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Working Group
"Pesticides and Beneficial Organisms"

OILB/SROP

Groupe de Travail
"Pesticides et Organismes Utiles"

SIDE-EFFECTS OF PESTICIDES ON BENEFICIAL ORGANISMS: COMPARISON OF LABORATORY, SEMI-FIELD AND FIELD RESULTS

Edited by **Heidrun Vogt**

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PREFACE

Pesticides suitable for use in integrated control programmes are urgently needed. One of the major aims of the Working Group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC), West Palearctic Regional Section (WPRS), therefore is to develop standard methods to test the side effects of pesticides on important natural enemies. Testing the side effects of pesticides on beneficial organisms has become obligatory in several countries and this has made the development of internationally approved standard guidelines even more important.

Recognizing that no single method could provide sufficient information to assess the side effects of pesticides, a combination of tests that includes several laboratory as well as semi-field and field methods is recommended. The distinction between pesticides with no or low toxicity and those with high toxicity can be done rather easily with laboratory experiments. However, results of laboratory tests can be used to predict effects in the field only in the case of no or low toxicity: Pesticides found to be harmless to a particular beneficial in the laboratory test are most likely to be of low risk to populations of the same organism in the field. No further testing in semi-field or field therefore is recommended, unless the pesticide is a multiple application product. In the case of pesticides with harmful effects in the laboratory, only field testing will reveal if or to what extent the harmfulness remains under practical conditions.

Guidelines to test the side effects of pesticides on beneficial organisms, developed according to standard characteristics, were published by the Working Group in two IOBC-Bulletins: (1) XI/4, 1988 and (2) XV/3, 1992. Results of testing the side effects of pesticides on beneficial organisms within six joint programmes and involving 7, 14, 21, 20, 22, 24 authors, respectively, were published elsewhere.

The present Bulletin compares results of the different types and tiers of standard tests developed by the Working Group with the aim of analyzing the importance of each testing tier, the validity of the methods and the origins of differences between laboratory, semi-field and field effects. The information included in this Bulletin will hopefully help the reader to understand the principles of the IOBC sequential testing scheme and to interpret the results of the tests and provide the research scientist with valuable knowledge for his own work.

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ACTIVITIES OF THE IOBC/WPRS WORKING GROUP "PESTICIDES AND BENEFICIAL ORGANISMS"

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Introduction

Parasitoids and predators of agricultural pests reduce the population of their prey or host and help to limit damage caused by the pest. Modern plant protection recommends therefore the reduction of the use of chemical pesticides to a minimum. If however the use of pesticides is indispensable, selective pesticides should be chosen. One of the major aims of the Working Group is therefore to coordinate international activities to develop standard methods to test the side effects of pesticides on the most important natural enemies and to choose selective pesticides suitable for use in integrated control programmes. The harmonization of testing guidelines with other international organizations such as the EPPO and the EC are important tasks.

Testing the side effects of pesticides on beneficial organisms has become obligatory in several countries and this made the development of internationally approved guidelines even more important and urgent. Moreover, the group aims to test the selectivity of pesticides and provide information to other IOBC Working Groups and to users of integrated control programmes in member countries. The role of natural enemies in plant protection as well as the development of resistance among these organisms to pesticides in nature are also studied.

Some of the activities and the results of joint experimental work conducted by members of the Working Group in the last 5 years were summarized in the following 6 publications: The results of the fourth, fifth and sixth joint pesticides testing programmes were published by Hassan et al.(1988, 1991, 1994). Guidelines to test the side effects of pesticides on beneficial organisms were published in two WPRS-Bulletins (1988, 1992) and rearing methods for 14 different natural enemies by Samsøe-Petersen et al.(1989). These 6 multi-author publications involved 20, 22, 24, 21, 29 and 14 authors, respectively.

The Working Group organizes meetings and regularly sends Circular Letters to coordinate research and to divide tasks between the members. The coordination of research prevents the unnecessary overlap in research programmes.

Each of the 26 testing members of the Working Group develops rearing and testing methods for one beneficial organism. Most of them took part in joint pesticides testing programmes, improved their testing techniques and started to develop some of the remaining, urgently needed semi-field and field methods, to complete the sequential testing procedure. At the same time, a large number

of members carried out ecological work and conducted field experiments to assess the impact of natural enemies on several pests and/or tested the effects of pesticides in the field.

Standard characteristics of test methods

Standard guidelines to test the side effects of pesticides on natural enemies were developed by members of the IOBC/WPRS Working Group according to standard characteristics. These characteristics were recently discussed and updated by the members within the last group meetings.

LABORATORY

(a). Laboratory, susceptible life stage (e.g. adults of parasitoids, developmental stages of mites, larvae of predatory insects):

1. exposure of organisms to fresh pesticide deposit applied on glass plate, leaf, sand, sandy soil; 2. exposure of beneficial fungus, nematodes and collembola in contaminated standard medium (e.g. based on broth, agar or soil); 3. even film of pesticide, standard amount of 1,5 to 2 mg fluid/cm2 on glass or leaf and 4 to 6 mg fluid/cm2 on sand are used; 4. laboratory reared or field collected organisms of uniform age; 5. highest recommended concentration of pesticide; 6. adequate exposure period before evaluation; 7. adequate ventilation; 8. water treated control in each experiment, toxic standard at least in one experiment per year; 9. assessment of the reduction in beneficial capacity (reproduction, parasitism) beside mortality; 10. four evaluation categories: 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99%).

(b). Laboratory, less susceptible life stage (e.g. parasitoids within their hosts, adults of mites, adults of predatory insects):

1. direct spray of organisms and substratum. The points 3 to 10 of test (a) are applicable.

(c) Laboratory, duration of harmful activity:

1. exposure to pesticide residues applied on plants or soil at intervals after treatment; 2. weathering in the field under rain cover with periodical exposure to direct sunshine or under simulated field conditions (appropriate season of use of the pesticides); 3. pesticide application according to Good Agricultural Practise; 4. experiments and assessment of toxicity as in test (a), (point 4 to 10); 5. repeating of test at intervals until loss of toxicity (category 1 result) or up to one month after treatment; 6. four evaluation categories: A = short lived (<5 days), B = slightly persistent (5-15 days), C = moderately persistent (16-30 days), D = persistent (>30 days).

(d) laboratory, extended laboratory:

1. experiments are carried out under rain cover or in the laboratory under standard simulated field conditions (fluctuating temperature, air humidity and light to simulate a summer day). 2. a susceptible life stage of organism is used ; 3. adequate ventilation and air exchange to prevent the accumulation of pesticide fumes. The points 4 to 12 of the semi-field test are applicable.

SEMI-FIELD

1. experiments are carried out in the field with climatic factors to be left unaffected as much as possible, where necessary, rain cover can be used; 2. appropriate time, crop and season for the

chemical, but chosing conditions to represent the worst case; 3. experiments to be repeated under different weather conditions; 4. beneficial organisms (possibly a susceptible life stage) to be present on the crop during spraying - if practical - or to be released as soon as possible after spraying; 6. laboratory reared or field collected organisms of uniform age; 7. highest recommended dose of pesticide; 8. application according to Good Agricultural Practise; 9. adequate exposure period before evaluation; 10. water treated control and toxic standard in each experiment; 11. assessment of the reduction in beneficial capacity (reproduction, parasitism, prey intake) besides mortality; 12. four evaluation categories: 1 = harmless (<25%), 2 = slightly harmful (25-50%), 3 = moderately harmful (51-75%), 4 = harmful (>75%).

FIELD

(a) Field, naturally occurring organisms:

1. crops or soil inhabited by naturally occurring beneficials are directly sprayed; 2. experiment to be repeated at different locations; 3. no release of beneficial organisms in the same year of the experiment; 4. sampling is carried out at intervals before and after treatment(s); 5. highest recommended dose rates and number of treatments following Good Agricultural Practise; 6. experiments are carried out at the appropriate time and season for the chemical; 7. adequate exposure period before evaluation; 8. water treated control and negative toxic standard in each experiment; 9. mortality, survival, population changes may be monitored; 10. plot design and number of collected individuals to exceed a certain limit to allow statistical analysis; 11. four evaluation categories: 1 = harmless (<25%), 2 = slightly harmful (25-50%), 3 = moderately harmful (51-75%), 4 = harmful (>75%).

(b) Field, released organisms:

1. laboratory reared or field collected beneficial organisms of uniform age are released in field plots and are directly sprayed. The points 4 to 11 of the field test are applicable.

Joint testing of pesticides

The joint testing of pesticides within organized programmes has become a standard feature of the Working Group. These testing programmes provide valuable information on the side effects of pesticides and give the testing members opportunity to improve testing techniques and develop better guidelines. Joint testing programmes, each including 20 chemicals were organized every two years.

The 8th programme was initiated this year. About 20 pesticides that are registered in member countries were chosen. This testing programme is not only meant to provide valuable information on the side effects of pesticides on beneficial organisms but it also gives the testing members an opportunity to compare results with other colleagues, to improve testing techniques, and to profit from the experience of the ad-hoc review committee of the Working Group.

Among the 122 pesticides tested till now, the following compounds were found to be harmless to nearly all the beneficial organisms tested or have limited persistence to the natural enemies tested: The insecticides and acaricides Dipel (*Bacillus thuringiensis*), Applaud (buprofezin), Shell Torque (fenbutatin-oxide), Azomate (benzoximate), Dimilin (diflubenzuron) - toxic to predatory larvae, Spruzit-Nova-flüssig (pyrethrum and piperonylbutoxide) - short lived, Pirimor Granulat (pirimicarb) - short lived, Cesar S.L. (hexythiazox), Apollo SOSC (clofentezine), Kelthane

(dicofol), Tedion V 18 (tetradifon); the fungicides Nimrod (bupirimate), Saprol (triforine), Sumisclex (procymidone), Dyrene flüssig (anilazine), Bayfidan (triadimenol), Anvil (hexaconazole), Calixin (tridemorph), Bayleton (triadimefon), Ronilan (vinclozolin), Orthocid 83 (captan), Cercobin-M (thiophanate-methyl), Ortho Difolatan (captafol), Derosal (carbendazim), Daconil 500 (chlorothalonil), Plondrel (ditalimfos), Pomarsol forte (thiram), Dithane Ultra (mancozeb) - with restrictions for predatory mites, Baycor (bitertanol), Delan flüssig (dithianon), Vitigran (copper-oxychlorid), Impact (flutriafol), Rovral PM (iprodion); the herbicides Illoxan (diclofop-methyl), Semeron (desmetryn), Betanal (phenmedipham), Kerb 50 W (propyzamid), Cycocel Extra (chlormequat), Luxan 2,4-D amine (2,4-D aminesalt), Ally (metsulfuron-methyl and Grasp (tralkoxydim), Basagran (bentazone) and the plant growth regulators Rhodofix (naphthyl acetic acid), Dirigol-M (alphanaphthyl-acetamid).

Field research and observation

Field work aimed to assess the impact of natural enemies on different crops as well as to study the effects of the use of pesticides on beneficial organisms under practical conditions was carried out by members of the Working Group. The occurrence of natural enemies resistant to pesticides and ways to assess their ecological impact are also being studied.

Some aims of the Working Group

1) Complete the development of sequential testing procedures (laboratory, extended laboratory, semi-field and field methods) for important natural enemies.
2) Ring testing and validation of IOBC standard methods to test the side effects of pesticides on beneficial organisms.
3) Global recognition of IOBC standard test methods.
4) Exchange ideas with EPPO, EC and BART experts on the framework and the methods used for the testing.
5) Compare results of laboratory, extended laboratory, semi-field and field methods.
6) Discuss the value of extended laboratory methods in the rating of pesticides.
7) Continue to organize Joint pesticide testing programmes and provide information for IPM.
8) Research on indicator species to extrapolate the risk on groups of natural enemies and non-target species.
9) Standardization of pesticides' rate to be used in the different types of tests.
10) Discuss new ideas for testing on entomopathogenic fungi (Entomophthorales, Deuteromycetes).
11) Exchange information and experiences on GLP.

Conclusions

The international guidelines developed by the Working Group to test the side effects of pesticides on beneficial organisms were adapted for use for registration purposes in several countries. The use of these standard methods allowed the exchange of results from one country to another and saved the cost of repeated testing.

An international net of laboratories to conduct the tests in 13 countries has been established. The cooperation with the EPPO and with the EC to harmonize testing guidelines has given fruitful results. EPPO and EC guidelines based on the IOBC test methods are in preparation.

The close cooperation between the members of the Working Group, especially in developing of test methods, the fast exchange of information as well as the joint activities, especially in testing of pesticides helped to create close personal contacts between the members and speed progress.

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COMPARISON BETWEEN FIELD AND LABORATORY TESTING METHODS TO EVALUATE THE PESTICIDE SIDE-EFFECTS ON THE PREDATORY MITES *Amblyseius andersoni* AND *Typhlodromus pyri*

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Abstract

Several laboratory and field testing methods have been proposed to evaluate the side-effects of pesticides on the phytoseiid mites *Amblyseius andersoni* (Chant) and *Typhlodromus pyri* Scheuten. In the present work the results of field and laboratory tests concerning insecticides sprayed on the above-mentioned species are compared.

Field tests were carried out on 2 *T. pyri* strains originating from vineyards of different areas of Northern Italy and on 3 *A. andersoni* strains originating from two orchards (apple and peach) and one vineyard of the Veneto region (North-eastern Italy). The laboratory tests were performed on the same strains. 12 insecticides commonly used in vineyards and fruit orchards were chosen for the tests. In field tests the effect of pesticides was classified in 4 classes (E) calculated by assessing the possible population reduction in the treated plots 40 days after treatments. The method used in the laboratory was that described by Overmeer and Van Zon (1982). The overall effect was subdivided in 4 categories.

A total of 33 trials were involved in the comparison. In 17 of them, the same toxicological class was reached both in the field and in the laboratory tests. In 13 trials the laboratory data overestimated the toxicity recorded in the field, in the remaining 3 cases the reverse was found. Overestimation in the laboratory tests was mainly due to the high toxicity of pesticides on juveniles and, in a few cases, even to the reduction in fecundity.

The number of field tests should be reduced by performing a number of laboratory tests. Data was plotted in a 2x3 design in which boundaries (decision thresholds) were suggested from practice. A good fit between laboratory and field tests was found. Taking T1=50%, T2=95% and T3=35% we obtained a good compromise between reducing the number of compounds that need retesting (10) and the number of errors (3). Two errors concerned products considered harmful in the laboratory but harmless in the field. The one exception was related to chlorpyriphos-methyl.

Introduction

During the last decades several laboratory and field testing methods have been proposed to evaluate the side-effects of pesticides on predators belonging to the Phytoseiidae family. After the review by Overmeer (1985) the interest in toxicological methods has increased and new methods have been developed especially for species of economic importance such as *Amblyseius andersoni* (Chant) and *Typhlodromus pyri* Scheuten.

Concerning field methods, a number of authors in Europe have tried to evaluate the side-effects of pesticides on phytoseiids in vineyards and orchards. Some experiments were carried out within a common programme in which different compounds were tested on a number of beneficial organisms. The results of these programmes allow interesting comparisons among different species (e.g. Hassan et al., 1991). In other cases, especially in viticulture, different procedures have been proposed (some of them have been reported in the Bulletins of the IOBC Working Group "Integrated pest control in viticulture"). In all these testing methods the pesticide effects are evaluated by comparing the phytoseiid densities on treated and untreated plots. Samplings are made at different dates before and after the treatments but their timing is quite different. However, the standardization of testing methods is needed especially in order to compare the results of tests on a species occurring in different regions. An attempt at this concerns *T. pyri* on the grapevine (Boller et al., 1988). As is well known, field tests are expensive and many factors can interact with negative implications in the interpretation of results. For example in the global effects of a pesticide, mortality effects cannot be distinguished from those linked to a strong reduction of fecundity.

The above-mentioned aspects have stimulated the proposal of laboratory or semi-field testing methods. One of the most clear advantage of laboratory testing methods is that both the mortality of different developmental stages and the fecundity of adult females can easily be measured. A test developed by Overmeer and Van Zon (1982) has been used by different authors in order to obtain data on the side-effects of a large number of pesticides on some susceptible and resistant strains of *T. pyri* and *A. andersoni* (Hassan et al., 1988, 1991). Concerning these species, this method was officially accepted by the IOBC Working Group "Pesticides and Beneficial Organisms" until 1992. In their latest bulletin (Hassan et al., 1992), however, a novel method is proposed. This method has the advantage of being universally applicable to all species of phytoseiids (Bakker and Calis, 1989; Bakker et al., 1992).

In Italy, the interest in the side-effects of pesticides on phytoseiids has for many years involved different crops like hazelnut (Ragusa, 1975), citrus (Viggiani, 1982), apple (Strapazzon and Dalla Montà, 1986; Duso et al., 1994), peach (Duso, 1985, 1992), grapevine (Duso and Girolami, 1985; Duso and Pavan, 1986; Duso et al., 1988). In most cases, the

experiments concerned *T. pyri* and *A. andersoni*. The effects of pesticides on the former species were studied on the grapevine while trials on *A. andersoni* were carried out on grapevine, peach and apple. Some years later a number of toxicological tests were made on these strains in the laboratory, using the Overmeer and Van Zon' method (Duso et al., 1992; Camporese et al., 1993).

In the present work the results of field and laboratory tests concerning insecticides are compared. A relation between them might reduce the amount of field tests by proposing sequential schemes (Bakker et al., 1992).

Materials and Methods

Phytoseiid strains used in the experiments

Field tests were carried out on 2 strains of *T. pyri* originating from vineyards in different areas of Northern Italy (Asti and Verona) but characterized by similar LC_{50}-values to parathion (Camporese and Duso, unpub. data). Parathion and methidathion were tested on the Verona strain (Duso, unpub. data) and the remaining compounds on the Asti strain (Duso et al., 1988). Laboratory tests were carried out on the Verona strain (Duso et al., 1992; Camporese and Duso, unpubl. data).

The *A. andersoni* strains originated from two orchards (apple and peach) and one vineyard of the Veneto region. Results concerning field experiments were taken from Duso and Pavan (1986) and unpublished data of the same authors for the grape strain, from Duso (1985, 1992) for the peach strain, from Strapazzon and Dalla Montà (1986) and Duso (unpubl. data) for the apple strain.

The laboratory tests were performed on the same strains (Duso et al., 1992).

Pesticides

Pesticides commonly used in vineyards and fruit orchards were chosen for toxicological tests. The following 12 insecticides were chosen for laboratory tests: acephate (150 g/hl of 42.5% a.i.), azinphos-methyl (200 g/hl of 25% a.i.), chlorpyriphos-methyl (200 g/hl of 25% a.i.), fenitrothion (150 g/hl of 48.5% a.i.), methidathion (250 g/hl of 19% a.i.), parathion (200 g/hl of 20% a.i.), pyridafenthion (200 g/hl of 40% a.i.), trichlorphon (300 g/hl of 40% a.i.), carbaryl (200 g/hl of 49% a.i.), methomyl (200 g/hl of 25% a.i.), deltamethrin (50 g/hl of 2.8% a.i.) and flucythrinate (50 g/hl of 10% a.i.). The number of insecticides used in field tests was variable. In some cases their concentration was slightly different.

Field testing method

Crops inhabited by naturally occurring predators were sprayed according to a statistical design (mainly randomized blocks). Samplings were carried out at intervals before and after (usually 10, 20 and 40 days) the treatments. Insecticides were applied only once in early or mid summer. Water treated control or harmless and harmful toxic standards were usually included. Population densities were monitored by counting eggs and active stages using a binocular; juveniles were distinguished from adults. Tests were made when the number of predators allowed a statistical analysis (e.g. at least 1 active form per leaf). The occurrence of prey was negligible or low. In the present work the effect of insecticides is classified under 4 classes (E) calculated by assessing the possible population reduction in the treated plots on the control 40 days after treatments:

Class 1: < 25%
Class 2: 25 - 50%
Class 3: 51 - 75%
Class 4: > 75%

The possible population reduction at 10 days after treatments has been included for a comparison.

Laboratory testing method

The method used was that described by Overmeer and Van Zon (1982). The effect of each pesticide was calculated by the following formula:

$E = 100\% - (100\% - M) \times R$

in which:

$M = ((Mt-Mc)/(100-Mc)) \times 100\%$

R = average egg production per treated surviving female divided by the average egg production per female in the control group.

The pesticides were classified on the basis of the following E-values:

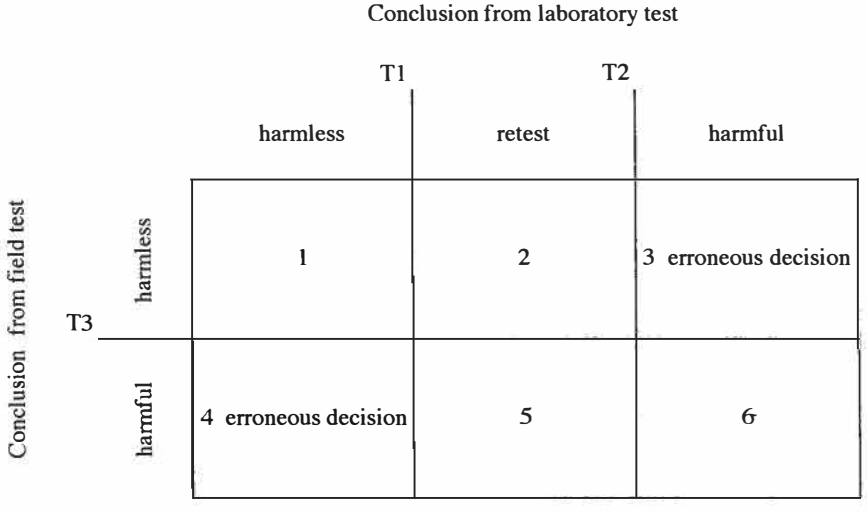
Class 1: < 30%
Class 2: 30% - 79%
Class 3: 80% - 99%
Class 4: > 99%

Comparison between field and laboratory methods

According to the sequential decision making scheme proposed for phytoseiids by Bakker et al. (1992) a compound is classified as harmless or harmful. In this scheme, laboratory tests have two possible outcomes, i.e. further field tests are necessary or not. The decision to stop testing can be made when a compound proves to be harmless or harmful in the laboratory. Using this scheme several data concerning field and laboratory testing results were plotted in a 2x3 design in which decision thresholds (T1, T2, T3) were defined according to practical experience (Fig. 1). In determining these thresholds, the correspondence between laboratory and field tests should be maximized and the number of erroneous decisions minimized.The variation of T1, T2 and T3 determines changes on the frequencies of erroneous decisions and on the ratio between frequencies in 1,3,4,6 vs. frequencies in 2+5.

In the present work the following decision thresholds (boundaries of the design) were chosen: T1=50%, T2=95% and T3=35%.

Figure 1: The sequential decision making scheme proposed for phytoseiids by Bakker et al. (1992).



Results

T. pyri (grapevine)

The insecticides used and the evaluation of their effects are reported in Table 1. Concerning field tests the possible reduction of phytoseiid densities is reported at 10 and 40 days after treatments but only the latter has been considered for the evaluation. Concerning laboratory tests, M and R values are reported.

In field tests the insecticide effects were classified within the whole range of categories while in the laboratories most of them fell in the 4th class. Parathion proved to be harmless in the field and slightly harmful in the laboratory. Fenitrothion obtained the same values (moderately harmful) in both situations. The effect of quinalphos, phosalone, chlorpyriphos-methyl and pyridafenthion was more severe in the laboratory (especially due to their effect on juvenile survival) than in the field. Methidathion and deltamethrin reached the same toxicity values.

Table 1: The results of field and laboratory tests on *T. pyri* are compared.

	Field test			Laboratory test			
Insecticide	T+10 d	T+40 d (E)	Class	M	R	E	Class
Parathion	10%	20%	1	46%	0.65	65	2
Fenitrothion	37%	52%	3	81%	0.17	97	3
Quinalphos	48%	30%	2	82%	0.00	100	4
Phosalone	48%	45%	2	100%	0.00	100	4
Chlorpyriphos-m.	68%	70%	3	100%	0.00	100	4
Pyridafenthion	98%	68%	3	100%	0.00	100	4
Methidathion	90%	85%	4	100%	0.00	100	4
Deltamethrin	100%	94%	4	100%	0.00	100	4

A. andersoni (grapevine)

None of the insecticides tested resulted as being harmless in the field (Table 2). Parathion reached low toxicity values (25%) confirming the harmlessness found in the laboratory. The effect of azinphos-methyl in the field was also slightly overestimated. In contrast, quinalphos and acephate effects proved to be more severe in the laboratory (especially due to their effect on juveniie survivai). The remaining compounds reached the same toxicity values in both situations.

Table 2: The results of field and laboratory tests on a strain of *A. andersoni* occurring on the grapevine are compared.

	Field test			Laboratory test			
Insecticide	T+10 d	T+40 d (E)	Class	M	R	E	Class
Parathion	33%	25%	2	0%	0.76	24	1
Azinphos-methyl	50%	52%	3	57%	0.63	73	2
Carbaryl	87%	62%	3	18%	0.17	85	3
Quinalphos	26%	46%	2	85%	0.54	92	3
Fenitrothion	76%	62%	3	78%	0.31	94	3
Chlorpyriphos-m.	65%	67%	3	81%	0.20	96	3
Acephate	59%	62%	3	100%	0.00	100	4
Methidathion	70%	82%	4	100%	0.00	100	4
Pyridafenthion	70%	83%	4	100%	0.00	100	4
Trichlorphon	74%	83%	4	100%	0.00	100	4
Deltamethrin	94%	90%	4	100%	0.00	100	4
Flucythrinate	100%	92%	4	100%	0.00	100	4

A. andersoni (peach)

The insecticide effects were classified within the whole range of toxicity classes both in the field and in the laboratory (Table 3). Parathion proved to be harmless in both situations and similar values (slightly harmful) were found concerning azinphos-methyl. Carbaryl, quinalphos, chlorpyriphos-methyl, phosalone and acephate were more toxic in the laboratory than in the field. The remaining compounds reached the same toxicity values in both situations.

Table 3: The results of field and laboratory tests on a strain of *A. andersoni* occurring on the peach are compared.

	Field test			Laboratory test			
Insecticide	T+10 d	T+40 d (E)	Class	M	R	E	Class
Parathion	0%	15%	1	15%	0.84	28	1
Azinphos-methyl	27%	32%	2	16%	0.50	58	2
Carbaryl	14%	33%	2	18%	0.17	85	3
Quinalphos	22%	30%	2	75%	0.60	85	3
Chlorpyriphos-m.	17%	30%	2	84%	0.42	93	3
Phosalone	40%	37%	2	100%	0.00	100	4
Acephate	42%	72%	3	100%	0.00	100	4
Trichlorphon	44%	82%	4	100%	0.00	100	4
Deltamethrin	94%	100%	4	100%	0.00	100	4

A. andersoni (apple)

Azinphos-methyl proved to be harmless in both situations and similar values (slightly harmful) were found concerning fenitrothion (Table 4). The effect of chlorpyriphos-methyl was more severe in the field while opposite results were found concerning methidathion.

Table 4: The results of field and laboratory tests on a strain of *A. andersoni* occurring on the apple are compared.

	Field test			Laboratory test			
Insecticide	T+10 d	T+40 d	Class	M	R	E	Class
Azinphos-methyl	12%	16%	1	6%	0.86	19	1
Chlorpyriphos-m.	88%	65%	3	21%	0.94	26	1
Fenitrothion	65%	46%	2	40%	0.81	51	2
Methidathion	16%	20%	1	71%	0.08	98	3

Discussion

Comparison between toxicity classes

In the comparison between the results of laboratory and field testing methods, 33 trials were involved. In 17 of them the same toxicological class was reached both in the field and in the laboratory tests. In 9 of the latter cases the classification fell in the 4th class, the remaining in the 1st, 2nd and 3rd classes.

In 13 of the total (33) trials the laboratory data overestimated the toxicity recorded in the field. This overestimation was due mainly to the high toxicity of pesticides on juveniles and in a few cases even to the reduction in fecundity. It has been shown that protonymphs (used at the beginning of laboratory tests) are more vulnerable than adult females (Overmeer and Van Zon, 1981). Therefore the pesticide impact in the field is affected by the population age-structure. In the case of *T. pyri* and *A. andersoni* the ratio between adults and juveniles during

summer periods, in which experiments were carried out, frequently reached values of 2:1 or 3:1. The lower toxicity values obtained for some compounds in the field, when compared to the laboratory, can also be influenced by the presence of refuges, particular leaf morphology or the exposure to non-lethal concentrations of chemicals. Another factor involved in the overestimation of pesticide effects can be linked to the irritant effects which cause predator mortality on the glue barrier.

In 3 of the total trials, field results were more severe than those obtained in the laboratory. However, in two cases (parathion and azinphos-methyl applied on *A. andersoni* on the grapevine) the toxicity values were slightly higher than the upper limit of the class. The third case concerns chlorpyriphos-methyl (applied on *A. andersoni* on the apple). The results obtained using this insecticide in the field might be explained by the high vapour tension of the compound. In the laboratory, phytoseiids were placed on treated Petri dishes in absence of ventilation. A better estimation of the toxicity of this kind of compound might be obtained by using the recent "coffin cell" method, in which ventilation is assured (Bakker et al., 1992).

An improvement in these results was attempted by considering the field toxicity as the mean between the initial toxicity (after 10 days from treatments) and persistence values. This calculation was recently proposed in apple orchards where fungicides, insecticides and acaricides were applied; the results appeared to be more realistic than considering persistence only (Duso et al., 1994). However, in the present comparison the correspondence between laboratory and field data was not improved.

The lack of close relations between laboratory and field toxicity classes for a number of compounds suggests that the use of an additional laboratory test, carried out on adult females (the so-called Test b), is also needed. Mortality effects will be more balanced and fecundity reduction well estimated. Additional data is needed on this topic in particular. With regard to insecticides, one of the main difficulties is to find compounds characterized by low toxicity. In the present work only a few products were classified in the 1st and 2nd classes in the laboratory despite the moderate to high resistance of these phytoseiid strains to some organophosphates.

Can the number of field trials be reduced by laboratory tests ?

One of the main objectives of this comparison should be to highlight definite correlations between field and laboratory results in order to reduce the use of the former and thus the relative costs.

A correlation between mortality in the laboratory (assessed after 7 days) and initial toxicity in the field (estimated after 10 days) might be an attempt to search for a procedure for sequential tests. Concerning *T. pyri*, a reduction in the population density of over 48% in the

sequential tests. Concerning *T. pyri*, a reduction in the population density of over 48% in the field after 10 days corresponded to a total mortality (mainly of protonymphs) in the laboratory. In the case of *A. andersoni* (peach), a reduction in the field of over 40% corresponded to 100% mortality in the laboratory. Unfortunately, the results obtained on *A. andersoni* on the grapevine and apple were not so clear.

Another correlation might involve the reduction in fecundity (measured on females developed from protonymphs exposed to fresh residues of pesticides) with the persistence effect in the field (assessed 40 days after treatments). However, a reduction in fecundity was observed for the majority of compounds. Concerning the 33 trials, the persistence effect was stronger than initial toxicity in 16 cases while the reverse was observed in 12 cases and no clear variations were recorded in 5 cases. In 7 of the 12 above-mentioned cases phytoseiid reimmigration from untreated plants can be retained important.

According to the sequential decision making scheme proposed by Bakker et al. (1992) we plotted the data (33 cases) in a 2x3 design in which boundaries (decision thresholds) were suggested from practice. A good fit between laboratory and field tests was found. Taking T1=50%, T2=95% and T3=35% we obtained a good compromise between reducing the number of compounds that need retesting (10) and the number of errors (3). Two errors concerned products considered as harmful in the laboratory but harmless in the field. The one exception was related to chlorpyriphos-methyl.

The present work deals with two phytoseiid species easily reared in the laboratory and thus a large number of predators is available for laboratory testing. It should be mentioned that other species, economically important in European vineyards or orchards, e.g. *Amblyseius aberrans* (Oudemans), are not reared so easily. Knowledge of the side-effects of pesticides on these species is retained important by advisory services. Therefore, field tests concerning these species still remain important.

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EFFECTS OF PESTICIDES ON THE PREDATORY MITE *TYPHLODROMUS PYRI* SCHEUTEN - A COMPARISON OF FIELD AND LABORATORY RESULTS

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Abstract

Investigations on the toxicity of several fungicides and acaricides on *Typhlodromus pyri* were carried out under field conditions. The results obtained from plant protection practice in grape plantations commercially used in Hungary and from small scale field tests were compared with results of IOBC Working Group "Pesticides and Beneficial Organisms" from laboratory and field tests.

The fungicide Rézoxiklorid 50 WP (a.i. 50 % copper oxychloride) and the acaricide Apollo 50 SC (a.i. 500 g/l clofentezin) were harmless, whilst the fungicide Dithane (a.i. 80 % mancozeb) was harmful to the predatory mite *T. pyri* in all type of tests. The fungicide Tilt 250 EC (a.i. 250 g/l propiconazole) is moderately harmful in the IOBC tests but it seems to be harmless in the small scale tests in Hungary. This might be be due to the lower concentration used in Hungary.

Introduction

T. pyri is the most abundant species among the predatory mites living in vineyards in Hungary (Dellei and Szendrei, 1988; Györffyné Molnár, 1986; 1987; 1990b). It was found that naturally occurring populations of *T. pyri* can be preserved by the use of an appropriate plant protection technology, i.e. timing of pesticide applications and suitable spraying techniques (Györffyné Molnár, 1990a; Englert, 1992) combined with choosing pesticides which are harmless to predatory mites (Hassan et al., 1983; 1986; 1988; 1991; Duso et al., 1992).

Material and Methods

Sampling and spraying

We determined the overwintering predatory mite population by dissection of 50 buds/ha, in February 1992 due to decide on a table for experiments. During the growing period of grape, from the growth stage "two leaves" until the falling of leaves, we collected 4 x 25 leaves weekly in general and counted the adults and nymphs of predatory mites on them in the laboratory.

In 1992 the farmers used only fungicides (Table 1) on the chosen plantation, which was 16 ha large. The spraying volume was 1000 l/ha. The fungicides were applied by means of a NOVATUR 1507-type spraying machine.

Table 1. Fungicide combinations and their application time in 1992

1. 27.05.1992	Dithane M45 (80 % mancozeb)	1.5 kg/ha
	Karathane LC (350 g/l dinocap)	0.3 l/ha
2. 07.06.1992	Mikal 75WP	
	(50% fosetil-AL + 25% folpet)	4 kg/ha
	Tilt 250 EC	
	(250 g/l propiconazole)	0.2 l/ha
3. 23.06.1992	Mikal 75WP	
	(50% fosetil-AL + 25% folpet)	4 kg/ha
	Tilt 250 EC	
	(250 g/l propiconazole)	0.2 l/ha
	Thiovit (80% sulfur)	3 kg/ha
4. 20.07.1992	Dithane M45 (80 % mancozeb)	1.5 kg/ha
	Szulfur 900 FW (900 g/l sulfur)	3 kg/ha
	Karathane LC (350 g/l dinocap)	0.3 l/ha
5. 30.07.199	Rézoxiklorid 50 WP	
	(50% copper oxychloride)	4 kg/ha
	Szulfur 900 FW (900 g/l sulfur)	3 kg/ha
	Karathane LC (350 g/l dinocap)	0.3 l/ha
6. 22.08.199	Rézoxiklorid 50 WP	
	(50% copper oxychloride)	4 kg/ha
	Szulfur 900 FW (900 g/l sulfur)	3 kg/ha

We chose small scale plots (45 m^2) from the same plantation to test acaricides on the natural population of *T. pyri* a year later in 1993. The number of nymphs and adults of predatory mites were counted on 5x10 leaves one day before pesticide application and at the 7th, 14th and 29th days after spraying.

The pesticides were used in 4 replicates according to following doses (l/ha):

Andalin DC-25	(250 g/l flucycloxuron)	0,6 l/ha
Apollo 50 SC	(500 g/l clofentezine)	0,4 l/ha
Neoron 500 EC	(500 g/l bromopropylate)	1,5 l/ha
Omite 57 E	(570 g/l propargite)	1,2 l/ha

Interpretation of results

We made a comparison between fungicide combinations applied and the changes of the predatory mite population before and after spraying. We used IOBC categories (Hassan et al., 1991) to compare the toxicity of the pesticides. Laboratory and field data for the comparison were

obtained from the publications of IOBC Working Group "Pesticides and Beneficial Organisms" (Hassan et al., 1983; 1986; 1988; 1991).

Results and Discussion

T. pyri composed more than 80% of the overwintering populations of the predatory mites. Among the phytophagous mites *Eryophies vitis* (13%) and *Tydeus californicus* (6%) were the most abundant species in 1992. The populations of this prey mites remained low throughout the growing season. Field observations showed that *T. pyri* has a high tolerance to low prey densities, as it can use pollen and plant juices alone to complete its development (Eichhorn and Hoos, 1990)

The number of predatory mites (*T. pyri)* was reduced significantly following the 1st (Dithane M45 + Karathane LC) and 4th (Dithane M45 + Karathane LC + Sulfur 900FW) spraying in the large scale field test. The 5th spraying included also the fungicide Karathane LC beside Sulfur 900 FW and Rézoxiklorid 50 WP but did not reduce the number of *T. pyri* (Table 2).

The increase in foliage is the most intensive from middle of May until flowering of the vine. This phenophase of the vine started at the time of the 2nd spraying. For this reason it is difficult to separate the effect of increase in foliage from that of the pesticide application with regard to the decrease of the mite population following the 1st spraying. The predatory mite population stabilized after the 2nd pesticide application and decreased significantly only after the 4th spraying in which the pesticide combination was nearly the same as in the 1st one (Dithane M45 + Karathane LC + Sulfur 900 FW).

We may conclude by the comparison of changings in the predatory mite population (Table 2) with the fungicide combinations applied (Table 1), that Dithane M45 must be the toxic component of the combinations. The IOBC results also confirm this observation (Table 4)

There was a slightly reduction in the number of predatory mites after the 2nd and 3rd spraying. The fungicide Mikal 75 WP (50% fosetil-AL + 25% folpet) was the dominant component in both combinations applied. Among the active ingredients of the fungicide Mikal 75 WP folpet (Ortho-Phaltan) was tested by the IOBC Working Group and it was found to be harmless to predatory mites (Hassan et al., 1988). The other component (fosetil-AL) as well was found to be harmless in the field (Boller et al., 1989).

It is important to know not only the toxic effect of the active ingredients of the pesticides but those of their isomers, too. For example, the active ingredient of the fungicide Karathane (dinocap) has two isomers and one of them is a typical acaricide (Worthing and Hance, 1991). Different countries use different formulations for the same active ingredients and this may lead to different results in the toxicity tests.

According to IOBC laboratory testing Thiovit is moderately harmful (category 3) for *Amblyseius finlandicus* (Hassan et al, 1983). We consider it as slightly harmful (category 2) to *T. pyri* in the field, if we suppose that this is the toxic component of the combination and not the fungicide Mikal 75 WP.

The fungicide Tilt 250 EC was moderately harmful to *A. finlandicus* both in laboratory and field tests carried out by the IOBC Working Group, but it seems to be harmless in our field evaluation (Table 4). This can be due to the lower concentration (0,02 %) used in Hungary, whilst it was tested in a higher concentration (0,08 %) by the IOBC.

Table 2. Changes in the mite population during the vegetation period in large-scale plot experiment (16 ha) in 1992

Date 1992	Number of mites on 100 leaves Prey	*T. pyri*	Number of fungicide application
Apr. 29	153	2	
May 07.	18	1051	
May 14.	19	788	
May 25.	33	204	
May 27.			1.
June 04.	4	94	
June 07.			2.
June 11.	6	75	
June 22.	11	62	
June 23.			3.
June 30.	2	39	
July 08.	1	43	
July 20.			4.
July 22.	0	29	
July 30.	0	3	5.
Aug. 06.	13	41	
Aug. 11.	3	17	
Aug. 22.			6.
Aug. 26.	5	35	
Sept.01.	3	37	
Sept.09.	8	32	
Sept.15.	4	98	

Table 3. Effect of acaricides on *T. pyri* in small-scale plot experiments in 1993. Number of *T. pyri* on 5 x 10 leaves/replicates

Treatment	Dose l/ha	Before Spraying	After Spraying 7th	14th	29th day
Omite 57E	1.2	77 ± 2	70 ± 23	29 ± 11	97 ± 9
Apollo 50 SC	0.4	71 ± 14	109 ± 9	96 ± 16	109 ± 18
Andalin DC 25	0.6	32 ± 8	67 ± 11	46 ± 14	50 ± 14
Neoron 500 EC	1.5	82 ± 10	16 ± 9	11 ± 7	37 ± 11
Untreated		75 ± 12	112 ± 10	95 ± 18	189 ± 12

Table 4. Comparison of toxicity data obtained in different tests.

Pesticide	IOBC results lab.	IOBC results field	Hungarian field results large-	Hungarian field results small-scale
Dithane M45 [a]	4	4	4	-
Karathane LC	-	-	1	-
Mikal 75 WP	-	-	1?	-
Szulfur 900 FW	-	-	1	-
Thiovit	3[c]	-	2?	-
Tilt 250 EC	3	3[c]	1?	-
Rézoxikloríd 50 WP [b]	1	1	1	-
Andalin DC-15	-	-	-	1
Apollo 50 SC	1	1	-	1
Neoron 500 EC	-	-	-	4
Omite 57 E	-	-	-	1

[a] = Dithane Ultra (80% mancozeb) was tested by IOBC.
[b] = Vitigran (45% copper oxychloride) was tested by IOBC.
[c]= *Amblyseius finlandicus* was in the IOBC test.
? = The pesticide needs further investigations.

Among the acaricides tested in small-scale plots the results with Apollo 50 SC are consistent with those of IOBC Working Group. This acaricide was found to be harmless to predatory mites in all type of tests. We have found harmless, too, the acaricides Andalin DC-25 (250 g/l flucycloxuron) and Omite 57 E (570 g/l propargite). Neoron 500 EC (500 g/l bromopropylate) was harmful in our test on *T. pyri*.

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TESTING THE SIDE EFFECTS OF PESTICIDES ON THE PREDATORY MITE *TYPHLODROMUS PYRI* (ACARI, PHYTOSEIIDAE) IN FIELD TRIALS

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Abstract

Predatory mites, especially the species *Typhlodromus pyri* (Oudemans), are widely used for the control of the red spider mite, *Panonychus ulmi* (Koch) and the apple rustmite, *Aculus schlechtendali* (Nalepa), in apple orchards. Therefore, it's necessary to test the side effects of pesticides on this important antagonist. Laboratory and semi-field trials have their limits, especially in the case of testing the side-effects of cumulated treatments. That's one of the main reasons why there's a growing interest for field trials on this important beneficial arthropod, simulating the effects in practice. These are done in realistic situations, by applying not only the highest dose rates, but also in the case of fungicides by spraying a number of cumulative treatments of the same or of differents compounds, according to the maximum possible number of applications that might be used by fruitgrowers. During the last four years, a number of experiments in field trials has been carried out in Belgian orchards.

1. Introduction

Integrated Pest Management (IPM) is now getting more and more the standard system in apple orchards in most European countries. Depending on the specific and local problems the accent is put on the reduction of broad spectrum pesticides, the application of selective and/or biological compounds, the use of biotechnics, the introduction of antagonists, mainly predatory mites, frequent monitoring, the protection of a specific and typical fauna in the orchard to create a more "stable" system, or, in most cases, a combination of these measures.

Some countries are introducing strains of *Typhlodromus pyri*, which are more or less resistant to organophosphates and carbamates, sometimes even to sulphur. Other countries or regions stimulate the introduction of sensitive strains of the same species of predatory mite to force the fruitgrower to be careful with his applications against diseases and insects. Introduction in the orchards takes place by putting out infested shoots in the summertime, or by attaching strips of textile with high numbers of hibernating females in the winter or early spring.

1.1 General considerations

Because of this widely use of *Typhlodromus pyri*, it's obvious that the side effects of old and new compounds must be tested on this antagonist. Until a few years ago, these tests were mainly done on glassplates in laboratory, according to the test methods of the IOBC group "Pesticides and Beneficial Organisms" (Hassan (1985), Hassan (1988), Hassan (1992)). These are very hard tests on what is supposed to be the most sensitive stage of the predatory mite. If no hazard is

recognized, this allows the prediction of no/very low risk in the environment with a high level of certainity. However, this approach leads to specific **problems**:

a) The most sensitive stage of an organism is often depending on the mode of action of the compound.
b) The given food is untreated.
c) Both sexes may react different to the compound.
d) Cumulative effects of the repeated use of a compound, or the cumulative effects of the alternation of two or more compounds, can not be predicted by this method.
e) Behaviour might be affected or some other sublethal effects might pass unnoticed.
f) Pesticides with a difficult mode of action like entomophagous fungi, pre-insecticides or growth regulators are not easily tested this way.

This explains the growing interest for field trials on this predatory mite, but also for other important beneficials, simulating the use of the compound in practice. This is always done in realistic situations, by applying the highest dose rate, but also in the case of fungicides by spraying a number of cumulative treatments of the same or differents compounds, according to the number of applications used by the fruitgrowers.

This approach has great **advantages** compared with laboratory or semi-field trials:

a) Applications can be made at the same moment or at the same phenological stage of the crop as will be done in practice.
b) Field tests offer the chance to detect sublethal or indirect effects.
c) The duration of possible pesticide effects and the rapidity of recovery can be observed.
d) Prey/predator ratio's might be taken into account.
e) Cumulative treatments of the same or different compounds, according to GAP (good agricultural practice) is possible.
f) Field trials are relevant, carried out under realistic conditions and allow risk assesment.

Nevertheless, field trials also show some considerable **disadvantages** and difficulties:

a) The population of beneficials in an orchard or a field is often very heterogeneous. Sampling before the treatment is therefore essential.
b) The population of beneficials is often very heterogeneous within the tree or plant. This causes problems for adequate sampling.
c) The densities of beneficials in the field are often very low, leading to plots of a very big size.
d) Experiments are sometimes carried out for a longer period, which might give confusion, e.g. in the case of recolonisation.
e) Sensitivity to a compound may differ between fields, even within one region, according to the origin of the introduced beneficials or previous used spraying programs. In long terms, this also leads to the fact that previous obtained data on e.g. toxicity of a compound to predatory mites,

may not be correct anymore, due to changes in the sensitivity of the population during the last years.

f) Due to the unique situation with respect to geographical location, species communities, changing agricultural practices like pesticide use patterns, and particulary weather conditions, field trials allow poor reproducibility.

g) Field tests are vulnerable to disruption by adverse weather conditions.

h) The choice of spraying equipment or technique may influence the results. Mistblowing for instance may give different results than spraying, even if the same amount of product is used per ha.

i) Even the same stage of a beneficial may have a different sensitivity for a certain compound on different moments in the season, e.g. the sensitivity of adult females of *Typhlodromus pyri* may differ between spring, summer and autumn.

j) The results are sometimes difficult to interpret.

1.2 Differences between environmental fate and selectivity

In all European countries, according to EC directive 91/414, a committee of scientists, specialised in different aspects of ecotoxicology, studies the effects of old and new molecules on the environment and on the beneficial organisms that are important as plant protection agents. This might give cause to some contradictions. Even in the case of a very high selectivity for important beneficials, some new compounds will not reach the market or only under very strict conditions because of disadvantages for the environment. IPM is a rational plant protection system, based on anti-resistance strategies (Sterk and Highwood, 1992) and building up a more "stable system", based on multi-species ecosystems, but it is not always synonymous with complete environmental safe systems. With regard to compounds that are not selective for certain important antagonists, they may be allowed within IPM, however very restrictively, if an alternative doesn't exist. Hereby the predator/prey ratio should be considered. If a product has some toxic effect on predatory mites, but also shows activity on noxious mites, it still may fit in an IPM program. Harmful compounds might be used on moments when important beneficials like anthocorids and phytoseiids are not abundant or not active, e.g. in wintertime or early in the season, or by a way that they are not affected, e.g. trunc treatments.

2. Side-effects of pesticides on beneficial mites

2.1 Standard methods to test the side-effects of pesticides on beneficials organisms

Members of the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" have developed standard guidelines to test the side-effects of pesticides on beneficial organisms. There was agreement in this group that a combination of laboratory, semi-field and field tests is needed to show the side-effects of pesticides on beneficial organisms and that the beneficials chosen for the test should be relevant to the crop on which the pesticide is to be used.

2.2 Sequentiel testing schemes

Recognizing that no single test method would provide sufficient information to show the side-effects of a pesticide on a beneficial organism, a combination of tests that includes laboratory, semi-field and field methods to be carried out in a particular sequence is recommended by the

IOBC/WPRS Working Group. Pesticides found to be harmless to a particular beneficial in the laboratory test are supposed to be also harmless to the same organism in the field and no further testing in semi-field or field experiments is therefore required. This approach will be taken over in the frame of European regislatury testing requirements.

2.3 Classification for field trials

According to the principles of the IOBC Working Group "Pesticides and Beneficial Organisms" four evaluation categories (% mortality or reduction in beneficial capacity) are used: 1 = harmless (< 25 %), 2 = slightly harmful (25 - 50 %), 3 = moderately harmful (51- 75 %) and 4 = harmful
(> 75 %). As mentioned before, harmful doesn't mean the product may not be used at all in IPM but if yet only with restrictions. The mode of action of the compound and a carefully chosen moment of application may fit within IPM-schemes.

3. Side-effects of pesticides in field trials on beneficial mites in orchards in Belgium

3.1 Introduction

Typhlodromus pyri (Oudemans) and *Euseius finlandicus* (Oudemans) are the most common predatory mites in orchards in Belgium. Due to the high densities on sour cherry trees and its susceptibility for pesticides, *Euseius finlandicus* provides good material for testing side-effects of pesticides in field trials. More important however is the apple predatory mite, *Typhlodromus pyri*. Most of the trials on beneficial mites are now carried out on this antagonist.

3.2 Methods and material

3.2.1 General considerations

The trials are realized on trees with a sufficient number of predatory mites. At least a mean value of 1 predatory mite on 2 leaves is required. Relatively young bush trees are very suitable for such trials as they are usually left untreated by the growers. It's best to choose orchards where the predatory mite has been introduced in the last years. Because of the abundance of spider mites and apple rustmites the densities of the predatory mite will be much higher in these recently infested orchards than in stabilised plots.

All predatory mites in the testing orchards are from the same origin, an organophosphates and carbamates resistant strain from the Netherlands.

3.2.2 Description of the experiment: single applications

Trees with a sufficient population of predatory mites are selected and the number of predatory mites is precounted on 50 leaves. The numbers of possible preys, in this case *Panonychus ulmi* (Koch), *Tetranychus urticae* (Koch), *Tydeus* spec. and eriophiyds, mainly the apple rustmite, *Aculus schlechtendali* (Nalepa), are also recorded to avoid host population density influences. The trees are sprayed with the test compound by means of a knapsack mist blower until run-off. The control plot is treated with water. After +/- 1 and/or 2 weeks after the treatment the remaining mobile stages of predatory mites and possible preys are counted again on 50 leaves. The results are calculated with the Henderson-Tilton formula and divided into four categories corresponding

to the set standard for field methods of the IOBC Working group "Pesticides and Beneficial Organisms".

$$\text{Henderson - Tilton WG \%} = 100 \times \left(1 - \left(\frac{K1}{K2} \times \frac{R2}{R1}\right)\right)$$

K1 = Total number of target species before treatment in the control plot
K2 = Total number of target species after treatment in the control plot
R1 = Total number of target species before treatment in the test plot
R2 = Total number of target species after treatment in the test plot

3.2.3 Description of the experiment : multiple applications

Fungicides are generally more selective than insecticides or acaricides, but because of their repeated use the negative effects are cumulated. Dose rate, period and frequency of the treatments are important factors in the selectivity. Essential is also the predator/prey ratio for those fungicides which also have acaricidal activity. A distinction should be made between those with effects on predatory mites, but also on noxious mites, and those who are only toxic for predatory mites. Examples for the first group are for instance sulphur and tolylfluanide.

Like in the trials with single applications, trees with sufficient numbers of predatory mites are selected and the number of predatory mites is precounted on 50 leaves. The numbers of possible prey are also recorded to avoid host population density influences and to check acaricidal activities of fungicide treatments.

The trees are sprayed with the test compound by means of a knapsack mist blower at 300 liter per ha. The control plot is treated with water. In most trials the remaining mobile stages of predatory mites and possible preys are counted again after 2 treatments on 50 leaves. This is repeated after 4 treatments, in some trials also after 6 treatments if the number of predatory mites was still high enough. In a large scale trial in Zelk in 1993, the numbers of predatory mites were even counted in 1994 in spring. In one trial in 1993 in Kozen, assessments were made after 1, 2 and 3 applications.

The results are each time calculated with the Henderson-Tilton formula and divided into four categories corresponding to the set standard for field methods. These are compared with the results obtained in the laboratory tests of the Joint Pesticide Testing Programmes carried out by the IOBC Working group "Pesticides and Beneficial Organisms", if available, after a single treatment of the resistant strain of *Typhlodromus pyri*. The formulations and dose rates in these trials are not always the same as used in the field trials.

In this article only the results of multiple applications with the same compound are discussed. The results of the trials on predatory mites with alternations of different fungicides will be published in the future.

4. Results

4.1 Single applications

The results are the final results of trials, carried out in 1990, 1991, 1992 and 1993 in different orchards on different apple varieties on different moments. Most trials were carried out in late summer or early autumn, when the densities of predatory mites were at a maximum.

4.1.1 Acaricides

Trade name	Active ingredient	Formu-lation	Dose % formulated compound	IOBC category field	IOBC category lab.
Massai	tebufenpyrad	20 WP	0.025	2/3	
			0.050	4	
Naja	fenpyroximate	050 SC	0.050	1	
			0.080	1/2	
			0.160	4	
Magister	fenazaquin	200 SC	0.075	3	
			0.100	4	
Sanmite	pyridaben	150 EC	0.050	4	
Sirbon	brofenprox	100 EC	0.010	4	
			0.020	4	
Torque	fenbutatinoxide	055 SC	0.045	1	
Talstar	bifenthrin	100 EC	0.030	4	
Rufast	acrinathrine	150 EC	0.020	4	

4.1.2 Insect growth regulators

Trade name	Active ingredient	Formu-lation	Dose % formulated compound	IOBC category field	IOBC category lab.
Cascade	flufenoxuron	100 WDC	0.050	1	
Andalin	flucycloxuron	25 L	0.050	1	
Match	lufenuron	050 EC	0.100	1	
Admiral	pyriproxifen	100 EC	0.100	1	

4.1.3 Insecticides

Trade name	Active ingredient	Formu-lation	Dose % formulated compound	IOBC category field	IOBC category lab.
Confidor	imidacloprid	70 WG	0.007	1	
		70 WG	0.014	1	
		200 SL	0.075	1	
Chess	pymetrozine	25 WP	0.080	1	
Deltanet	furathiocarb	400 EC	0.030	2/3	
Zolone Flo	fosalone	500 Flow	0.120	2	
Kilval	vamidothion	400 EC	0.120	3	4
Disonex	diazinon	162 EC	0.180	1	1

4.1.4 Discussion

From the new group acaricides, only fenpyroximate shows a good selectivity for *Typhlodromus pyri* (Oudemans). Tebufenpyrad, fenazaquin, brofenprox and acrinathrine are all rather toxic in these trials, like the toxic standard bifenthrin. .

The insect growth regulators, even the strong acaricides flufenoxuron and flucycloxuron, have no toxic effect at all on the predatory mites.

Imidacloprid, which will be registrated under several trade names like Confidor, Admire and Orbit, is harmless for *Typhlodromus pyri* (Oudemans). The first days after the treatment however, some of the predatory mites seems paralyzed, but they soon recover. Pymetrozine, another systemic aphicide, is also harmless.

As well known, there's a strong gradient in toxicity between organophoshates, going from vamidothion being more toxic, to diazinon which is almost harmless.

A single treatment with carbamates like furathiocarb is also possible.

4.2 Multiple applications with fungicides

As mentioned before, only the results of multiple applications with the same compound will be discussed. Trials on predatory mites with alternations of different fungicides during the whole season have been carried out, but will be discussed in the future.

Also some trials have been made to see the impact of the mode of application, namely the differences in toxicity for predatory mites between mistblowing and spraying of the same amount of active ingredient of tolylfluanide and thiram per ha, but the research is not yet finished.

4.2.1 Kozen I

This trial was carried out in 1992 in an apple orchard in Kozen. The predatory mites were introduced in 1991. Six treatments were carried out with a mistblower at 300 liter per ha. The number of predatory mites in the plots were counted before the treatment and after 2, 4 and 6 treatments.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Euparen M	tolylfluanide	50 WG	1125
Euparen	dichlofluanide	50 WG	1125
Microsulfo	sulphur	80 WG	2400
Delan	dithianon	750 SC	562
Orthocide	captan	83 WP	1660
Orthocide + Aseptaman	captan + manganese	83 WP	1660 + 120
Orthocide + Pomarsol	captan + thiram	83 WP 80 WG	1120 + 800
Orthocide + Dithane M 22	captan + maneb	83 WP 80 WP	1120 + 800
Dithane M 45	mancozeb	80 WP	2400
Dithane M 22	maneb	80 WP	2400
Thianosan	thiram	80 WG	2400
Polyram combi	metiram	80 WG	2400

Trade name	IOBC category after 2 treatments	IOBC category after 4 treatments	IOBC category after 6 treatments	IOBC category laboratory
Euparen M	2	1	1	
Euparen	1	1	1	2
Microsulfo	1	2	1	3
Delan	1	1	1	1
Orthocide	1	1	1	
Orthocide + Aseptaman	1	1	1	
Orthocide + Pomarsol	1	2	1	
Orthocide + Dithane M 22	2	2	2	
Dithane M 45	2	3	3	4
Dithane M 22	3	3	3	4
Thianosan	1	1	1	4
Polyram combi	1	3	2	4

4.2.2 Kozen II

This trial was carried out in 1992 in an apple orchard in Kozen. The predatory mites were introduced in 1991. Four treatments were carried out with a mistblower at 300 liter per ha. The number of predatory mites in the plots were counted before the treatment and after 2 and 4 treatments.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Euparen M	tolylfluanide	50 WG	750
Euparen M	tolylfluanide	50 WG	1125
Orthocide	captan	83 WP	1432
Thianosan	thiram	80 WG	2400
Thionic	ziram	76 WG	2280

Trade name	IOBC category after 2 treatments	IOBC category after 4 treatments	IOBC category laboratory
Euparen M 750	1	2	
Euparen M 1125	2	2	
Orthocide	2	2	
Thianosan	2	3	4
Thionic	1	3	

4.2.3 Zelk

This trial was carried out in an apple orchard in 1993 in Zelk. The predatory mites were put in the orchard in summer of 1992. Six treatments were carried out with a mistblower at 300 liter per ha. The number of predatory mites in the plots were counted before the treatment and after 2, 4 and 6 treatments, and in the spring of 1994.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Euparen M	tolylfluanide	50 WG	750
Euparen M	tolylfluanide	50 WG	1125
Microsulfo	sulphur	80 WG	2400
Orthocide	captan	83 WP	1660
Dithane M 45	mancozeb	80 WP	2400
Thianosan	thiram	80 WG	2400
Polyram combi	metiram	80 WG	2400

Trade name	IOBC cat. after 2 treatments	IOBC cat. after 4 treatments.	IOBC cat. after 6 treatments	IOBC cat. 05/ 1994	IOBC cat. 06/ 1994	IOBC cat. lab.
Euparen M 750	1	1	1	1	2	
Euparen M 1125	1	3	2	3	2	
Microsulfo	1	1	1	2	2	3
Orthocide	1	1	1	1	1	
Dithane M 45	2	3	3	3	4	4
Thianosan	1	2	3	3	2	4
Polyram combi	2	2	3	3	4	4

4.2.4 Kozen III

This trial was carried out in an apple orchard in 1993 in Kozen. The predatory mites were introduced in the orchard in the summer of 1991. Three treatments were carried out with a mistblower at 300 liter per ha with a weekly interval. The number of predatory mites in the plots were counted before the first treatment and after each treatment.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Euparen M	tolylfluanide	50 WG	1125
Bavistin	carbendazim	50 WP	375
Orthocide	captan	83 WP	1660
Nustar	flusilazole	20 DF	45
Topsin M	thiofanaat-methyl	70 WP	1275
Ronilan	vinclozolin	500 SC	750
Thianosan	thiram	80 WG	2400

Trade name	IOBC cat. after 1 treatment	IOBC cat. after 2 treatments	IOBC cat. after 3 treatments	IOBC cat. laboratory
Euparen M	1	1	1	
Bavistin	1	1	1	
Orthocide	1	1	1	
Nustar	1	1	1	
Topsin M	1	1	1	

Ronilan	1	1	1	
Thianosan	1	1	1	4

4.2.5 Kozen IV

This trial was carried out in an apple orchard in 1993 in Kozen. The predatory mites were introduced in the orchard in the summer of 1991. Four treatments were carried out with a mistblower at 300 liter per ha with a weekly interval. The number of predatory mites in the plots were counted before the first treatment and after the 2nd and 4th treatment.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Polyram Combi	metiram	80 WG	2400
Thionic	ziram	76 WG	2280
Orthocide	captan	83 WP	1660
Pallitop	nitrothal-isopropyl + metiram	48 + 3.2 WP	360 + 24
Pallitop	nitrothal-isopropyl + metiram	48 + 3.2 WP	720 + 48
Dithane M 45	mancozeb	80 WP	2400
Thianosan	thiram	80 WG	2400
Thianosan + Dithane M 45	thiram + mancozeb	80 WG 80 WP	1200 + 1200
Thianosan + Orthocide	thiram + captan	80 WG 83 WP	1200 + 830

Trade name	IOBC cat. after 2 treatments	IOBC cat. after 4 treatments	IOBC cat. laboratory
Polyram Combi	1	2	4
Thionic	1	3	
Orthocide	1	1	
Pallitop	1	1	
Pallitop	1	1	
Dithane M 45	1	3	4
Thianosan	1	1	4
Thianosan + Dithane M 45	1	3	
Thianosan +Orthocide	1	2	

4.2.6 Kozen V

This trial was carried out in an apple orchard in 1993 in Kozen. The predatory mites were introduced in the orchard in the summer of 1991. Four treatments were carried out with a mistblower at 300 liter per ha with a weekly interval. The number of predatory mites in the plots were counted before the first treatment and after the 2nd and 4th treatment.

Trade name	Active ingredient	Formulation	Gram a.i. per ha
Euparen	dichlofluanide	50 WG	1125
Rubigan	fenarimol	120 EC	60
Trimidal	nuarimol	090 EC	90
Topaz	penconazole	100 EC	30
Delan	dithianon	750 SC	562
Rovral	iprodione	500 SC	1000

Trade name	IOBC cat. after 2 treatments	IOBC cat. after 4 treatments	IOBC cat. laboratory
Euparen	1	1	2
Rubigan	1	1	1
Trimidal	1	1	1
Topaz	1	1	1
Delan	1	1	1
Rovral	1	1	1

4.2.7 Discussion

As was to be expected the classical contactfungicides against scab, captan and dithianon, were not or only slightly toxic for the predatory mite, *Typhlodromus pyri* (Oudemans), even if applied 3 times or more. The dicarboximiden iprodione and vinclozolin, which were used against *Botrytis* at the end of the flowering and before harvest to prevent fruit rot in storage, are also selective for this predatory mite. All these fungicides can be used without any restriction in (belgian !) IPM apple orchards. Also the DMI-fungicides flusilazole, fenarimol, penconazol and nuarimol are harmless or only slightly toxic. The difference in selectivity between the benzimidazols thiofanat-methyl and carbendazim, which is sometimes mentioned, is not proved in these trials. Among the dithiocarbamates mancozeb, maneb and ziram have the highest toxicity for *Typhlodromus pyri* (Oudemans). After 4, sometimes even after 2 treatments, class 3, meaning moderately harmful, is already reached. Metiram and especially thiram are less toxic. In an orchard with a high density of predatory mites, 4 yearly applications are certainly possible.

In contrast with data from other countries, sulphur, tolylfluanide, certainly the lower dose rate, and dichlofluanide seem to be rather harmless for *Typhlodromus pyri* (Oudemans) in Belgian orchards. Four applications with these fungicides each season, are tolerable. This number might

even be higher because they also show considerable miticidal activity. In contrast is the fact that the same formulation of sulphur was very toxic for this species of predatory mite, even applied only once, in Swiss vineyards (6th Joint Testing Program, IOBC, Entomophaga, In press). As mentioned earlier (c.f. 1.1) we have to be aware that differences in sensitivity to the same compound occur between predatory mite strains mainly due to pretreatment effects.

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SIDE-EFFECTS OF PESTICIDES ON THE PARASITIC WASP *ENCARSIA FORMOSA*: COMPARISON OF RESULTS FROM LABORATORY TESTING METHODS WITH PRACTICAL EXPERIENCES IN GLASSHOUSE VEGETABLES.

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Abstract

A number of conventional pesticides (insecticides, fungicides, acaricides) and some insect growth regulators were tested in the laboratory to evaluate their side-effect on the parasitic wasp *Encarsia formosa*. The validity of the lab results for prediction of the compatibility with biological control of the greenhouse whitefly, *Trialeurodes vaporariorum*, was checked.

Introduction

Glasshouse experiments to assess the side-effects of pesticides on beneficial organisms are time consuming, expensive and sometimes very difficult to interpret. Therefore laboratory testing methods were developed for *E. formosa* (Oomen, 1985). A number of tests form the components of a sequential decision making scheme. (Fig. 1). This scheme involves a residual contact test (test 1), in which one day old adult parasitoids are exposed to a fresh dry residue on glass plates in the laboratory, a direct contact test (test 2) in the laboratory, in which mature pupae stuck on paper are sprayed with the test compound; a residual contact persistence test (test 3) in which one day old adults are exposed to naturally aged residues on leaves in the laboratory, and a glasshouse test (test 4) in which the effect of the test compound sprayed on whole plants in the glasshouse, with a settled population of the greenhouse whitefly and *E. formosa* is compared with the effect of 2 reference compounds.

The effect on *E. formosa* of a number of pesticides, of which a majority relevant for use in glasshouse vegetable crop protection, tested in sequential lab tests, was compared with the practical experiences with the same pesticides on *Encarsia* in glasshouses. The lab tests were carried out by ourselves, and where mentioned, by Oomen; the practical experiences with the pesticides in glasshouses were given by producers of biocontrol agents (Biobest BVBA, Ciba Bunting, Koppert BV).

By comparing laboratory testing methods with glasshouse experiences, the reliability of the former methods for predicting compatibilities of pesticides with whitefly biocontrol can be shown.

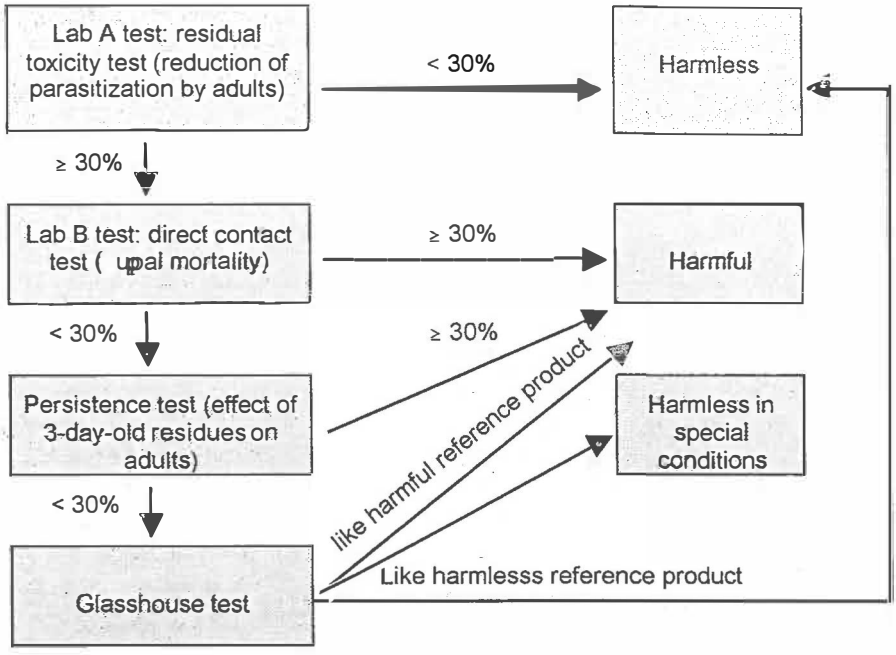

Fig. 1. Sequential testing scheme for *Encarsia formosa*.

Materials and methods

The laboratory testing methods, in agreement with the IOBC Guidelines (Hassan, 1985) and described by Oomen (1989) were used. The test compounds are formulations which are commercially available in many European countries. For each product, the highest recommended concentration for practical application, was tested.

Test insects originated from the Koppert BV company, The Netherlands. They were reared on tobaccoplants in the laboratory.

Results and discussion

The side-effects of pesticides in the laboratory test 1 are categorized in 4 evaluation categories numbered from 1 to 4, no. 1 being the harmless (parasitization reduction less than 30%), no.2 the slightly harmful (parasitization reduction between 30 and 79%), no.3 the moderately harmful (parasitization between 80 and 99%) and no.4 the harmful products (parasitization reduction > 99%). In test 2, the same evaluation categories are used, but in this case the eclosion reduction of wasps from the black scales is used instead of the parasitization capacity. In test 3 (persistence), 4 evaluation categories based on mortalities, are used. The critical

level in the sequential scheme (Fig. 1) is 30%.

The glasshouse experience is expressed as compatible or incompatible with *E. formosa,* as given by the biocontrol companies Biobest, CibaBunting and Koppert.

The laboratory data (Table 1) concerning test 1 can be separated in 2 distinct categories: the evaluation category no. 1 and the no. 4 categories. All no. 1 compounds are feasible for IPM in glasshouse vegetables. The majority of the compounds scoring evaluation category no.4 are not usable in IPM programs, but there are a few exceptions, the products with short persistance like pirimicarb, mevinphos and heptenophos. For test 1, a few products have to be classified in category no. 2 (fenpyroximate, tebufenpyrad WP formulation) and no.3 (dicofol, dichlofluanid, tebufenpyrad EC formulation, teflubenzuron); according to practical experiences tebufenpyrad and teflubenzuron can be used together with *Encarsia*, but dichlofluanid, dicofol may cause problems with biological control of the whitefly. These compounds need further sequential testing for evaluation of their side- effects on *Encarsia.*

The test 2 results can also be categorized in 2 groups, the evaluation categories 1 and 2, and the categories 3 and 4. Products scoring harmless (no.1) sometimes were not compatible with *E. formosa* in the glasshouse (mevinphos, heptenophos, pyrazophos, sulfur, dietofencarb+carbendazim), while products scoring slightly harmful (no.2) could still be used together with the parasitoid (tebufenpyrad). Products that have a score of 3 and 4 (pyridaben, amitraz, cypermethrin, deltamethrin) can be categorized as incompatible with *E. formosa.* The test 2 on the more protected stage of the parasitoid can thus best be used to demonstrate the harmfulness of a compound.

The harmless products belong to the groups of fungicides, acaricides and insect growth regulators, while the harmful products are to be found in the groups of the organophosphates, the carbamates and the pyrethroids.

Regarding the aforementioned results, the testing of products with expected adult toxicity, should be better started in the sequential testing scheme with test 2, while products with expected low or no toxicity to adult wasps should be submitted to test 1 first, which has also been already proposed by Dr. Oomen (1993).

Table 1. Evaluation of the effects of pesticides (lab tests 1 and 2, persistence test) according to the IOBC classification categories, and compatibility with *E. formosa* according to practical experiences in glasshouses.

Pesticide Trade Name	Pesticide Common Name	Concen-tration (%AI)	Classification Category			Glasshouse compatibility with *E. formosa*
			Test 1	Test 2	Persistence	
Organophosphates						
Asepta (358g/l)	Bromophos*	0.1	4	-	-	NO
Ekamet (500g/l)	Etrimfos*	0.1	4	4	-	NO
Hostaquick (550g/l)	Heptenophos	0.055	4	2	1	YES(1w)

Phosdrin (100g/l)	Mevinphos*	0.058	4	1	1	NO
Actellic (50%)	Pirimiphos-methyl*	0.2	4	-	4	NO
Hostathion (424g/l)	Triazophos*	0.1	4	3	-	NO
Carbamates						
Prosevor (85%)	Carbaryl*	0.125	4	3	2	NO
Croneton (500g/l)	Ethiofencarb*	0.05	4	1	-	NO
Dicarzol (50%)	Formetanate	0.05	4	2	4	-
Vydate CHL (250g/l)	Oxamyl (drip irrigation)	250g/ha	-	-	-	YES
Vydate L (245g/l)	Oxamyl (spray)*	0.0368	4	2	-	NO
Pirimor G(50%)	Pirimicarb*	0.05	4	-	1	YES
Unden (50%)	Propoxur*	0.05	4	-	2	NO
Pyrethroids						
Talstar (100g/l)	Bifenthrin	0.005	4	2	4	NO
Baythroid (50g/l)	Cyfluthrin	0.0015	4	2	-	NO
Cymbush (100g/l)	Cypermethrin*	0.005	4	3	3	NO
Force (50g/l)	Tefluthrin*	0.001	4	-	-	NO
Insecticides with acaricidal activity						
Thiodan (35%)	Endosulfan*	0.05	4	-	2	NO
Rody (10WP)	Fenpropathrin	0.005	4	2	4	NO
Lannate (25%)	Methomyl*	0.03	4	-	4	NO
Sanmite (150g/l)	Pyridaben	0.015	4	3	-	NO
Acaricides						
Mitac (200g/l)	Amitraz*	0.04	4	3	1	NO
Neoron (50%)	Bromopropylate*	0.04	-	-	-	YES

Apollo (500g/l)	Clofentezine	0.02	1	1	2	YES
Plictran (25W)	Cyhexatin*	0.05	4	1	4	YES/NO
Torque (50%)	Fenbutatin oxide*	0.025	1	-	-	YES
Naja (50g/l)	Fenpyroximate	0.06	2	1	-	-
Pyranica (200EC)	Tebufenpyrad	0.016	2	2	-	YES
Masai (20WP)	Tebufenpyrad	0.016	2	2	-	YES
Fungicides						
Benlate (50WP)	Benomyl*	0.03	-	-	-	YES
Baycor (25WP)	Bitertanol	0.0925	1	1	-	YES
Orthocid (83%)	Captan*	0.15	1	-	-	YES
Morestan (25%)	Chinomethionat	0.025	4	1	-	YES/NO
Alto (100SI)	Cyproconazole	0.008	3	1	-	-
Euparen (50WP)	Dichlofluanid	0.075	3	1	1	YES/NO
Kelthane (35%)	Dicofol*	0.05	3	1	-	YES
Sumico (25%+25%)	Diethofencarb + carbendazim	0.05 + 0.05	4	1	-	YES
Nissorun (10%)	Hexythiazox	0.05	1	1	-	YES
Rovral (50WP)	Iprodione*	0.075	1	-	-	YES
Fungaflor (200g/l)	Imazalil*	0.01	-	-	-	YES
Dithane (80WP)	Maneb*	0.4	4	1	-	YES
Omnex (10WP)	Penconazole	0.0025	1	-	-	YES
Sumisclex (50WP)	Procymidone	0.075	1	-	-	YES
Afugan (300g/l)	Pyrazophos*	0.025	4	1	4	NO
Netzschwefel (80WP)	Sulfur	0.32	4	1	4	NO

Topsin (70%)	Thiophanate-methyl*	0.075	1	-	-	YES
Saprol (190g/l)	Triforine	0.0285	1	1	-	YES
Ronilan (500g/l)	Vinclozolin*	0.05	1	-	-	YES
Insect growth regulators						
Applaud (250g/l)	Buprofezin	0.0075	1	-	-	YES
Trigard (75WP)	Cyromazine	0.05	1	-	-	YES
Polo (500SC)	Diafenthiuron	0.04	1	1	-	YES
Dimilin (25WP)	Diflubenzuron	0.0125	1	1	-	YES
Andalin (250g/l)	Flucycloxuron	0.015	1	1	-	YES
Cascade (160g/l)	Flufenoxuron	0.005	1	1	-	-
Nomolt (150g/l)[2]	Teflubenzuron	0.016	3	1	1	YES
Miscellaneous						
Delfin (85%)	Bt.kurstaki	0.085	1	-	-	YES
Evisect (50WP)	Thiocyclam*	0.015	2	1	1	-

*: lab tests carried out by Dr. Oomen
(-): not tested or unknown
(YES/NO): disagreeing company views (YES: pesticide compatible with *E. formosa;* NO: pesticide incompatible with *E. formosa*)
(1w): compatible with *E. formosa* after 1 week.

Conclusions

The laboratory methods (test 1 to check the parasitization capacity of the wasps after exposure to a pesticide; test 2 to check the contact toxicity of the pesticide on the protected stage of *E. formosa* in the black scale; test 3 to check the persistence of a product) for testing the side- effects of pesticides on the parasitic wasp *E. formosa*, are very suitable for an accurate evaluation of most pesticides. Only a very few exceptions have to be tested in a "field" experiment, in this case a glasshouse, for the final rating of the side-effect.

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EFFICACY OF DIFFERENT CONCENTRATIONS OF SEVEN PESTICIDES ON *PHYTOSEIULUS PERSIMILIS* A.-H. (ACARINA: PHYTOSEIIDAE) AND ON *TETRANYCHUS URTICAE* K. (ACARINA: TETRANYCHIDAE) IN LABORATORY AND SEMIFIELD TEST

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Summary

Five insecticides and two acaricides were tested in the recommended field concentration as well as in adequate dilutions to evaluate their effect on different developmental stages of the two-spotted spidermite *T. urticae* and its antagonist the predatory mite *P. persimilis.* The main points of interest were to find out the sublethal concentration for the pest and the beneficial, investigations on the escaping effect as well as on the feasibility of semifield test methods.

The acaricides Abamectin and Flucycloxuron proved to be "harmful" to both mite species. Flucythrinate and Methomyl were more harmful for *P. persimilis* than for *T. urticae,* Sojaoil and Diazinon were more harmless for *P. persimilis* than for *T. urticae* and Propoxur was "harmless" for both species.

Introduction

Integrated pest control and the use of natural enemies for the control and reduction of greenhouse pests has gained in importance during the last two decades (v. Lenteren, 1989). Due to different reasons, the use of these beneficial organisms has to be combined with the application of insecticides, acaricides and fungicides. Often more than one pest species occur in a greenhouse, so that pests like aphids and thrips cannot be controlled satisfactory by their antagonists (s.a. Zhang & Sanderson, 1990), because of the use of harmful pesticides against other pests.

With the *laboratory test* the pesticides were tested in dilutions of the recommended field concentration in regard to those concentrations, which kill the pest species to a certain degree of control but do not hazard the beneficials. The results of the laboratory test were basic values for further tests under semifield conditions.

Methods and Material

Test methods were a *laboratory residual test* for both species and only for *P. persimilis* a *semifield residual test,* followed by a *semifield persistence test.* The testing procedures corresponded with the standard methods of the IOBC working group "Pesticides and Beneficial Organisms" for testing side effects of pesticides on the predatory mite *P. persimilis* (Oomen, 1987).

Each testing unit of the *laboratory test* (trial conditions: +25 °C; 80 - 90 % rel. humidity; 16 hours light) consisted of a single bean leaf of *Phaseolus vulgaris* (variety Saxa), which was placed upside down on a layer of wet, water-saturated cotton wool in a perforated Petri dish with a diameter of 9 cm. For these trials the bean leaves were sprayed in a Potter-Tower (Burkhard manufacturing; working pressure: 12 lb/sq inch; distance of 1,5 cm between spray table and tube) for 1,5 mg wet deposit per cm^2 .

Mortality of the juveniles was checked up to 4 days after application. The egg production per female was recorded from the first day of egg laying up to the end of the observation period after 8 days. The juvenile mortality, the average egg production per female and the escaping rates were parameters to evaluate the efficacy of the tested pesticides. Additionally the hatching rate of the *P. persimilis* eggs, laid during the test, was recorded. The obtained data were used to calculate the efficacy values as well as for statistical procedures, which included an ANOVA (variance analysis; SPSS-Programme). For the *semifield residual test* potted plants of *Phaseolus vulgaris* were cut back to one primary leaf. The testing scheme resembled that of Sterk & Vanwetswinkel (1988), with the difference that only juveniles were used. The *semifield persistence test* followed the scheme of the laboratory test quite apart from the fact, that here primary leaves of *P. vulgaris* with different old pesticide residues were used. Both semifield tests took place under greenhouse conditions with 20 - 27 °C, 55 - 75% rel. humidity and 16 hours light. The spraying was done with a commercial handsprayer. For *P. persimilis* and *T. urticae* testings 4 respectively 20 replicates for untreated and treated groups each containing 15 and 10 individuals per pesticide respectively and concentration were used as well for the *laboratory test* as for the *semifield persistence test*. 2 to 10 replicates were used for the *semifield residual test*.

Results

Laboratory residual test

After the assessment of efficacy of the tested concentrations of the pesticides using the *laboratory test*, the very same were classified in the four categories of harmfulness as agreed by the IOBC-working group. Additionally the following four levels, which were important for the choice of the compounds and their concentrations respectively, for further testing with the semifield methods were used:

(1) harmles for both species
(2) more harmless for *P.persimilis* than for *T. urticae*
(3) more harmful for *P. persimilis* than for *T. urticae*
(4) harmful for both species (in high dilutions see number (2) above)

"Harmless and harmful to both species" corresponded with the IOBC-levels, whereas "more harmless and more harmful" represent in-between values which are relative figures. Absolute and corrected data respectively (Abbott, 1925) are shown in table 1.

Table 1: Classification according to the four categories of harmfulness (IOBC - Working group "Pesticides and Beneficial Organisms")

active ingredient	concentration a.i. in ppm	IOBC categories of harmfulness (E[3]) *P. persimilis*	*T. urticae*	ratio of harmfulness of *T.urt.* to *P.pers.*
Sojaoil	rec. field concentr.	2 (64,9)	3 (91,4)	P.p. < T.u.
Flucythrinate	63,75	4 (100)	3 (93,4)	P.p. > T.u.
	31,9	4 (100)	2 (59,5)	
	12,75	4 (100)	2 (33,2)	
	6,39	4 (100)	1 (0)	
	1,28	4 (100)	1 (12,6)	
	0,64	4 (47,1)	1 (0)	
	0,13	2 (20,8)	1 (0)	
Diazinon	400,0	1 (27,3)	3 (89,5)	P.p. < T.u.
	200,0	1 (14,4)	2 (76,3)	
Propoxur	374,0	1 (12,3)	1 (26,2)	P.p. = T.u.
	187,0	1 (0)	1 (21,4)	
Methomyl	406,5	4 (100)	3 (97,3)	P.p. >T.u.
	203,3	4 (100)	3 (89,9)	
	101,6	4 (100)	1 (28,6)	
	40,7	3 (86,9)	2 (52,9)	
	20,3	3 (92,9)	1 (17,9)	
	10,2	2 (77,9)	2 (58,2)	
	4,1	2 (43,1)	2 (32,6)	
Abamectin	4,5	4 (100)	4 (100)	P.p. = T.u. (P.p. >T.u.)[2]
	2,25	4 (100)	4 (100)	
	1,13	3 (86,9)	4 (100)	
	0,45	2 (46,2)	4 (100)	
	0,23	1 (12,7)	4 (100)	
	0,11	3 (16,7)	4 (100)	
	0,05	1 (0)	2 (62,4)	
	0,02	1 (0)	1 (12,7)	
Flucycloxuron	150,0	4 (100)	100%[1]	P.p. = T.u. (P.p. >T.u.)[2]
	75,0	4 (100)	100%	
	37,5	4 (100)	100%	
	15,0	2 (45,9)	99%	
	7,5	1 (15,4)	96%	
	1,5	1 (26,1)	43%	
	0,75	1 (19,2)	8%	

1) Mortality after the ovo-larvicid test

2) more harmless for *P. persimilis* than for *T. urticae* at higher dilutions of the rec. field concentration

3) efficacy of the tested pesticides in % (Oomen, 1987)

Both tested acaricides and one insecticide were chosen for further investigations using those concentrations which resulted in IOBC-levels "moderately to slightly harmful" for *P. persimilis* and were thus tested with the *semifield residual* and *semifield persistence test.*

Semifield test

After the *semifield persistence test* Methomyl at concentrations between 40,7 and 10,2 ppm proved to be not persistent. The two acaricides were at least slightly persistent down to a dilution of 1/10th of the recommended field concentration, due to a "slightly harmful to harmful effect" even of the 10 day old pesticide residues (table 2).The evaluation after the *semi- field residual test* revealed a higher effect on the beneficial than after the laboratory test, though the *semifield residual test* is a less severe test than the *laboratory test.*

Table 2: Classification according to the four categories of harmfulness and the four categories of persistence respecively (IOBC-workinggroup) after the laboratory, semifield residual and semifield persistence test with *P. persimilis*

active ingredient	concentration a.i. in ppm	IOBC classification after the Laboratory test	Sem. residual test	Sem.persis-tence test
Methomyl	40,7	3	-	1
	20,3	3	2	1
	10,2	2	2	1
Abamectin	1,13	4	1	2 (at least)[1)]
	0,45	2	2	2
	0,23	1	1	1 - 2
Flucycloxuron	15,0	2	1	2 (at least)[1)]
	7,5	2	4	1

1) at least slightly persistent after the semifield persistence test with 10 day old residuals

Discussion

Results of the *semifield residual test* mostly did not correspond with those of the *laboratory test.* Besides the different climatic conditions in the greenhouse compartment compared to the climatic chambers, which could influence the trials, problems with the "escaping" occured, which is the running off of the testing animals from the testing unit. Natural escaping was partly caused by male predators searching for new females, which mainly occured between the 4th and 7th day of the observation period. Escaping could also been caused by the repellent effect of a pesticide, which was often concealed by its lethal effect at the recommended field concentration. Only at higher dilutions the repellent effect came to the light (table 1). The fact of the escaped

mites raised the question how to take them into consideration for the calculation of the efficacy of the pesticides. In other works the escapers were either counted dead (Zhang & Sanderson, 1990) or were not mentioned at all (Helle & Overmeer, 1985; Kniehase & Zobelein, 1990). The best way to handle the escapers for assessing the efficacy is the omission of the escapers by putting the number of dead and alive mites 100 %. This scheme seems to be most favourable for a correct evaluation. Escaping has been proved to be also a main problem in the *laboratory test* (comp. Blümel, Bakker & Grove, 1993). But while the escapers of the *laboratory test* and the *semifield persistence test* could be found drawned in the moist cotton layer of the testing unit, they could not been detected at the day of assessment in the *semifield residual test*. Only eggs, larvae, juveniles and 1 - 2 adult females could be found on the day of assessment. Therefore it was difficult to note the number of dead individuals or the number of escaped predators. Only those mites could be counted dead, that died shortly after they were placed on the surface of the pesticide treated leaf. In this case the dead larvae still could be found after 7 days. For all others it is not known wether the escaped animals were affected by the pesticide or not.
Therefore the proposed formula for the calculation of the effect of the pesticide (Sterk & Vanwetswinkel, 1988) was not appropriate and another scheme of calculation was used: instead of counting the number of all mite stages present on the testing unit, in this work the mean number of descendants was used. The descendants included eggs as well as larvae and juveniles. The "number of descendents" corresponded more to the "average egg production" of the *laboratory test*.
A further problem was the 7 day long period between the beginning and the evaluation of this testing scheme: According to the description of Sterk & Vanwetswinkel (1988) only one assessment of the trial plants 7 days after the treatment was neccessary. Kniehase & Zobelein (1990) checked their plants twice (day 2 and 7) during the observation period of 10 days using a binocular. But this can be critical in regard to the danger of a disturbance of the mites causing uncontrolled escaping.

The *semifield residual test*, which should be a step between the *laboratory* and the *field test*, does not give evidence about what happens to the mites during the observation period. No exact statement can be made about the start of the egg laying period or the period of main escaping and therefore data are lost for the assessment. In regard to the specified problems I would propose to omit the *semifield residual test* and let the *laboratory test* be completed by a *persistence test* and followed by a *field test* in the greenhouse as in Oomens description of the testing scheme. Similar conclusions were drawn by Bakker et al. in 1992.

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EFFECTS OF PESTICIDES ON *CHRYSOPERLA CARNEA* STEPH. (NEUROPTERA, CHRYSOPIDAE) IN THE LABORATORY AND SEMI-FIELD

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Abstract

The toxicity of 152 pesticides (products) on larvae and pupae of *Chrysoperla carnea* were tested in the laboratory and 55 of them in the semi-field. The tests were performed according to the common principles of the IOBC/WPRS working group "Pesticides and Beneficial Organisms". The data show a relatively high consistency of the results proving the reliability of the test methods. The hypothesis that products shown harmless in the laboratory do not need further testing in the semi-field and/or field is confirmed in general. However, a few exceptions are observed and we conclude that a careful, case-by-case evaluation is necessary.

Introduction

Toxic effects of 152 pesticides (formulated products) on larvae and pupae of *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) have been assessed during the last 15 years at our institute. The majority of the products were tested within the joint pesticide testing programmes of the IOBC/WPRS working group "Pesticides and Beneficial Organisms". The results of approximately 100 pesticides have been published so far in FRANZ et al. (1980) and HASSAN et al. (1983, 1987, 1988, 1991, in press).

This paper presents an extensive table compiling all data of laboratory and semi-field tests with larvae and pupae of *C. carnea*. Laboratory and semi-field data are presented in an easily comparable way.

Material and methods

All tests were carried out according to the methods described by BIGLER (1988) and BIGLER and WALDBURGER (1988). The methods were developed with respect to the standardized guidelines of the IOBC/WPRS working group "Pesticides and Beneficial Organisms" (HASSAN, 1985). The most important points are summarized for a better understanding of the results.

Laboratory method

Glass plates are treated with the pesticides (2 mg/cm^2), 30 larvae L_1, (2-3 days old, reared at 22°C) are placed singly on the fresh, dried pesticide film, a water treated glass plate is used as control, glass plates are placed in a ventilated growth chamber at 22±1°C, 70% RH, 16L:8D, larvae are fed daily with fresh pea aphids (*Acyrtosiphon pisum*) until pupation, pupae are collected 2-4 days before emergence of the adults, fecundity and fertility are assessed twice a week during 4 weeks starting 2 weeks after emergence of adults, mortality of larvae and pupae and the number of fertile eggs are taken into account to assess the total effect.

Semi-field method

One test unit consists of 25 broad bean plants (*Vicia faba*) at growth stage 4-6 leaves (10-12 cm high) that are treated to the point of "run-off", 200-300 pea aphids, 1 ml of eggs of *Ephestia kuehniella* and 20 larvae L_1 (2-3 days old, reared at 22°C) are added to the test units as soon as the pesticide film has dried, 3 units per pesticide are set up, a water treated control is included in each series, aphids and eggs of *E. kuehniella* are added two or three times within 3 days if aphids are killed by the pesticide, the test units are exposed under a shelter to outdoor conditions, each unit is covered with a cotton cloth after the first pupae are observed and the emerged adults are collected.

In both test procedures we measured the total larval and pupal mortality. Fecundity and fertility were assessed in the laboratory tests only. In agreement with the IOBC/WPRS working group, but with a few exceptions, each product was tested at the highest recommended concentration.

Most of the 152 pesticides listed in Table 1 (64 insecticides, 48 fungicides, 35 herbicides, 5 plant growth regulators) were tested as formulated products with one single active compound. Mixtures of more than one active ingredient were not selected for the joint pesticide testing programmes of the IOBC/WPRS working group.

Results and discussion

The results of all laboratory and semi-field tests are summarized in Table 1. In laboratory tests we found 71.1% of insecticides, 26.2% of fungicides, 20.7% of herbicides and one out of five plant growth regulators with an effect over 50%. In the semi-field the values above 50% effect dropped to 50% for insecticides and 6.3% for fungicides. Only five herbicides were tested in the semi-field because of the difficulties when the plants were killed within the first days of the test. The data show that insecticides are by far the most dangerous pesticides for *C. carnea* whereas other pesticides are harmful in the semi-field in a few cases only.

Table 1: Toxicity of pesticides on *Chrysoperla carnea* (Laboratory: values of 30 larvae assessed as larval and pupal mortality, fecundity and fertility of the adults that were exposed to the pesticide as larvae and pupae, Semi-field: mean of 3 x 20 larvae, no fecundity and fertility tests performed)

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Insecticides and acaricides										
Acephate	Orthen Orthen	WP WP	50% 50%	0,15% 0,15%	100,0	100,0	91,8 85,0	4	4	4 4
Acocyclotin	Peropal Peropal	WP WP	25% 25%	0,10% 0,10%	96,7 100,0	95,8 100,0		3 4	3 4	
Amitraz	Maitac	EC	200 g/l	0,30%	46,4	29,3	0	2	2	0
Azinphos-methyl	Gusathion Gusathion	EC EC	25% 25%	0,20% 0,20%	100,0	100,0	35,0 52,1	4	4	2 3
Bacillus thuringiensis	Dipel	WP	20%	0,10%					1	
Bacillus thuringiensis	Delfin	WG	85%	0,10%			12,7			1
Bacillus thuringiensis tenebr.	Novodor FC	SC	10'000 IU/mg	1,00%	3,3	0	0	1	1	1
Benzoximate	A Azomate		200 g/l	0,15%	55,0	47,8		2	2	
Bromophos-methyl	Asepta Nexion	EC	600 g/l	0,27%	100,0	100,0	92,0	4	4	4
Butocarboxim	Drawin 755	EC	50%	0,10%	96,7	96,3	26,0	4	4	2
Buprofezin	Applaud	WP	250g/l	0,03%	10,3	31,3		1	2	
Chlorfenvinphos	Birlane Birlane	EC EC	800 g/l 880 g/l	0,33% 0,33%	88,4	87,8	8,2 18,0	3	3	1 1
Chlorpyriphos-ethyl	Dursban	WP	25%	0,25%	100,0	100,0	100,0	4	4	4
Clofentezine	Apollo SOSC Apollo SOSC	SC SC	500 g/l 500 g/l	0,04% 0,04%	22,2 30,8	3,1 37,0		1 2	1 2	
Cyfluthrin	Baythroid 50 Baythroid 50	EC EC	50 g/l 50 g/l	0,05% 0,125%	43,3 25,0	33,7 0	27,3 75,5	2 1	2 1	2 4
Cyhexatin	Plictran 25W	WP	25%	0,10%	100,0	100,0		4	4	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Cypermethrin	Ambush	EC	100 g/l	0,04%	100,0	100,0	57,7	4	4	3
Cyromazine	Trigard	WP	75%	0,067%	89,7	100,0	54,0	3	4	3
Deltamethrin	Decis	EC	25%	0,06%	74,2	89,0	29,8	2	3	2
	Decis	EC	25%	0,06%			23,5			1
	Decis	EC	25%	0,06%			25,5			2
	Decis	EC	25%	0,06%			20,8			1
	Decis	EC	25%	0,06%			21,4			1
Demeton-S-methyl	Metasystox (i)	EC		0,10%	100,0	100,0		4	4	
Dialiphos	Torak E	EC	432 g/l	0,25%	65,2	72,9	12,2	2	2	1
Diazinon	Basudine Vloeibaar	EC	230 g/l	0,21%	100,0	100,0	43,8	4	4	2
Dicofol	Kelthane	WP	190 g/l	0,15%					1	
Dimethoate	Perfektion	EC	500 g/l	0,21%	100,0	100,0	85,4	4	4	4
Diflubenzuron	Dimilin	WP	25%	0,05%	100,0	100,0	97,1	4	4	4
Endosulfan	Thiodan 35	EC	200 g/l	0,10%					1	
Ethiophencarb	Croneton	EC	300 g/l	0,10%	96,4	96,4	51,0	3	3	3
	Croneton	EC	300 g/l	0,10%			46,8			2
Etrimfos	Ekamet	EC	500 g/l	0,20%	100,0	100,0	89,6	4	4	4
Fenbutatin-oxid	Shell Torque	SP	150 g/l	0,05%					1	
Fenitrothion	Folithion	EC	550 g/l	0,10%	100,0	100,0		4	4	
Fenpropathrin	Rody	WP	10%	0,05%	100,0	100,0	76,0	4	4	4
Fenvalerate	Sumicidin	EC	36 g/l	0,075%					3	
Fenoxycarb	Insegar	WP	25%	0,06%	100,0	100,0	50,0	4	4	2
Flubenzimine	Cropotex	WP	50%	0,10%	100,0	100,0	28,6	4	4	2
Fluvalinate	Klartan	SC	240 g/l	0,06%	23,3	17,2	41,7	1	1	2
	Klartan	SC	240 g/l	0,06%			40,8			2

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentration tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Heptenophos	Hostaquick	EC	565 g/l	0,10%			25,5			2
	Hostaquick	EC	550 g/l	0,10%	100,0	100,0	16,4	4	4	1
	Hostaquick	EC	550 g/l	0,10%			21,6			1
Hezythiazox	César SL	WP	200 g/l	0,025%	8,3	39,3		1	2	
Insecticidal soap	Neudosan	AL	51%	2,0%	13,8	13,8		1	1	
Insecticidal soap	Stockler Natural	AL	50%	2%	26,1	45,3		1	2	
Lambda-cyhalothrin	Karate	EC	5%	0,075%	24,1	24,1		1	1	
Lindane	Asepta Lindane	WP	90%	0,10%					1	
Methamidophos	Tamaron	WP	600 g/l	0,15%	100,0	100,0	83,3	4	4	4
Methidathion	Ultracid	WP	180 g/l	0,075%	100,0	100,0		4	4	
Methomyl	Lannate	WP	150 g/l	0,10%	100,0	100,0		4	4	
Mevinphos	Phosdrin	EC	350 g/l	0,58%	100,0	100,0	63,8	4	4	3
	Phosdrin	EC	350 g/l	0,58%	92,9	88,2		3	3	
Oxamyl	Vydate L		245 g/l	0,15%	100,0	100,0	67,3	4	4	3
Parathionethyl + Mineral oil	Folidol oil	EC	100 g/l 560 g/l	1,0%	100,0	100,0		4	4	
Permethrin	Ambush	EC	250 g/l	0,02%	100,0	100,0		4	4	
	Ambush	EC	250 g/l	0,076%	10,3	41,7		1	2	
	Ambush	EC	250 g/l	0,08%	65,5	62,4		2	2	
Phosalone	Rubitox	EC	350 g/l	0,20%					1	
Phosmet	Imidan	WP	50%	0,25%	100,0	100,0	20,4	4	4	1
Phosphamidon	Dimecron	SC	20%	0,25%	100,0	100,0	100,0	4	4	4
Pirimicarb	Pirimor	SG	50%	0,10%	0	0		1	1	
Propoxur	Unden			0,15%	100,0	100,0		4	4	
Pyrethrum + Piperonyl-butoxid	Spruzit-Nova flüssig	EC	2,1% 16,5%	0,10%					1	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Pirimiphos-methyl	Actellic 50		50%	0,20%	100,0	100,0		4	4	
Rape seed oil	Telmion	FW	85%	2,0%	95,8			3		
Teflubenzuron	Nomolt	SC	13,75%	0,20%	100,0	100,0	100,0	4	4	4
	Nomolt	SC	13,75%	0,10%	100,0	100,0	100,0	4	4	4
Tetradifon	Tedion V18	EC	80 g/l	0,20%	25,0	20,5		1	1	
Thiocyclamhydrogenoxalate	Evisect	WP	50%	0,03%	42,3	58,5	65,5	2	2	3
	Evisect	WP	50%	0,03%			52,0			3
Triazophos	Hostathion	EC	424 g/l	0,24%	100,0	100,0	38,3	4	4	2
Trichlorphon	Dipterex	WP	80%	0,10%	39,1	11,1		2	1	
Vamidothion	Kilval	EC	400 g/l	0,125%	100,0	100,0	38,3	4	4	2
Verticillium lecanii	Micro germin A+F	WP	7,4%	0,40%	0	0	0	1	1	1
Fungicides										
Anilazine	Dyrene flüssig	EC	480 g/l	0,40%	28,3	32,6		1	2	
Bitertanol	Baycor	WP	25%	0,37%	15,4	36,6		1	2	
Bupirimate	Nimrod	EC	25%	0,04%					1	
Captafol	Ortho Difolatan	WP	960 g/l	0,20%					1	
Captafol + Pyrazophos	Furesan	WP	34,3% 18,4%	0,75%		100,0	98,0	4	4	
Captan	Orthocid 83	WP	750 g/l	0,15%	20,0	0		1	1	
Carbendazim	Derosal	WP	60%	0,05%					1	
Chinomethionate	Morestan	WP	25%	0,10%	82,1	83,5	31,9	3	3	2
Chlorothalonil	Daconil 500	SP	500 g/l	0,30%	3,6	3,5		1	1	
Copper-oxychloride	Vitigran	WP	45%	1,00%	76,7	84,4	10,0	2	3	1
Cyproconazole	Alto	SL	10%	0,08%	13,3	5,5	5,5	1	1	1
Dichlofluanid	Euparen	WP	50%	0,20%	11,5	6,2		1	1	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Dithalimfos	Plondrel			0,075%					1	
Dithianon	Delan flüssig	SC	223 g/l	0,20%	14,5	37,6		1	2	
Difenoconazole	Score	EC	250 g/l	0,05%	3,7	10,4		1	1	
Ethirimol	Milgo E	EC	280 g/l	0,18%	10,6	0		1	1	
Fenarimol	Rubigan Vloeibar	EC	120 g/l	0,12%	54,1	72,6	0	2	2	1
Fenpropimorph	Corbel	EC	750 g/l	0,17%	40,7	85,9	9,8	2	3	1
	Corbel	EC	750 g/l	0,17%	44,8	37,1	19,6	2	2	1
Fenpropimorph + Propiconazol		EC	400 g/l 125 g/l	0.25%	21,4	26,9		1	1	
Flutriafol	Impact S.	SC	12%	0,16%	10,5	49,0	16,7	1	2	1
Folpet	Ortho Phaltan	WP	50%	0,33%	75,0	80,3	6,1	2	3	1
Hexaconazole	Anvil	SP	50 g/l	0,03%	17,6	34,9		1	2	
Iprodion	Rovral PM	WP	50 %	0,15%	7,5	26,9		1	1	
Lecithin	Bio-Anti Mildew		25%	0,15%	10,0	7,3		1	1	
Lime sulphur	Nevikén	WP	7%	3,0%	100,0	100,0	20,0	4	4	1
Mancozeb	Dithane 22	WG	80%	0,50%	20,7	27,0		1	1	
Mancozeb	Dithane Ultra	WG	80%	0,20%	0	0	0	1	1	1
Maneb	Dithane M22	WP	80%	0,20%					1	
Metiram	Polyram Combi	WG	80%	0,42%	16,7	15,0		1	1	
Myclobutanil	Systane	EC	125 g/l	0,24%	0	0		1	1	
Nuarimol	Trimidal	EC	90 g/l	0,08%	13,5	0		1	1	
Penconazole	Topas	EC	100 g/l	0,04%	0	5,0	0	1	1	1
Penconazole	Omnex	WP	10%	0,025%	4,2	0		1	1	
Prochloraz	Sportak forte	EC	400 g/l	0,187%	14,8	0		1	1	
Procymidone	Sumisclex	WP	50%	0,15%	10,4	24,7		1	1	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentration tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Propiconazol	Tilt	EC	250 g/l	0,08%	7,4 17,9	27,2 0	9,8	1 1	1 1	1
Propineb	Antracol	WP	70%	0,20%	2,8	22,2		1	1	
Pyrazophos	Afugan	WP	30%	0,05%			39,6		3	2
Sulphur	Thiovit	WP	80%	0,40%	33,3	72,0		2	2	
Sulphur	Netzschwefel Bayer	WP	80%	0,40%	93,3	95,6	0	3	3	1
Tebuconazole	Folicur	EC	250 g/l	0,25%	36,7	25,3	18,4	2	1	1
Thiophanat-methyl	Cercobin-M Cercobin Cercobin	WP WP SC	70% 70% 500 g/l	0,10% 0,10% 0,10%	17,9	27,8	14,3 0	1	1 1	1 1
Triadimefon	Bayleton Spezial	WG	5%	0,05%	13,8	0,9		1	1	
Triadimefon	Bayleton	WP	25%	0,10%	22,7	11,9		1	1	
Triadimenol	Bayfidan Bayfidan	EC EC	250 g/l 250 g/l	0,05% 0,05%	48,4	51,6		2	1 2	
Triforine	Saprol	EC	190 g/l	0,15%	6,9	32,0		1	2	
Thiram	Pomarsol forte	WP	80%	0,20%	0,4	0		1	1	
Vinclozolin	Ronilan	WG	50%	0,05%	47,8	54,1		2	2	
Herbicides										
Amine salt	Luxamine 2,4-D		500 g/l	0,432%	10,3	26,5		1	1	
Amitrol + Diuron	Ustinex PA	WP	30% 56%	1,0%	100,0	100,0		4	4	
Atrazin	Gesaprim 50	WP	50%	0,67%	17,7	26,6		1	1	
Bentazone	Basagran	EC	480 g/l	0,40%	10,3	1,3		1	1	
Bromacil	Hyvar X	WP	80%	0,20%	20,6	16,7		1	1	
Bromofenoxim	Faneron	WP	50%	1,70%	21,4	15,8		1	1	
Bromoxynil	Certrol B	EC	235 g/l	0,33%	0	0		1	1	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Clopyralid	Lontrel 100	SC	100 g/l	0,30%			14,3			1
	Lontrel 100	SC	100 g/l	0,12%	24,1	15,0	31,1	1	1	2
Desmetryne	Semeron	WP	25%	0,25%	0	0		1	1	
Diclofop-methyl	Illoxan	EC	36%	0,75%					1	
Difenzoquat	Avenge		200 g/l	1,0%					1	
Dinoseb	Aretit flüssig	EC	14%	1,25%	100,0	100,0		4	4	
Ethofumesat	Tramat 500	SC	500 g/l	1,0%	10,0	0		1	1	
Fluazifop butyl	Fusilade	EC	12,6%	0,25%	3,7	13,7		1	1	
Fluroxypyr	Starane 180	EC	180 g/l	0,50%	10,0	3,7		1	1	
Glufosinate-ammonium	Basta	SL	200 g/l	0,50%	62,1	74,2	25,0	2	2	2
Glyfosat	Roundup	EC	360 g/l	1,0%	51,7	41,1		2	2	
Haloxifop	Gallant super	EC	104 g/l	0,375%	16,7	17,5		1	1	
Ioxynil	Topper	EC	240 g/l	0,24%	0	34,7		1	2	
Isoproturon	Arelon flüssig	SC	500 g/l	0,75%	7,7	0		1	1	
Metabenzthiazuron	Tribunil	WP	70%	0,67%	0	0		1	1	
Metamitron	Goltix	WG	70 %	2,50%	10,0	10,0		1	1	
Metsulfuron-methyl	Ally	WP	20%	0,067%	0	0		1	1	
Monolinuron	Aresin	WP	50%	0,75%	91,7	91,7		3	3	
Phenmedipham	Betanal	EC	16%	2,25%					2	
Propachlor	Ramrod	SP	44,8%	1,0%	50	22,0		2	1	
Propyzamid	Kerb 50W	WP	50%	0,75%					1	
Quizalofop-ethyl	Targa	EC	100 g/l	0,30%	37,0	58,4		2	2	
Sethoxydim	Fervinal Plus	EC	133 g/l	0,79%	25,0	34,8	19,0	1	2	1
Sethoxydim	Fervinal Plus	EC	133 g/l	0,79%			4,4			1
Simazin	Gesatop 50	WP	50%	0,375%	0	0		1	1	

Table 1: continued

Active ingredient	Product	Formu-lation	Content of active ingredient	Concentra-tion tested	Toxicity %			Toxicity category IOBC*		
					Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]	Laboratory Mortality[1]	Total effect[2]	Semi-field Mortality[1]
Tralkoxydim	Grasp	EC	100 g/l	0,50%	42,7	59,3		2	2	
Tridemorph	Calixin	EC	750 g/l	0,075%	21,4	23,0	0	1	1	1
	Calixin	EC	750 g/l	0,075%			26,7			2
Plant growth regulators										
Alpha Naphthylacetamid	Dirigol N	WP	50%	0,020%	0	9,0		1	1	
Alpha Naphthylaceticacid	Rhodofix	WP	1%	0,15%	0	0		1	1	
Chlormequat	Cycocel Extra	SL	70%	0,70%	0	0		1	1	
Carbaryl	Prosevor	EC	85%	0,125%	100,0	100,0		4	4	
Ethephon	Terpal C	SL	37,7%	0,625%	6,9	33,0		1	1	

[1]larval and pupal mortality (after Abbott)
[2]assessed as larval and pupal mortality, fecundity and fertility (see Bigler, 1988)
* a few results of the first pesticides tested were available only as toxicity category but not as % toxicity.
Laboratory: 1 : 0-30%, 2 : 30-79%, 3 : 80-99%, 4: >99%
Semi-field: 1 : 0-25%, 2 : 26-50%, 3 : 51-75%, 4: >75%

The semi-field test was performed in general for those products that showed in the laboratory test a total effect of more than 50%. Some products were tested more than once either in the laboratory or in the semi-field. All multiple tests, except two out of 15, show the consistence of the data. Differences of less than 20% between tests of the same product and concentration are acceptable to our opinion. The acaricide Clofentezin (Apollo) and the fungizide Fenpropimorph (Corbel) show both similar mortalities in consecutive laboratory tests, but there is a high difference in the total effect observed. This is explained by high variations of the fecundity between the test series due to low numbers of females. The only inexplicable difference of two semi-field tests remains with the herbicide Tridemorph (Calixin). The insecticides Deltamethrin (Decis) and Heptenophos (Hostaquick) are often used as references in the semi-field tests. The mortalities of five tests with Decis performed over several years vary from 20.5% to 29.8% and three tests with Hostaquick vary from 16.4% to 25.5%. The average difference between multiple tests of the 15 products is 7.4%. These results show the reliability and consistency of the tests.

An important issue is the examination of the hypothesis of the IOBC/WPRS working group that "pesticides found to be harmless to a particular beneficial in the laboratory test are most likely to be harmless to the same organism in the field and no further testing in the semi-field or field experiments is therefore recommended" (HASSAN, 1985). Figure 1 presents the results of 55 pro-

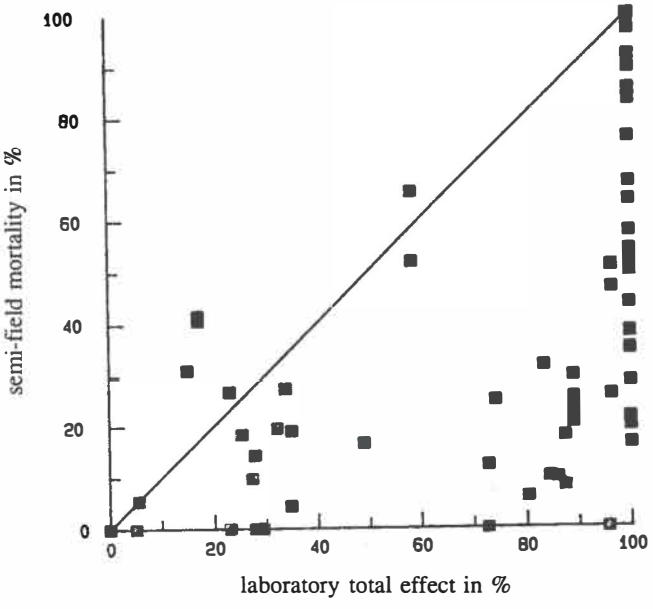

Figure 1: Comparison of pesticide toxicities, assessed in laboratory and semi-field tests, on larvae and pupae of *Chrysoperla carnea*

ducts evaluated in the laboratory and in the semi-field. Out of 55 pesticides tested in 72 experiments only five tests (6.9%) show a higher toxicity in the semi-field than in the laboratory. The five test results belong to the four pesticides Fluvalinate (Klartan), Thiocyclamhydrogenoxalate (Evisect), Clopyralid (Lontrel 100) and Tridemorph (Calixin). All these products were tested twice in the semi-field whereby Fluvalinate was the only one showing a higher toxicity in both semi-field tests compared to the laboratory. The other three products had a higher toxicity in the semi-field in one out of two tests. The five test results are located on the upper side of the diagonal line in Figure 1. Out of 18 laboratory tests with less than 50% effect, four were more toxic in the semi-field than in the laboratory. However, none of them reaches values in the semi-field that would ask for further testing in the field. In Figure 1 we can distinguish two main toxicity groups with regard to the laboratory tests. A low toxicity group with less than 40% effect and a high toxicity group with over 70% effect. This is caused by our selection of products for the semi-field tests and should not be interpreted as an inherent characteristic of the products in general. It should be noticed however, that the effect in the semi-field is lower than in the laboratory in all cases (51 tests) of the high toxicity group (over 70%). The hypothesis that only products with more than 50% effect in the laboratory (toxicity category 1 in HASSAN, 1985) must be tested further in the semi-field and field can be confirmed by our data.

The comparison of toxicity data from the laboratory and semi-field (and field) needs careful interpretation because of the different methods used and the specific modes of action of the pesticides. The larvae and pupae are constantly exposed to the pesticide in the laboratory tests whereas in semi-field experiments they may escape the pesticide when they move to the ground or eventually to untreated plant parts. We observed in the semi-field tests with several pyrethroids (e.g. Deltamethrin, Cypermethrin, Permethrin) the well known "knock-down" effect causing the larvae to fall on the ground. The paralysed larvae lie for two to five days on the soil and recover afterwards. In the meantime, the pyrethroids are degraded so far that a portion of the larvae can reestablish on the plants and complete their development (see also data in Table 1). Under field conditions the situation may be different. Knocked-down larvae on the soil may be killed by epigaeic arthropod predators or washed into the soil by rain and drown. We observed in our own field experiments that rain has a negative impact on survival of larvae and pupae of *C. carnea*.

The situation is even more complicated when Insect Growth Regulators (IGR) are tested. The chitin inhibitors Diflubenzuron (Dimilin) and Teflubenzuron (Nomolt) killed all young larvae at the first moult (as L_1) in the laboratory experiments. The juvenile hormone analogue Fenoxycarb (Insegar) killed all larvae at the end of a long lasting (> 30 days) third larval instar in the

laboratory test. In the semi-field experiments we did not observe any delayed emergence of the adults in the Insegar treatment. The mortality in Dimilin and Nomolt treatments in semi-field tests was almost complete. In one test with Nomolt we observed seven pupae (out of 60 L_1 set up) but none developed to the adult stage. Similar experiences are reported by VOGT (1992). In laboratory experiments, the mortality of young larvae (L_1, L_2,) exposed to Dimilin or Nomolt was 100% and 63% respectively. If L_3 larvae were exposed to these IGRs, most of them were able to build cocoons, but they did not succeed to pupate. In our own field experiments with Nomolt, where we used young L_2, we measured in four experiments from 1989 to 1992 larval and pupal mortalities of 97.5 to 100%.

Conclusions

The 152 pesticides tested at the highest recommended concentration according to the IOBC/WPRS working groups' rule show a wide range of toxicities to larvae and pupae of *C. carnea*. Multiple experiments of the same product demonstrate quite consistent results proving a relatively good reliability of the methods. Based on our long experience, we postulate that laboratory tests used for registration purposes should be carried out with a higher number of larvae and repetitions (e.g. three or four times 25 larvae per product and concentration). The number of repetitions of the semi-field test should be increased from three to four. These improvements would make the results more reliable and statistics could be applied to the data, but this would cause higher costs.

The hypothesis that products showing no or low toxicities in laboratory tests need no further testing in semi-field or field experiments is confirmed in general by our data. However, a few exceptions may occur and careful interpretation is necessary.

The comparison of laboratory, semi-field and field data need detailed information on the test methods and the mode of action of the pesticide. In order to elucidate the remaining questions and to study the effects of pesticides on eggs and adults we need additional test methods.

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EFFECTS OF PESTICIDES ON *Chrysoperla carnea* STEPH. (NEUROPTERA, CHRYSOPIDAE) IN THE FIELD AND COMPARISON WITH LABORATORY AND SEMI-FIELD RESULTS

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Abstract

The effects of 7 insecticides on *Chrysoperla carnea* were investigated under field conditions by treating young dwarf apple trees on which larvae form a rearing had been released. The survival of the larvae and their further development were checked by using baitcards containing *Sitotroga cerealella*-eggs. The insect growth regulators Cascade (a.i. Flufenoxuron), Dimilin (a.i. Diflubenzuron), Nomolt (a.i. Teflubenzuron) and Insegar (a.i. Fenoxycarb) revealed to be moderately to very harmful. Amongst the neurotoxic insecticides, Baythroid (a.i Cyfluthrin) was highly toxic while Evisect (a.i. Thiocyclamhydrogenoxalat) and Imidan (a.i. Phosmet) only slightly affected the larvae. The results are discussed in comparison with laboratory and semi-field tests.

Introduction

One of the main criteria for integrated plant protection is the use of selective pesticides which preserves the natural enemies. Therefore, knowledge about the effects of pesticides on beneficials is indispensable. Whereas much experience exists with laboratory and semi-field methods for *Chrysoperla carnea* (Bigler and Waldburger, elsewhere this volume), a field test, which completes the sequential testing scheme, has been established for this species only recently (Vogt, 1992, Vogt et. al., 1992, Wetzel et al., 1991). Field investigations are necessary for all pesticides which have turned out to be harmful under laboratory or semi-field conditions in order to examine if or to which degree the harmfulness remains under practical conditions. This paper presents the results of field tests with 7 pesticides belonging to different insecticidal types such as neurotoxins, chitin synthesis inhibitors, juvenoids and pyrethroids. All the tests have been carried out at the institute in Dossenheim. A comparison of the data with laboratory and semi-field results is included.

Material and Methods

The tests were carried out according to the method described by Vogt et al. (1992) with some modifications in the most recent tests. For better comprehension the main principles of the test method are shortly described: Laboratory reared *C. carnea* larvae of uniform age are released on young dwarf apple trees free of prey insects (see details in table 1). Afterwards the trees are treated with the pesticides using a knap-sack handsprayer. The control plants are sprayed with water. 24 hrs after the pesticide treatment special baitcards containing eggs of *Sitotroga cerealella* Olivier (Lep., Gelechiidae) as food for the lacewing larvae are attached to the trees and placed as well on the ground around the tree trunk. These baitcards allow to recapture surviving larvae. One day later the baitcards are checked. The larvae in the baitcards are counted and released again on the tree. If necessary the baitcards are replaced by new ones. The checking of the baitcards is repeated the

Table 1. Specifications to material and methods of the field tests

Insecticide	Date of the field test	No. of replicates	No.of larvae per tree Stage of larvae	No. of bait-cards per tree a) tree top b) ground	Enclosures or barriers
Insegar 0,04 % (25 % Fenoxycarb WP)	15.5.-4.6.1990 (20 d)	n=12 Co n=11 Ins [a]	400 old L2 young L3	a) 15 b) 15	barriers digged in the ground
Evisect S 0,03 % (50% Thiocyclam WP)	19.6.-28.6.1990 (9d)	n=10	400 10 % L1 90 % L2	a) 15 b) 10	none
Dimilin 0,05 % (25 % Diflubenzuron WP)	17.7.-1.8.1990 (15 d)	n=10	400 40 % L1 60 % L2	a) 15 b) 10	none
Baythroid 50 0,05 % (50 g/l Cyfluthrin)	4.6.-10.6.1991 (6d)	n=6	300 6% L1 94 % L2	a) 15 b) 10	none
Cascade 0,05 % (10 % Flufenoxuron WDC)	17.7.-5.8.1991 (19 d)	n=6	300 1 % L1 99 % L2	a) 15; 20 [b] b) 10	none
Nomolt 0,09 % [c] (15 % Teflubenzuron SC)	13.7.-2.8.1993 (20 d)	n=4	350 26 % L1 74 % L2	a) 20 b) 10	special barriers (fig. 1)
Imidan 0,25 % (50 % Phosmet WP)	13.7.-2.8.1993 (20 d)	n=4	350 26 % L1 74 % L2	a) 20 b) 10	special barriers (fig. 1)

a) 1 tree had to be omitted because of too many ants (Co = Control, Ins = Insegar)
b) from day 16 (23.7.1991) on
c) dosage corresponds to the 7th joint testing programme of the IOBC group with Nomolt 0,1 % a.i. 13,75 %

following days. With "classical pesticides" the evaluation ends when the number of recaptured larvae has become and remains low for several days, usually after 4-5 days. In the case of insect growth regulators (IGRs) the evaluation stops when the number of cocoons found in the baitcards has decreased to a minimum. Usually a test with an IGR lasts for 14 up to 21 days. All cocoons are collected and kept in a climatic chamber (approximately 25° C and 70 % RH, L: 16 h, D: 8 h). The emergence of adults is assessed and if possible a test of reproduction follows.

Whereas usually no enclosures were used around the tree trunks, in the case of the Insegar test a barrier was digged into the ground around each tree. Beginning in 1992 problems arised with ants as predators of the lacewing larvae as well as of the *Sitotroga* eggs. In several methodical experiments we have constructed a special barrier (fig.1) which prevents ants and other predators, e.g. earwigs, climbing up the trees and on the other hand allows the green lacewing larvae their natural behavior, moving down and up the tree trunk. For the tests carried out in 1993 these special barriers were used resulting in increased recapture rates as well as in less deviations of the replicates.

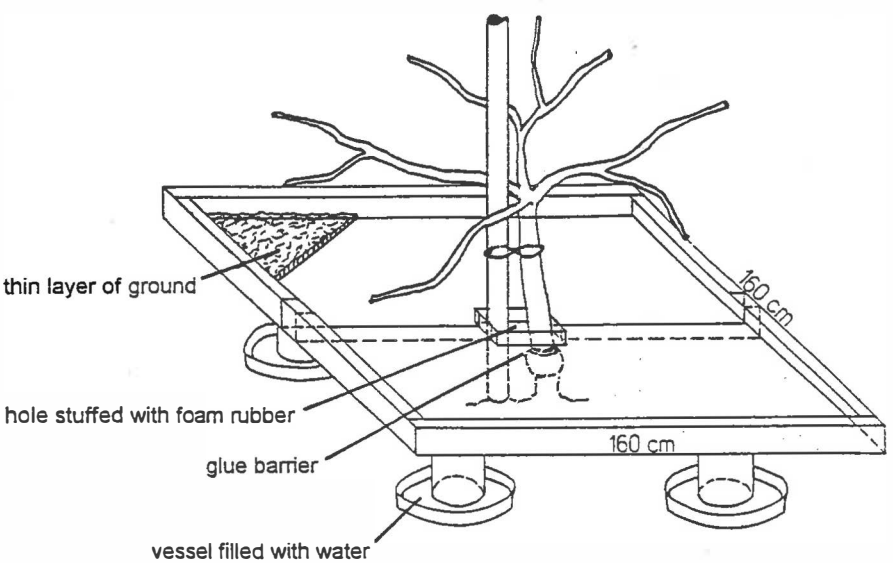

Fig. 1. Special barrier made of sheet-steel used in the field test

Test insects

The *C. carnea* larvae as well as the *Sitotroga* eggs were kindly provided by Dr. Hassan and his team (BBA Darmstadt) from a mass rearing. The *C. carnea* rearing in this institute is supplemented each year with field catches.

Classification of the pesticides

The mean number of recaptured larvae per tree and the mean number of cocoons per tree, respectively, are used for the calculation of the percentage effect according to the formula

$$E\,\% = 100 \times \frac{U - T}{U}$$

U= larvae (cocoons) per tree in untreated
T= larvae (cocoons) per tree in treated

The classification of the toxic effcts follows the guidelines of the IOBC working group "Pesticides and Beneficial Organisms": class 1 = harmless = < 25 % effect, class 2 = slightly harmful = 25-50 % effect; class 3 = moderately harmful = 51-75 % effect, class 4 = harmful = >75 % effect.

Results

Insegar 0,04 % (a.i. 25 % Fenoxycarb WP)

There were no differences with regard to the recapture of larvae (table 2). This corresponds to the mode of action of the juvenoid, which does not impair the larvae until pupation. Pupation on the Insegar treated trees was delayed for 1 week. The total number of cocoons found on the Insegar treated trees within three weeks after the application of the juvenoid was significantly lower than on the Control trees (table 3). The checking of the baitcards was stopped after three weeks. The larvae still found at this time were collected and reared under controlled conditions. 84,4 % (27 out of 32) larvae from the control and 79,4 % (50 out of 63) from the Insegar trees, respectively, pupated. Thus, there was only a slight difference at this date. This could be a hint, that in the meantime the active ingredient Fenoxycarb had been degraded under field conditions or that the larvae themselves had been able to metabolize the substance. This is supported by the fact that the pupation of larvae from earlier collections during the checking of the field trial was clearly impaired by Insegar. On the whole, whereas in the control 91 % (143 out of 157) of all larvae collected at different dates pupated, in the case of Insegar the percentage was only 68 % (127 out of 187). The percentage of permanent larvae, intermediate forms between larvae and pupae, pupae without cocoons dying later and larvae dying before pupation amounted to 27,8 % in the case of Insegar versus 7 % in the control. When looking at these results we have to keep in mind that the larvae had been reared in uncontaminated glass petri dishes after their collection from the treated trees. This might have lessened the effect of Insegar. With regard to the emergence of adults a classification was not possible because the parasitization of the cocoons was very high.

According to this test in the field Insegar is moderately harmful to *C. carnea* larvae.

Evisect S 0,03 % (a.i. Thiocyclam 50 % WP)

A significant reduction in the recapture of the larvae on the Evisect treated trees was observed at the first two checking dates. Afterwards, with the exception of day 7, the number of larvae recaptured increased again and there were no more any differences between the control and the treated trees (fig. 2). This is caused by the knock-down effect of the neurotoxically acting insecticide: the larvae are impaired temporary and recover again. Over the whole period of the test

Table 2. Mean number of larvae recaptured per tree during the whole period of the test and percent reduction

Insecticide	Treated	Untreated	Reduction	IOBC classification
Insegar	276,4 ± 56,1	268,0 ± 23,4	no reduction	no classification before pupation
Dimilin	214,0 ± 28,0	279,5 ± 22,9	23,4 % 1)	
Cascade	111,3 ± 17,0	250,7 ± 39,1	55,6 %	
Nomolt	245,7 ± 32,5	556,3 ± 50,4	55,8 %	
Evisect	158,6 ± 28,0	220,5 ± 50,0	28,1 %	2
Baythroid	4,8 ± 2,9	118,2 ± 26,2	95,9 %	4
Imidan	497,7 ± 47,5	556,3 ± 50,4	10,5 %	1

1) reduction at several checking dates between 38 and 60 %

Table 3. Mean number of cocoons per tree during the whole period of the test and percent reduction

Insecticide	Treated	Untreated	Reduction	IOBC classification
Insegar	6,0 ± 1,5	12,6 ± 4,4	52,4 %	3
Dimilin	4,3 ± 2,3	10,6 ± 3,0	59,4 %	3
Cascade	5,0 ± 2,0	13,7 ± 3,4	63,5 %	3
Nomolt	2,7 ± 2,8	24,5 ± 1,3	89,0 %	4
Imidan	21,7 ± 4,8	24,5 ± 1,3	11,4 %	1

the reduction in recapture amounts to 28,1 % (table 2) and if the first two checkings are left out to 8 %, respectively. The larvae from the last evaluation date were collected and their further development was observed under controlled conditions. 206 out of 213 larvae from the Evisect treated trees (= 96,7 %) and 149 out of 157 larvae from the control trees (= 94,6 %), respectively, pupated.

According to this test in the field Evisect is harmless to sligthly harmful for *C. carnea* larvae.

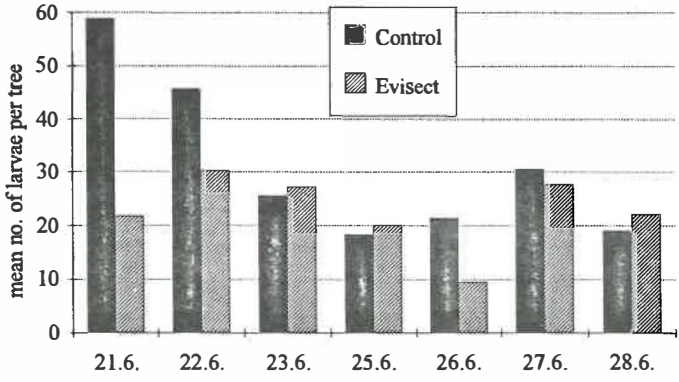

Fig. 2. Recapture of *C. carnea* larvae during the field test with Evisect 0,03 %

Dimilin 0,05 % (a.i. 25 % Diflubenzuron DC)

From the 3rd checking of the baitcards onward the recapture rates were mostly lower on the Dimilin trees than on the Control trees, revealing that this chitin synthesis inhibitor harmed the larvae during moulting into the next larval instar. Whereas over the whole period of the test the mean reduction in recapture was only 23,4 % (table 2), on several dates it amounted to values between 38 and 60 %. In the end, the number of cocoons was reduced by almost 60 % (table 3).

Out of the cocoons found in the baitcards the intact ones were reared in the climatic chamber and their further development was assessed (table 4).

Table 4. Further development of the cocoons collected in the field test with Dimilin

	Total no. of cocoons for rearing	No. of dead pupae in the cocoons	No. of parasitized pupae	No. of adults
Control	89	34 = 38,2 %	29 = 32,6 %	32 = 36,0 %
Dimilin	40	29 = 72,5 %	9 = 22,5 %	2 = 5,0 %

The percentage of dead pupae in the cocoons was rather high even in the control. An explanation could be that the pupae were hurt when collecting the cocoons or that they were killed by host-feeding of the parasitoids emerging in the rearing (unfortunately, the cocoons had not been kept individually). Nevertheless, the percentage of dead pupae was distinctly higher in the case of Dimilin. Additionally to the above mentioned factors, we have to consider disturbances in the prepupal moulting caused by this IGR which result in a higher mortality during pupation. Thus, the effect on emergence of adults, according to the formula of Abbott (1925), would be 86,1 % (IOBC-classification 4). Investigations on reproduction were not possible because of the low number of adults in the Dimilin variant.

With regard to pupation, Dimilin had turned out to be moderately harmful in the field. Considering the high mortalities of the pupae as well as possible impairments on reproduction (cf. Vogt 1992) its effects are probably even worse.

Baythroid 50 0,05 % (a.i. Cyfluthrin 50 g/l)

The pyrethroid Baythroid was highly toxic. Almost no larvae were found in the baitcards (table 5). The effect of this insecticide amounted to 95,9 % (table 2). Thus, Baythroid is very harmful to *C. carnea* larvae in the field.

Table 5. Mean no. of recaptured larvae per tree after treatment with Baythroid

Date	Days after start of the test	Control	Baythroid 0,05%
6.6.91	2	44,2 ± 14,1	2,3 ± 2,0
7.6.91	3	22,8 ± 8,8	0,7 ± 1,1
8.6.91	4	30,8 ± 7,4	1,3 ± 1,2
10.6.91	6	20,3 ± 5,1	0,5 ± 0,5
6.6.-10.6.91		118,2 ± 26,2	4,8 ± 2,9

Cascade 0,05 % (a.i. Flufenoxuron 100 % WDC)

Beginning from the second checking (day 3 after the insecticide treatment) onwards the number of larvae recaptured on the Cascade treated trees decreased continuously. This reveals, that Cascade caused a rather high mortality of the larvae during their moulting into the next larval stage. In the end, significantly less cocoons were found (table 3). The cocoons collected in the field were further observed under controlled conditions, whereby each cocoon was kept individually. Whereas in the case of Casacade 42,8 % of the pupae died within the cocoon or during emergence, mortality amounted to 16,2 % only in the control (table 6). The effect on emergence of adults, according to the formula of Abbott (1925), is 54,4 %. A reproduction test was not possible because of the low

number of adults in the case of Cascade due to the high mortality caused by the IGR itself and the high parasitization rate of the pupae (table 6).

Table 6. Further development of the cocoons collected in the field test with Cascade

	Total no. of cocoons for rearing	No. of dead pupae in the cocoons or during emergence	No. of parasitized pupae	No. of adults
Control	74	12 = 16,2 %	33 = 44,6 %	29 = 39,2 %
Casacde	28	12 = 42,8 %	11 = 39,3 %	5 = 17,8 %

According to this test in the field, Cascade has to be classified as moderately harmful to *C. carnea* larvae.

Nomolt 0,09 % (a.i. Teflubenzuron 15 % a.i. SC) [1]

Like in the case of Cascade, beginning from the second checking onward the number of larvae recaptured on the Nomolt treated trees decreased continuously. Thus, Nomolt, too, caused a high mortality of the larvae during their moulting into the next larval stage. In the end, the number of cocoons was extremely low in the case of Nomolt compared with the control (table 3). In addition, only one adult emerged from the Nomolt cocoons (table 7):

Table 7. Further development of the cocoons collected in the field test with Nomolt

	Total no. of cocoons for rearing	No. of dead pupae in the cocoons	No. of parasitized pupae	No. of adults
Control	78	14 = 17,9 %	4 = 5,1 %	58 = 74,4 %
Nomolt	10	7 = 70 %	2 = 20 %	1 = 10 %

The test revealed that Nomolt is very harmful to *C. carnea* larvae in the field.

Imidan 0,25 % (a.i. 50 % Phosmet WP)

In the field Imidan did not kill the green lacewing larvae. Approximately the same number of larvae as well as cocoons were found on the treated and on the water treated trees (table 2). Out of all cocoons collected from the baitcards, in both variants 78 were intact, i.e. not sucked out, and their further development was assessed in the climatic chamber. In both variants 58 adults emerged (=74,4 %). The remaining cocoons were either parasitized (Control: 5 %, Imidan: 9 %) or the pupae

[1] corresponds to the dosage in the 7th IOBC joint testing programme: Nomolt 0,1 % (13,75 % a.i. SC)

died in the cocoons or shortly after emergence (Control: 20,5 %, Imidan 16,7 %). Investigations on the reproduction resulted in no differences between Control and Imidan insects.

According to this test in the field, Imidan 0,05 % is harmless for *C. carnea* larvae.

Comparison and discussion of field, semi-field and laboratory results

The comparison of the sequential testing (table 8) confirms in most cases that the laboratory residual test indeed is, as intended by the IOBC-working group, a worst case test and that the effects are less severe in semi-field and field. Whereas in the laboratory test the beneficials are exposed constantly to an even pesticide film, in the semi-field and field experiment they can hide and the plants probably are not sprayed as evenly. Furthermore, in the field the pesticides, being exposed to weathering, can be degraded more quickly. On the other hand, contact toxicity might be more relevant in the case of the field test, because the larvae are exposed to the direct spray.

In the case of the neurotoxins three different situations resulted: the effect of Evisect was about 30 % less severe in the field, the effect of Imidan about 90 %. Whereas the decrease in harmfulness of Evisect in the field corresponds to the experiences with other pesticides, in the case of Imidan it is considerable. An explanation might be the heavy rainfall one day after the application and/or the shorter persistence of the insecticide under field conditions.

Baythroid revealed very severe effects in the laboratory as well as in the field in our investigations. It is known from some pyrethroids, however, that the response of larvae exposed to direct sprays is greater than that observed for larvae placed on fresh dry residues after spraying (Ford and Salt, 1987).

The chitin inhibitors Dimilin, Cascade and Nomolt killed all young larvae during their first moult in the laboratory experiment.The effects in the semi-field test were nearly the same. Under field conditions as well these products harmed the larvae to a great degree resulting in significantly lower numbers of cocoons, the most severe effects appearing with Nomolt. In addition, the number of adults emerging from these cocoons was distinctly lower than in the control. Thus, the total effect of these chitin synthesis inhibitors is greater than the one calculated according to the assessment of cocoons. Furthermore, it is well known, that reproduction of adults that were exposed as larvae to chitin inhibitors such as Dimilin and Cascade is impaired (Charmillot and Pasquier, 1992, Ahmad, 1992, Vogt, 1992).

As to the juvenoid Insegar, its 100 % harmfulness in the laboratory decreased to 52,4 % in the field. Several facts are to be considered to explain this decrease. As the field test was started in the midth of may, it took place within a time of considerable shoot growth, in correspondance to the use of Insegar in practice, which is one treatment before and/or one after blossom, the latter usually at the beginning of may. The growth of the shoots and the unfolding of new leaves result in a "diluting" effect, so that the larvae are less exposed to the residue. Furthermore, in the field the juvenoid might be degraded more quickly than under laboratory conditions. Nevertheless, a more than 50 % effect remained in the field. In addition, pupation was delayed for one week.

Table 8. Effect of 7 insecticides on larvae of *C. carnea* in laboratory, semi-field and field tests (the results of laboratory and semi-field tests were kindly provided by F. Bigler, Swiss Federal Research Station for Agronomy, Zürich, if not mentioned otherwise.)

Mode of action	Insecticide			Effect % *IOBC classification*		
	Active ingredient	Product	Concentration tested %	Laboratory [a]	Semi-Field [b]	Field [c]
Neurotoxin	Thiocyclam-hydrogenoxalate	Evisect	0,03	58,5 *2*	65,5 *3*	28,1 *2*
		Evisect	0,03		52,0 *3*	
Neurotoxin	Cyfluthrin	Baythroid 50	0,05	100 [e] *4*	-	95,9 *4*
Neurotoxin	Phosmet	Imidan	0,25	100 *4*	20,4 *1*	10,5 *1*
Chitin inhibitor	Diflubenzuron	Dimilin	0,05	100 *4*	97,1 *4*	59,4 + 86,1 [d] *3 - 4*
Chitin inhibitor	Flufenoxuron	Cascade	0,05	100 [e] *4*	-	63,5 + 54,4 [d] *3*
Chitin inhibitor	Teflubenzuron	Nomolt	0,1	100 *4*	100 *4*	89,0 + 86,5 [d] *4*
Juvenoid	Fenoxycarb	Insegar	0,06	100 *4*	50,0 *2*	-
			0,04	100 [f] *4*	95,4 [f] *4*	52,4 *3*

[a] larval and pupal mortality plus reduction of fecundity and fertility of the adults that were exposed as larvae and pupae
[b] larval and pupal mortality
[c] neurotoxins: reduction of larval recapture, insect growth regulators: reduction of cocoons
[d] decrease in emergence of adults
[e] results from Vogt
[f] results from Rumpf (1990)

When looking at the different results of the semi-field tests, which took place under outdoor conditions under a shelter, we have to consider that the methods were not identical. The experiment of Bigler and Waldburger (this volume) was carried out on bean plants and the larvae were released after spraying. The experiment of Rumpf (1990) took place on potted apple trees and the larvae were released before spraying. These differences in test conditions probably have influenced the experiments.

The results of the field tests with the IGRs give rise to a critical evaluation of these insecticides. Dependent on the active ingredient, the harmfulness in the field ranged between 60 and 90 %. In comparison, most broad spectrum neurotoxins remain very harmful in the field (Boller et al. 1989, Hassan et al., in press, Hassan et al., in prep., Wetzel et al. 1991). Besides, they are not only harmful to larvae but also to insect adults and they can induce pest outbreaks (cf. Croft 1990a). The IGRs are more selective and less toxic to natural enemies, but they are not selective "per se". They differ in selectivity and as the results presented here and elsewhere confirm, they can have harmful effects on arthropod predators and parasites (cf. Zaki and Gesraha, 1987, Croft, 1990b, Grenier and Plantevin, 1990, Narayana and Babu, 1992, Lischke 1993). Furthermore, most IGRs have shown to be rather persistent (cf. Charmillot et al., 1989, Hull et. al, 1991, Weiss and Vogt, in press). The selectivity of IGRs is not only determined by their inherent properties (physiological selectivity) but to a great degree by the timing of their application (coincidance with sensitive stages of non-target organisms, i.e. ecological selectivity) as well as by the frequency of their application. More investigations in the field are necessary to elucidate how to use IGRs within integrated plant protection in order to achieve a high selectivity.

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EVALUATION AU CHAMP DES EFFETS SECONDAIRES DES PESTICIDES SUR *FORFICULA AURICULARIA* ET *ANTHOCORIS NEMORALIS* : VALIDATION DES RESULTATS DE LABORATOIRE.

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ABSTRACT

The toxicity of 13 pesticides against two predators of pear psylla, *Forficula auricularia* and *Anthocoris nemoralis*, is evaluated under laboratory and field conditions. In most cases, these pesticides present an equivalent or lower toxicity under field than under laboratory conditions. For two pesticides, abamectine and fenoxycarbe, the low population of *F. auricularia* observed in the field can be due to the high toxicity of these products on the prey, *Cacopsylla pyri*. These results indicate that complementary sampling methods must be used, to assess both mortality and population level of the predators, and that the predator/prey ratio has to be taken in acount for field evaluations of pesticides.

INTRODUCTION

La séquence de tests standardisés définie par l'OILB - groupe de travail "Pesticides et organismes utiles"- pour évaluer l'effet des pesticides sur les auxiliaires prévoit dans une première étape des tests de laboratoire, standardisés et reproductibles. Pour la plupart des auxiliaires, seule cette première étape est définie, homologuée par le groupe de travail, et utilisée régulièrement au sein du groupe dans le cadre de programmes conjoints d'expérimentation. Les tests en plein champ sont plus difficilement réalisables, et les méthodes en sont encore souvent en phase de mise au point. C'est le cas pour deux espèces prédatrices qui exercent une régulation sur les populations de psylles du poirier (*Cacopsylla pyri* (L)): *Forficula auricularia* et *Anthocoris nemoralis*. Elles sont inclues dans les programmes d'expérimentation au laboratoire et ont été soumises dans ce cadre à des études de sensibilité à divers pesticides (Hassan et al. 1987, 1994). Mais nous ne disposons pour ce qui est du plein champ que de quelques données, acquises en vergers de

poiriers lors d'études des effets à court terme des pesticides (Stäubli et al. 1984), ou lors de la mise au point d'une méthode d'évaluation au champ pour *F. auricularia*. Nous nous proposons de comparer ces données aux résultats obtenus en conditions de laboratoire, comme première évaluation pour ces deux espèces de la validité de la séquence de tests appliquée par l'OILB.

METHODES EXPERIMENTALES

Tests de laboratoire

Les tests de laboratoire sont conduits selon les protocoles établis dans le cadre du groupe de travail pour *F. auricularia* (Sauphanor et al. 1992) et *A. nemoralis* (Stäubli & Pasquier 1988). Le principe général est une exposition des insectes, dans un premier temps de jeunes stades larvaires (stade sensible) d'âge homogène issus d'élevages de masse, au résidus frais séché du pesticide appliqué sur support verre. L'action du traitement est suivie sur l'ensemble du développement de l'insecte, ainsi que, pour *A. nemoralis,* sur la fécondité des femelles survivantes. L'effet total est dans ce cas calculé par la relation d'Overmeer & Van Zon(1982):

E (%) = 100% - (100% - M) x R

M : mortalité Abbott

R : Fecondité insectes traités / Fécondité insectes témoin

Les résultats sont exprimés en pourcentage de toxicité et par classe (OILB) : 1=non toxique (<30%), 2=peu toxique (30-79%), 3=moyennement toxique (80-99%), 4=toxique (>99%).

Test au champ

La méthode en cours de mise au point pour évaluer les effets des pesticides sur la faune auxiliaire, dont *F. auricularia* et *A. nemoralis* en verger de poiriers (Sauphanor et al 1993), est basée sur le dispositif utilisé pour les tests d'homologation des traitements sur *C. pyri* et sur le carpocapse des pommes *Cydia pomonella* L. (ANPP 1987, OEPP 1988).

Les parcelles élémentaires (PE) de 4 ou 5 arbres contigus sur le rang, sont disposées en bloc de Fischer à 5 répétitions avec une rangée de garde entre les blocs et un arbre de garde entre les parcelles sur le rang.

Les traitements sont appliqués à l'aide d'un motopulvérisateur à jet projeté, à la dose de 1000 l de bouillie/ha .

L'échantillonage, sur les 3 arbres centraux de chaque PE, se conforme aux normes recommandées pour le contrôle des ravageurs dans le cadre des essais d'homologation. Il inclut des contrôles visuels (sur 5 rameaux par PE) et des frappages hebdomadaires permettant le dénombrement des populations de psylles et de pucerons, mais aussi de certains auxiliaires, dont les oeufs et les formes mobiles d'*A. nemoralis*.

Des bandes pièges constituées de bandes de carton ondulé disposées à la base des troncs de chaque arbre d'observation sont relevées chaque semaine et permettent la capture et le dénombrement par stade des forficules. Les captures s'élèvent en moyenne à une vingtaine d'individus par arbre et par relevé dans les parcelles témoin.

Des entonnoirs de 0,5 m² (Steiner1977) sont accrochés sous la frondaison des arbres de chaque parcelle élémentaire et relevés deux fois par semaine. Ils sont dotés de tubes collecteurs à fond grillagé et remplis de tourbe humide favorisant la survie des insectes capturés vivants.

Lors de ces échantillonages, les larves des deux espèces de prédateurs, lorsqu'elles sont présentes en effectif suffisant, sont mises en élevage au laboratoire pour évaluer leur taux de survie préimaginale après exposition aux traitements au champ.

Pour les principaux effectifs observés, les traitements sont comparés par analyse de variance.

L'ensemble du dispositif d'observation permet d'appréhender les interactions pesticides / auxiliaires / ravageurs, et de déceler des effets particuliers de certains pesticides sur un auxiliaire. Les résultats présentés ici prennent en compte uniquement la réduction des captures, dans les bandes-pièges pour *F. auricularia* et par frappage pour *A. nemoralis*.

Nous nous référons également pour cette dernière espèce aux résultats obtenus lors d'essais à court terme (Stäubli et al., 1984).

Les résultats sont exprimés en pourcentage de toxicité et par classe (OILB) : 1=non toxique (<25%), 2=peu toxique (25-50%), 3=moyennement toxique (51-75%), 4=toxique (>75%).

RESULTATS ET DISCUSSION

Pour deux spécialités, le fenoxycarbe et l'abamectine, de fortes différences de toxicité sur *A. nemoralis* sont enregistrées d'un test à l'autre au laboratoire. La cause en est pour le fenoxycarbe une période d'observation plus courte lors du 3ème test que lors des deux premiers. Pour l'abamectine, différentes formulations étaient testées, mais à la même concentration de matière active.

Si l'on excepte la moindre toxicité au champ de la deltamethrine sur *F. auricularia*, sur une population constituée toutefois en grande partie d'adultes (stade moins sensible), les pyréthrinoïdes testées sont très toxiques au laboratoire et au champ sur ces deux prédateurs. Il en est de même pour 2 des 3 organophosphorés testés, l'heptenophos apparaissant non toxique sur *A. nemoralis* et *F. auricularia*.

Parmi les Régulateurs de Croissance des Insectes, le diflubenzuron à la concentration testée n'exprime de forte toxicité que sur *F. auricularia* en conditions de laboratoire. Le fenoxycarbe, non toxique sur cette espèce, est moins toxique au champ qu'au laboratoire sur *A. nemoralis*.

Tableau 1. Toxicité comparée de différents pesticides en condition de laboratoire et de plein champ sur ***A. nemoralis & F. auricularia***

			Forficula auricularia				*Anthocoris nemoralis*			
			Laboratoire, L2		Verger		Laboratoire		Verger	
Matière active	Produit commercial	Concentration testée	Mortalité (%)	Classe	Toxicité* (%)	Classe	effet total ** (%)	Classe	Toxicité* (%)	Classe ***
parathion	Penncap 240g/l	0,125%	100	4	97,2	4			98,4	4
diazinon	Basudine 250g/l	0,12%	100	4	90,2	4	54-31	2-2	*68 - 66*	*3 - 3*
heptenophos	Hostaquick 550g/l	0,10%	8	1			44-41	2-2		1
bifenthrine	Talstar 80g/l	0,05%	100	4	88,9	4	100-100	4-4	99,5	4
fenvalerate	Sumicidin 100g/l	0,075%					100	4	*96 - 100*	*4 - 4*
permethrine	Ambush 25%	0,03%					100	4	*94 - 97*	*4 - 4*
deltamethrine	Decis 25g/l	0,06%	100	4	53,3	3	100	4	*88 - 90*	*4 - 4*
pyrèthre+ rotenone+PBO	Biophytoz L 15+30+15g/l	0,70%	26	2	0	1				
fenoxycarbe	Insegar 25%	0,06%	3,3	1	33,1	2	100-100-66	4-4-2	74,5	3
diflubenzuron	Dimilin 25%	0,05%	100	4	36,1	2	8-0	1-1	0,0	1
amitraze	Maitac 200g/l	0,30%	10,4	1	0	1	90	3	*43 - 26*	*2 - 2*
abamectine	Vertimec 18g/l	0,06%	46,7	2	66,7	3	0-100-100-37	1-4-4-2	75,0	3
soufre	Thiovit 79 %	0,30%					88-88	3-3	*41 - 50*	*2 - 2*

*Réduction d'effectif par rapport au témoin

**Chaque valeur correspond à un essai

***Données en italique : essais à court terme (STÄUBLI et al 1984); toxicité sur larves - sur adultes

Dans la grande majorité des cas analysés, la toxicité des traitements en conditions de laboratoire est supérieure ou égale à la toxicité des mêmes traitements en condition de plein champ, conformément aux principes de la séquence de tests (Tableau 1). Les exceptions à cette règle sont l'abamectine et le fenoxycarbe, semblant plus toxique au champ qu'au laboratoire sur *F. auricularia*, et le diazinon, plus toxique au champ qu'au laboratoire sur *A. nemoralis*. Dans ces deux cas, les différences restent de faible amplitude.

Lorsque les études au champ portent sur des populations naturelles d'auxiliaires et de ravageurs, comme c'est le cas ici, la chute des effectifs d'auxiliaires peut être liée plus à la raréfaction de la proie sous l'effet du traitement qu'à l'action directe du traitement sur l'auxiliaire. C'est ce que l'on observe en verger de poiriers pour l'abamectine et le fenoxycarbe, réduisant très fortement les populations de psylles et aboutissant de ce fait à une réduction des effectifs de forficules plus forte au champ qu'au laboratoire. On constate en effet que la chute de forficules dans les entonnoirs est équivalente dans les parcelles témoin et fenoxycarbe, indiquant l'absence de toxicité directe de ce produit sur forficules. Les chutes de forficules dans les parcelles abamectine sont deux fois plus nombreuses que dans les parcelles témoin, indiquant une toxicité moyenne.

La raréfaction de la proie pourrait également être à l'origine des baisses d'effectif de *A. nemoralis* dans les parcelles fenoxycarbe. Contrairement aux résultats des essais à court terme (Stäubli et al. 1984) classant ce produit non toxique au champ sur *A. nemoralis*, une forte réduction des populations du prédateur est observée sur les parcelles traitées au fenoxycarbe dans les essais à moyen terme (Sauphanor et al. 1993). Mais la toxicité du produit est dans ce cas confirmée par la baisse du rapport numérique prédateur / proie, dont la prise en compte sur ce type d'essai apparaît donc essentielle, et par la mise en élevage au laboratoire de larves collectées au champ après l'application des traitements (réduction de 50% du taux de survie préimaginale par rapport au témoin).

D'une manière générale, outre la présence souhaitable de la proie pour certains auxiliaires, la méthodologie apparait plus difficile à définir au champ qu'au laboratoire. Il est nécessaire de prévoir une période d'exposition suffisante pour permettre l'évaluation de pesticides à effet différé, tels les régulateurs de croissance des insectes, mais cela peut se traduire pour certains organismes par des recolonisations des parcelles. Une très forte hétérogénéité des résultats peut en outre être enregistrée entre différents essais conduits selon le même protocole, avec des notes de toxicité variant de 1 à 4 pour une espèce donnée (Stäubli et al. 1984). Les tests en plein champ doivent pour celà être considérés comme un élément de la séquence de tests, dont les résultats sont à confronter avec ceux

obtenus lors des étapes précédentes. Ils peuvent dans certains cas mettre en lumière des modes d'actions particulier des produits et conduire à adapter la méthodologie utilisée au laboratoire.

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INITIAL AND EXTENDED LABORATORY TESTS FOR THE ROVE BEETLE *ALEOCHARA BILINEATA* GYLL.

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Abstract

Results of tests with 53 pesticides from the initial and the extended laboratory tests for the rove beetle *Aleochara bilineata* are compared. The initital laboratory tests is very efficient for the detection of toxic effects exerted through contact on adults and/or eggs of *A. bilineata*, but it will not reveal effects on larval stages that can be demonstrated by the otherwise less sensitive extended test.

The two test methods

The laboratory tests for *Aleochara bilineata* were described in detail by Naton (1989) (extended) and Samsøe-Petersen (1987) (initial) and briefly in e.g. Hassan 1992. Thus they shall not be described here in any detail. Only the basic conditions and the important aspects in which they differ will be briefly summarized in the following.

Similarities and differences

The larvae of this species are parasites on fly pupae. Both tests take place on moist sand to which adult beetles are released (in principle) after a spray, that results in a deposit equal to 600 l/ha, i.e. 6 µl/cm².

In the initial laboratory test the sand has a depth of 1 cm and forced ventilation is applied, while in the extended laboratory test the depth is 5 cm and there is no forced ventilation. This implies that exposure through contact with the pesticide deposit is greatest in the initial test, while exposure via inhalation is greatest in the extended test.

The parameters measured in the initial test are survival/mortality and number of eggs laid by individual females during 4 days of exposure as well as hatch of the eggs after exposure. In the extended test fly pupae are added and the parameters measured after a 5 weeks exposure period are the number of parasitized pupae and the number of living offspring of 10 pairs of beetles.

Thus the initial test gives detailed information about effects on survival, egg production and hatch of eggs, while the extended test gives a measure of the effects on the total reproductive capacity (fecundity) - including effects on reproductive behaviour. In addition the recording of the total number of parasitised pupae can add informations about the mode (part of life cycle) of action of the pesticides when compared to the number of emerging adults of the next generation (living offspring).

Interpretation based on the combination of results

Combining results from both tests can give information about the importance of exposure through contact/inhalation - even though the interpretation has to be based on assumptions about the relative importance of each exposure route. (These assumptions may be verified by additional experiments with the initital test changing the exposure from spray deposits to vapours.) Furthermore a combination of results can lead to interpretations concerning the stage of this species that is most susceptible to pesticides; i.e. adults, eggs or larvae.

Results from comparable tests with 53 pesticides

Both tests were used for testing most of the pesticides of the 4th, 5th and 6th joint pesticide testing programmes of the Working Group, and the results of these tests are presented in Table 1-6. Results from tests with pesticides that were not used in both tests were published by Naton (1989), Moreth & Naton (1992) and Samsøe-Petersen (1987, 1993 and 1994a and b) in combination with the results presented here.

In Tables 1-6, the numbers of the joint testing programmes are indicated in parenthesis following the name of the product in order to facilitate comparisons with these publications. In a few cases the dosage applied in the two tests was not comparable. These are indicated by footnotes to the tables.

There are two tables for each of the major groups of pesticides: Insecticides, fungicides and herbicides. As many fungicides and herbicides showed no considerable effects in any of the tests, these are listed in separate tables (3 and 5).

In the remaining tables the effects measured are presented in a logical order starting with the mortality of parents, followed by reduction in egg production, hatch of eggs and total parasitization ending with succesfull parasitization (living offspring).

Table 3. Fungicides with no measurable or considerable effect on *Aleochara bilineata* in neither the initial nor the extended laboratory tests, arranged in aphabetical order of the active ingredient.

Product	Active ingredient	Conc. tested, % a.i. in 6 µl/cm²
Baycor (5th)	bitertanol	0.0925
Delan flüssig (5th)	dithianon	0.0446
Milgo-E (4th)	ethirimol	0.0504
Rubigan Vloeibaar (4th)	fennarimol	0.0144
Ortho-Phaltan 50 (4th)	folpet	0.165
Anvil (6th)	hexaconazole	0.0015
Polyram-Combi (4th)	metiram	0.35*
Topas (4th)	penconazole	0.004
Sumisclex (6th)	procymidone	0.075
Tilt (4th)	propiconazole	0.02
Bayfidan (6th)	triadimenol	0.0125
Calixin (6th)	tridemorph	0.0563
Saprol (6th)	triforine	0.0285

Numbers in parenthesis indicate numbers of "joint pesticide testing programmes" performed by the Working Group.

* There was a slight difference between the concentrations used for the two tests.

Discussion

The general impression of the comparison of results from the two laboratory tests is, that the effects measured in the initial test are equal to or exceeding the effects measured in the extended test. This is in accordance with the possibility of reduced exposure through contact with the pesticides in the deeper sand layer in the latter experimental design.

Table 1. Effects of insecticides on *A. bilineata* in the initial and/or extended laboratory tests. The compounds are grouped according to their chemistry and mode of action. This table shows results from organophosphorous compounds and one carbamate.

			Initial				Extended		
Product	**Active ingredient**	**Concentration % a.i. in 6 µl/cm²**	**Morta-lity No.**	**Reduction in egg prod. %**	**Red. in hatch %**	**IOBC Class. Initial**	**Red. in tot. no. paras. pupae %**	**Red. in living off-spring %**	**IOBC Class. Extended**
Birlane Fluid	chlorfenvinphos	0.492*	9	100		4	100*	100*	4
Birlane EC 40 (4th)	"	0.132	9	99.7	.	4	98	99	3
Phosdrine W10 (4th)	mevinphos	0.058	9	95	.	3	68	68	3
Perfekthion (4th)	dimethoate	0.084	9	84	37	4	93	94	3
Tamaron (6th)	methamidophos	0.09	8	91	.	3	53	28	2
Dursban Spritzp. (4th)	chlorpyriphos	0.063'	9	96	100	4	100 '	100'	4
Basudine Vloeib. (4th)	diazinon	0.038	9	98	.	3	-	100	4
Dimecron 20 (4th)	phosphamidon	0.05#	9	73	22	3	+23#	+51#	1
"	"	0.005	-	-	-		5	+2	1
Torak E (5th)	dialiphos	0.108	9	89	NS	3	72	76	3
Asepta Nexion (4th)	bromophos	0.1¤	7	88	NS	3	72¤	78¤	3
Hostathion (4th)	triazophos	0.1	9	99.5	.	4	100	100	4
Vydate L (5th)	oxamyl	0.0368	9	93	.	3	+54	+6	1

Numbers in parenthesis indicate numbers of "joint testing programmes" performed by the Working Group.
NS = Not significant.
. = Numbers too low to allow statistical analysis.
- = Not measured.
* This product was applied at a rate of 2.5 µl/cm² in the extended lab.test.
' This product was applied at a conc. of 0.05% a.i. in the extended lab.test.
This product was applied at a conc. of 0.02% a.i. in the extended lab.test.
¤ This product was applied at a conc. of 0.037% a.i. in the extended lab.test.

Table 2. Effects of insecticides on *A. bilineata* in the initial and extended laboratory tests. The compounds are grouped according to their chemistry and mode of action. This table shows results from 2 pyrethroids, 5 insect growth regulators and 3 miscellaneous insecticides.

			Initial				Extended		
Product	**Active ingredient**	**Concentration % a.i. in 6 µl/cm²**	**Morta-lity No.**	**Red.in egg prod. %**	**Red. in hatch %**	**IOBC Class. Initial**	**Red. in tot. no. paras. pupae %**	**Red. in living offspring %**	**IOBC Class. Extended**
Rody (6th)	fenpropathrin	0.005	9	100	.	4	39	36	2
Ambush C (4th)	cypermethrin	0.004*	9	100	.	4	100*	100*	4
Dimilin (6th)	diflubenzuron	0.0125	2	NS	NS	1	+12	+19	1
Trigard (6th)	cyromazine	0.0503#	0	NS	NS	1	8#	58#	3
Cropotex (5th)	flubenzimine	0.05	0	NS	pos	1	18	39	2
Apollo SOSC (5th)	clofentezine	0.02	0	NS	28	1	+11	+32	1
Insegar (5th)	fenoxycarb	0.015	0	NS	59	2	9	3	2
Cesar S.L. (5th)	hexythiazox	0.005	0	NS	11	1	7	5	1
Evisect (5th)	thiocyclam	0.015	9	97	.	4	100	100	4
Neudosan (6th)	kali-seife	0.958	1	NS	NS	1	7	9	1

Numbers in parenthesis indicate numbers of "joint testing programmes" performed by the Working Group.
NS = Not significant.
pos = Minor increase, significant at the 5% level.
. = Numbers too low to allow statistical analysis.
* This product was applied at a conc. of 0.008% a.i. in the extended lab.test.
# This product was applied at a conc. of 0.015% a.i. in the extended lab.test.

Table 4. Effects of fungicides on *Aleochara bilineata* in the initial and/or the extended laboratory tests.

			Initial				Extended		
Product	**Active ingredient**	**Concen-tration % a.i. in 6 µl/cm²**	**Morta-lity No.**	**Red.in egg prod. %**	**Red. in hatch %**	**IOBC Class. Initial**	**Red. in tot.-no. paras. pupae %**	**Red. in livin offspring %**	**IOBC Class. Extended**
Vitigran (5th)	Cu-oxychloride	0.45	0	pos	NS	1	39	33	2
Euparen (5th)	dichlofluanid	0.075	0	NS	18	1	43	48	2
Corbel (4th)	fenpropimorph	0.128	5	93	.	3	3	2	1
Impact (5th)	flutriafol	0.02	0	NS	NS	1	21	17	1
Rovral PM (5th)	iprodione	0.075	0	NS	NS	1	30	24	1
Antracol (5th)	propineb	0.14	0	NS	pos	1	19	37	2
Dithane M 22 (5th)	maneb	0.4	2	42	44	2	19	31	2

Numbers in parenthesis indicate numbers of "joint pesticide testing programmes" performed by the Working Group.
NS = Not Significant.
pos = Minor increase, significant at the 5% level.
. = Numbers too low to allow statistical analysis.

Table 5. Herbicides with no measurable or considerable effect on *Aleochara bilineata* in neither the initial nor the extended laboratory tests, listed alphabetically with respect to the active ingredient.

Product	Active ingredient	Conc. tested, % a.i.in 6µl/cm²
Gesaprim 50 (4th)	atrazine	0.35
Fusilade (4th)	fluazifop-butyl	0.063
Basta (5th)	glufosinateammonium	0.1
Roundup (4th)	glyphosate	0.36
EXP 30004A (6th)	ioxynil	0.058
Ally (5th)	metsulfuron-methyl	0.013
Targa (6th)	quizalofop-ethyl	0.03
Grasp (6th)	traloxydim	0.03*

Numbers in parenthesis indicate numbers of "joint pesticide testing programmes" performed by the Working Group.
* This product was applied at a conc. of 0.05 % a.i. in the extended lab.test.

Table 6. Effects of herbicides on *Aleochara bilineata* in the initial and/or extended laboratory tests.

			Initial				Extended		
Product	Active ingredient	Concen-tration % a.i. in 6 µl/cm²	Morta-lity No.	Red.in egg prod. %	Red. in hatch %	IOBC Class. Initial	Red. in tot. no. paras. pupae %	Red. in living off-spring %	IOBC Class. Extended
Luxan 2,4-D (5th)	2,4-D aminesalt	0.2	0	NS	23	1	19	53	2
Tribunil (5th)	metabenzthiazuron	0.469	0	NS	100	4	16	48	2
Ustinex PA (3rd)	diuron + amitrol	0.36+0.30	0	NS	56	2	-	28	1(2)

Numbers in parenthesis indicate numbers of "joint pesticide testing programmes" performed by the Working Group
NS = Not Significant.
- = Not measured.

For some compounds the decrease in effect in the extended test is remarkable. Among these are Dimecron 20 and Vydate L (from Table 1), Rody (Table 2), Corbel (Table 4) and Tribunil (Table 6).

With Dimecron 20 and Vydate L a strong positive effect was recorded in the extended test, while the initial test showed very strong negative effects. Dimecron 20 was applied at different rates in the two tests: In the initial test it was used at a concentration of 0.05% a.i. in the 6 µl/cm² while the extended test was performed twice with 0.02% and 0.005% respectively. In the initial test a clear toxic effect on egg production was measured at the high rate. In the extended test egg production and survival of the larvae was enhanced strongly at the intermediate rate while the lowest rate showed no effects in this test. The enhancement measured is difficult to interpret, and this is one of the cases where information about the dose response in the laboratory tests would have been very useful. With Vydate L all the adults died in the initial test and only very few eggs were laid. The reduced contact with the compound in the extended test seems to have been enough to minimize the toxic effect. Actually the number of parasitized pupae in the extended test was increased in the treated units, but at the end of the test the number of surviving offspring was equal in treated and untreated.

The results with Rody and even more with Corbel seems to indicate that the strong effects measured in the initial test are easily reduced by the reduced exposure. Again the interpretation would be enhanced by knowledge of the dose response relationships. The effect of Corbel in the initial test is discussed in detail by Samsøe-Petersen (1994a), who showed that the introduction of refuges (areas without pesticide) reduced the effect of Corbel dramatically.

Tribunil exerted a 100% reduction in hatch of eggs in the initial laboratory test - without affecting survival of adults or number of eggs laid during the exposure period. This effect showed a considerable decrease in the extended test - even though the vitality of the emerged larvae seems to have been reduced, resulting in a halving of the number of offspring.

The strong effects in the initial test are in accordance with the assumption of the Working Group, that results from the initial laboratory test showing no considerable effects (NS or < 30% reduction) imply that the pesticide is not toxic to the species. Consequently the compound should not affect it under any circumstances, when applied at a dose equivalent to the one tested. There are however exceptions to this picture, and these shall be discussed in more detail. First of all it is important to clarify why deviations from the basic assumptions mentioned above are recorded. Secondly these results may improve our understanding of the action of these pesticides on *Aleochara bilineata*.

To facilitate the comparisons, the results with pesticides diverting from the simple interpretation of reduced exposure in the extended test are collected in Table 7, in which the outcome of each test is presented by means of the "IOBC-classes". In this way comparisons are facilitated and numerical differences that may not be "biologically significant" are smoothed out. The classification of the results of the initial test is based on the reduction in mean number of hatched larvae (that cannot always be deduced from the separately calculated reductions in average egg production and average hatch), while that of the extended test is based exclusively on the reduction in the number of living offspring.

Table 7. Pesticides from Table 1-6 for which the results of the initial and extended laboratory tests call for discussion. The results are expressed in accordance with the "IOBC-classification". The pesticides are listed continuously as they were taken from Tables 1-6.

Product		Initial *	Extended **
Insect Growth Regulators	Trigard	1	3
	Cropotex	1	2
	Apollo SCOC	1	pos
Fungicides	Vitigran	1	2
	Euparen	1	2
	Impact	1	1
	Rovral PM	1	1
	Anthracol	1	2

* 1= reduction < 30%, 2= reduction 30-79%
** 1= reduction < 25%, 2= reduction 25-50%, 3= red. 51-75%

Among the insect growth regulators Trigard and Cropotex clearly affect the development of larvae without affecting the other stages in the life cycle of *A. bilineata*. This is the more striking as Trigard was applied at a dosage of approximately one third in the extended test. As the larval stages are not included in the intital test the usefulness of the extended test is clearly illustrated by these results as discussed by Samsøe-Petersen (1993). For pesticides expected to affect developmental stages of insects (like these insect growth regulators) it is logical that an evaluation of possible side effects on *A. bilineata* should not be based on the initial laboratory test.

Apollo reduced the hatch of eggs in the initial test but increased survival of offspring in the extended test. The magnitude of these effects was very similar and relatively low (about 30%). The "biological significance" could thus be questioned, but on the other hand these results could be interpreted to indicate that this product may affect *A. bilineata*. Again informations about the dose response relationships would be very valuable in order to interpret the results.

The fungicides that showed considerably stronger effects (as judged by the IOBC-classes) on the beetles in the extended test than in the initial test were Vitigran, Euparen and Anthracol. The magnitude of the effects measured in the extended test was limited (max. 48% reduction), and again the "biological significance" of these effects could be questioned. But it is still worrying that the extended test revealed unexpected effects that were not shown by the initial test. Informations about the vapour pressures of these compounds have not been available so the possibility of effects by inhalation in the extended test cannot be evaluated.

In summary the initital laboratory test is very efficient for the detection of toxic effects exerted through contact on adults and/or eggs of *A. bilineata*. Among the 53 pesticides tested with both methods the initial test recorded toxic effects stronger than or at the same level as the extended test for 48 compounds. In five cases the highest effect was recorded in the extended test and among these two could be immediately explained by the intended mode of action of the pesticides. Thus the combination of results from the initial and the extended laboratory tests is a valuable tool in the registration and interpretation of effects of pesticides on *A. bilineata*, but in several cases the lack of dose response information impedes the interpretation.

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COMPARISON OF EFFECTS OF PESTICIDES ON ADULT CARABID BEETLES IN LABORATORY, SEMI-FIELD AND FIELD EXPERIMENTS

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Abstract

The effects of three pesticides, chlorpyrifos, lambda-cyhalothrin and lindane on carabid beetles were tested in laboratory, two different designed semi-field tests and in field tests. The pesticides used caused different effects of toxification on carabid beetles depending on the type of test design used and on the pesticide. The results obtained in the three test tiers were compared with each other. Reasons for different effects in these tests were analysed and discussed.

Introduction

Testing effects of pesticides on beneficial organisms is carried out to select selective pesticides that are needed to protect the predator-prey interactions in agricultural ecosystems. Within the IOBC testing scheme (Hassan, 1989) laboratory tests are conducted as a first step to investigate the harm of pesticides. If a product turns out to be harmful further steps are semi-field and field tests. This sequence of test methods should be as efficient and as realistic as possible in regard to the field situation within each step to avoid unnecessary testing. Comparing the results of laboratory, semi-field and field tests is important for the development of test methods. Laboratory and semi-field tests should represent a worst case situation but at the same time be as realistic to the field situation as possible. Therefore, when developing test methods the field situation has to be kept in mind. A field test should give a clear picture of the distribution and dynamics of carabid beetles. This is quite complicated using carabid beetles as test organisms, because there is no adequate method available to measure the real density of these beetles. They easily dig into the soil, they may stay inactive for long periods and may move over quite long distances. All this may be induced by natural conditions as well as by the use of pesticides.

In this paper, results from experiments using different pesticides and test methods (Heimbach, 1992; Abel and Heimbach, 1992; Heimbach et al., 1992b) to investigate effects of pesticides on *Poecilus cupreus* L. (Coleoptera: Carabidae) are compared, showing the problems arising in the practical use and interpretation of these tests.

Material and Methods

Laboratory and semi-field tests were carried out using adults of *Poecilus cupreus* (Coleoptera: Carabidae) from a laboratory rearing. Effects on natural occurring populations of carabid beetles (*P. cupreus* occurred only in low densities) were investigated in field tests. The laboratory and the two types of semi-field tests were carried out according to IOBC guidelines described by Heimbach (1992) (laboratory test, beetles in small boxes filled with quartz sand with usually 5 replicates, 6 beetles each), Abel and Heimbach (1992) (semi-field test, metal boxes of 600 cm^2, filled with wet loamy sand without plants, dug into the soil of the field with usually 6 replicates, 6 beetles each) and Heimbach et al. (1992b) (semi-field test, metal frames of 1 m^2 sunk into the soil of the field including crop plants with usually 4 replicates, 10 beetles each). The number of replicates and type of soil differed in some of the experiments.

Field experiments in summer were conducted in fields with winter wheat or sugar beet on 0.75 to 1.2 ha sized plots without replications in 1989 and 1990, in 1991 and 1992 3 untreated and 6 treated replicates. Untreated plots were of the same size as the treated ones. In the centre of each plot pitfall traps (5 in 1989, 10 in 1990, 6 in 1991 and 1992) were buried and the carabid beetles population caught before and after application to assess the activity density. The pitfall traps were emptied about once a week without disturbing the surrounding soil surface. The activity of the beetles was analysed until 2 weeks after application in 1989, 4 weeks in 1990 and 1991 and 5 weeks in 1992. In addition only few ground-photoeclectors were used. But the number of beetles caught with these eclectors was not sufficient for an interpretation. In some experiments dead beetles or beetles showing symptoms of toxification were collected by hand within comparable areas of treated and untreated parts of the field up to about one week after application.

Field experiments in autumn (sprayed in October) were conducted in fields with winter cereals on plots of about 0.06 ha (4 replicates), using 2 ground-photoeclectors per plot (each covering 0.25 m^2, a total of 2 m^2 for each treatment). The eclectors were replaced about every fortnight. The photoeclectors were used until 6 weeks after application in 1991 and 7 weeks in 1992. The use of a D-Vac did not result in sufficient numbers of beetles.

Lindane and chlorpyrifos (registered rate in Germany 800 g a.i./ha and 960 g a.i./ha) were applied just after sowing of sugar beets in April and lambda-cyhalothrin (registered rate in Germany 10 g a.i./ha) as an aphicide in cereals in May/June. All applications were carried out using a qualified spraying equipment with nozzles, pressure and speed according to good agricultural practice. Laboratory and some of the semi-field experiments were sprayed using a hand hold spraying equipment for small plot experiments. Field and some of the semi-field experiments were sprayed using a tractor mounted spraying boom of 12 m length. Water amounts of 300 to 400 l per ha were used.

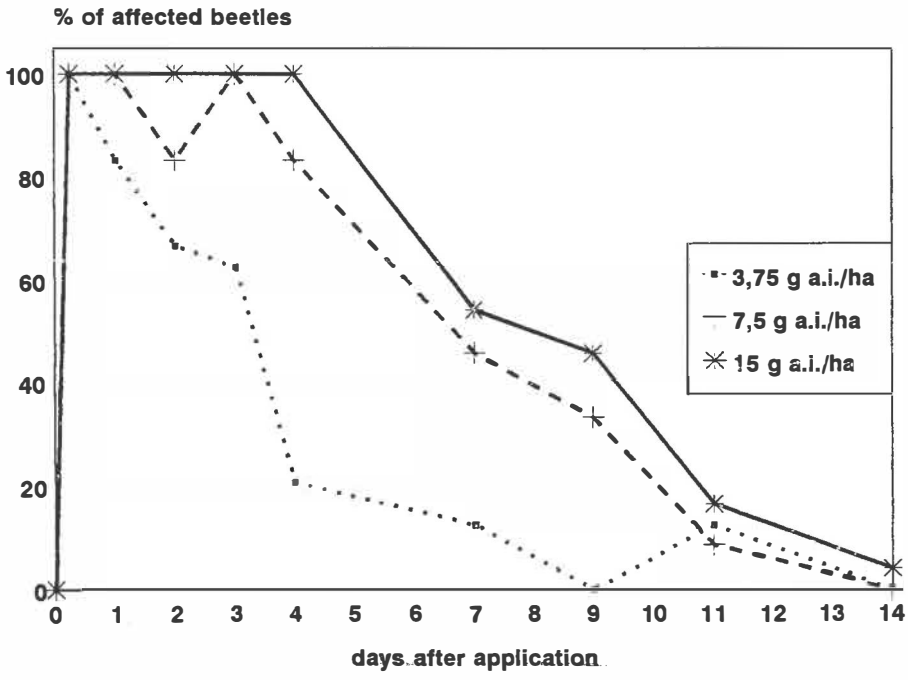

Figure 1: Effects of three different rates of lambda-cyhalothrin on adult *Poecilus cupreus* in a laboratory test (4 replicates, 6 beetles each)

Results

Laboratory experiments

As an example, results of an experiment of 3 different rates of lambda-cyhalothrin are shown in Fig. 1. The knockdown effect which is typical for pyrethroids occurred very fast and with the same intensity at all rates, but recovery of the beetles took longer the higher the rate was. After 14 days no differences between the 3 rates were detected any more.

In a laboratory study at 4 different post treatment temperatures (Fig. 2) the knockdown effect was very similar but the recovery strongly depended on temperature. At lower temperatures it took much longer until beetles began to recover.

In a further experiment lindane (Tab. 2) and chlorpyrifos (Tab. 3) turned out to be very toxic though the test was conducted on a loamy sand (not on quartz sand as described in the test guideline) and the beetles were exposed only to fresh residues of these pesticides and were not sprayed topically together with the substratum as usual.

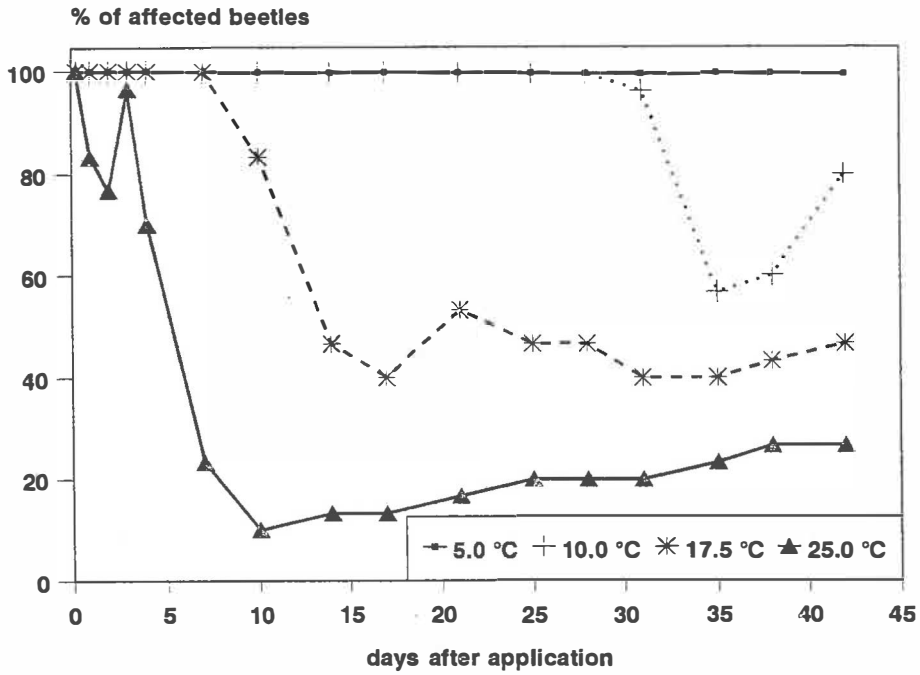

Figure 2: Effects of 10 g a.i./ha lambda-cyhalothrin on adults of *Poecilus cupreus* in a laboratory test using 4 different post treatment temperatures

Semi-field experiments

In most cases lambda-cyhalothrin showed distinct knockdown effects following summer and autumn applications (Tab. 1) except of one experiment (18/10/90) in which no symptoms of toxification were detected. The time needed for the recovery following the knockdown of the beetles was shorter after summer application than after autumn application. In most experiments more beetles were visible in the treated areas after application than in the untreated ones. At the end of the experiments the beetles were picked by hand from the soil. In almost all experiments more beetles were found to be healthy in untreated areas in both types of semi-field test-design especially after autumn applications. In the experiments with frames the number of inserted beetles which were found back at the end of the studies was usually much lower in the treated areas compared to untreated ones. In contrary the number of beetles found back in boxes was very high and did not differ distinctly between treated and untreated ones. After autumn applications only very few surviving beetles were detected in the treated frames even using reduced amounts of the

Table 1: Effects of different rates of lambda-cyhalothrin in summer and autumn applictions on Poecilus cupreus in semi-field tests using frames and boxes

date of appl. type of test	treatment	% affected of visible beetles (No. visible) after 6/7d		after 11/13d		% healthy	% dead	% affected	% missing
29/4/91	**summer**								
winter rye						**test finished after 21/24 d**			
frames,EC39	untreated	0	(1)	0	(1)	**95**	0	0	5
(40 beetles)	10 g a.i./ha	92,3	(13)	0	(6)	**25**	0	0	75
boxes	untreated	0	(0)	0	(1)	**91,7**	2,8	0	5,6
(36 beetles)	10 g a.i./ha	33,3	(6)	12,5	(8)	**88,9**	11,1	0	0
25/6/91									
sugar beet						**test finished after 15/17 d**			
frames,EC46	untreated	0	(8)	0	(2)	**91,7**	4,2	0	4,2
(24 beetles)	10 g a.i./ha	0	(9)	0	(0)	**58,3**	0	0	41,7
boxes *	untreated	0	(23)	0	(27)	**91,7**	0	0	8,3
(72 beetles)	10 g a.i./ha	17,6	(17)	20	(20)	**86,1**	9,7	1,4	2,8
26/5/93									
sugar beet						**test finished after 15/16 d**			
frames,EC41	untreated	0	(4)	0	(0)	**45**	0	0	55
(40 beetles)	10 g a.i./ha	71	(7)	25	(4)	**42,5**	2,5	0	55
boxes	untreated	0	(6)	0	(4)	**92**	0	0	8
(30 beetles)	10 g a.i./ha	65	(23)	0	(16)	**93**	0	0	7
18/10/90	**autumn**								
winter wheat						**test finished after 6 d**			
frames,EC12	untreated	0	(33)	-		**82,5**	0	0	17,5
(40 beetles)	10 g a.i./ha	0	(31)	-		**77,5**	0	0	22,5
25/10/90									
winter barley						**test finished after 6 month**			
frames,EC11	untreated	0	(6)	0	(12)	**72,5**	15	0	12,5
(40 beetles)	10 g a.i./ha	96	(25)	100	(19)	**0**	30	0	70
16/10/91									
winter barley						**test finished after 28 d**			
frames,EC12	untreated	0	(4)	0	(12)	**72,5**	0	10	17,5
(40 beetles)	10 g a.i./ha	100	(7)	89	(18)	**2,5**	12,5	62,5	22,5
boxes	untreated	17	(6)	0	(6)	**90**	0	10	0
(36 beetles)	10 g a.i./ha	100	(30)	100	(31)	**0**	16,7	83,3	0
22/10/92									
winter barley						**test finished after 6 month**			
frames,EC13	untreated	0	(0)	0	(0)	**82,5**	2,5	0	15
(40 beetles)	2,5 g a.i./ha	100	(5)	100	(3)	**55**	5	0	40
	5 g a.i./ha	100	(7)	90	(10)	**25**	17,5	0	57,5

EC = growth stage of crop * untreated boxes 36 beetles

pyrethroid (22/10/92) at the end of these studies at the next spring. With the exception of one experiment (26/5/93) the total number of beetles found back at the end of the experiments was sufficient for data interpretation.

When beetles were exposed to fresh residues of lindane in different semi-field experiments the results varied considerably (Tab. 2). In boxes highly toxic effects were observed. In two out of three frame experiments nearly no mortality occurred and in only one experiment toxic effects were noticed. Residues of chlorpyrifos (Tab. 3) killed about 100 % of the beetles without differences between boxes and frames in further similar experiments.

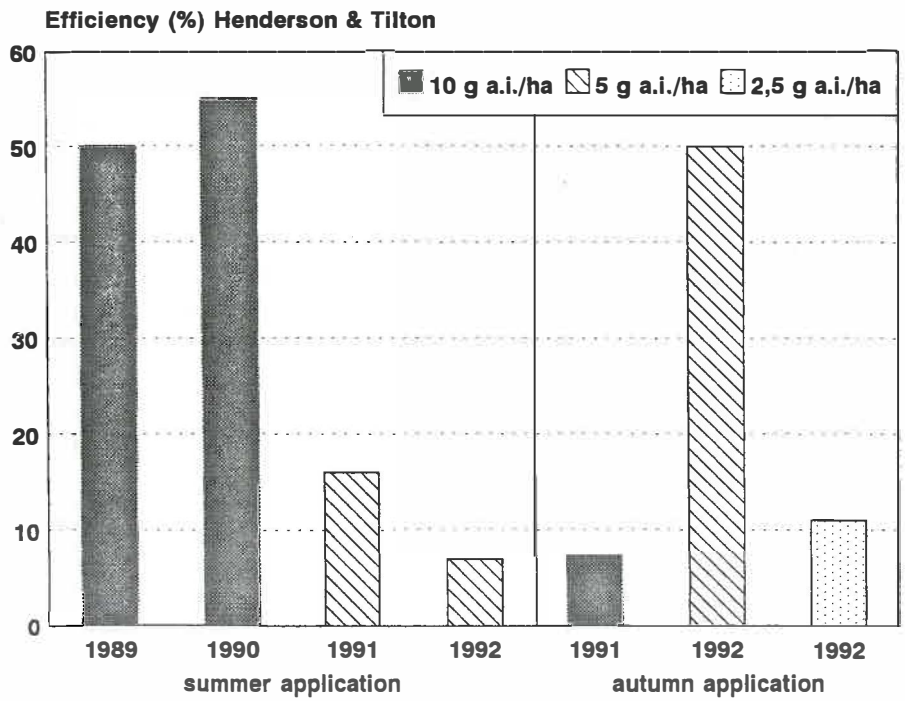

Figure 3: Efficiency of different rates of lambda-cyhalothrin applied in summer on the activity of carabid beetles (pitfall traps) and applied in autumn on the density of carabid beetles (ground-photoeclectors) in field experiments

Field experiments

The full application rate of lambda-cyhalothrin reduced the activity density to about 50 % in summer 1989 and 1990 (Fig. 3). The reduced rate (5 g a.i./ha) resulted in only slight effects in

Table 2: Effects of lindane sprayed at 800 g a.i./ha on carabid beetles in a sugar beet field (pitfall traps until 7/5/90) and of lindane residues in semi-field (sugar beet) and laboratory tests

date of appl. type of test	substrate	spp. (No.=)	treatment	% mortality	% reduction of activity ** (No. in control)	No. of dead beetles found
laboratory	loamy sand	P.c. (20)	untreated	10		
			treated	85		
4/4/89,EC00 semi-field frames	silty loam	P.c. (40)	untreated	0		
			treated	0		
7/5/89,EC21 semi-field frames	loamy sand	P.c. (40)*	untreated	20		
			treated	60		
2/4/90,EC00 semi-field boxes	loamy sand	P.c. (36)	untreated	3		
			treated	78		
semi-field frames	silty loam	P.c. (40)	untreated	0		
			treated	5		
field	silty loam	Pt. spp.	treated	-	90 (105)	94 (0)
		Carab.	treated	-	93 (395)	95 (0)

P.c. = Poecilus cupreus, Pt.spp. = Pterostichus spp., Carab. = all Carabidae
* = only 20 beetles in untreated, ** Abbott, 1925

Table 3: Effects of chlorpyrifos sprayed at 960 g a.i./ha on carabid beetles in a sugar beet field (pitfall traps until 7/5/90) and of chlorpyrifos residues in semi-field (sugar beet) and laboratory tests

date of appl. type of test	substrate	spp. (No.=)	treatment	% mortality	% reduction of activity ** (No. in control)	No. of dead beetles found
laboratory	loamy sand	P.c. (20)	untreated	10		
			treated	100		
4/4/89,EC00 semi-field frames	silty loam	P.c. (40)	untreated	0		
			treated	98		
2/4/90,EC00 semi-field boxes	loamy sand	P.c. (36)	untreated	3		
			treated	97		
semi-field frames	silty loam	P.c. (40)	untreated	0		
			treated	98		
field	silty loam	Pt. spp.	treated	-	78 (105)	23 (0)
		Carab.	treated	-	89 (395)	23 (0)

P.c. = Poecilus cupreus, Pt.spp. = Pterostichus spp., Carab. = all Carabidae
** Abbott, 1925

summer 1991 and 1992. In most experiments distinct higher numbers of dead or toxicated carabid beetles were collected by hand on sprayed plots compared to similar areas within untreated plots. The occurrence of dead and toxicated beetles lasted more than one week in some of the experiments. The reduction of activity density was quite different in two autumn experiments. The full rate (10 g a.i./ha) had only little effects in 1991 similar to the effects of just a quarter of this rate in 1992, whereas the half of the full rate reduced the activity to about 50 % in 1992.
Lindane and chlorpyrifos had quite strong effects on carabid beetles (Tabs. 2, 3) according to the number of dead beetles collected by hand and to the number of beetles caught with pitfall traps.

Discussion

Laboratory tests should represent reasonable worst case situations to allow regulatory decisions whether a product is harmless or potentially harmful and has to be tested in tier II or III test systems. This decision is quite easy if organisms are killed during a test as with lindane and chlorpyrifos. Both pesticides have been shown to be toxic in laboratory tests to a variety of arthropods including carabid beetles (Hassan et al., 1983; 1988).
The interpretation of laboratory test results is much more complicated if the beetles show symptoms of toxification but recover as after application of lambda-cyhalothrin. Although in the laboratory total recovery of beetles is observed within some days after application without any mortality, dead beetles and reduced activity of beetles can be found in the field for several weeks after application. The results of the laboratory test with different post treatment temperatures indicate that for a worst case situation in the laboratory not only the exposition of the organisms to the pesticide and the type of substratum has to be taken into account but also the temperature (Heimbach and Baloch, 1994), which might not be reasonable for all pesticides. The importance of the temperature is also demonstrated by the summer and autumn applications of lambda-cyhalothrin in semi-field tests, in which longer lasting and more intensive effects could be observed in autumn. But in these experiments also the crop cover differed according to the season. Also other field studies with lambda-cyhalothrin application in autumn showed long lasting effects on different organisms even at lower rates than 10 g a.i./ha (Brown et al., 1988; Krause et al., 1990).

The results of the two different types of **semi-field** design varied in the case of lindane quite drastically. Little or no effects were observed in frames but high mortality occurred in boxes. The type of soil might be the reason for this differences as the soil influenced the toxicity of lindane in other laboratory tests (Heimbach et al., 1992a). Therefore effects are more likely to occur on

loamy sands which were used in the boxes and also in the case of one frame experiments in which the substratum was loamy sand and in which some mortality was observed.

The mortality of the beetles was very high after application of chlorpyrifos independent from the semi-field test design. This pesticide turned out to be very toxic for carabid beetles in semi-field experiments even if the pesticide had been sprayed one week or more before introduction of the beetles in some other experiments (Floate et al., 1989; Heimbach et al., 1994). A gauze cover on frames or its absence influenced the effects on carabid beetles in semi-field tests on chlorpyrifos (Heimbach et al., 1994) but frames without a cover represented a more realistic field situation.

Lambda-cyhalothrin had toxic effects in both types of semi-field tests in all of our experiments except of one. Brown et al. (1988) found mortality of 2 out of 4 tested species of carabid beetles in semi-field tests in which 5 g a.i./ha was applied in autumn.

Comparing the total recovery rate in the untreated frames at the end of the two different designs of semi-field experiments (Tab. 1) shows that the number of beetles is distinctly higher in the boxes in half of the experiments. This might be caused by the fact that beetles cannot escape by burying into the soil. This might happen in the frames and be the reason for high beetle losses in one experiment (26/5/93) in which beetles were observed to hide in burrows of the earthworm *Lumbricus terrestris* L. which might enable them to walk down up to 3 m into the soil. In most cases it was easier to observe beetles in the boxes due to the absence of a plant cover and a smaller area.

In addition to a better rate of introduced beetles found back at the end of the experiments in boxes, the food uptake of the beetles represents a further advantage of boxes which was usually not possible in frames (Heimbach and Abel, unpublished) because other organisms like mice, birds, other natural predators or earthworms ate or removed the *Musca* pupae used as a food supply.

On the other hand the occurrence of predators and competitors of *Poecilus cupreus* is an advantage of the frame test, if the frames are not covered with a gauze, because it is more likely that in the frames sublethaly affected beetles are killed and eaten by these predators which cannot invade the boxes. This predation on sublethaly affected beetles seems to be the reason for much lower rates of surviving beetles found in treated frames at the end of the experiments compared to boxes. Accordingly more dead beetles were picked up in treated boxes compared to treated frames during the experiments. But beetles are able to recover in boxes even if they are sublethaly affected though they would die if they would not be kept apart from competitors and predators.

With lambda-cyhalothrin the observations during the first 14 days do not show distinct differences between the two types of test design regarding the percentage of affected beetles although loamy sand was used in boxes whereas mostly sandy loam was used in frames. Also other laboratory experiments with pyrethroids (fenvalerate and lambda-cyhalothrin) showed that for these pesti-

cides the type of soil was generally much less important than for lindane, parathion and endosulfan (Heimbach et al., 1992a; Wehling and Heimbach, 1994).
Boxes represent a better worst case situation because important factors influencing the exposure and availability of a pesticide as the type of soil, soil moisture (Sprick, 1992) and plant cover can be simulated easily, whereas frames have the advantage to represent a more realistic field situation including important biological parameters like predation if they are not covered with a gauze. The results indicate that only one experiment will usually not give enough information for a hazard assessment of a pesticide.

In **field** tests it is quite difficult to assess the density of carabid beetles as they hide and bury easily into the soil. Pitfall traps represent only an activity density (Basedow et al., 1987) and beetles do not fall into traps if they are skilful. In consequence, a reduction of activity after a pesticide application may be induced by mortality or just by reduced activity. A real pesticide induced effect may be overcompensated by hyperactivity (Croft, 1990) or reduced skilfulness in treated areas. Additionally the appearance of a new generation of a species that had been in the protected pupa stage in the soil during application (Sprick, 1992) has to be considered. The period of time during which an effect is found is influenced by the plot size and the number and activity of beetles in the neighbourhood of the plot. Nevertheless, effects lasting for several weeks should be regarded as a result of a negative impact of the pesticide.
Other methods analysing the density of carabid beetles (e.g. ground-photoeclector, D-Vac, watering or sampling of soil cores) are quite ineffective because only small areas can be analysed and the numbers of beetles found is accordingly very low. Also hiding in the soil, burying or hyperactivity if induced by a pesticide will influence the results. There are no useful methods to get data about the mortality or changes of the real density after a pesticide application. The picking up of dead beetles from the soil surface also is quite inaccurate because unaffected predators in the field may eat or remove dead beetles. Altogether field tests have to be interpreted with care.
The effects of lindane and chlorpyrifos application in the field are similar to results of Basedow (1989) and Floate et al. (1989) who found severe effects after the use of these pesticides. Lambda-cyhalothrin showed effects on the beetle activity varying from year to year and depending on the application rate. Brown et al. (1988) found quite long lasting effects of this pyrethroid on different species of carabid beetles when 5 g a.i./ha were applied in autumn in cereal fields. The deviation between results of different experiments does not seem to be too distinct for a pesticide that causes sublethal effects and only little direct mortality. In general one should expect varying results when pesticides are used for which the efficiency is affected to a high degree by biological parameters as predators (e.g. lambda-cyhalothrin) or other parameters as sorption of the product to soil or evaporation (e.g. lindane, Heimbach et al., 1992a; Siebers et al., 1993). In

summary, field tests should be replicated at different locations and dates to get a reliable view of the effects of a pesticide and more than one method should be used to analyse effects.

Comparing all types of tests with chlorpyrifos resulted in quite similar values of high effects in all of them. Higher numbers of dead beetles and reduced activity were found in the field test with lindane compared to the field test with chlorpyrifos although lindane turned out not to be very toxic in frames on the same field at the same time. Lindane was more toxic under field conditions compared to the semi-field frames for several reasons:
- the exposure of the natural carabid population might have differed from beetles introduced into the frames because of different behaviour of these beetles,
- the beetles in the frames were exposed only to residues in this experiment and it is known that lindane evaporates very fast (up to 80 % loss within 24 h, Siebers et al., 1993),
- species and physiological status of laboratory reared beetles exposed in frames and the field population were different. This may influence the sensitivity of test organisms (Hof, 1993; Heimbach, unpublished) (e.g. well fed laboratory reared *P. cupreus* in the frames but hungry beetles which just had survived the winter in the field test).

A comparison of results of different test designs after application of lambda-cyhalothrin demonstrates the importance of including sublethal effects in the laboratory test as described in the laboratory test guideline (Heimbach, 1992). Otherwise, a product might be considered to be harmless according to laboratory results although it might be harmful in the field. Such sublethal effects have also to be considered in semi-field test designs which exclude some important biological parameters as in boxes. But even flat frames influence and restrict certain predators, reducing the potential effect of a product applied.

Conclusions

The comparison of the three test tiers in laboratory, semi-field and field shows that the laboratory test for *Poecilus cupreus* (Heimbach, 1992) fulfils the requirements for a tier I test.
The reasonable worst case situation may differ in semi-field tests according to the chemical and physical properties of the pesticide, the type of soil, temperature etc. Also the importance of sublethal effects has to be considered. Usually, one experiment cannot be sufficient for a hazard assessment, especially if the less standardizable semi-field frame method (Heimbach et al., 1992b) is used. The box method (Abel and Heimbach, 1992) has advantages for products for which the efficiency is highly dependent on the climate and type of substratum whereas frames give more realistic results for products causing sublethal effects as knockdown.

As field experiments are very difficult to be interpreted and results may vary quite considerably they should be repeated at different locations or dates preferring reasonable worst case situations. In order to help interpreting the results of field tests, it would be an advantage if semi-field tests would be carried out at the same date and field as field tests.

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EFFECTS OF TWO INSECTICIDES (LAMBDA-CYHALOTHRIN AND ENDOSULFAN) ON SPIDERS IN LABORATORY, SEMI-FIELD AND FIELD TESTS

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Introduction

To assess the effects of pesticides on the environment test-designs with different organisms (mostly single-species-tests) have to be developed. Usually the first step is to establish a biotest under standardized conditions in laboratory. This test should simulate the worst case. An intermediate step is to carry out a semi-field test by exposing organisms in the field. This test-design considers the influence of abiotic factors, like temperature, soil type, soil and air humidity, but only to a limited extent the biotic ones like availability of prey, competition and population dynamics. The last step is to establish a field test with trapping at weekly or even daily intervals. The sensitivity and behaviour of the species together with the bioavailability of the pesticide have to be considered to understand effects in the field.
In this studies spiders were chosen, because they are known to be beneficial in a number of crops. The aim of this investigation was to compare the efficiency of two insecticides, the pyrethroid *Karate (lambda-cyhalothrin)* and the chlorinated hydrocarbon *Thiodan 35fl. (endosulfan)* in lab, semi-field and field tests on spiders. Additionally some biotic and abiotic factors influencing the efficiency were also investigated.

Laboratory studies

In the field most of the spiders are not hit directly by the sprayed pesticide. Depending on their behaviour and habitat they are exposed to the residues on different substrates and under different climatic conditions. To help explain effects in the field, a laboratory study with field collected spiders *Pardosa spp.* (Lycosidae) was established to investigate the influence of different types of substrate and different substrate humidities on the bioavailability of the two insecticides. In parallel a chemical residue analysis was carried out (Wehling, Heimbach and Siebers, *in prep.*). The performance of this laboratory test is based on the method for testing *Poecilus cupreus* (Carabidae) developed by Heimbach and Brasse (1991) and is described in detail by Wehling et al., 1993.

The results presented assess the acute and residual toxicity of *Karate* and *Thiodan 35fl.*. Effects were assessed in terms of mortality and sublethal effects up to 14 days after treatment. The symptoms caused by these two insecticides were classified in the following way: 0= normal; 1= slightly damaged; 3= medium damaged; 4= badly damaged; 5= dead.

Bioavailability of different substrates

In the first trial the bioavailability of *Karate (15g a.i./ha)* and *Thiodan (213g a.i./ha)* on different substrates (maize-leaves, quartzsand, loamy sand and sandy loam) was investigated. The spiders were exposed immediatly after treatment and two days later.
The strongest effects could be found on quartzsand after treatment with both insecticides (figs. 1-4). The pyrethroid *Karate* excites peripheral nerves, particulary sensory cell bodies and motor nerve terminals (Beeman, 1982) and causes all kinds of intoxication (loss of co-ordinated movement, periods

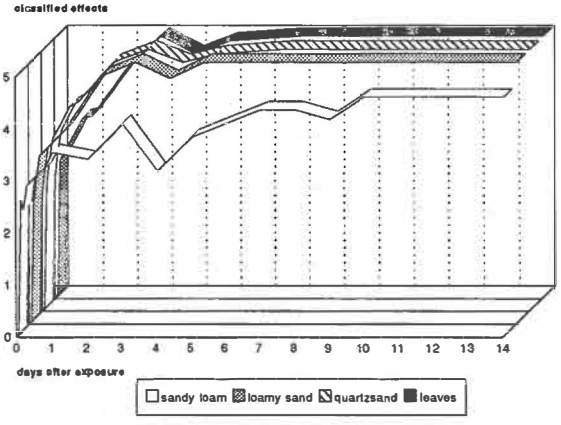

Figure 1: Average of the classified effects on *Pardosa spp.* exposed to fresh residues of Karate (15g a.i./ha) on different substrates

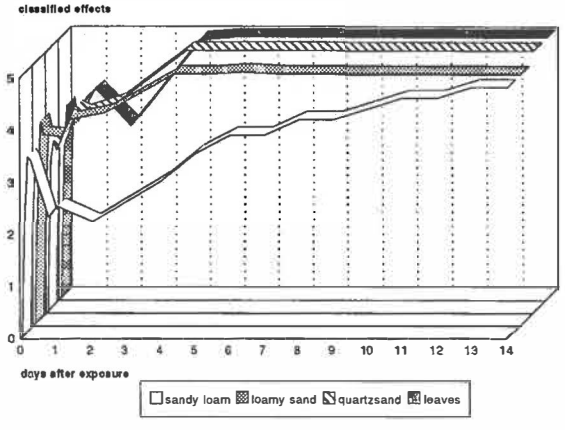

Figure 2: Average of the classified effects on *Pardosa spp.* exposed to two day old residues of Karate (15g a.i./ha) on different substrates

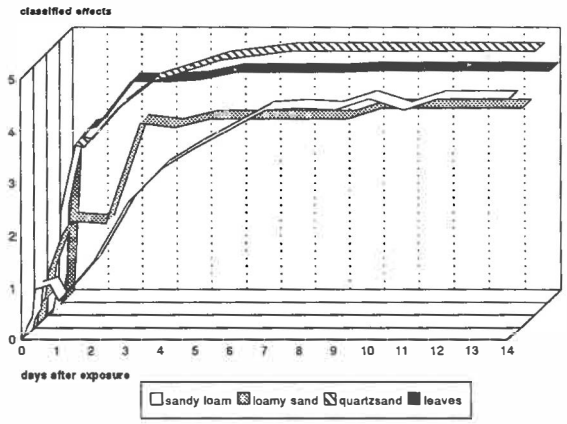

Figure 3: Average of the classified effects on *Pardosa spp.* exposed to fresh residues of Thiodan fl. (213g a.i./ha) on different substrates

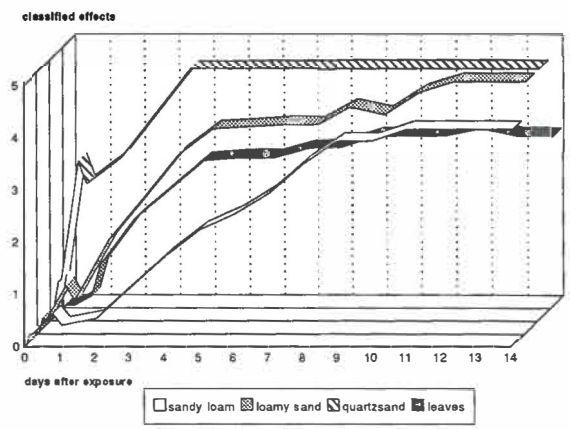

Figure 4: Average of the classified effects on *Pardosa spp.* exposed to two day old residues of Thiodan 35fl. (213g a.i./ha) on different substrates

of convulsive activity and periods of paralysis). These symptoms occurred after exposing spiders on fresh and two day old residues independent of the substrate type. Chemical residual analysis did not show any loss of active ingredient during two days. This explains why there is no decrease in biological effects of the two day old residues. Only in the case of sandy loam a decrease in biological effects could be observed. This is because of the high content of clay and humus which have a high adsorption potential for most pesticides. In contrary *Thiodan* having a high vapour pressure, volatilized quickly, especially from maize-leaves and quartzsand as the chemical residual analysis showed. Scheunert (1992) states that generally volatilization from leaves is very intensive in the beginning and more temperature-independent than from soil. The fast volatilization of *Thiodan* correlated with a decrease in biological effects especially on maize-leaves when spiders were exposed on two day old residues.

Biovailability from different surface-humidities

In the second trial the bioavailability of *Thiodan (160g a.i./ha)* from quartzsand and loamy sand each substrate with a wet and with a dry surface was determined. Spiders were exposed to the residues immediately after treatment and two days later.

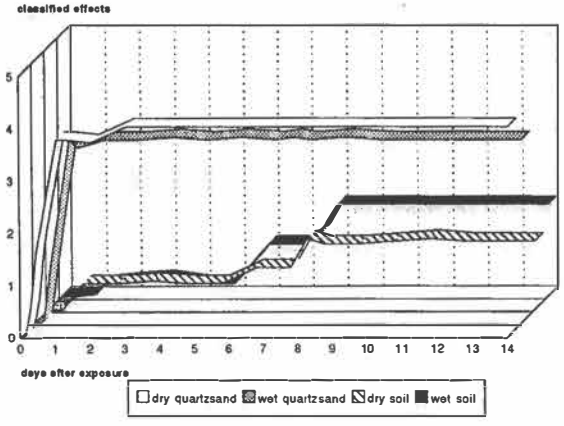

Figure 5: Average of the classified effects on *Pardosa spp.* exposed to fresh residues of Thiodan 35fl. (160g a.i./ha) using different substrates and surface humidities

More effects could be observed on dry than on wet quartzsand (fig. 5 and 6), though the loss of *Thiodan* was more intensive on wet sand. Scheunert (1992) also found a higher volatilization from wet sand surface, but Everts et al. (1991) found more effects on dry surfaces. Everts et al. (1991) also described strong effects on very wet substrates, which might be caused by open contaminated water (Critchley, 1972).
In all cases the spiders on loamy sand were less affected than those on quartzsand and the effects decreased with the age of residues. Using soil the influence of humidity on toxicity was not that clear. More loss of the pesticide was found on dry soil which could be correlated with less biological effects on spiders on fresh residues. On two day old substrates no biological effect, different from the control,

could be observed, even though there was no loss of the residue.
The bioavailability of a pesticide is influenced by adsorbtion and desorption, degradation, leaching and volatilization. Therefore a lot of factors have to be considered to interprete the results of these two trials. The adsorption capacity of substrates and the possibility of remobilisation of pesticides by adding water, which is more likely on sandy soil, are important as well as the volatilization which is higher on wet sand and rough soil-surfaces (Scheunert, 1992). The volatilization of a pesticide causes on the one hand a lower potential of contact-toxicity but on the other hand may cause a higher fumigant-toxicity. Further more, the drying out of the organisms caused by excretion of fluids after treatment has to be considered. This drying out was visible in our trials and is also described by Ingram (1955) and Casida and Maddrell (1971). The water-loss of the organisms can probably be compensated by a higher availability of water on wet surfaces. This could also be a reason for less biological effects on wet substrates.

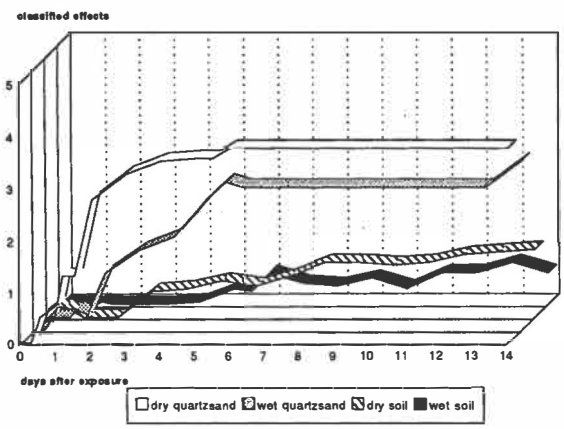

Figure 6: Average of the classified effects on *Pardosa spp.* exposed to two day old residues of Thiodan 35fl. (160g a.i./ha) using different substrates and surface humidities

Semi-field studies

A semi-field test was carried out in the autumn in winter barley to investigate the effects of *Karate (10g a.i./ha)* and *Thiodan (213g a.i./ha)* under field conditions. Plastic enclosures without a bottom of 172 cm^2 size were pushed into the soil and 4 subadult *Pardosa spp.* (Lycosidae) were released (8 replicates per treatment). It was necessary to place a gauze over the enclosures after the application to prevent the spiders from escaping. The field with the cages containing the spiders was treated with a tractor-mounted-sprayer. Assessments of sublethal effects and mortality were carried out up to 14 days after treatment. No effect could be observed after treatment with *Thiodan* at any time. In the case of Karate 2 h after treatment nearly 50% of the spiders showed symptoms of intoxication and at the end of the trial 90% of them were dead. Under these semi-field conditions *Karate* caused strong effects on the spiders. A recovery which usually is observed in laboratory tests after knock-down could

not be found, which maybe due to the effect of the low autumn temperatures, because the effects of *Karate* have been shown to be negativly correlated with temperature (Coats et al., 1989; Heimbach and Baloch, 1993). In contrast *Thiodan* is very toxic under laboratory conditions even in residue tests. The different effects of these two insecticides under field conditions can be explained by their different chemical–physical characters. *Thiodan* has a high vapour pressure and evaporates very qickly from rough soil surface (Scheunert, 1992) so it can disappear without causing any effects. Besides the effects of *Thiodan* are positively correlated with temperature (Müller, 1989; Scheunert, 1992) thus the efficiency under field conditions in autumn could be too low to effect large spiders such as Lycosids. But in the next section it is shown that *Thiodan* can effect spiders in the field in autumn and summer.

Field studies

Over several years field trials were established in winter wheat and winter barley (summer and autumn applications) to assess the influence of *Thiodan* (213g a.i./ha) and *Karate* (10g or 5g a.i./ha) on spider populations. Different sampling methods were used to determine the abundance and activity–density of spiders: Pitfall-traps, ground- photoeclectors (emergence–traps) and suction sampler (D–Vac). In the autumn in winter barley, the plots were 0.25 ha in area with four replicates per treatment. For summer application in winter wheat (1988-1990) the treated plots were about 0.7-1 ha in area without any replication except in 1991 where there were two replicates per treatment. In the 1991 trials *Karate* was treated at three different times of the day to assess the influence of different conditions (for example temperature, air–humidity and acticity–dynamics).

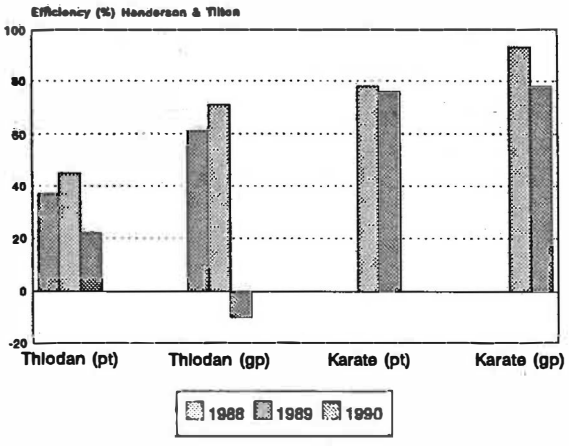

Figure 7: Efficiency of Karate (10g a.i./ha) and Thiodan 35fl. (213g a.i./ha) on spiders: Summer application (pt= pitfall-traps, gp= ground- photoeclectors)

Karate caused a significant reduction of individual and species numbers after both summer and autumn applications (figs. 7–9). The efficiency (Henderson and Tilton 1955) was nearly 80% in all years and lasted up to 35 days after application in 1991 (fig. 9) independent of the time of application. *Thiodan* also showed effects on spiders (fig. 7). But the reduction of individual numbers was not

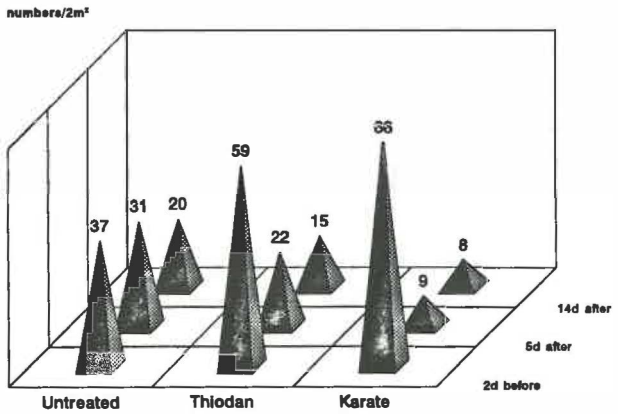

Figure 8: Number of spiders (D-Vac) after an autumn application with Thiodan 35fl. (213g a.i./ha) and Karate (10g a.i./ha)

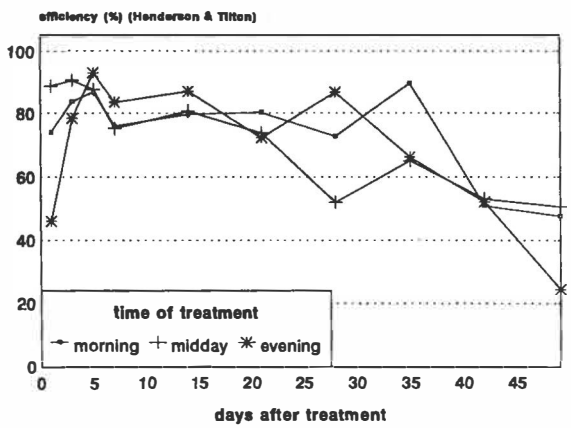

Figure 9: Effects of Karate (5g a.i./ha) on spiders in the field treated at three times of the day (summer application 1991)

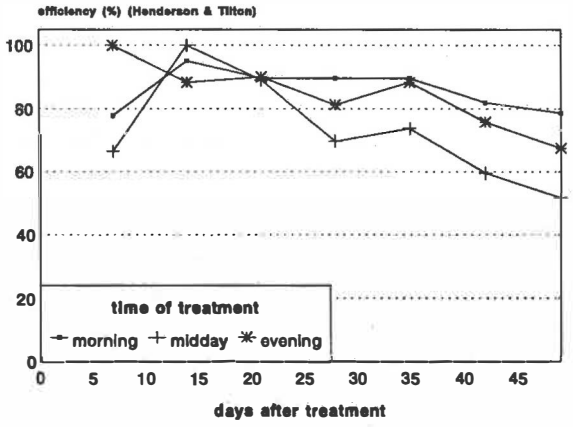

Figure 10: Effects of Karate (5g a.i./ha) on *Oedothorax apicatus* in the field treated at three different times of the day (summer application 1991)

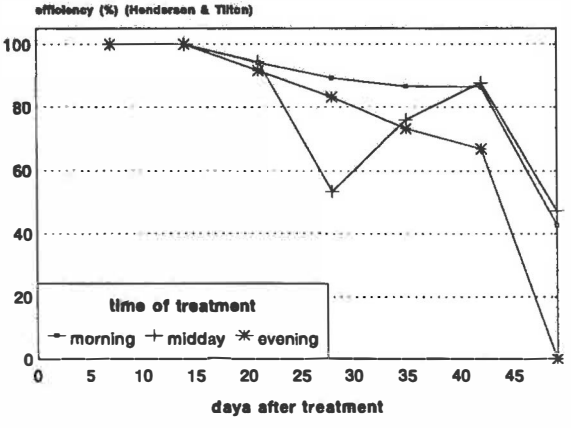

Figure 11: Effects of Karate (5g a.i./ha) on *Erigone atra* in the field treated at three different times of the day (summer application 1991)

significant. In 1990 the diversity index (Shannon and Wiener, 1948 in Mühlenberg, 1989) changed after *Thiodan* application, some species of the low vegetation zone of cereals like *Bathyphantes graciles* and *Lepthyphantes tenuis* were strongly reduced. This does not show that these two species are more sensitive than the others however. The sensitivity of a species is not only determined by the toxicity of a chemical, but also by the behaviour of the species and its exposure to the chemical (Jepson et al. 1990). These species build their web in the upper zone, thus they are more exposed to *Thiodan* and to its fumigant action than species living on the soil.
To estimate effects in the field the dispersive ability of the test species has to be taken into account. Treated plots in cereal fields are progressively reinvaded by spiders which inhabit untreated control plots within the same field and the borders of the field (Thomas, 1988; Thomas et al., 1990; Jepson and Thacker, 1990). In our 1991 trial recolonization appeared 3–5 weeks after application, but was different with regard to species (figs. 10 and 11). *Oedothorax apicatus* is a predominant nocturnal surface hunter (Thornhill, 1983) and the females do not balloon as adults (Thomas et al., 1990). So their dispersive ability is restrained and the efficiency is still high 50 days after application (fig. 10). In contrast *Erigone atra* is described as a frequent and common aeronaut (Locket and Millidge, 1953) and is able to reinvade treated plots quickly, which resulted in decreasing efficiency after a comparable time (fig. 11).

Conclusions

By carring out different types of tests it has been shown that a lot of biotic and abiotic factors like behaviour, temperature, type of substrate, air and surface humidity do influence the effect of pesticides on spiders. Thus results from all kinds of tests have to be interpreted and generalized very carefully using all knowledge of the physical and chemical properties of the pesticides as well as the biology of the test organisms. An order of precedence of different pesticides found in a laboratory–test can not always be transfered directly into the field.

Acknowledgment

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SIDE EFFECTS OF SULPHUR AND A NATURAL PYRETHROID ON *Trichogramma dendrolimi* MATSUMURA (HYM., TRICHOGRAMMATIDAE) IN APPLE ORCHARDS

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Abstract

To assess the side effects of insecticides on *Trichogramma dendrolimi* in apple orchards mass reared parasitoids were released on apple trees, which where then treated with a pesticide. Monitoring was carried out by offering fresh host eggs to the wasps for parasitization. The pesticides NAB, a sulphur product, and Spruzit, a natural pyrethroid, showed a reduction of parasitization of 0 to17% and 16 to 47%. The toxic standard E 605 (parathion) caused reduction of parasitization between 85 and 99%.

1. Introduction

The egg parasitoid *Trichogramma* is used world wide to control lepidopterous pests. In German apple orchards since 1984 investigations were carried out for releasing *T. dendrolimi* against the codling moth *Cydia pomonella* L. (Lep., Tortricidae) and the summer fruit tortrix *Adoxophyes orana* F. v. R. (Lep., Tortricidae) (Hassan, 1989, Hassan, 1993). One reason for the up to now unsatisfying results under practical conditions (Wetzel, 1994), could be the side effects of pesticides applied against other pests or diseases. Therefore a method for testing side effects of pesticides in the field has been developed (Wetzel & Dickler, in preparation). The results of the tests with NAB, a sulphur product, and Spruzit, a natural pyrethroid, are presented in this paper.

2. Material and method

2.1 Method (Wetzel & Dickler, 1992)

About 20.000 ready to emerge *T. dendrolimi* wasps were released in the center of an apple tree having a round canopy and a crown diameter between 1,5 - 2,0 m. The trees were protected from ants and earwigs by providing a sticky band around each trunk. One to two days after emergence of the adults the pesticide was applied at the recommended concentration and an amount of water of 1000 l/ha. Each trial includes a toxic standard (parathion) and a water treated control. Before and after the treatment eggs of *Sitotroga cerealella* Olivier (Lep., Gelechiidae) glued on 17 pieces of white cardboard (2 x 7 cm) (= eggcard, containing ca. 300 *S. cerealella*-eggs/cardboard) were offered for parasitization. The eggcards were placed at a minimum distance of 50 cm from the releasing point and were

changed about every other day. Percent parasitization of the eggs on the cardboards was examined in the laboratory. Each treatment was carried out with three replicates.

2.2 Material

T. dendrolimi (strain 26) (Hassan, 1992 b) was reared by Apple Company in Darmstadt according to the rearing method of Hassan (1981).

The used pesticides were:

NAB plus (Cohrs): Mixture of wettable sulphur, algae calcium and bentonit, a fungicide with effects on mites by contact.

Spruzit (Neudorff): 4% Pyrethrin and 16% Piperonylbutoxid, an insecticide with toxic effect by contact and weak toxic effect by respiration.

E 605 (Bayer): Parathion(-ethyl), an insecticide with toxic effect by contact, respiration and ingestion.

2.3. Evaluation of the pesticides

The evaluation of the pesticides used in these experiments is based on the categories for field tests of the IOBC/WPRS working group 'Pesticides and Beneficial Organisms' (Hassan, 1992 a).

2.4. Statistic

The statistical analysis was carried out in the computer center of the University of Heidelberg with the aid of the software package SAS. The significance was tested according to Scheffé at level P = 5. The statistical results given in the tables followed by the same letters are not significantly different.

3. Results

The results are given from four experiments testing the side effects of the pesticides NAB and Spruzit. In the figures 1 to 4 Sc stands for the introduction of *S. cerealella*-eggcards on the trees. The individual data of parasitized eggs and the statistical analyses are shown in the annex.

3.1. Side effect of NAB

3.1.1. Experiment I

Figure 1 illustrates the parasitization rate over a period of nine days. Before the application of the pesticide or water in all plots only a minimal parasitization could be found. After treatment, the number of parasitized eggs in the control plots increased on average to 620. In the NAB plots parasitization reached 246 and in the E 605 plots 15 parasitized eggs. The third evaluation day reveals the maximum of parasitization. On that day the amount of parasitized eggs in the NAB plots was found to be even higher than in the control plots. The

Figure 1

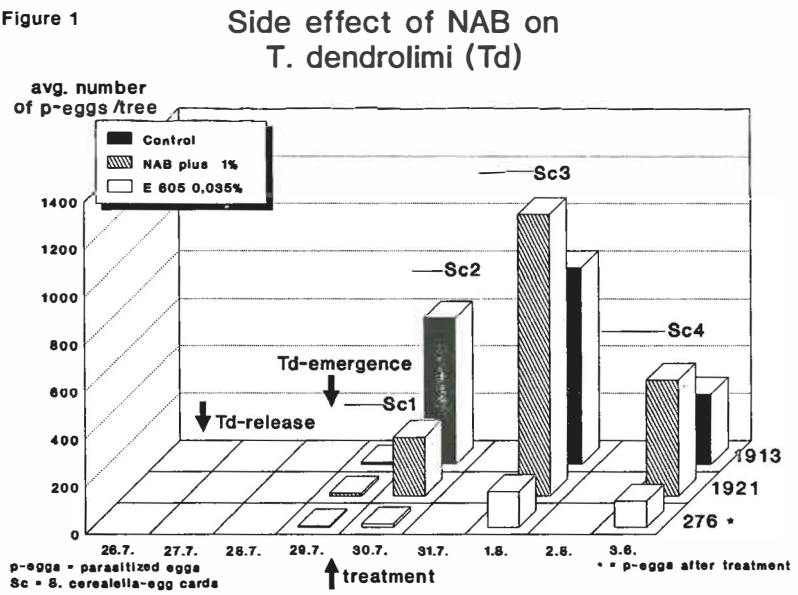


Figure 2

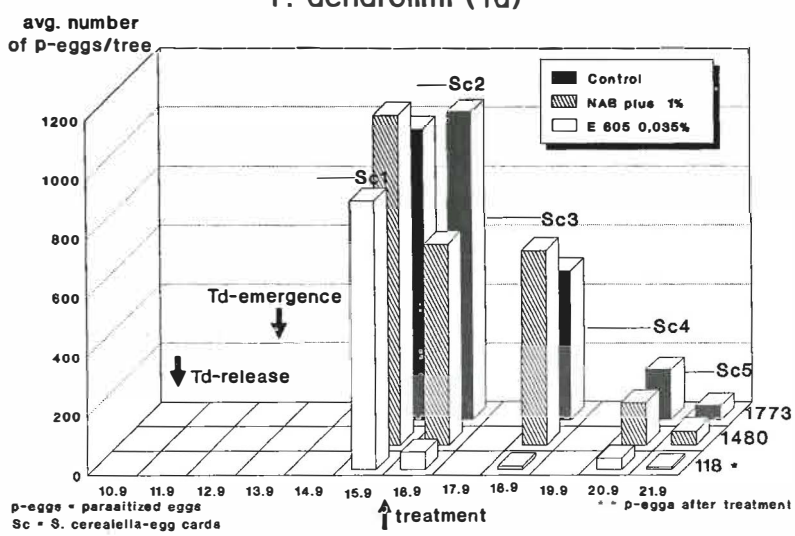


Figure 3

Side effect of Spruzit on T. dendrolimi (Td)

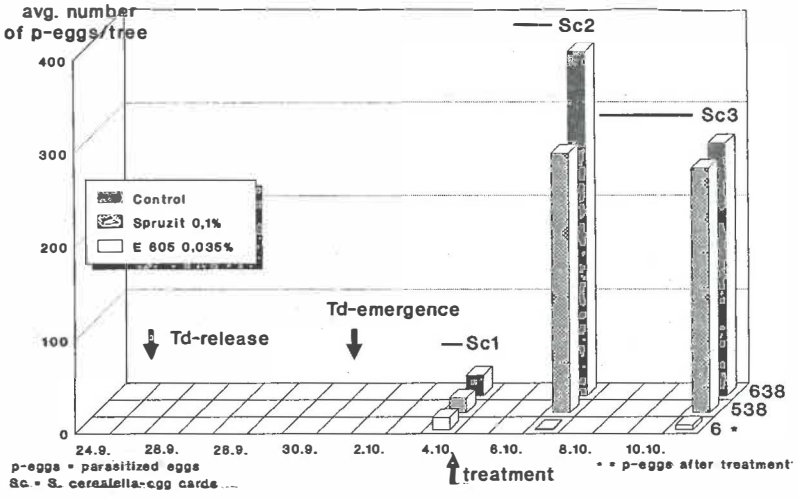
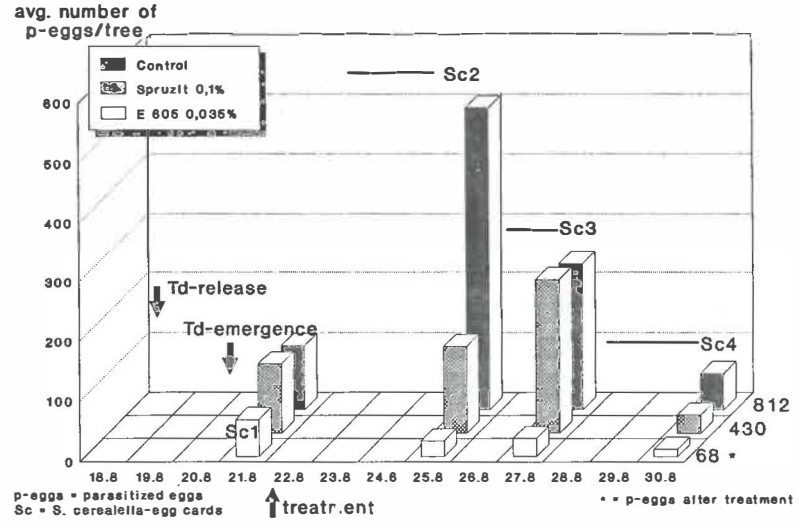


Figure 4

Side effect of Spruzit on T. dendrolimi (Td)



fourth day showed in all plots lower parasitization rates. Altogether the average number of parasitized eggs were: 1913 in the control plots, 1921 in the NAB plots and 286 in the E 605 plots. Parasitization in the E 605 plots was reduced by 85% in comparison to the control.

3.1.1. Experiment II

Figure 2 points out that the pesticide and water treatments took place exactly on the highest point of parasitization. Before the application in all plots parasitization rates of more than 900 parasitized eggs were reached. On the following day the amount of parasitized eggs decreased on the E 605 trees to an average number of 60. This level was no more exceeded during the whole trial. In the control plots parasitization persisted at the value of the day before and decreased on the third day of evaluation. The NAB plots differed from the control only on the second evaluation day. On that day parasitization was 35% lower. In total it was reduced 17% in comparison to the control (1480 parasitized eggs compared with 1773 parasitized eggs). The reduction in the E 605 plot was 93%.

3.2. Side effects of Spruzit

3.2.1.Experiment III

This experiment considered the side effect of the pyrethroid compound Spruzit (fig. 3). Due to the cold climate conditions during this trial, *T. dendrolimi* showed only low activity four days after emergence. Therefore the period between the changings of the eggcards was longer. The E 605 treatment resulted in nearly 0% parasitization. Only 6 parasitized eggs could be found after the treatment. The amount of parasitized eggs in the Spruzit plots had decreased about 25% on the second evaluation day. In total parasitization was reduced only by 15% compared to the control.

3.2.2. Experiment IV

The treatments in this experiment were carried out shortly after parasitization started (fig. 4). While the amount of parasitized eggs in the contol plots increased to an average number of 507, they stayed under the level of the beginning in the E 605 plots and reached a level of 144 parasitized eggs in the Spruzit plots. On the third evaluation day the number of parasitized eggs decreased in the control plot to 244, which is about equivalent to that in the Spruzit plots. Altogether, the Spruzit treatment caused a reduction of parasitization of 47%, the E605 treatment of 92%.

4. Discussion

According to the IOBC/WPRS guidelines (Hassan, 1992 a), the tested pesticides are rated as followed:

NAB - harmless, < 25% reduction of parasitization (experiment I and II),

Spruzit - harmless (experiment III) to slightly harmful, < 50% reduction of parasitization (experiment IV),
E 605 - harmful, > 75% reduction of parasitization (experiment I, II, II and IV).

The strong toxic effect of parathion corresponds to the results of laboratory tests (Suter, 1978) and emphasizes the suitability of this pesticide as toxic standard.

Spruzit turned out to be harmful in laboratory tests to *T. cacoeciae* (Hassan, 1984). According to the field tests presented here it only slightly injures *T. dendrolimi*, since the parasitization rate was only reduced up to 50%.
In experiment IV the wasps were more affected by the pesticide than in experiment III. The insects were already well spread over the tree and parasitization was strongly increasing. A lot of wasps probably died from contact toxicity (see fig. 4, second evaluation day), others may have been injured only temporary and could recover, since parasitization increased again on the next day. In the control plots however the highest point of parasitization had occured two days earlier.
Experiment III illustrates the decreasingly harmful effect of Spruzit, when the parasitoids are not sprayed directly but come in contact with the substance over the deposit. As the control of emergence showed, *Trichogramma*-adults were already present but not dispersed all over the tree. Probably most of them still had stayed in the releasing units at the time of the pesticide treatment. Therefore the contact toxicity of Spruzit could not have its full effect. Parasitization was reduced in this experiment by only 16%. E 605 however, as a poison of respiration, reduced the parasitization rate by 99%.

Also the moderatly harmful effect of the sulphur compound according to laboratory tests (Hassan et al., 1994) could not be confirmed. In the beginning of experiments I and II the number of parasitized eggs were reduced compared to the control; in experiment I by 60%, in experiment II by 35%. Afterwards the parasitoids seemed to recover and a parasitization rate higher than that in the control occured.

The lower toxicity of a pesticide in the field compared to the laboratory corresponds with the concept of the IOBC/WPRS working group "Pesticides and Beneficial Organisms" for conducting the tests. That means the toxicity becomes less from laboratory tests to semi-field and field tests, because the trial conditions are less severe. The field experiments also demonstrated that results can differ depending on the developmental stage of the insects, or as in this case, on activity, which is closely related to the temperature. Therefore it is advantageous to use a time-table in which the activities are listed in hour-degrees (Wetzel & Dickler, in preparation). For that reason, and because the pesticide effect depends on climate conditions, any trial should be repeated several times.

To get satisfying results by the inundative release of *Trichogramma* to control lepidopterous pests in apple orchards, it is necessary to have informations about the side effects of pesticides used simultaneously. NAB will minimally effect *T. dendrolimi*. Spruzit however leads to a reduction of parasitization up to 50 %.

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6. Annex

Experiment I

Amount of parasitized eggs, +/-standard deviation

Observation	Control	1% NAB	0,035% E 605
1	11 +/-3	13 +/-2	6 +/-1
Treatment			
2	620 +/-64	246 +/-99	15 +/-8
3	996 +/-117	1187 +/-289	161 +/-97
4	297 +/-176	488 +/-175	120 +/-63
Σ after treatment	1924 a	1921 a	276 b

Numbers in the table with the same letter are not significantly different.

Expreiment II

Amount of parasitized eggs, +/-standard deviation

Observation	Control	1% NAB	0,035% E 605
1	987 +/-259	1116 +/-103	910 +/-414
Treatment			
2	1044 +/-61	680 +/-112	59 +/-30
3	507 +/-145	649 +/-114	12 +/-1
4	172 +/-14	141 +/-45	38 +/-16
5	50 +/-44	46 +/-59	9 +/-9
Σ after treatment	1773 a	1480 a	118 b

Numbers in the table with the same letter are not significantly different.

Experimet III

Amount of parasitized eggs, +/-standard deviation

Observation	Control	01% Spruzit	0,035% E 605
1	22 +/-6	16 +/-8	12 +/-5
Treatment			
2	368 +/-55	277 +/-143	1 +/-1
3	270 +/-45	261 +/-33	5 +/-6
Σ after treatment	638 a	538 a	6 b

Numbers in the table with the same letter are not significantly different.

Experiment IV

Amount of parasitized eggs, +/-standard deviation

Observation	Control	0,1% Spruzit	0,035% E 605
1	107 +/-9	115 +/-44	61 +/-24
Treatment			
2	507 +/-175 a	144 +/-18 b	26 +/-23 c
3	244 +/-88	256 +/-123	30 +/-38
4	61 +/-39	30 +/-13	12 +/-17
Σ after treatment	812 a	430 ab	68 b

Numbers in the table with the same letter are not significantly different.

COMPARISON OF THREE DIFFERENT LABORATORY METHODS AND ONE SEMI-FIELD TEST METHOD TO ASSESS THE SIDE EFFECTS OF PESTICIDES ON *Trichogramma cacoeciae*

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ABSTRACT

The side effects of 139 pesticides on *Trichogramma cacoeciae* using three different types of laboratory test methods was studied. The combination of tests used included: (a) initial toxicity test on adult parasitoids (susceptible life stage), (b) direct spray of parasitoid pupa within host eggs (less susceptible life stage) and (c) duration of harmful activity (persistence) on adults. The combination was chosen to include two different developmental stages of the natural enemy that greatly vary in their susceptibility as well as in their vulnerability to pesticides. The persistence test reveals the duration of the harmful activity and helps to asses the impact of the chemical. In addition, the initial toxicity of 9 pesticides on *Trichogramma* adults was compared using laboratory and semi-field tests.

The results showed that the preparations greatly differ in their initial toxicity as well as in their persistence. 23 insecticides/ acaricides, 10 fungicides, 7 herbicides and plant growthregulators were harmful to the adult parasitoid but harmless to moderately harmful to the *Trichogramma*-pupa within the host eggs. 13 insecticides/ acaricides, 5 fungicides, 6 herbicides and plant growthregulators were harmful in the initial toxicity test but were short-lived to moderately persistent and therefore are much more useful for use in integrated control. Short lived preparations are likely to have much less impact on the natural enemy than persistent ones. The experiments to compare the initial toxicity in laboratory and semi-field tests revealed very little differences. This can be attributed to the similar mode of exposure in these tests.

INTRODUCTION

A combination of several standard laboratory tests together with semi-field and field methods was recommended by the Working Group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC), West Palearctic Regional Section (WPRS) to assess the side effects of pesticides on beneficial arthropods. The methods used in the tests were published by Hassan 1977, 1980 and 1992. The present study aims to evaluate the role of three different types of laboratory test methods and the semi-field test in assessing the side effects on *Trichogramma cacoeciae*. The comparison is also an attempt to interpret the results of the different types of tests.

MATERIALS AND METHODS

The experiments were carried out using standard IOBC/WPRS methods within the 6 joint pesticide testing programmes of the Working Group. The preparations and the concentrations tested are given in Table 1 - 3.

(a) **Laboratory, susceptible life stage** (adults of parasitoids):

The initial toxicity was tested by exposing the adult parasitoids to a fresh dry pesticide film applied on glass plates at the recommended concentration. The exposure cage consisted of twosquare glass plates and an aluminium frame (13 cm long, 1.5 cm high and 1 cm wide). Each of three sides of the frame contained 6 ventilation holes (1 cm diameter), covered with black tight material. Two portable openings on the fourth side of the frame were used to introduce the *Trichogramma*, host eggs and food. The cage was held together with two clamps. The glass plates were sprayed with the pesticide at the recommended concentration as indicated in Table 1. The experiment started with a 24 h period of forced exposure. At the end of the 24 h exposure, the parasitoids, if still alive, were given host eggs to measure their parasitization capacity. Eggs of the Angoumois grain moth *Sitotroga cerealella* (Oliv.) were offered on the 2nd, 3rd and 5th day of the experiment. The capacity of parasitism per *Trichogramma*-adult female and the reduction in capacity compared with the control (treated with water) was used to measure the effect of the chemical. Three replicates were used for each treatment. The pesticides were then classified in four categories as shown in Table 1.

(b) **Laboratory, less susceptible life stage** (parasitoids within their hosts):

Seven day old *T. cacoeciae* pupae within *Sitotroga*-eggs were directly sprayed and the emerging parasitoids, if any, were tested for their capacity to parasitize host eggs. The same experimental cage but with untreated glass plates and the method of assessment of parasitism described above were used. Three replicates each with about 300 eggs were used for each treatment.

(c) **Laboratory, duration of harmful activity** (persistence toxicity on adults):

The technique used to test the persistence of pesticide residues involves the spraying of potted vine plants, maintaining them under field or field simulated environment and exposing adult *Trichogramma* to samples of the treated leaves, taken at different time intervals after application. Exposure tests were carried out 3, 10, 17, 24 and 31 days after the treatment of the vine plants. Three replicates were used for each treatment.The same experimental cage as in (a), using untreated glass was utilized. The sampled leaves were spread inside the cage to cover the entire lower surface. The reduction in parasitism compared to control was plotted on a probit scale against time. The persistence is the time required for the pesticide residue to loose effectiveness so that a reduction in parasitism of less than 50 %, compared with the control, is reached.

(d) **Semi-field test** (adults of parasitoids)

The semi-field method involved the spraying of potted apple trees, enclosing the crown of each treated tree in a cage (80 x 80 x 80 cm) consisting of a metal frame with cloth walls and releasing

of 24 hours old adult parasitoids. Stripes of paper with honey and agar were placed on the foliage. The natural enemy was exposed to the treated tree for a period of one day before the assessment of survival was started. Assessment was carried out by distributing stripes of paper on the foliage with eggs of the Angoumois grain moth *Sitotroga cerealella* (Oliv.) glued on them. About 5000 *Trichogramma* adults were released per cage and 5000 host eggs were added on the second, third and fifth day after the beginning of the exposure (a total of 1500 eggs per cage). Three replicates were used for each treatment. The number of host eggs parasitized during the course of the experiment is counted at least 9 days after parasitism. The reduction in parasitism compared to water treated trees was assessed and the pesticides were classified according to the categories given in Table 1 (Dickler, Hassan 1979, Hassan 1977 & 1992).

RESULTS AND DISCUSSION

The laboratory tests

The results of testing 60 insecticides / acaricides (Table 1), 45 fungicides (Table 2), 34 herbicides / plant growth regulators (Table 3) on *Trichogramma* using the three different methods showed that the chemicals differed markedly in their initial as well as their residual toxicity. In each Table, the preparations were listed according to their increasing toxicity in the initial contact test. In each evaluation category, the duration of harmful activity (persistence) was considered to be more important than the effect of the preparation on the parasite within its host (less susceptible life stage).

Short lived preparations are much more suitable for use in modern plant protection. Persistent chemicals affect natural enemies for longer periods of time and are therefore likely to have a much greater impact on the natural enemy in the field.

The semi-field test

Comparison between the results of the laboratory and the semi-field experiments using 9 pesticides are given in Table 4. The two insecticides Dimilin (diflubenzuron) and Zoecon 619 and the fungicide Aspor-C (zineb) were harmless / slightly harmful to *Trichogramma* both in the laboratory and in the semi- field tests. Kelthane (dicofol) was moderately harmful to the parasite in both types of tests. Despirol (kelevan) was moderately harmful in the laboratory but harmful in the semi-field test. Plictran 25 W (cyhexatin), Afugan (pyrazophos), Torak (dialifos) and Rubitox WP (phosalon) were harmful in the laboratory as well as in the semi-field test.

The results indicate that differences between the results of the two types of tests were found with three out of a total of nine pesticides. In all cases, the toxicity was higher in the laboratory than in the semi-field test. However, the difference in all cases was only one category.

The differences in the results of the laboratory and semi-field tests were smaller than expected. This can be attributed to the similar mode of exposure in the two methods. In both cases, the adult parasitoids were exposed to a fresh dry pesticide film. The differences could be partly due to the nature of the exposure surface and the larger size of the cage. The chemicals were applied on glass plates in the laboratory and on apple foliage in the semi-field. The size of the laboratory cage

Table 1: Results of three different laboratory tests on *Trichogramma cacoeciae* (1) initial toxicity on adults (susceptible life stage), (2) pupae within *Sitotroga*-eggs (less susceptible life stage) and (3) persistence toxicity on adults (duration of harmful activity).

Preparation (Active ingredient)	Conc. tested %	Adult	Pupa within host egg	Persistence
I n s e c t i c i d e s / a c a r i c i d e s				
1 Dipel (Bacillus thuringiensis)	0.10	1	-	-
2 Torque (fenbutatin-oxid)	0.05	1	-	-
3 Dimilin (diflubenzuron)	0.05	1	-	-
4 Apollo SOSC (clofentezine)	0.04	1	-	-
5 Cesar (hexythiazox)	0.025	1	-	-
6 Insegar (fenoxycarb)	0.06	1	-	-
7 Applaud (buprofezin)	0.03	1	-	-
8 Dimilin (diflubenzuron)	0.05	1	-	-
9 Trigard (cyromazine)	0.067	1	-	-
10 Neudosan (Kali-Seife)	2.0	1	-	-
11 Delfin WG (Bacillus thuring.)	0.10	1	-	-
12 Novodor FC (Bac.thuring.tenebr.)	0.50	1	-	-
13 Micro Germin (Verticillium lec.)	0.2	1	-	-
14 Nomolt (teflubenzuron)	0.10	1	1	-
15 AAzomate (benzoximate)	0.15	2	-	-
16 Kelthane (dicofol)	0.15	3	1	2
17 Evisect S (thiocyclam)	0.03	3	3	2
18 Cropotex (flubenzimine)	0.10	3	1	4
19 Pirimor- Granulat (pirimicarb)	0.10	4	1	1
20 Croneton (ethiophencarb)	0.10	4	1	2
21 Tedion V 18 (tetradifon)	0.20	4	1	2
22 Asepta Lindane (lindane)	0.10	4	3	2
23 Dimecron 20 (phosphamidon)	0.25	4	3	2
24 Spruzit-Nova-flüssig (pyrethrum+)	0.10	4	4	2
25 Unden (propoxur)	0.15	4	4	2
26 Basudine vloeibar (diazinon)	0.21	4	4	2
27 Phosdrine W 10 (mevinphos)	0.58	4	4	2
28 Dipterex WP 80 (trichlorphon)	0.10	4	2	3
29 Thiodan 35 Spritzp. (endosulfan)	0.10	4	3	3
30 Hostaquick (heptenophos)	0.10	4	4	3
31 Peropal (azocyclotin)	0.10	4	1	3
32 Plictran 25 W (cyhexatin)	0.10	4	1	4
33 Rubitox Spritzp. (phosalone)	0.20	4	1	4
34 Ambush (permethrin)	0.02	4	1	4
35 Orthen (acephate)	0.15	4	2	4
36 Maitac (amitraz)	0.30	4	2	4
37 Decis (deltamethrin)	0.06	4	2	4
38 Gusathion (azinphos-methyl)	0.20	4	3	4
39 Kilval (vamidothion)	0.125	4	1	4
40 Vydate L (oxamyl)	0.15	4	1	4
41 Rody (fenpropathrin)	0.05	4	1	4
42 Klartan (fluvalinate)	0.06	4	1	4
43 Baythroid 50 (cyfluthrin)	0.05	4	2	4
44 Karate (lambda-cyhalothrin)	0.075	4	2	4
45 Tamaron (methamidophos)	0.15	4	4	4
46 Torak E (dialiphos)	0.25	4	3	4
47 Lannate (methomyl)	0.10	4	4	4
48 Sumicidin (fenvalerate)	0.075	4	4	4

49	Actellic 50 (pirimiphos-methyl)	0.20	4	4	4
50	Ultracid (methidathion)	0.075	4	4	4
51	Folithion (fenitrothion)	0.10	4	4	4
52	Hostaquick (heptenophos)	0.10	4	4	4
53	Ekamet (etrimfos)	0.20	4	4	4
54	Asepta Nexion (bromophos)	0.27	4	4	4
55	Birlane EC 40 (chlorfenvinphos)	0.33	4	4	4
56	Dursban Spritzp. (chlorpyrifos)	0.25	4	4	4
57	Ambush C (cypermethrin)	0.04	4	4	4
58	Perfekthion (dimethoate)	0.21	4	4	4
59	Hostathion (triazophos)	0.24	4	4	4
60	Imidan (phosmet)	0.25	4	2	-

Initial toxicity: 1 = harmless (<30%), 2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%), 4 = harmful (>99%).
Persistence: 1 = short-lived, 2 = slightly persistent, 3 = moderately persistent,
4 = persistent

Table 2: Results of three different laboratory tests on *Trichogramma cacoeciae* (1) initial toxicity on adults (susceptible life stage), (2) pupae within *Sitotroga*-eggs (less susceptible life stage) and (3) persistence toxicity on adults (duration of harmful activity).

Preparation (Active ingredient)	Conc. tested %	Adult	Pupa within host egg	Persistence
F u n gi c i d e s				
1 Nimrod (bupirimate)	0.04	1	-	-
2 Cercobin-M (thiophanat-methyl)	0.10	1	-	-
3 Ortho Difolatan (captafol)	0.20	1	-	-
4 Orthocid 83 (captan)	0.15	1	-	-
5 Bayleton (triadimefon)	0.10	1	-	-
6 Ronilan (vinclozolin)	0.05	1	-	-
7 Derosal (carbendazim)	0.05	1	-	-
8 Daconil 500 (chlorothalonil)	0.30	1	-	-
9 Milgo-E (ethirimol)	0.18	1	-	-
10 Ortho-Phaltan 50 (folpet)	0.33	1	-	-
11 Topas (penconazole)	0.04	1	-	-
12 Baycor (bitertanol)	0.37	1	-	-
13 Delan flüssig (dithianon)	0.20	1	-	-
14 Vitigran (copper-oxychlorid)	1.00	1	-	-
15 Impact (flutriafol)	0.16	1	-	-
16 Rovral PM (iprodion)	0.15	1	-	-
17 Saprol (triforine)	0.15	1	-	-
18 Sumisclex (procymidone)	0.15	1	-	-
19 Dyrene flüssig (anizaline)	0.40	1	-	-
20 Bayfidan (triadimenol)	0.05	1	-	-
21 Anvil (hexaconazole)	0.03	1	-	-
22 Calixin (tridemorph)	0.075	1	-	-
23 Alto 100 SL (cyproconazol)	0.08	1	-	-
24 Score EC 250 (difenoconazol)	0.05	1	-	-
25 BioBlatt Mehltaumittel (lecithin)	0.15	1	-	-
26 Dithane Ultra (mancozeb)	0.10	2	-	-
27 Pomarsol forte (thiram)	0.20	2	-	-
28 Rubigan Vloeibaar (fenarimol)	0.12	2	-	-
29 Antracol (propineb)	0.20	2	-	-
30 Omnex WP 10 (penconazol)	0.025	2	-	-
31 Tilt (propiconazole)	0.08	3	1	1
32 Dithane Ultra (mancozeb)	0.20	3	1	2
33 Trimidal EC (nuarimol)	0.08	3	1	2
34 Plondrel (ditalimfos)	0.075	3	1	3
35 Netzschwefel Bayer (sulphur)	0.40	3	1	4
36 Corbel (fenpropimorph)	0.17	4	1	1
37 Euparen (dichlofluanid)	0.20	4	1	3
38 Sportak (prochloraz)	0.187	4	1	3
39 Euparen (dichlofluanid)	0.15	4	1	3
40 Nevikén (lime-sulphur)	3.00	4	2	3
41 Polyram-Combi (metiram)	0.42	4	1	4
42 Afugan WP 30 (pyrazophos)	0.05	4	1	4
43 Thiovit (sulphur)	0.40	4	1	4
44 Morestan (chinomethionate)	0.10	4	1	4
45 Dithane M 22 (maneb)	0.50	4	1	4

Initial toxicity: 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99%).
Persistence: 1 = short-lived, 2 = slightly persistent, 3 = moderately persistent, 4 = persistent

Table 3: Results of three different laboratory tests on *Trichogramma cacoeciae* (1) initial toxicity on adults (susceptible life stage), (2) pupae within *Sitotroga*- eggs (less susceptible life stage) and (3) persistence toxicity on adults (duration of harmful activity).

Preparation (Active ingredient)	Conc. tested %	Adult	Pupa within host egg	Persistence
Herbicides / Plant growth regultors				
1 Betanal (phenmedipham)	2.25	1	-	-
2 Hyvar X (bromacil)	0.20	1	-	-
3 Gesatop 50 (simazin)	0.375	1	-	-
4 Fusilade (fluazifop-butyl)	0.25	1	-	-
5 Luxan 2,4-D amine (aminesalt)	0.432	1	-	-
6 Tribunil (metabenzthiazuron)	0.67	1	-	-
7 Ally (metsulfuron-methyl)	0.076	1	-	-
8 Dirigol-N (alphanaphthyl-acetamid)	0.02	1	-	-
9 Exp.30004 A (ioxynil)	0.24	1	-	-
10 Lontrel 100 (clopyralid)	0.12	1	-	-
11 Targa (quizalofop-ethyl)	0.30	1	-	-
12 Grasp (tralkoxydim)	0.50	1	-	-
13 Basagran (bentazone)	0.40	1	-	-
14 Tramat 500 (ethofumesat)	1.00	1	-	-
15 Starane 180 (fluroxypyr)	0.50	1	-	-
16 Arelon flüssig (isoproturon)	0.75	1	-	-
17 Goltix 70 WG (metamitron)	2.50	1	-	-
18 Illoxan (diclofop-methyl)	0.75	2	-	-
19 Ustinex PA (amitrol+diuron)	1.00	2	-	-
20 Gesaprim 50 (atrazin)	0.67	2	-	-
20 Basta (glufosinate-ammonium)	0.50	2	-	-
21 Roundup (glyphosate)	1.00	2	-	-
22 Faneron (bromofenoxim)	1.70	3	1	-
23 Gallant Super (haloxyfop)	0.50	3	1	1
24 Semeron (desmetryne)	0.25	4	1	2
25 Cycocel Extra (chlormequat)	0.70	3	1	3
26 Kerb 50 W (propyzamid)	0.75	3	1	4
27 Fervinal Plus (sethoxydim)	0.79	4	1	1
28 Avenge (difenzoquat)	1.00	4	1	2
29 Rhodofix (1-naphthyl-acetic acid)	0.15	4	1	2
30 Certrol B (bromoxynil)	0.33	4	1	2
31 Ramrod (propachlor)	1.00	4	3	2
32 Aretit flüssig (dinoseb)	1.25	4	4	4
33 Prosevor (carbaryl)	0.125	4	4	4
34 Aresin (monolinuron)	0.75	4	2	-

Initial toxicity: 1 = harmless (<30%), 2 = slightly harmful (30-79%),
3 = moderately harmful (80-99%), 4 = harmful (>99%)
Persistence: 1 = short-lived, 2 = slightly persistent, 3 = moderately persistent, 4 = persistent.

Table 4: Comparison of laboratory and semi-field results (*Trichogramma cacoeciae*), tests carried out by DICKLER and HASSAN

Pesticide (Category) (Active ingr.)	Conc. %	Reduction in parasitization in % laboratory		semi-field	
Dimilin (diflubenzuron)	0.05	32.1	(2)	0	(1)
Zoecon 619 *	0.10	71.6	(2)	0	(1)
Aspor-C * (zineb)	0.20	5.9	(1)	13.1	(1)
Kelthane * (dicofol)	0.15	96.3	(3)	60.0	(3)
Plictran 25 W* (cyhexatin)	0.10	100.0	(4)	83.1	(4)
Despirol * (kelevan)	0.05	97.0	(3)	87.0	(4)
Afugan (pyrazophos)	0.05	100.0	(4)	89.6	(4)
Torak * (dialifos)	0.10	100.0	(4)	92.1	(4)
Rubitox WP (phosalon)	0.20	100.0	4)	93.4	(4)

Laboratory: 1= harmless (<30%), 2= slightly harmful (30-79%)
3= moderately harmful (80-99%), 4 = harmful (>99%)
Semi-field: 1= harmless (<25%), 2 = slightly harmful (25-50%),
3 = moderately harmful (51-75%), 4 = harmful (>75%)

* not registered in Germany at this time

was 13 x 13 x 1,5 cm, the semi-field cage was 80 x 80 x 80 cm. To improve the laboratory method, the air in the cage was continuously changed to prevent the accumulation of pesticide fumes. Each cage was connected to an aquarium pump through a tube system. The entire air in the cage was exchanged every 1 to 2 minutes.

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SIDE-EFFECTS OF PESTICIDES ON *Opius concolor* Szèpl (HYMENOPTERA, BRACONIDAE), A PARASITOID OF THE OLIVE FLY.

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Abstract. Studies on the side-effects of pesticides on *O. concolor* are presented. Attending to the literature and to our own laboratory results, adults of this parasitic wasp are quite susceptible to pesticides, but pupae, the most protected life stage, are, on the other hand, very resistant, even to broad spectrum ones. This different sensitiveness of the two stages probably play an important role in explaining field results, and should be kept in mind when interpreting laboratory results.

Introduction. Olive groves have been gaining more and more attention in recent years from pest experts in order to achieve a more reasonable approach to the management of its pest complex (see, for example, Haskell, 1992). Among the available techniques to reach this goal, arthropod biological control agents are under scope.

Opius concolor Szèpligetti (Hymenoptera, Braconidae) is one of the beneficial insects that can be found in this crop. This wasp is native from the southern Mediterranean Basin, where it occurs naturally as an endoparasitoid of the olive fly, *Bactrocera oleae* (Gmelin) (Diptera, Tephritidae), which is one of the major insect pests of olives in the Mediterranean area (Viggiani, 1986). Since its discovery in Tunisia in 1910, there have been many attempts to introduce this beneficial in new areas. Releases were increased from late fifties, when successfully mass reared in *Ceratitis capitata* Wiedemann (Diptera, Tephritidae), and thus, it could be established in some new locations (the hottest ones, viz. Crete, Sicily, Sardinia, etc.). Since its establishment was not possible in other places tested (viz. Spain), its use there is based on inundative releases made periodically either in spring (against the wintering generation of the fly) or early in autumn. In both cases (wasp present all through the year or introduced periodically), as the use of pesticides is still the most common weapon used against olive pests (Kapatos, 1989; Jacas *et al.*, 1992[a]), the effects of pesticides on *O. concolor* can not be neglected and it would be very convenient to know a bit more about these side-effects.

Results and discussion. First attempts to assess the impact of some pesticides on *O. concolor* are those of Liotta (1978) and Maniglia (1978), who studied the effect of some nervous poisons used against the fly (dimethoate, fenthion and carbaryl) on this wasp in laboratory, but under conditions that would fit better with those considered as an extended lab test by the IOBC Working Group. They exposed adult wasps to small potted treated olive trees kept under laboratory conditions. In their report, they concluded that all products were highly toxic to *O. concolor*, being dimethoate the least harmful one, and that they had a considerable lasting effect, up to 31 days.

Later on, Genduso (1981) observed under true field conditions the emergence of *O. concolor* adults from olives obtained from trees treated with dimethoate. He explained these results because

of the high protection that the fly puparium offers to *O. concolor* and to the fact that pupae remained inside the fruit, so that exposure was minimal. Something similar was described a few years earlier by Roberti and Monaco (1967) for some olive fly ectoparasitoids, whose only protection was the olive since these pupate outside the fly puparium.

In 1990, Croft and Theiling, attributed some 85 records of pesticide impact to *O. concolor*, making this species the twentieth most commonly tested one. Moreover, *O. concolor* was found to be the most susceptible species out of the twenty two considered. No information detailed about the sources used for such a large number of records are given there, so it is not possible to discuss the methodology used, formulations, etc. We just know that the pesticides considered were organophosphates and carbamates, so it is not rare that *O. concolor* resulted so affected and thus classed as a very sensitive species.

From 1989 onwards, we began working on developping a sequential scheme to test side effects of pesticides on *O. concolor* since there was no standardized method developed neither for braconids, nor for natural enemies of olive pests. First results of our laboratory studies on the most exposed life stage of this wasp, the adults (lab a)(Jacas *et al.*. 1992[a,b]), demonstrated the high suscetibility of this wasp to pesticides (being males more sensitive than females). Young adult wasps (less than 24-h-old) were exposed to a fresh residue of pesticide on a glass surface (1.5 mg fluid/cm^2; maximum recommended dose, calculation being made on a rate of 1.200 l/ha) treated under the Potter Tower and evaluation was based on the time required to kill 50% of insects (LT_{50}, Finney, 1971) and on the parasitic behaviour of survivors (for this reason only females were chosen for the test); these determinations being made in a sequential way, so that only products that did not affect survival passed to the evaluation of parasitism, the rest passing directly to the following step: the test on the most protected life stage.

Broad spectrum insecticides were very harmful to adult wasps (categories 3-4), being the non-neurotoxical insecticides, such as *Bacillus thuringiensis*, the JHA Fenoxycarb, or the IGI Cyromazine, and fungicides and herbicides far more harmless (table 1). As this method took into account not just direct mortality (by means of LT_{50}), but also the parasitic performance, sulphur, a fungicide that showed a very little detrimental effect on survival of *O. concolor* (category 1) could be discarded, because of its effects on parasitism (category 3). The method has been improved and will be published soon (Jacas and Viñuela, 1994).

Simultaneously to the development of a method for the adults, another method to test the effects of pesticides on parasitized fly pupae has been developed (lab b). In that test, 11-day-old puparia are directly sprayed with the pesticides under the Potter Tower. Results have been really very interesting because none of the products used affected the emergence of parasitoids (table 1). Even products that proved to be very harmful to adults of this wasp (viz. organophosphates, carbamates and pyrethroids), resulted in being harmless to pupae, that really constitute the most protected life stage of the wasp. This phenomenon could explain the field results of Genduso (1981) with dimethoate, a product that we classed as harmful to adults (category 4) and harmless (category

Table 1. Results of pesticides tested against *Opius concolor* adult females (lab.a) and pupae (lab.b).

Active ingredient	Trade name	Firm	Conc. (%a.i.)	Laboratory a	Laboratory b
chlorpyriphos-methyl	Reldan	Dow Elanco	0.0424	4	1
dimethoate	Sistematón EC	Agrocrós	0.060	4	1
fenitrothion	Folithion EC	Bayer	0.075	4	1
formothion	Anthio EC	Sandoz	0.050	4	1
heptenophos	Hostaquick	Hoechst	0.055	4	1
malathion	Malafín EC	Agrocrós	0.150	4	1
phosalone	Zolone EC	Rhône-Poulenc	0.070	4	1
phosmet	Imidan WP	ICI	0.125	4	1
trichlorfon	Dipterex SP	Bayer	0.320	4	1
alpha-cypermethrin	Fastac EC	Shell	0.004	4	1
cyfluthrin	Baythroid 50	Bayer	0.0015	4	1
lambda-cyhalothrin	Karate	ICI	0.0038	4	1
endosulfan	Thimul 35 EC	Rhône-Poulenc	0.088	4	1
carbaryl	Sevin WP	Rhône-Poulenc	0.170	4	1
Bacillus thuringiensis var. *kurstaki*	Bactospeine SC	Agrocrós	17 10^6 IU/l	1	1
	Delfin DG	Sandoz	32 10^6 IU/l	1	-
fenoxycarb	Inségar WP	Maag	0.150	1	1
flufenoxuron	Cascade	Shell	0.005	1	-
cyromazine	Trigard WP	Ciba-Geigy	0.015	2	1
Bordeaux mixture	C.Bord.Vallès	I.Q. Vallès	0.200	2	1
copper-oxychloride	Cupravit WP	Bayer	0.196	4	1
cuprous oxide	Oxiram WP	Ciba-Geigy	0.300	3	1
sulphur	Microtox WP	Agrocrós	0.432	3	1
mancozeb	Dithane M-45	Rohm & Haas	0.208	1	-
	Dithane DG	Rohm & Haas	0.205	1	-
mancozeb + copper sulphate	Cuprodithane	Rohm & Haas	0.048 + 0.120	1	-
	idem Fuerte	Rohm & Haas	0.144 + 0.033	1	-
zineb	Fitonil Forte	Ciba-Geigy	0.200	1	1
dithianone	Delan 75 WP	Shell	0.075	1	-
cyproconazol	Alto	Sandoz	0.0186	1	-
difenoconazol	Score	Ciba-Geigy	0.125	2	1
penconazol	Omnex	Ciba-Geigy	0.0025	2	1
tebuconazol	Folicur	Bayer	0.0938	3	1
ethofumesat	Tramat 500	Schering	0.500	1	-
fluroxypyr	Starane 180	Dow Elanco	0.090	2	1
haloxyfop	Gallant	Bayer	0.052	2	1
metamitron	Goltix WG	Bayer	1.750	2	1

1) to pupae. It therefore appears that the puparium of the fly is a very good shield that protects the wasp against treatments, and this could play an important role in achieving the ecological selectivity of treatments through the application of pesticides when most of the population of the beneficial is protected inside its host (who is also protected inside the olive fruit or in the soil), as we hope to prove in the future when completing the sequential scheme.

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LABORATORY TESTS ON THE ENTHOMOPATHOGENIC FUNGUS *BEAUVERIA*

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Abstract

We present and discuss methods used in the frame of the OILB Working Group "Pesticides and Beneficial Organisms" in testing programmes to assess fungal sensitivity. Particular emphasis is given to points related to fungal biology and persistence. The efficiency of the techniques is evaluated and their pertinence discussed. Some limitations intrinsic to laboratory work are reviewed which have to considered when choosing one particular strain out of all the possible isolates. We choose two strains pathogenic against *Otiorhynchus sulcatus* F.. Results from the Vth, VIth and VIIth joint pesticide testing programmes of the Working Group are compared.

Introduction

Fungi in their majority are saprophytes in their environment, they act as opportunistic pathogens. Even if the saprophytic stage of some of them has not been described yet. Entomopathogenic fungi are responsible for endemic and epizootic diseases of insects and mites, controlling sometimes naturally insect populations. They are also used as biocontrol agents. Their mode of action is quite different from other microorganisms. Viruses and bacteria need to be ingested by the pest before action.Their effect is septicemic or toxic. At the opposite entomopathogenic fungi penetrate actively through the arthropod's cuticle inside the host. The infecting cell is usually a asexual spore. It has to germ, the germ tube penetrates through the cuticle, partly mechanically, partly by enzymatic processes (proteases, lipases, chitinases, etc). Sometimes specialized organs may be formed as appressorium, haustorium. This mechanism of infection occurs only in favourable micro and macro environmental conditions. As fungi are mesophilic organisms, temperatures between 10°C and 40°C are favourable for their growth (optimal growth between 20°-35°C), nevertheless a relative humidity above 85% is required to remove the spore dormancy and allow germination. Entomopathogenic fungi are widely distributed among fungal taxons belonging to Oomycetes, Zygomycetes, Ascomycetes and Adelomycetes.

Main advantages to use fungi as biocontrol agents

The introduction of virulent strains induces less perturbation in the environment for the species already are part of the ecosystem. They are harmless to mammals and plants and specific, up to insects species in some instances. Their persistence in the environment is related to climatic and ecological factors. Some entomopathogenic fungi have been tested as compatible with pesticides and beneficial organisms (coccinellids, nematodes, bees etc). Their potential for integrated control is not to be neglected.

Disadvantages

At present we lack good formulations and efficient methods for storage.

Isolates to be assessed in the testing programmes

As entomopathogenic fungi are occasional parasites they have a saprophytic state in the environment (soil, water, organic materials, etc.). It is of interest to test them "in vitro" before screening them in semi-field or in field conditions. In culture fungi multiplicate by mycelium but also by diverse kind of asexual spores eg. arthrospores, chlamydospores, different kind of conidia, etc.. These spores make possible their identification. Some fungi (Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes) have also a sexual multiplication. This stage allows classification. As the cellular fungal membrane is mainly made of chitin and chitosan, fungi are very resistant to biological and chemical products.

Testing antifungals has always been difficult whatever the purpose. Different techniques have been used to test antifungal drugs against human pathogenic fungi (Vanbreuseghem et al 1967). MIC (Minimal Inhibitory Concentration), versus MFC (Minimal Fungicidal Concentration) are difficult to perform, all forms of sporulation are not as sensitive: mycelium seems to be the most sensitive stage, resting spores the less sensitive one. In medical mycology solid media give pour results because most of the fungistatics/fungicides are insoluble in water and deposit in the bottom of the Petri dish. Fungi are aerobic and can grow at the very top of the medium without contact with the antifungal drug, wether it is in the medium or on a disc on the medium. These are the reasons why most in vitro tests are performed in liquid media (NCCLS standard methods 1992).

Which fungus has to be tested in our testing programmes? We have to choose between:

Natural pathogens

Some natural epizootics occur spontaneously with Entomophthorales (Zygomycetes). These epizootics are difficult to control, various factors being involved, different genera, species or strains succeeding or interacting in a unstable environment and climate for a variable insect population.

Commercial preparations

Some fungi are commercially used as control agents in agriculture; as *Aschersonia, Beauveria, Hirsutella, Metarrhyzium, Nomuraea, Verticillium,* etc. For this purpose the most virulent strains against a particular pest in a defined environment are screened. As illustration biocontrol of the soil larvae of the black vine weevil (*Otiorrhynchus sulcatus* F., Coleoptera, Curculionidae) can be obtained by entomopathogenic fungi well developping in soil like strains of *Beauveria brongniartii* (Coremans-Pelseneer et al. 1988, 1989) or *Metarrhizium anisopliae* (Zimmerman 1988).

Useful saprophytic fungi

Non pathogenic strains of phytopathogens like *Fusarium* are already dispersed intentionally in the farming ecosystem. They also could be included in the testing frame.

Pragmatically, in the testing programmes, we will choose between fungal genera, species, strains, for particular isolates regarding the target pest insect.

Sensitive stage to be assessed

A rapid screening method based on the most sensitive stage of the fungus (growing mycelium) would enable to yield useful results (Tuset 1985, Coremans-Pelseneer & Tillemans 1988). The pathogenicity of the resulting spores could be assessed in a second stage if needed. Two procedures are already described for this purpose: Hokkanen and Kotiluoto (1992), Keller (1993).

We tested *B. brongniartii* as biological control against the black vine weevil (*Otiorhynchus sulcatus*) and we observed the persistence of this fungus in soil. We tested the pesticides proposed in the Vth, VIth, and VIIth OILB/SROP programmes against two strains of this fungus. We used in parallel two techniques: Tuset's and liquid medium. Considering the difficulty peculiar to test fungi against pesticides, we screened the two strains on both solid and liquid media in a first step.

Material and methods

Strains

2 strains of *B. brongnartii* (isolates: 4792, 5015), 14 days old at 25°C, on 10x diluted Sabouraud medium, (0.2% dextrose, 1.0% neopeptone "Difco", 2% agar).

Media

Solid medium (Tuset 1985): potato dextrose agar (20% potato, 1.5% dextrose, 2% agar) 25 ml per Petri dish, 1 dilution/product, 3 up to 3x5 repetitions.

Broth medium: Sabouraud's broth (2% dextrose, 1% neopeptone "Difco") 5 ml per tube, 3 dilutions/product (0.1x, 1x and 10x) 2 repetitions/dilution.

Testing procedure

1 mm^3 culture is set on each medium. Concentration 1x is the highest concentration allowed in practice.

Results obtained for three testing programmes

5th Testing programme

Only one fungicide, maneb (Dithane M 22^R), is classified as class 4 (harmful) against *B. brongniartii*, because it is fungicide at the concentration used in practice. Three products, one fungicide, propineb (Antracol^R), and two herbicides, dichlofluanid (Euparen^R) and metabenzthiazuron (Tribunil^R), are classified in class 3 (moderately harmful) (table 1, V^{th} testing programme).

6th Testing programme

None of the substances tested were fungicide against our fungal strains, and one, ioxynil (EXP 30004a^R), is classified in class 3 (table 2, VI^{th} testing programme).

7th Testing programme

An insecticide, heptenophos (Hostaquick^R), and three fungicides (azols) had to be classified class 3 or 4 (see discussion) (table 3, VII^{th} testing programme).

A summary of the results obtained for the three tested programmes is given in table 4.

Table 1. Results from the Vth Testing programme

Active ingredient	Product	Firm	Active ingred. %or g/l	Conc tested % product	Laboratory - % inhibition Solid.med. Strain 4792	Solid.med. Strain 5015	Broth med. Strain 4792	Broth med. Strain 5015	IOBC cat.
Insecticides									
dialiphos	Torak E	Sopra	432 g/l	0,25	49	49	0	10	1-2
oxamyl	Vydate L	du Pont de Nem	245 g/l	0,15	0	0	0	0	1
thiocyclam	Eviset S	Sandoz	50%WP	0,03	0	0	99	80	1-3
clofentezine	Apollo SOSC	Maag	500 g/l	0,04	0	0	0	0	1
hexyhiazox	Cesar	Procida	200 g/l	0,025	/	/	0	0	1
fenoxycarb	Insegar	Maag	25%WP	0,06	25	10	0	0	1
flubenzimine	Cropotex	Bayer	50%WP	0,1	79	/	20	20	1-2
fungicides									
bitertanol	Bayer	Bayer	25%WP	0,37	49	/	35	49	2
dithianon	Delan flussig	Cela merck	223 g/l	0,2	10	20	10	10	1
copper-oxychlor	Vitigran	Hoechst	45%WP	1	10	25	0	0	1
flutriafol	Impact	ICI	12%SC	0,16	75	/	50	50	2
iprodion	Rovral PM	Rhone	50%WP	0,15	20	20	0	0	1
		Poulenc	50%WP	0,15	20	20	0	0	1
maneb	Dithane M22	Rohn Haas	80%WP	0,5	100	100	99	100	4
propineb	Antracol	Bayer	70%WP	0,2	100	99	75	75	2-4
dichlofluanid	Euparen	Bayer	50%WP	0,15	75	75	75	75	2
Herbicides									
2-4D amine salt	Luxan 2,4-D amine	Luxan	500 g/l	0,432	10	25	0	0	1
glufosinate ammonium	Basta	Hoechst	200 g/l	0,5	10	10	0	0	1
metabenzthiazuron	Tribunil	Bayer	70%WP	0,67	90	/	75	25	1-3
metsulfuron-methyl	Ally	Maag	20%WP	0,067	15	/	0	0	1
alphanaphthyl-	Dirigol-M	Siegfried		0,02	0	0	0	0	1

IOBC classification 1 = harmless (<30%)
2 = slightly harmful (30-79%)
3 = moderately harmful (80-99%)
4 = harmful (>99%)

Table 2. Results from the VIth Testing programme

Active ingredient	Product	Firm	Active ingred. %or g/l	Conc tested % product	Laboratory - % inhibition Solid.med. Strain 4792	Solid.med. Strain 5015	Broth med. Strain 4792	Broth med. Strain 5015	IOBC cat.
Insecticides									
buprofezin	Applaud	ICI	250 g/l	0,03%	0	0	0	0	1
diflubenzuron	Dimilin	Duphar	25% WP	0,05%	0	0	0	0	1
cyromazine	Trigard	Ciba geigy	75% WP	0,067%	0	0	0	0	1
fenpropathrin	Rody	Shell	10% WP	0,05%	0	0	0	0	1
methamidophes	Tamaron	Bayer	600 g/l	0,15%	0	0	0	0	1
Fungicides									
triforine	Saprol	Shell Agrar	190 g/l	0,15%	40	45	50	50	2
sulphur	Netzshwefel	Bayer	80%WP	0,4%	0	0	0	0	1
procymidone	Sumiselex	Bayer	50%WP	0,15%	25	0	25	0	1
anilazine	Anilazine	Bayer	480 g/l	0,4%	75	/	50	/	2
tidimenol	Baryfidan	Bayer	250 g/l	0,05%	99	0	50	10	2-3
hexoconazole	Anvil	ICI	50 g/l	0,03%	75	/	75	/	2
Herbicides									
ioxynil	Exp-30004A	Rhone Poulenc	240 g/l	0,24%	80	/	90	/	3
quizalofop-ethyl	Targa	Rhone Poulenc	100 g/l	0,03%	25	0	25	0	1
tralkoxydim	Grasp	ICI	100 g/l	0,5%	0	0	0	0	1
lime-sulphur	Neviken	PVV	7%	3%	50	75	50	75	2
tridemorph	Calixin	BASF	750 g/l	0,075%	50	50	50	/	2
bentazone	Basagran	BASF	480 g/l	0,4%	0	0	0	0	1

IOBC classification 1 = harmless (<30%)
2 = slightly harmful (30-79%)
3 = moderately harmful (80-99%)
4 = harmful (>99%)

Table 3. Results from the VIIth Testing programme

Active ingredient	Product	Firm	Active ingred. %or g/1	Conc tested % product	Laboratory - % Inhibition Solid.med. Strain 4792	Solid.med. Strain 5015	Broth med. Strain 4792	Broth med. Strain 5015	IOBC cat.
Insecticides									
Bacillus thuringiensis	Delfin WG	Sandoz	85%	0,1%	0	0	0	0	1
fulvinate	Klartan	Sandoz	240 g/1	0,06%	0	0	0	0	1
Bacillus thuringiensis	Novodor	Novo Nordisk	53%	0,5%	25	25	0	0	1
cyfluthrin	Baythroid 50	Bayer	50 g/1	0,125%	50	50	75	75	2
heptenophos	Hostaquick	Hoechst	550 g/1	0,1%	100	100	100	100	4
teflubenzuron	Nomolt	Shell	13,75%	0,1%	0	0	0	0	1
Fungicides									
cyproconazol	Alto 100 SL	Sandoz	10%	0,08%	100	100	100	99	3-4
diflenoconazol	Score EC 250	Ciba geigy	250 g/1	0,05%	100	100	100	99	3-4
lecithin	Bio Blatt Mechltaumittel	Neudorff	25%	0,15%	50	50	55	55	2
penconazol	Omnex	Ciba geigy	10%	0,025%	100	100	75	75	3
tebuconazol	Folicur	Bayer	250 g/1	0,25%	100	100	99	99	3-4
Herbicides									
ethofumesat	Tramat 500	Schering	500 g/1	1%	25	25	0	0	1
fluroxypyr	Starame 180	Dow Elanco	180 g/1	0,5%	25	0	0	0	1
haloxyfop	Gallant Super	Dow Elanco	52 g/1	0,5%	50	50	75	75	2
isoproturon	Arelon fl.	Hoechst	500 g/1	0,75%	25	25	0	0	1
metamitron	Goltix 70	Bayer	70%	2,5%	25	50	0	0	1

IOBC classification 1 = harmless (<30%)
2 = slightly harmful (30-79%)
3 = moderately harmful (80-99%)
4 = harmful (>99%)

Discussion and Conclusion

What concerns the techniques follows:

Solid medium is easy to manipulate, many replicates can be done; we made 3 up to 15 growth observations. Diameter measurements as well as quantitative and qualitative sporulation analysis are easy to make with Tuset's medium. On the other hand, this medium is heavy what concerns the number of Petri dishes to be cultivated. Dilutions are difficult to perform at 50°C (before medium solidification).

Broth medium is mainly used to perform MIC, dilutions are easy to make, it is a good check to estimate fungicidal action of a substance by washing the inoculum and providing new medium (MFC). Moreover only mycelium is produced in liquid medium and it is the most sensitive stage of the fungus.The liquid medium has also the advantage to be close to the NCCLS standard methods (1992).

In both media we observed few contaminations by bacteria or other fungi. In a first step we introduced a check in which the tested product was heat sterilized with the tested medium, but we abandoned this check by reason of the possibility of degradation for some substances. Most results could be assessed at their best after 7 days. We got good correlations between both media and between the two strains of *B. brongniartii* tested.

What concerns the results:

We are surprised that most pesticides are harmless against *B. brongniartii* even in liquid medium, which is the worst condition for the fungus, because it is immersed in the tested stuff. This weak toxicity could sometimes be observed up to 10fold the recommended concentration for some products. Testing three programmes, 30 out of 54 substances are classified as harmless (class 1) against two *B. brongniartii* strains: Table 4 summarizes the results obtained for the Vth, VIth, and VIIth IOBC testing programmes. Using the results obtained for two strains of *B. brongniartii* tested both on solid and liquid media we express our results according to the IOBC classification. Most pesticides are classified as harmless or slightly harmful in our experimental conditions.

Table 4. Number of products tested against two strains of *B. brongniartii*

		class 1	class 2	class3	class 4	Tot tested
Vth Test Prog.	I	4	2	1	-	7
	F	4	3	-	2	9
	H	4	-	1	-	5
	Total	12	5	2	2	21
VIth Test Prog.	I	5	-	-		5
	F	2	4	-		6
	H	3	2	1		6
	Total	10	6	1		17
VIIth Test Prog.	I	4	1	-	1	6
	F	-	1	1.	3	5
	H	4	1	-	-	5
	Total	8	3	1	4	16

Class 1 = Harmless (< 30%) Class 3 = Moderately harmful (80-99%)
Class 2 = Slightly harmful (30%-79%) Class 4 = Harmful (> 99%)

I = Insecticide F = Fungicide H = Herbicide

We have to point out that in the 7th testing programme out of five fungicides tested four are azols. This group of substances is fungistatic against most eumycetes preventing one of the steps of membrane ergosterols formation. As far as this mechanism is not implicated, the azols seem to be little or not toxic against mammals for instance. If we removed the azol from the medium, the fungus still grew. Of course it should be very interesting to go on with the pathogenicity of the fungus after contact with the pesticide. For this purpose Hokkanen and Kotiluoto (1992) stated to test fungal virulence against *Tenebrio molitor*. We got poor and weak reproducible results by testing our two *B. brongniartii* strains on *T. molitor* (unpublished data). Therefore, as mentioned before, another approach could be to test pesticides against parasitized insects (Keller 1993). Pesticides can also be added to soil supporting fungal inocula with our without insects (Hokkanen and Kotiluoto 1992). Another parameter to be considered here is the fungal persistence and/or development in soil.

For all these reasons we recommend the use of both solid and liquid media to assess pesticide action against beneficial entomopathogenic fungi, at least to evaluate class 1 and 4 in a first step.

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TESTING THE TOXICITY OF PESTICIDES TO EARTHWORMS IN LABORATORY AND FIELD TESTS

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Key words: *Tubifex tubifex, Eisenia fetida, Lumbricus terrestris,* molluscicides, nematicides, herbicides, fungicides, insecticides, funnel test, ecotoxicology, PIEC, predicted initial environmental concentration.

Abstract

As testing the toxicity of pesticides to earthworms in the field is known to be labour intensive, we compared the results of tree laboratory tests with results from field tests. We tested the acute toxicity of 300 pesticides and related compounds in a short term screening test using *Tubifex tubifex.* The results were compared with some available *Eisenia fetida* LC50 values worked out with the *Eisenia* test according to the OECD protocol No. 207. With a third test, the funnel test, which we consider a simulated field test, 12 pesticides have been tested. Twelve pesticides were also investigated in field tests.

With these four tests a stepwise system is proposed. The **first step** uses the annelids *T. tubifex* and *E. fetida* as test animals. *Tubifex* LC50 have been determined in a simple laboratory test and their corresponding ratios LC50/PIEC have been calculated (PIEC = **P**redicted **I**nitial **E**nvironmental **C**oncentration). The correlation of *T. tubifex* LC50 with published LC50 values of *E. fetida* was R = 0.648 (n = 32, F = 21.74, p = 0.00006). The seven-day *Tubifex* test, a fast and low cost test, is an ideal supplement to the common *Eisenia* test, to avoid erroneous assessments by the latter. *T. tubifex* is usually more sensitive, because of maximum exposure in the watery medium. Therefore the *Tubifex* test tends to overestimate the risk; but the ocurrence of dead earthworms or a reduction of populations after the application of a pesticide in the field can most often be explained by its low LC50/PIEC ratio. On the contrary, the *Eisenia* test, due to the test medium may underestimate the risk. If both the *Eisenia* and the *Tubifex* tests show no detrimental effect, no such effect is probable in the field and a field test is unnecessary. If one test, especially the *Tubifex* test shows detrimental

effects, a further test is required.

In the **second step** with the 21-day funnel test, which simulates exposure conditions in the field, the real earthworm *Lumbricus terrestris* is used. It examines effects of the pesticides on activity and behaviour of the earthworms. The test allows to simulate different exposure scenarios such as application of the pesticide on the soil surface, incorporation into the soil or contamination of the food. In this test the activity or the behaviour or the LD50 may be examined.

For the final hazard assessment field tests, as a **third step**, are often necessary. Field tests should be done with all products that have shown risks in previous laboratory tests. Laboratory tests can show a hazard only, i.e. a possible risk in the field. Results of field tests, however, are often difficult to interprete and difficult to reproduce, since the exposure of the earthworms to the pesticides is unpredictable because of weather conditions, mainly rainfall after the application. A toxic standard shows whether the results of a particular experiment are reliable. Statistical analyses of data show the often great variance of the data, because the tests run under variable climatic and edaphic conditions.

In single field tests a reliable prediction of the effect of the practical use of pesticides in different soils, weather conditions etc. has not been possible; therefore the interest in laboratory tests to reduce the number of pesticides for which field testing is required is great. The presented sequence of tests is considered a possible solution. The proposed three part sequence is meant to give a more valid prediction of ecotoxicological effects on earthworms in the field than with any single test alone. While the presentation of the results does not yet follow the categories of IOBC with respect to the evaluation of harmfulness of the pesticides to earthworms, we intend to do so in the future.

INTRODUCTION

Agricultural practices may have negative effects on earthworms in the field (Greig-Smith *et al.*, 1992). Since intensive tillage may have strong effects (Wyss & Glasstetter, 1992; Lee, 1985), the remaining earthworm populations must not be reduced further by pesticide usage. Legislations in the European Union and at national levels call for testing schemes in the process of registering pesticides for use in agriculture (EU Directive 91/414, Annex VI). Edwards & Bohlen (1992)

collected the published information, which is, however, far from sufficient for many active ingredients and for a still larger number, especially for newer ones, it is still unpublished. Since field tests are laborious and therefore expensive, various laboratory tests with annelids have been devised to attempt to predict the effect of the pesticides on earthworms in the field (Reinecke, 1992). The most commonly used test species is the compost worm *Eisenia fetida* Sav. (OECD 1984; EEC 1985). Other test species are *Tubifex tubifex* Müll. (Ammon, 1985) and *Lumbricus terrestris* L. in the funnel test (Bieri *et al.*, 1989; Bieri, 1992). The aim of this paper is to compare results of the *Tubifex* test, the *Eisenia* test, the funnel test and a field test as recommended by Bieri & Ammon (1990).

MATERIALS AND METHODS

The ***Tubifex* test** according to Ammon (1985) determines the LC50 of *T. tubifex* in a watery medium with a sand-soil mixture sediment. The test substances, usually formulated pesticide products, are dissolved in unchlorinated tapwater in a range of concentrations of 0.0 to 1000 ppm in four replications in pill vials. To each vial five *T. tubifex* are added. After two and seven days the living worms are counted and the LC50 is calculated.

The ***Eisenia* test** is performed according to OECD protocoll 207 (OECD 1984, Greig-Smith *et al.* 1992). Data for the *Eisenia* test were taken from the literature as far as available.

The **funnel test** according to Bieri *et al.* (1989) determines the activity and weight changes of *L. terrestris* in a micro field plot in the laboratory (Högger *et al.* 1992). Funnels with a surface of $0.0113 m^2$ (=12 cm diam.) are filled with unsterilized field soil. A transparent tube of 30 cm length is fitted to the stem of the funnel to serve as burrow for the worm. The tube is covered with a removable black pipe. Pesticides are sprayed or sprinkled on the surface of the soil or mixed into it. Five wheat seeds or clover leaves are added to the surface as feed and indicators of worm activity. One weighed large juvenile worm is added to a hole in the soil in the middle of the funnel. The funnel is covered with a screen to prevent the escape of the worm. There are twenty replicates per treatment. Every two to three days the number of withdrawn bait is counted. After 21 days the worms are weighed and then placed into water for another two days. The worms are weighed again.

The **field test** is performed on 10 x 10 m grassplots (Edwards & Brown, 1982). It contains an untreated plot, one with a known toxic standard and three test pesticides at normal and fourfold normal application rate. Earthworm populations are determined before application of the pesticides, three weeks and 3-4 months afterwards by expelling them with formalin or a mustard powder supension in rings of 1/4 m^2 area (Högger, 1993). We used two replicates per treatment and three sampling units per plot. Worms are counted and weighed for each sample. *L. terrestris, Nicodrilus nocturnus* and *N. longus* dominated in our test field populations (Jäggi *et al.*,1993).
Results are expressed as ratios of the earthworm weights after the treatment to the weights before the treatments. The scale is set to a ratio of 1.0 for the untreated control. The analysis of variance and the LSD tests were calculated with transformed values of the ratios ($y = (x+0.5)^{1/2}$), where x = ratio of weights.
While the presentation of the results does not yet follow the categories of OIBC with respect to the evaluation of harmfulness of the pesticides, we intend to do so in the future, especially if additional *Eisenia* LC50 become publicly available.

RESULTS

Tubifex test

An important character of a pesticide is the ratio of its toxicity towards an organism and the concentration in the environment. This property measures the possible effectiveness towards wanted and unwanted targets. The concentration in the soil environment is determined by the concentration of the active ingredients in the product, the application rate and the target mass, which has been defined as the top 2.5 cm soil layer with a density of 1.5 and named Predicted Initial Environmental Concentration (= PIEC; Kula C, 1992). Table 1 shows these values for 300 pesticides and related products. The ratio *Tubifex* LC50/PIEC is used as the initial measure of the potential effect of a pesticide on earthworms, because it allows a relative grouping of the products in a range from relatively nontoxic to extremely toxic. Absolute class limits do not exist as the heavy horizontal lines in Table 2 might suggest. The order of magnitude of the toxicity/PIEC and the relative position on the scale are more important. In analogy to the toxicity assessment towards *Eisenia fetida* (Kula C, 1992), a ratio smaller than about 10 indicates potentially harmful products, whereas products with

a ratio greater than 100 are most probably not harmful in the field. For comparison the available relative toxicity assessments based on literature data, predominantly *E. fetida* toxicities (Edwards & Bohlen, 1992), are included. These differ in many cases from the assessment by the ratio *Tubifex* LC50/PIEC alone (Fig. 1). The *Tubifex* test is obviously very sensitive because of maximum exposure in the watery medium. It ranks as toxic many ingredients which may be found nontoxic in further testing.

Tests in soil media may give in many cases results closer to those obtainable in the field, because of more realistic exposure. As a first approximation the standard *Eisenia* test is used, where the pesticides are mixed into the substrate. Corresponding *Eisenia* LC50/PIEC can be calculated (EEC, 1993). Table 2 shows in the left part 32 pesticide ingredients arranged according to increasing *Eisenia* LC50/PIEC ratio whith their corresponding relative toxicity. In this ranking some ingredients, such as Benomyl, Carbaryl and Methiocarb are obviously misplaced. The arrangement in the right part of Table 2 according to increasing *Tubifex* LC50/PIEC ratios corresponds better with the assessments of Edwards & Bohlen (1992). Differences occur mainly for active ingredients with a large ratio *Eisenia* LC50/ *Tubifex* LC50. The *Tubifex* test does never underestimate toxicity by a large factor. The correlation of the toxicities measured with the two test organisms in different media is not very strong, depending on the scale of calculation (Fig. 2). The number and selection of value pairs has also an influence on the correlation coefficient (see also: Reinecke, 1992).. Both tests together give a more reliable first assessment of toxicity than either test alone. For ingredients with apparent high toxicity in either test further testing is required in the laboratory and/or in the field.

Funnel Tests

In a first funnel test the effect of the mode of application of known earthworm toxic compound **Dinoseb** on the activity of *Lumbricus terrestris* is presented (Fig. 3). When the pesticide was mixed into the soil analogous to the procedure in the standard *Eisenia* test, there was about a 50% reduction of activity as measured by the total number of eaten or disappeared wheat seeds. Spraying the same amount on the surface, either with or without subsequent simulated rainfall, reduced the activity by about 90%. The last two modes provide obviously a higher exposure, similar to the one in the field.

Commercial **molluscicide** baits which contained 3.5% Metaldehyde, 1% Methiocarb and 5% Bensultap were applied at 5 granules per funnel. Wheat seeds served as control. Activity of *L. terrestris*, as measured by disappeared bait, was reduced initially by all pesticides. The baits with the insecticidal compounds Methiocarb and Bensultap had a permanent effect, whereas in the Metaldehyde bait the activity increased with time (Fig. 4). This may be due to the temporary effect of the fungicidal conservation agent in the latter. The *Tubif*ex LC50/PIEC for 80% Metaldehyde is 440 and for the used 3.5% bait 10.6. The final weight loss was about 12% in the methiocarb and bensultap treatments and 2% and 3% in the metaldehyde and control treatments, respectively (Fig. 5). Metaldehyde baits are considered harmless and Methiocarb and Bensultap may be harmful. The latter two need further testing in the field.

The granular **nematicides** 5% Carbofuran (Curaterr 5G) and 2% Terbufos (Aragran 2G) at 30 kg/ha and 10% Aldicarb (Temik 10G) at 20 kg/ha were compared in the funnel test (Fig. 6). The experiment was performed twice. The activity of *L. terrestris*, as measured by the disappeared wheat seeds, was reduced in all treatments including control, but greatest in the nematicide treatments. The pesticide applications resulted in a greater weight loss than the control. Out of 20 worms per treatment only 13 to 15 survived (Fig. 7). The reason for the large number of dead worms in the control is unclear. Nevertheless, all three granular nematicides reduced the activity of the earthworms at about the same rate.

Field Tests

The field tests with **molluscicide baits** contained an untreated control, a toxic control, i.e. 50% Benomyl at 4 kg/ha, 29% Dinoseb at 6 l/ha or 46% DNOC at 10 l/ha, single applications of the three molluscicide baits 3.5% Metaldehyde (Limax G), 5% Bensultap (Malice G) and 1% Methiocarb (Mesurol G) at 10 and 40 kg/ha each. The experiment was repeated in three years on neighbouring sites (Fig. 8). A reduction of the relative ratio of the earthworm weights after the treatments to the weights before the treatments to less than 0.75, relative to the control, is considered biologically significant. The statistical analysis of variance of the data (F-test), however, showed no treatment effects after 3 weeks (short term), but significant treatment effects after 3 to 4 months (long term). The year effects were significant in the short term, but not in the long term (Table 3).

The treatment means had no significant differences to the control in the short term, but they were significantly different from the control in the long term. However, short and long term means had the same tendency.

In conclusion, only the Metaldehyde bait applied at the normal rate had no detrimental effect on the earthworms overall in three years. Results in single years vary (Table 3).

The field tests with granular **nematicides** were performed in two years with a toxic control, i.e. 29% Dinoseb at 10 l/ha, 5% Curaterr (Curaterr 5G) and 2% Terbufos (Aragran 2G) at 34 and 136 kg/ha and 10% Aldicarb (Temik 10G) at 23 and 92 kg/ha (Fig. 9). Treatment effects over two years were not significant in the short and long term. Year effects were not significant in the short term, but significant in the long term (Table 3). The only significant treatment mean effect was produced by the four-fold application rate of 2% Terbufos in the short term. In the long term all treatments including the toxic control had no longer an effect. The results appear inconclusive. There may be measurable effects of the granular nematicides, at least in the short term.

The field test with **herbicides** attempted to compare the effects of 46% DNOC at 20 l/ha, 18% Glufosinate at 5 and 20 l/ha, 17% Linuron at 7 and 28 l/ha and 20% Metsulfuron-methyl at 40 and 160 g/ha during one year (Fig. 10). There were no treatment effects in the short or long term, not even for the mean of the toxic control DNOC. Except in metsulfuron-methyl the populations were higher after treatment than before treatment in all treatments. The results are therefore inconclusive. Lack of rainfall after the applications may be the reason for the failure, although the herbicides produced a strong effect on the grass sward.

DISCUSSION

Toxicity testing of pesticides has the aim to minimize unwanted effects of pesticide usage on earthworms in the field. In general, LC50 tests measure an acute toxicity and rightly are used as first steps in a testing scheme. More subtle effects are thought to emerge from reproduction tests e.g. with *E. fetida* or the funnel test with *L. terrestris*. Such effects may then be similar to those

measured by a field test on a mixed population of species over several months.

The ***Tubifex* test** measures an inherent toxicity of the compounds because of maximum exposure in the watery environment. Results indicate often a high toxicity of the products. So far there are no indications that a toxicity has been judged too low. In the standard ***Eisenia* test** the products are evenly mixed into the sand-soil-peat mixture where some compounds may be adsorbed or decomposed. Besides possible different sensitivities of the two test organisms, the fate of the compounds in the artificial medium might have lead to large *Eisenia* LC50/*Tubifex* LC50 ratios and this may explain the obvious misplacement of the known earthworm-toxic compounds Benomyl, Carbaryl and Methiocarb in Table 2. In the funnel test the exposure is more realistic, especially for species which move at least temporarily on the soil surface such as *L. terrestris* (Fig. 3).

The ***Eisenia* test** remains the compulsory test in the EU for the time being. In the meantime, we recommend the *Tubifex* test as a supplementary, cheap, and sufficiently reliable initial test. Because of the obvious misidentification of toxic compounds as untoxic (Table 2, left), which the *Tubifex* test identified correctly as toxic, the latter test is an insurance against false assessments.

The **funnel test** is a behavioral test and measures worm activity and weight losses as indirect indication of toxicity under controlled laboratory conditions. The exposure is nearest to the one under field conditions, where pesticides are mostly applied to the soil or plant surface and not mixed evenly into the soil as in the standard *Eisenia* test. Contaminated plant material may be used as food as well and repellent effects may become apparent. With an appropriate range of concentrations an LC50 can be determined. It is, however, somewhat more laborious than the *Eisenia* test and the test animals must be extracted from natural field sites beforehand. For the latter reason it is performed best during spring and fall.

In **field tests** experimental conditions, such as temperatures and rainfall, are unpredictable. In field tests with earthworms the variability of earthworm populations between plots and years is a major problem. Statistical analysis of data is often difficult, because of the low number of replicates, which depends on available manpower. The fate of the pesticides which reach the soil surface is often uncertain in an experiment (evaporation, decomposition, dilution, adsorption etc.). On plant surfaces they will often remain for a while to produce the desired control effect. Rainfall of 10 to 20 mm

will often wash pesticides into the worm burrows or cause the worms to escape from flodded burrows to the soil surface, where they may be exposed to high concentrations of pesticides applied shortly before. Such events produce the unpopular mass occurrence of earthworm cadavers. Irrigation of testplots may be necessary to simulate these events (Kula H, 1992).
Field results are only interpretable if they include those of a toxic standard, which shows a distinct effect over at least 3 months. The apparent "no-effect" of some tested compounds in the literature is therefore of little value.

A single field test for earthworm toxicity gives relatively little information compared to the amount of information available from the efficacy trials up to that point in the development of pesticides under different agronomic, edaphic and climatic conditions. It may therefore be very economic to record observations on earthworm toxicity in these development stages as well.

One aim of testing is also to be able to use confidentially as many of the current pesticides as possible in the near future. Erroneous laboratory tests should not cause the withdrawal of usefull pesticides. A combination of tests such as the ones described in this paper, including valid field tests will avoid a wrong assessment. Therefore, field tests despite all their difficulties cannot be replaced by laboratory tests at present.

Ecotoxicity testing schemes often consider only effects of single applications of single pesticides, but not of the repeated applications of several different pesticides in a season in practical agriculture. It appears however impractical to test thoroughly for such cumulative and possibly interactive effects in the laboratory. The funnel test may be a tool for such purposes for earthworms. Otherwise extensive field tests are necessary. A partial approach may be the comparison of organically and conventionally farmed fields taking into account the inherent differences imposed by the two farming systems, especially tillage and applications of manures (Pfiffner, 1993). In addition, a simple field test does not account for secondary poisonings in the food chain, e.g. of predatory birds. Pesticide residues in dead or dying earthworms on the surface of the soil can be high enough, so that a risk cannot be excluded (Lee, 1985).

No single laboratory test with a single organism predicts the risk of pesticides to earthworms in the field. Valid field tests are still necessary for final proof of "no essential effect" under conditions of normal use. According to the EU Directive 91/414 registration is denied if the acute toxicity/-

exposure ratio (LC50/PIEC) for earthworms (*E. fetida*) is <10, unless an appropriate risk assessment shows that earthworms are not at risk in the field.

One goal of the testing schemes, to reduce the necessary number of expensive final field tests substantially, is reached for those pesticides, which show no detrimental effects in both the *Eisenia* and *Tubifex* tests.

CONCLUSIONS

1. The *Eisenia* and the *Tubifex* test together and their toxicity/PIEC ratios are appropriate tools for an initial assessment of earthworm toxicity of pesticides.
2. If both tests show a high risk there is a high probability for a risk in the field. If only one test, especially the *Tubifex* test, shows a risk, there still may be a risk in the field. If both tests show little or no risk, the probability for a risk in the field is small.
3. The *Eisenia* and the *Tubifex* tests together reliably identify those pesticides for which no further testing is required.
4. Further and comparable data on *Eisenia* LC50 and toxicity/PIEC must be published to allow an open assessment of earthworm toxicity of pesticides.
5. The funnel test simulates field exposure of the earthworms more closely than other laboratory tests. It is, however, even more labour-intensive than the *Eisenia* test.
6. Good field tests, which include a toxic control, are the final proof for earthworm toxicity, although the results are still difficult to reproduce.
7. Events of toxic effects of pesticides in the field may be confirmed with a simple laboratory test.
8. Advantages and disadvantages of the four tests:

   ***Tubifex* test:** Advantages: very sensitive: no toxic compounds are classified as untoxic, fast, cheap, very repeatable, test animals available in pet shops or can be bred. Disadvantages: many compounds are classified as too toxic, not usefull as single test.

   ***Eisenia* test:** Advantages: widely adopted, simple, test animals easily bred in the laboratory, fairly repeatable. Disadvantages: data mostly unpublished, fairly laborious, some toxic compounds are classified as nontoxic, uses a non-field species, not useful as single test.

   Funnel test: Advantages: Exposure similar to field exposure, realistic short term results for a real field earthworm, can also measure an LC50. Disadvantages: fairly laborious, test animals must

be extracted from field sites.

Field test: Advantages: Final proof of effects of pesticides on earthworms in the field. Disadvantages: difficult to repeat although often necessary, depends on weather conditions, laborious, needs large experimental area.

More detailed results of these studies are contained in a report, written in German, available from the first author.

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Table 1: Earthwormtoxicology of Pesticides: Tubifex LC50, relative Toxicity, Application Rate, Predicted Initial Environmental Concentration (PIEC) and Ratio Tubifex LC50/PIEC †

Relative Toxicity according to Edwards & Bohlen (1992); values without decimals provisional : 0.00 = nontoxic; 1.00 = slightly toxic; 2.00 = moderately toxic; 3.00 = very toxic; 4.00 = extremely toxic.
PIEC : 1 kg AI / ha result in 2.7 ppm in the top 2.5 cm soil with density 1.5.

Product	Active Ingredient	Tubifex LC50 ppm AI	Rel. Tox. after Edw.&Bohlen	Application rate kg or l./ha	PIEC ppm	Tubifex LC50/ PIEC
Fungicides						
Dyrene	Anilazine, 48%	4.40	1.00	4	5.18	0.85
Benlate	Benomyl, 50%	0.57	4.00	0.25	0.34	1.69
Nimrod	Bupirimate, 25%	14.00	0	4	2.70	5.19
Captafol	Captafol, 80%	1.40	0.00	2	4.32	0.32
Captan	Captan, 83%	4.40	1.00	3	6.72	0.65
Derosal	Carbendazim, 60%	0.26	3.00	0.3	0.49	0.53
Daconil	Chlorothalonil, 41%	0.57	2.00	3	3.32	0.17
Serinal	Chlozolinate, 16%	160.00		1.5	0.65	246.91
Alto	Cyproconazol, 9.35%	26.00		0.8	0.20	128.74
Score	Difenoconazol, 24.8%	7.70		0.5	0.33	23.00
Acrobat	Dimetomorph, 7.5%	25.00		2.5	5.01	4.99
Beret	Fenpiclonil, 4.8%	11.00		2.5	0.32	33.95
Corbel	Fenpropimorph, 79%	13.00		1	2.13	6.09
Brestan K	Fentinacetate, 60%	0.46		0.5	0.81	0.57
Mapro	Fluazinam, 38.4%	0.20		0.5	0.52	0.39
Punch	Flusilazol, 25.9%	7.30		1	0.70	10.44
Impact	Flutriafol, 12.1%	18.00		1	0.33	55.10
Ortho-Phaltan	Folpet, 80%	1.70	0.00	3	6.48	0.26
Lemanor	Hexaconazol, 22.9%	18.00		1	0.62	29.11
Rovral	Iprodion, 50%	150.00		2	2.70	55.56
Dithane-Ultra	Mancozeb, 80%	24.50	0.00	3	6.48	3.78
Maneb	Maneb, 80%	8.00	0.00	3	6.48	1.23
Basitac	Mepronil, 40%	12.50		1	1.08	11.57
Enovit M	Methylthiophanate, 70%	1.00	3.00	0.7	1.32	0.76
Polyram	Metiram 80%	15.00		3	6.48	2.31
Monceren	Pencycuron, 23%	>1000.00		1.2	0.75	1341.92
Sportak	Prochloraz, 42%	16.00		1	1.13	14.11
Tilt	Propiconazol, 25.3%	5.70	3.00	0.5	0.34	16.69
Folicur	Tebuconazol, 23.4%	18.00		1	0.63	28.49
Tecto flowable	Thiabendazol, 42.7%	1.40	3.00	0.35	0.40	3.47
Pomarsol forte	Thiram, 80%	2.60	0.00	6	12.96	0.20
Bayleton	Triadimefon, 25%	52.00	0.00	0.5	0.34	154.07
Ronilan	Vinclozolin, 41.3%	520.00		1.5	1.67	310.88
Zineb	Zineb, 80%	240.00		2	4.32	55.56
Ziram	Ziram, 90%	15.00	0	6	14.58	1.03
Herbicides						
2,4,5 T NPT fl	2,4,5 T, 100%	1300.00		6	16.20	80.25
Gesin	2,4-D, 40%	>1000.00	1.00	2.5	2.70	370.31
Bandur	Aclonifen, 85%	67.00		5	11.48	5.84
Lasso	Alachlor, 43%	13.00		10	11.61	1.12
Grasip	Alloxydim, 75%	490.00		3	6.08	80.66
Hoestar	Amidosulfuron, 75%	1750.00		0.04	0.08	21604.94
Asulox	As ulam ,35%	>1000.00	0.00	4	3.78	264.55
Atrazin	Atrazin, 50%	540.00	0.00	3	4.05	133.33
Galipan	Benazolin, 20%	>1000.00		2	1.08	925.93
Laddok T	Bentazon 25.5%	62.00		5	5.74	10.81

Product	Active Ingredient	Tubifex LC50 ppm AI	Rel. Tox. after Edw.&Bohlen	Application rate kg or L/ha	PIEC ppm	Tubifex LC50/ PIEC
Herbicides (continued)						
Faneron	Bromphenoxim, 50%	23.00		5	6.75	3.41
Mikado	Chlomesulon, 26.3%	1000.00		2	1.40	712.25
Maloran	Chlorbromuron, 50%	24.00		4	5.40	4.44
Tenoran	Chloroxuron, 50%	170.00		7	9.45	17.99
Focus	Cycloxydim, 21%	17.00		3	1.70	9.99
Reglone	Diquat, 33%	11.60	0	5	4.46	2.60
Sevton	DNBP, 40%	0.77	2.00	6	6.48	0.12
Bruelex fl	DNOC, 46%	1.50	1.00	45	55.89	0.03
Extar forte	DNTBP, 25%	0.15		20	13.50	0.01
Nortron	Ethofumesate, 21%	6.30		5	2.84	2.22
Puma	Fenoxypropethyl, 5.61%	3.50		3	0.45	7.70
Furore Super	Fenoxypropethyl, 7.2%	1.45		1.5	0.29	4.97
Fusilade extra	Fluazifopbutyl, 12.6%	8.40		3	1.02	8.23
Fusilade	Fluazifopbutyl, 25%	5.60		3	2.03	2.77
Racer	Flurochloridon, 24.5%	15.50		3	1.98	7.81
Starane	Fluroxypyr, 35.6%	11.00		1.5	1.44	7.63
Flex	Fomesafen, 21.7%	1400.00		0.75	0.44	3185.98
Basta	Glufosinate, 18%	17.00		5	2.43	7.00
Roundup	Glyphosate, 41%	88.00	0.00	10	8.37	10.51
Arelon fl	Isoproturon, 50%	1900.00		3	4.05	469.14
Venzar	Lenacil, 80%	1000.00	0.00	5	10.80	92.59
Linopan	Linuron, 50%	55.00	0.00	2.5	3.38	16.30
Divopan	MCPB, 35%	150.00	0.00	4	3.78	39.68
Goltix	Metamitron, 70%	1000.00		5	9.45	105.82
Butisan S	Metazachlor, 43.1%	36.00		3	3.49	10.31
Tribunil	Methabenzthiazuron, 70%	57.00	0.00	5	9.45	6.03
Dual	Metolachlor, 96%	21.00		3	7.78	2.70
Sencor	Metribuzin, 70%	140.00	0.00	1	1.89	74.07
Ally	Metsulfuronmethyl, 20%	505.00		0.04	0.02	23379.63
Aresin	Monolinuron, 50%	180.00	0.00	2.5	3.38	53.33
Na TCA	Na TCA, 100%	>1000.00	0.00	8	21.60	46.30
Napa	Napropamid, 42%	44.00		3.5	3.97	11.09
Dasul	Nicosulforon, 4.2%	53.00		1.25	0.14	373.90
Gramoxone	Paraquat, 25%	48.00	0.00	3	2.03	23.70
Stomp	Pendimethalin, 32%	6.40	2.00	5	4.32	1.48
Betanal	Phenmedipham, 16%	17.50	0.00	6	2. 59	6.75
Topik	Piroxofop-propinyl, 22.2%	18.00		0.25	0.15	120.12
Agil	Propaquizafop, 10.5%	5.20		1	0.28	18.34
Kerb 50W	Propyzamid, 50%	200.00		1.5	2.03	98.77
Boxer	Prosulfocarb, 78.9%	12.00		5	10.65	1.13
Lentagran	Pyridate, 45%	170.00		2	2.43	69.96
Targa	Quizalofopethyl, 10%	2.90		3	0.81	3.58
Titus	Rimsulfuron, 25%	1700.00		0.04	0.03	62962.96
Gesatop	Simazine, 50%	540.00	2	6	8.10	66.67
Sodiumchlorate	Sodiumchlorate, 100%	>1000.00	0.00	300	810.00	1.23
Igran	Terbutryne, 50%	7.50		4	5.40	1.39
Tyllanex	Terbutylazine, 80%	1000.00		1.8	3.89	257.20
Harmony	Thiameturonmethyl, 75%	1000.00		0.03	0.06	16460.91
Splendor	Tralkoxydim, 9.85%	5.60		3	0.80	7.02
Triallate	Triallate, 48%	17.00	0.00	3	3.89	4.37
Express	Tribenuron-methyl, 75%	5000.00		0.04	0.08	61728.40
Garlon 3A	Triclopyr, 44.4%	250.00	0.00	2	2.40	104.27
Trifluralin	Trifluralin, 48%	50.00	0.00	3	3.89	12.86

Product	Active Ingredient	Tubifex LC50 ppm AI	Rel. Tox. after Edw.&Bohlen	Application rate kg or L/ha	PIEC ppm	Tubifex LC50/ PIEC
Insecticides, Acaricides und Nematicides						
Orthene	Acephate, 50%	440.00	2.00	3	4.05	108.64
Temik	Aldicarb, 10%	6.50	4.00	23	6.21	1.05
Gusathion	Azinphosmethyl, 90.1 tech	130.00	2.00	1.2	0.81	160.49
Novodor	Bacillus thuringiensis	5300.00		8	21.60	245.37
Delfin	Bacillus thuringiensis	10000.00		1.5	4.05	2469.14
Garvox	Bendiocarb, 3%	1.60	4.00	10	0.81	1.98
Talstar	Bifenthrin, 10%	1.28		0.2	0.05	23.70
Nexion	Bromophos, 3%	75.00	1.00	0.3	0.02	3086.42
Sevin	Carbaryl, 50%	0.15	4.00	0.6	0.81	0.19
Curaterr 5 G	Carbofuran, 5%	0.28	3.00	34	4.59	0.06
Marshall	Carbosulfan, 5%	1.40		14	1.89	0.74
Reldan	Chlorpyrifos, 42%	5.60	3.00	2	2.27	2.47
Cymbush	Cypermethrin, 10%	0.82	1	0.75	0.20	4.05
Decis	Deltamethrine, 2.5%	0.11		0.4	0.03	4.07
Torak	Dialifos, 20%	6.00	0.00	1	0.54	11.11
Diazinon	Diazinon, 25%	15.00	1.00	1	0.68	22.22
Kelthane	Dicofol, 20%	6.70	0.00	2	1.08	6.20
Pentac	Dienochlor, 50%	2800.00		2	2.70	1037.04
Dimilin	Diflubenzuron, 25%	850.00	0.00	0.25	0.17	5037.04
Perfekthion	Dimethoate, 40%	175.00	1	2.5	2.70	64.81
Melophen	Endosulfan, 35%	6.00	2.00	0.6	0.57	10.58
Sumialpha	Esfenvalerate, 2.9%	0.36		0.6	0.05	7.66
Croneton	Ethiophencarb, 46%	0.70	0	1	1.24	0.56
Ekamet	Etrimphos, 50%	11.00		1	1.35	8.15
Nemacur	Fenamiphos, 90.5%=tech.	76.00	1.00	60	16.20	4.69
Dyfonate G	Fonofos, 5%	0.14	2.00	14	1.89	0.07
Deltanet G	Furathiocarb, 5%	5.50		47	6.35	0.87
Hostaquick	Heptenophos, 50%	19.00		1	1.35	14.07
Karate	Lambda-Cyhalothrin, 5.5%	0.32		0.15	0.02	14.37
Gamasat L	Lindane, 20%	8.70	2.00	0.2	0.11	80.56
Tamaron	Methamidophos, 60%	10.00	1.00	0.6	0.97	10.29
Ultracid	Methidathion, 40%	15.50	2.00	1.5	1.62	9.57
Lannate	Methomyl, 25%	2.40	4.00	1	0.68	3.56
Phosdrin	Mevinphos, 25%	1.80		1.5	1.01	1.78
Arafos G	Oxamyl, 10%	23.00	2.00	50	13.50	1.70
Parathion	Parathion-ethyl, 46.7%	5.50	0.00	1	1.24	4.43
Ambush	Permethrin, 27%	18.00	0.00	0.12	0.09	205.76
Zolone	Phosalone, 33%	6.30	1.00	1.5	1.34	4.71
Pirimor	Pirimicarb, 50%	46.00		0.25	0.34	136.30
Unden fl	Propoxur, 20%	0.78	3.00	6	3.24	0.24
Plenum	Pymetrozin, 25%	400.00		0.8	0.54	740.74
Sano Plant	Pyrethrumextract, 10%	13.00		5	1.35	9.63
Nomolt	Teflubenzuron, 13.75%	10000.00		0.4	0.15	67340.07
Aragran	Terbufos, 2%	18.00	2.00	34	1.84	9.80
Evisect S	Thiocyclamhydrogenoxalate, 50%	1.70		0.25	0.34	5.04
Dipterex	Trichlorphon, 95%	0.58		3	1.11	0.52
Molluscicides						
Malice G	Bensultap, 5%	1.50		7.5	1.01	1.48
Metarex	Metaldehyde, 5%	146.00		7	0.95	154.50
Limalo	Metaldehyde, 80%	3800.00		4	8.64	439.81
Mesurol G	Methiocarb, 1%	2.20	3.00	10	0.27	8.15

† Because of space limitations in this volume only some examples out of the 300 available data are presented. Mixtures of two or more active ingredients among fungicides and herbicides are also omitted. The full table can be obtained by contacting the first author.

Table 2: Comparison of Toxicity Ranking of 32 Active Ingredients using Tubifex- und Eisenia LC50/PIEC Ratios and Relative Toxicity †

Active Ingredient	Tubifex LC50	Eisenia LC50	Eis/TubLC50	PIEC, ppm	EisLC/PIEC	Relat. Tox.††	Active ingredient	Tubifex LC50	TubLC/PIEC	Relat. Tox.††
Calciumcyanamide	64.00	115.00	1.80	475.20	0.24	1	Carbofuran	0.28	0.06	3.0
Aldicarb	6.50	3.30	0.51	6.21	0.53	4.0	DNOC	1.50	0.07	1.0
Na-chlorate	>1000.00	>750.00	0.75	810.00	0.93	0.0	Calciumcyanamide	64.00	0.13	1
DNOC	1.50	21.00	14.00	21.60	0.97	1.0	Propoxur	0.78	0.24	3.0
Methidathion	15.50	4.80	0.31	1.62	2.96	2	Folpet	1.70	0.26	0
Terbufos	20.80	6.60	0.32	1.41	4.68	2.0	Captafol	1.40	0.32	0.0
Carbofuran	0.28	28.00	100.00	4.59	6.10	3.0	Captan	4.40	0.65	1.0
Propoxur	0.78	27.00	34.62	3.24	8.33	3.0	Aldicarb	6.50	1.05	4.0
Endosulfan	6.00	9.40	1.57	0.57	16.49	2	Na-chlorate	>1000.00	1.23	0.0
Methamidophos	10.00	17.00	1.70	0.95	17.89	1	Bensultap	1.60	1.58	
Bensultap	1.60	30.00	18.75	1.01	29.70		Carbaryl	2.50	3.09	4.0
Trichloroacetic acid	>1000.00	1056.00	1.06	21.60	48.89	1.0	Benomyl	1.30	3.82	4.0
Parathion-ethyl	5.50	64.00	11.64	1.24	51.61	3	Parathion-ethyl	5.50	4.44	3
Folpet	1.70	339.00	199.41	6.48	52.31	0	Bupirimate	14.00	5.19	0
Atrazin	140.00	131.00	0.94	2.42	54.13	0	Cu-oxychloride	70.00	7.41	0.0
Benomyl	1.30	27.00	20.77	0.34	79.41	4.0	Methidathion	15.50	9.57	2
Captan	4.40	612.00	139.09	6.72	91.07	1.0	Methiocarb	2.70	10.00	3.0
Cu-oxychloride	70.00	900.00	12.86	9.45	95.24	0.0	Ethiofencarb	5.50	10.38	0
Captafol	1.40	516.00	368.57	4.32	119.44	0.0	Endosulfan	6.00	10.53	1
Paraquat	112.00	>200.00	1.79	1.50	133.33	0.0	Methamidophos	10.00	10.53	2
Bupirimate	14.00	401.00	28.64	2.70	148.52	0	Dialifos	6.80	13.88	0
Carbaryl	2.50	174.00	69.60	0.81	214.81	4.0	Terbufos	20.80	14.75	2.0
Chlormequat chloride	>1000.00	>1000.00	1.00	3.24	308.64		Trichloroacetic acid	>1000.00	46.30	1.0
Dialifos	6.80	218.00	32.06	0.49	444.90	0	Atrazin	140.00	57.85	0
Methiocarb	2.70	129.00	47.78	0.27	477.78	3.0	Paraquat	112.00	74.67	0.0
Ethiofencarb	5.50	262.00	47.64	0.53	494.34	0	2.4.5 T	1300.00	80.25	0
Triadimefon	52.00	>250.00	4.81	0.34	735.29	0.0	Triadimefon	52.00	152.94	0.0
Lindane	17.50	135.00	7.71	0.11	1227.27	2	Lindane	17.50	159.09	2
Pencycuron	>1000.00	>1000.00	1.00	0.75	1333.33		Chlormequat chloride	>1000.00	308.64	
2.4.5 T	1300.00	22000.00	16.92	16.20	1358.02	0	Metaldehyde	3800.00	439.81	
Metaldehyde	3800.00	60000.00	15.79	8.64	6944.44		Pencycuron	>1000.00	1333.33	
Chloracetamide	32.00	38.00	1.19				Chloracetamide	32.00		

† active ingredients for which an Eisenia LC50 is published and arranged according to increasing Eisenia LC50/PIEC ratio (left) and increasing Tubifex LC50/PIEC ratio (right)

††After Edwards & Bohlen 1992, values without decimals not sufficient evidence; 0.0=relatively nontoxic, 1.0=sligtly toxic, 2.0=moderately toxic, 3.0=strongly toxic, 4.0=extremely toxic

Table 3: Comparison of Results in the Tubifex-, Eisenia-, Funnel- und Field-Test for formulated Pesticides

Active ingredient and Concentration in Product	Product and Application Rate kg or L/ha	PIEC ppm	Tubifex LC50 ppm, 7 days	TubLC50/PIEC	Eisenia LC50 ppm, †3 14 days	EisLC50/PIEC	Funnel Test Effect†4 3 Weeks	Field Test †4 Reduction of weights †5					
								3 Weeks			4-5 Months		
								1991	1992	1993	1991	1992	1993
Dinoseb, 29%	Super Kabrol, 6 L	4.70	0.77	**0.16**			***		*			*	
	Super Kabrol, 10 L	7.83	0.77	**0.10**					0	**		0	0
DNOC, 46 %	Brülex,10 L	12.42	1.50	**0.12**	21.00	**1.69**	**			*			**
	Brülex,20 L	24.84	1.50	**0.06**	21.00	**0.85**	***			0			0
Benomyl, 50%	Benlate, 4 kg	5.40	0.57	**0.11**	27.00	**5.00**	**	*			*		
Metaldehyde, 3.5%	Limax G, 10 kg	0.95	10.00 †1	**10.52**	60000†2	**63152.89**		0	**	*	0	*	0
	Limax G, 40 kg	3.78	10.00 †1	**2.66**	60000†2	**15873.01**	0	0	**	0	**	*	0
Bensultap, 5%	Malice , 10 kg	1.35	1.60	**1.19**	30.00	**22.22**		0	0	**	**	*	0
	Malice , 40 kg	5.40	1.60	**0.30**	30.00	**5.56**	***	0	0	**	0	*	0
Methiocarb, 1%	Mesur ol G, 10 kg	0.27	2.70	**10.00**	129.00	**477.78**		0	*	*	*	*	0
	Mesurol G, 40 kg	1.08	2.70	**2.50**	129.00	**119.44**	***	0	0	**	**	*	*
Carbofuran, 5%	Curaterr, 34 kg	4.59	0.28	**0.06**	28.00	**6.10**	**		0	*		0	0
	Curaterr, 136 kg	18.36	0.28	**0.02**	28.00	**1.53**			0	**		0	0
Aldicarb, 10%	Temik, 23 kg	6.21	6.50	**1.05**	3.30	**0.53**	**		0	*		0	0
	Temik, 92 kg	24.84	6.50	**0.26**	3.30	**0.13**			0	**		0	**
Terbufos, 2%	Aragran, 34 kg	1.84	18.00	**9.80**	6.60	**3.59**	*		*	0		0	0
	Aragran, 136 kg	7.34	18.00	**2.45**	6.60	**0.90**			***	***		0	0
Glufosinate, 18%	Basta, 5 L	2.43	17.00	**7.00**			0			0			0
	Basta, 20 L	9.72	17.00	**1.75**						0			0
Linuron, 17%	Kariben, 7 L	3.21	17.00	**5.29**			0			0			0
	Kariben, 28 L	12.85	17.00	**1.32**						0			0
Metsulfuron- methyl, 20 %	Ally, 0.04kg	0.02	505.00	**23379.63**			0			*			0
	Ally, 0.16kg	0.09	505.00	**5844.91**						0			0

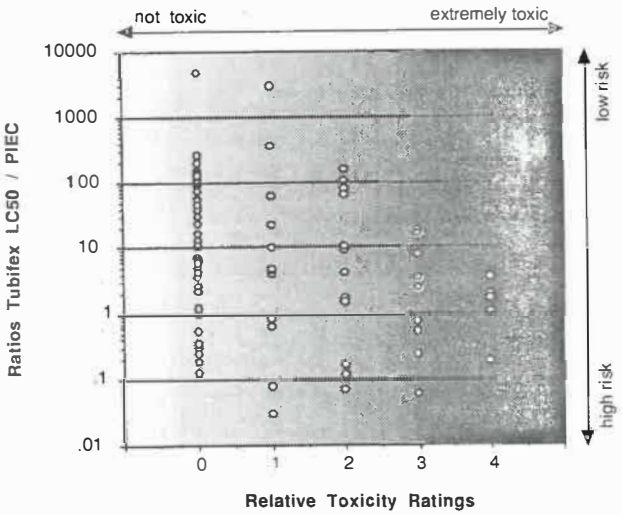
†1 incl. fungicidal conservation agents; Metaldehyde, 80%: Tubifex LC50 = 3800ppm

†2 Metaldehyde, 100%; †3 Data from the literature

†4 Effect: *** = > 75%, strongly toxic; ** = 40-75%, moderately toxic; * = 25-40%, slightly toxic; 0 = <25%, non-toxic (Heimbach, 1992)

†5 Results of field tests: Ratios of weights after treatment / before treatment, if control without pesticide is set = 1.0

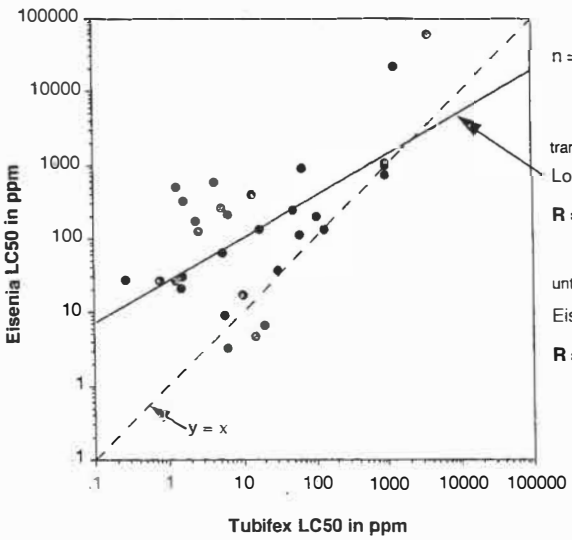
Fig. 1: Comparison of Relative Toxicity according to Edwards & Bohlen (1992) and the Ratio Tubifex LC50 / PIEC



n = 82 pesticides

Relative toxicity:
0 = relatively nontoxic (45 pesticides)
1 = slightly toxic (11 pesticides)
2 = moderately toxic (12 pesticides)
3 = strongly toxic (9 pesticides)
4 = extremely toxic (5 pesticides)

Fig. 2: Correlation of Tubifex- and Eisenia- LC50



n = 32 value pairs from table 2

transformed:
Log Eisenia LC50 = 1.4427 + 0.56429 Log Tubifex LC50

R = 0.648; R^2 = 0.420; F = 21.744; p = 0.00006

untransformed (regression line not shown)
Eisenia LC50 = - 1271.0 + 13.524 Tubifex LC50

R = 0.906; R^2 = 0.822; F = 138.5; p = 0.00001

Fig. 3: Funnel Test: Activity of L. terrestris after different Modes of Application of Dinoseb

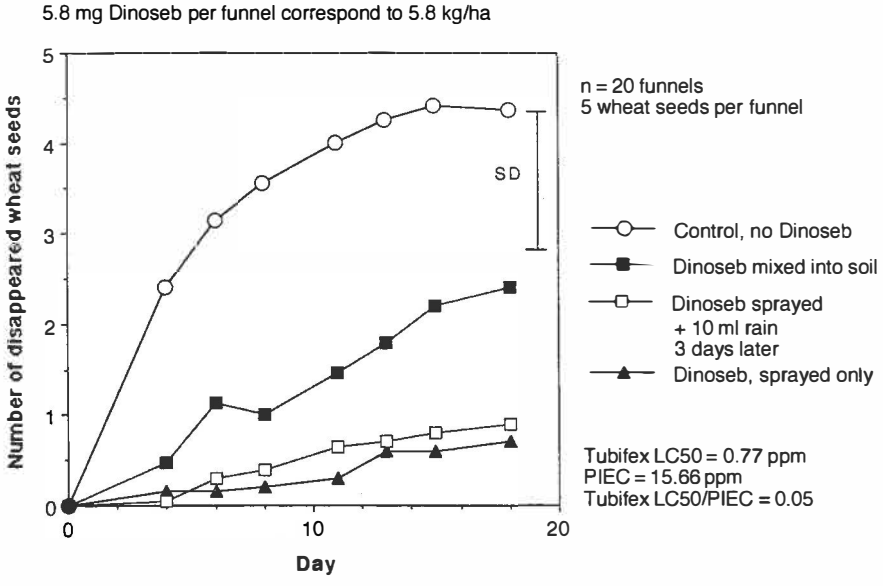


Fig. 4: Funneltest: Activity of L. terrestris under the Influence of Different Molluscicide Baits

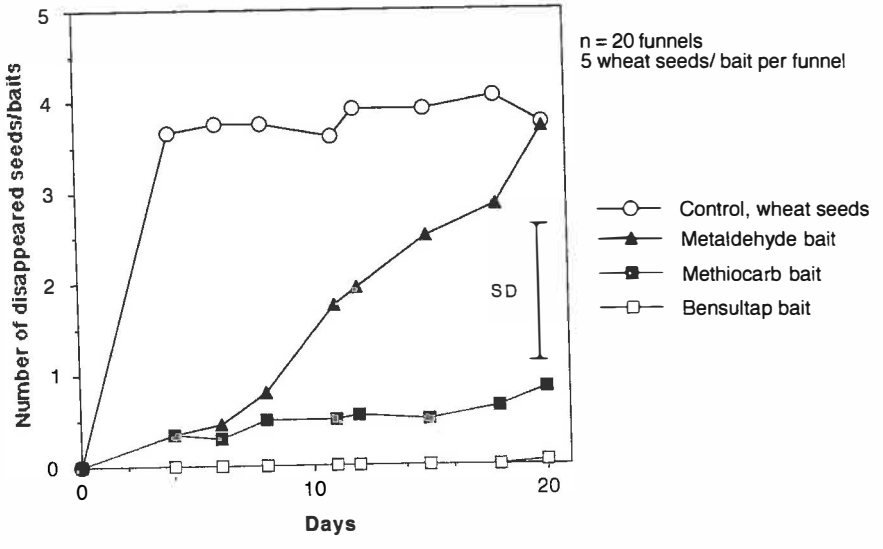


Fig. 5: Funneltest: Weight Changes of L. terrstris under the Influence of Molluscicides

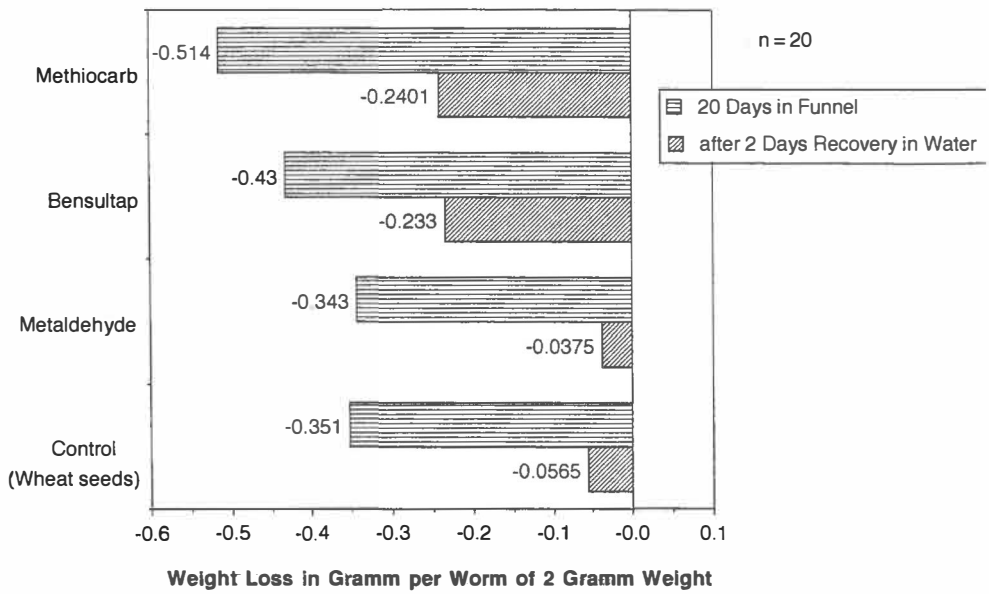


Fig. 6: Funneltest: Activity of L. terrestris under the Influence of Granular Nematicides

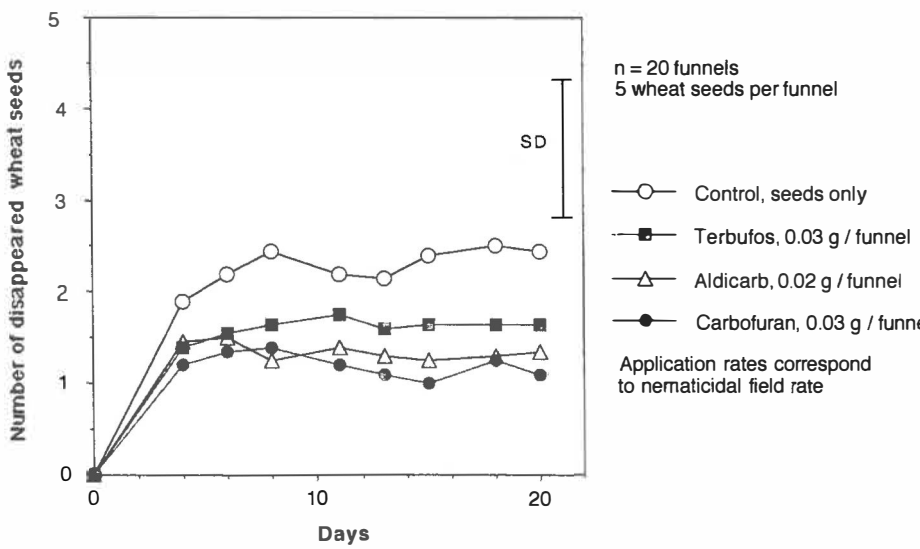


Fig. 7: Weight Changes of L. terrestris under the Influence of Granular Nematicides

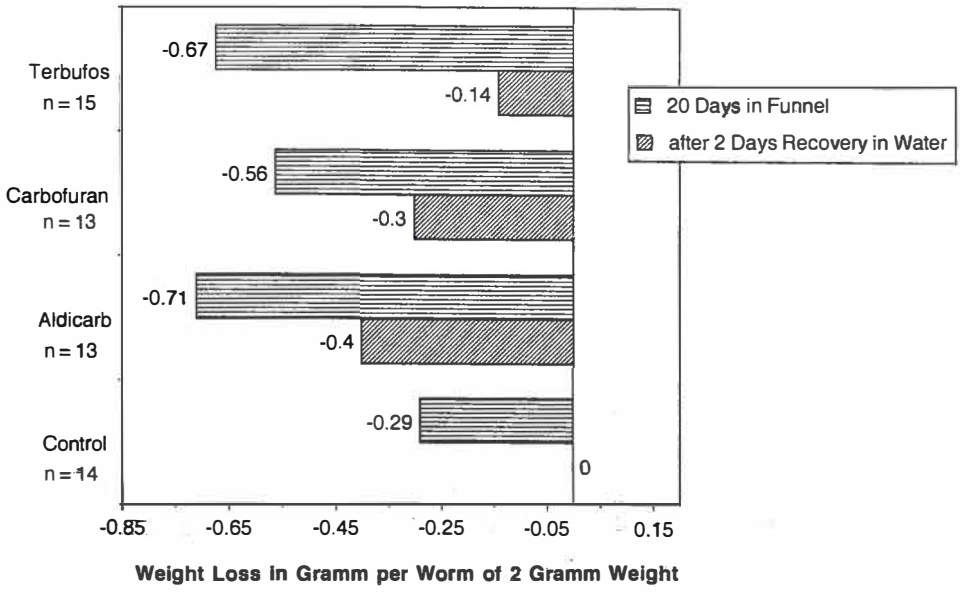
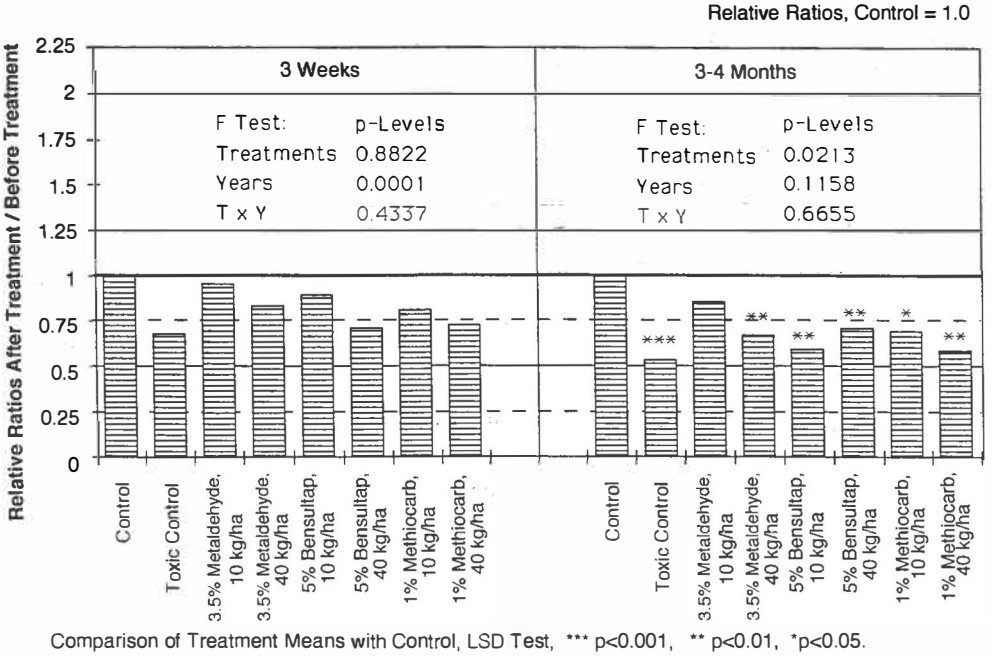
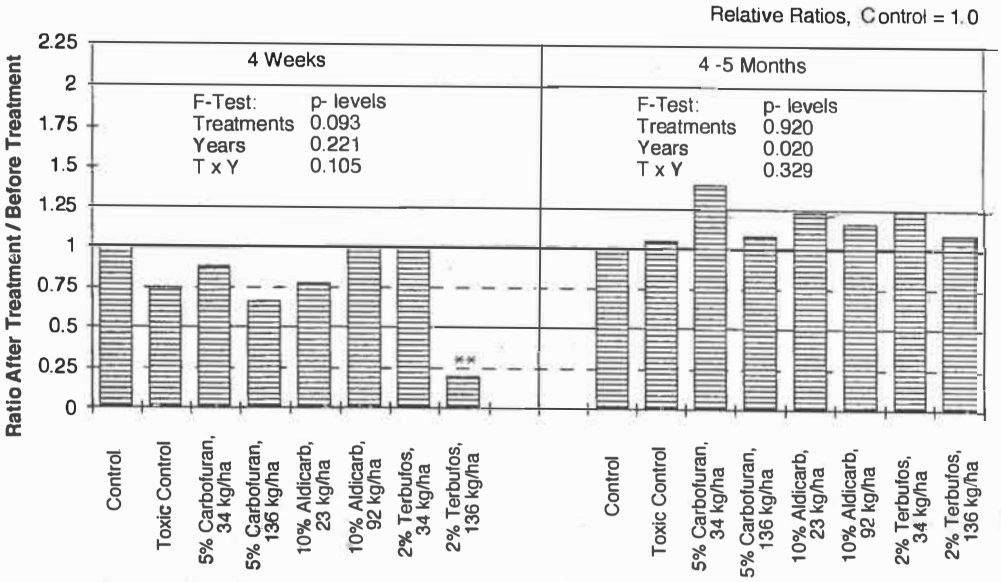


Fig. 8: Fieldtest: Effect of Molluscicide Baits on Earthworm Weights, 3 year Average.



Comparison of Treatment Means with Control, LSD Test, *** p<0.001, ** p<0.01, *p<0.05.

Fig. 9: Fieldtest: Influence of Granular Nematicides on Earthworms Weights, 2 Year Average.



** Mean significantly different from Control., $p < 0.01$, LSD Test.

Fig. 10: Fieldtest: Effect of Herbicides on Relative Earthworm Weights , 1 Year.