4.8
	tolyfluanide	Euparen M 50 WG	0.75
	imazalil	Fungaflor 500 EC	0.5
	pyrimethanil	Scala 400 SC	0.044
	Thiram	Pomarsol 80 WG	1.6
	Bitertanol	Baycor 500 SC	0.45

RESULTS AND DISCUSSION

Effects on Bumblebees

The microbial compounds tested were completely safe for bumblebees when bees were directly treated with the test compound (Table 4). The neonicotinoids imidacloprid and thiamethoxam were toxic to bumblebees in all trials. Spinosad was toxic if bees were treated directly or fed a mixture of product and sugarwater. However, this compound has been on the market for several years and no injury to bumblebees has been recorded in practice as long as bees are exposed only to dry residue. Fipronil is now used as a toxic standard for contact trials. It is also very harmful if given orally. Dimethoate and teflubenzuron are used as positive toxic standards for adult and larvae toxicity oral trials, respectively.

Effects on Beneficial Macro-organisms

As expected, both microbial compounds tested were very safe for all beneficial species evaluated (Table 5). Azadirachtin and spinosad were also safe (Categories 1 or 2), although spinosad was moderately toxic to adult *E. formosa* (Category 3). However, this effect was brief due to the short persistence of this compound. Both of the neonicotinoids, imidacloprid and thimethoxam, were very toxic. Chlorfenapyr was harmful for some species, but nontoxic for *O. laevigatus* or pupae of *E. formosa*. Fipronil was rather toxic for *O. laevigatus* and very toxic for *M. caliginosus*. Abamectin varied between non-toxic and harmful, but was nevertheless an interesting compound, due to its short persistence. Because pyriproxifen is known as a selective compound, the damaging effects on pupae of *E. formosa* were unexpected.

Table 4. Toxicity of pesticides for bumblebees *Bombus terrestris*.

Active Ingredient	% Mortality from Direct Contact ^a	% Reduction in Offspring ^a	
		Oral Feeding via Pollen:	Oral Feeding via Sugarwater:
<i>Paecilomyces fumosoroseus</i>	1	-	-
<i>Trichoderma harzianum</i>	1	-	-
<i>Bt. var. aizawai</i>	1	-	-
<i>Bt. var. kurstaki</i>	1	-	-
<i>Bt. var. israelensis</i>	1	-	-
<i>Cydia pomonella</i> GV	1	-	-
<i>Adoxophyes orana</i> GV	1	-	-
Imidacloprid	4	4	4
Thiamethoxam	4	4	4
Spinosad	4	1	4
Fipronil	4	4	4
Dimethoat	4	4	4
Teflubenzuron	1	4	4

^aClass 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%), and Class 4: harmful (>75%)

Effects on Beneficial Fungi

Captan and sulfur were less toxic for the beneficial fungi tested (Category 1 for sulfur and 2 and 3 for Captan) than was expected for these broad spectrum fungicides. The same was true for the strobilines azoxystrobin, kresoxim-methyl, and trifloxystrobin. Mepanipyrim, procymidone, and tolylfluanide also seem more or less compatible with these beneficial fungi, if there is gap of a few days between applications of fungi and fungicides. Pyrimethanil, thiram, and bitertanol were moderately or even very toxic. Imazalil was very toxic for these fungi and might act as a positive (toxic) standard in the future for these kind of tests.

Our results should not be generalized since tests were run with mycelium, not spores. Some of these test compounds might be very toxic if applied on the germination of blastospores or conidiospores. In previous trials, captan, tolylfluanide, and thiram, for example, were extremely toxic for germinating blastospores and conidiospores of *P. fumosoroseus* if, instead of a mycelium plug, a droplet of a spore suspension was placed on the treated agar. Sulfur strongly inhibited the formation of colonies after applying a blastospore suspension of the same fungus on treated agar (Aerts *et al.* 1997). In general, it seems that germinating spores are more sensitive to pesticides than is the mycelium.

Table 5. Toxicity of insecticides to beneficial arthropods.

Active Ingredient	<i>P. persimilis</i> Adult ?	<i>O. laevigatus</i> L1-l	<i>M. caliginosus</i> L1-L2	<i>E. formosa</i> Adult	<i>E. formosa</i> Pupae
<i>P. fumosoroseus</i>	1 ^a	1	1	1	1
<i>T. harzianum</i>	1	1	1	1	1
azadirachtin	2	2	2	2	1
chlorfenapyr	4	1	4	4	1
imidacloprid	4	4	4	4	3
thiamethoxam	3	4	4	4	3
spinosad	1	1	1	3	1
abamectine	4	3	2	3	1
fipronil	4	3	4	Not tested	Not tested
pyriproxifen	2	1	1	1	3
biphenrine	4	4	4	4	4

^aClass 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%), and Class 4: harmful (>75%)

Table 6. Toxicity of fungicides to mycelium of beneficial fungi.

Product	<i>P. fumosoroseus</i>	<i>V. lecanii</i>	<i>B. bassiana</i>	<i>T. harzianum</i>
captan	2 ^a	3	2	3
azoxystrobin	2	2	3	2
kresoxim-methyl	2	1	2	2
trifloxystrobin	2	1	1	1
mepanipyrim	1	1	1	2
procymidone	2	2	2	3
sulfur	1	1	1	1
tolyfluanide	2	3	1	2
imazalil	4	4	4	4
pyrimethanil	2	3	2	4
thiram	3	3	2	4
bitertanol	3	3	4	4

^aClass 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%), and Class 4: harmful (>75%)

Side-Effects Manual

The results of these and other trials carried out by Biobest N.V. can be found on the Web site www.biobest.be, or in the published side-effects manual (Sterk, 2001).

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