IOBC/WPRS

Working Group "Pesticides and Beneficial Organisms"

OILB/SROP

Groupe de Travail "Pesticides et Organismes Utiles"

GUIDELINES FOR TESTING THE EFFECTS OF PESTICIDES ON BENEFICIAL ORGANISMS:

DESCRIPTION OF TEST METHODS

Edited by S.A. Hassan

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INTRODUCTION

Selective pesticides that can be used to control pests without adversely affecting important natural enemies are needed for modern pest management. Testing the side effect of pesticides on beneficial organisms is therefore gaining increasing attention by research workers in different parts of the world. To encourage international cooperation, the Working Group "Pesticides and Beneficial Organisms" of the International Organization for Biological Control (IOBC), West Palearctic Regional Section (WPRS) was formed in 1974.

Standard methods to test the side -effects of pesticides on beneficial organisms were developed according to common characteristics. Whereas harmlessness of pesticides can easily be shown by laboratory experiments, harmfulness can only be confirmed under practical conditions in the field. Pesticides found to be harmless to a particular beneficial in the laboratory test are most likeky to be harmless to the same organism in the field and no further testing in semi-field or field experiments is therefore recommended.

Short description of 19 laboratory, 12 semi-field and 5 field test methods as well as the result of testing of about 100 pesticides on 9 to 20 beneficials were published elsewhere. The present IOBC- Bulletin is the second of a series that includes the full text of guidelines to test the side effects of pesticides on beneficial organisms, the first IOBC-Bulletin (XI/4) was published in 1988..

S. A. HASSAN
(Group convenor)

LIST OF PARTICIPANTS

F.M. BAKKER
Universiteit Amsterdam
Dept. Pure & Applied Ecology
Sect. Population Biology/MITDX
Kruislaan 302
1098 SM Amsterdam, Niederlande

MARKUS BIERI
Bahnhofstr. 30
8803 Rüschlikon, Schweiz

SYLVIA BLÜMEL
Bundesanstalt für Pflanzenschutz
Trunnerstr. 1 - 5
1020 Wien, Österreich

H. BOGENSCHÜTZ
Forstliche Versuchs- und
Forschungsanstalt
Baden-Württemberg
Abteilung Waldschutz
Wonnhaldestr. 4
W-7800 Freiburg, Germany

ANTONIO GARRIDOVIVAS
Dept. Proteccion Vegetal
Instituto Valenciano de
Investigaciones Agrarias
I.V.I.A.
Moncada, Valencia, Spanien

Sherif A. HASSAN
Institut für Biologischen
Pflanzenschutz der
Biologischen Bundesanstalt für
Land- und Forstwirtschaft
Heinrichstr. 243
6100 Darmstadt, Germany

UDO HEIMBACH
CHRISTIAN ABEL
Biologische Bundesanstalt
Inst. f. Pflanzenschutz in
Ackerbau u. Grünland
Messeweg 11/12
W-3300 Braunschweig, Germany

NEIL HELYER
HRI
Worthing Road
Littlehampton
Sussex BN 17 6LP, England

HEIKKI HOKKANEN
University of Helsinki
Department of Agricultural
and Forest Zoology
00710 Helsinki, Finnland

P. HUANG
NiedersächsischesForstliche
Versuchsanstalt
Abteilung B - Waldschutz
Grätzelstr. 2
W-3400 Göttingen, Germany

P.C. JEPSON
Dept. of Biology
Building 62
The University
Southampton S09 3TU, England

ISTVAN KISS
GABOR BAKONYI
Department of Zoology
and Ecology, University
of Agricultural Sciences
2103 Gödöllö, Ungarn

GAVIN LEWIS
Ecology Section ICI Agrochemicals
Jealott's Hill Research Station
Bracknell, Berkshire
RG12 6EY, England

L. MORETH
Bayerische Landesanstalt für
Bodenkultur und Pflanzenbau
Abteilung Pflanzenschutz
Menzinger Str. 54
W-8000 München, Germany

P. A. OOMEN
Plant Protection Service
Geertjesweg 15
P.O.Box 9102
6700 HC Wageningen, Niederlande

L. SAHSOE-PETERSEN
STatens Plantevaernscenter
Afd. F. Jordbrugszool
Lottenborgvej 2
2800 Lyngby, Dänemark

BENOIT SAUPHANOR
Station de Zoologie - INRA
Domains St. Paul BP 91
84140 Montfavet, Frankreich

INGO TORNIER
GAB Biotechnologie GmbH
Eutinger Str. 24
w-7532 Niefern-Öschelbronn,
Germany

AANA VAINIO
Agricultural Research Center
Institute of Plant Protection
31600 Jokioinen, Finnland

MARCUS VAN DE VEIRE
Faculty of Agricultural Sciences
University of Gent
Coupure 653
9000 Gent, Belgien

HEIDRUN VOGT
Biologische Bundesanstalt für
Land- und Forstwirtschaft
Institut für Pflanzenschutz
im Obstbau
Postfach 73
W-6915 Dossenheim, Germany

IOBC/WPRS WORKI NGGROUP
PESTICIDES AND BENEFICIAL ORGANI SMS

DESCRIPTION OF TEST METHODS

C O N T E N T S

MEETING OF THE WORKING GROUP "PESTICIDES AND BENEFICIAL ORGANISMS", UNIVERSITY OF SOUTHAMPTON, UK, SEPTEMBER 1991.

S.A. HASSAN

Insitute for Biological Pest Control,
Heinrichstr. 243, D-6100 Darmstadt,
Federal Republic of Germany.

The meeting of the Working Group took place at the University of Southampton on September 24 to 26, 1991. The invitation came from Dr. Paul Jepson, Department of Biology, Bassett Crescent East, Southampton. The following topics were discussed at the meeting:

(1) The standard characteristics of "extended laboratory" and "semi-field" test methods were discussed. It was proposed to rename some of the semi-field test methods to be "extended laboratory". In an "extended laboratory" method, a more realistic method of exposure, compared to the laboratory test, should be used in a standard manner (plant leaves, natural soil or plant cover) and the experiments may be carried out under controlled field simulated environmental conditions. A "semi-field" test is conducted under true field conditions (field environment, soil and crop cover). Commonly used methods of pesticides application and timing of treatment are adapted. Laboratory reared natural enemies, plot barriers and cages to confine the organisms may be used. These suggestions will be discussed in a more detailed way during the next meeting.

(2) The development of different types of test methods within sequential testing programmes for several beneficial organisms was dealt with and recommendations were discussed. In addition to the available standared laboratory test methods for "laboratory, exposed life stages" (e.g. adult of parasites and larvae of predators) for 21 beneficial organisms, the development of 5 methods (predatory mites, spiders, leafminer parasites, *Orius spp.*, *Opius concolor*) continues at the present time. Six laboratory methods to test the side effects of pesticides on "protected" or "laboratory, less exposed life stages" of beneficial organisms are available and 2 are in development (*Aleochara bilineata* and *Poecilus cupreus*). The number of methods to test the "duration of harmful activity" (persistence), available and in development, is 6 and 8 , respectively (*Aphidius matricaria*, *Phytoseiulus persimilis*, spiders, *Aleochara bilineata*, *Poecilus cupreus*, *Verticillium lecanii*, *Beauveria bassiana*, *Bembidion lampros*), the number for the "extended laboratory" tests is 5 and 5 (*Trichogramma cacoeciae*, *Aphidius matricaria*,

Verticillium lecanii, *Beauveria bassiana* und earthworms), the "semi-field" tests 5 and 11 (*Encarsia formosa*, *Typhlodromus pyri*, *Chiracanthium mildei*, *Metasyrphus corollae*, *Antocoris nemoralis*, *Aleochara bilineata*, *Bembidion lampros*, *Poecilus cupreus*, *Pterostichus melanarius Forficula auricularia* und *Steinernema feltiae*). Four guidelines to test the side effects in the "field" under practical conditions were developed and 10 are still in process (*Trichogramma cacoeciae*, *Chiracanthium mildei*, *Chrysoperla carnea*, *Antocoris nemoralis*, *Bembidion lampros*, *Poecilus cupreus*, *Beauveria bassiana*, *Forficula auricularia*, earthworms and nematods). These numbers show that priority by the working group is now given to the development of "extended laboratory", "true semi-field" and "field" methods without neglecting the remaining types of tests.

(3) The **publication** of new and improved testing guidelines in an IOBC/WPRS Bull. is planned for 1992. There is some urgency in publishing methods, especially of the semi-field and field types, as obligatory testing had now begun in Germany and would soon follow in other countries. The publication of the results of the 6th joint pesticides testing programme was also recommended.

(4) The presentation of IOBC/WPRS results on the side effects of pesticides in **"popular publications"** by testing members in different countries was discussed. Many members produce summaries of results in tabular form which may be used by extension workers, crop protection services and farming organisations. It was agreed that the exchange of information was very important and must be presented in a clear and easily understandable form. The use of databases may be a way forward in the future.

(5) Experiences with **GLP** were discussed. Some laboratories had separated fundamental research from work under GLP, whereas others had the whole laboratory operating under GLP. Factors such as increased expense and time in auditing were highlighted. It was concluded that laboratories with a research or crop protection function did not require GLP accreditation.

Standard characteristics of the different tips of test methods, result of meeting and discussion in writing:

(a). **Laboratory**, susceptible life stage (e.g. adult of parasites and larvae of predatory insects):
1. exposure of organisms to fresh pesticide deposit applied on glass plate, leaf or sand (soil); 2. exposure of beneficial fungus, nematodes and collembolan in contaminated standard medium (e.g. based on water, agar); 3. even film of pesticide, standard amount of 1.5 mg fluid/cm^2 on glass or leaf and 6 mg fluid/cm^2 on sand (soil) 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 controls; 9. mortality / reduction in beneficial capacity; 10. four evaluation categories: 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-98%), 4 = harmful (>99%).

(b). **Laboratory**, less susceptible life stage (e.g. parasites within their hosts, adults of mites, pupae or eggs of predatory insects):
1. direct spray of organisms. Point 3 to 10 in test (a) are applicable.

(c). Duration of harmful activity:
1. exposure to pesticide residues applied on plants or soil at intervals after treatment; 2. weathering under field or simulated field environment; 3. application similar to practise; 4. experiment up to one month after treatment; 5. four evaluation categories: 1 = short lived (<5 days), 2 = slightly persistent (5-15 days), 3 = moderately persistent (16-30 days), 4 = persistent (>30 days). Point 4 to 9 in test (a) are applicable.

(d). Extended laboratory method.
1. exposure to freshly dried pesticide film applied on plant leaves, standard natural soil, crops or treas; 2. experiments are carried out under field or controlled field simulated environmental conditions; 3. laboratory reared or field collected organisms of uniform age; 4. recommended concentration and dose of pesticide; 5. application should comply with good agricultural practise; 6. barriers or cages to confine beneficial organisms; 7. adequate exposure period before evaluation; 8. water treated controls; 9. mortality / reduction in beneficial capacity; 10. experiments should include negative (toxic) standard; 11. four evaluation categories: 1 = harmless (<25%), 2 = slightly harmful (25-50%), 3 = moderately harmful (51-75%), 4 = harmful (>75%).

(e). Semi-field, initial toxicity:
1. crops or soil inhabited by beneficial are directly sprayed; 2. experiments are carried out under true field conditions at the appropriate time and season for the chemical, climatic factors to be left unaffected as much as possible 3. experiments to be repeated at different locations. Point 3 to 11 in test (d) are applicable.

(f). Field:
1. crops or soil inhabited by beneficials are directly sprayed; 2. experiments are carried out at the appropriate time and season for the chemical; 3. experiment to be repeated at different locations; 4. laboratory reared or field collected organisms may be released to supplement naturally occurring populations; 5. sampling is carried out at intervals before and after treatment(s); 6. recommended dose rates and number of treatments (following good agricultural practice); 7. adequate exposure period before evaluation; 8. water treated controls; 9. mortality / reduction in beneficial capacity, population changes or recovery may be monitored; 10. plot design and number of 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%).

A DISCUSSION OF METHODS USED IN SEMI-FIELD STUDIES TO EVALUATE PESTICIDE TOXICITY TO BENEFICIAL INVERTEBRATES

Paul Jepson and Mike Mead-Briggs

Agrochemical Evaluation Unit
Department of Biology
School of Biological Sciences
Bassett Crescent East
Southampton SO9 3TU
United Kingdom

ABSTRACT

This paper summarises some of the methods that have been used at Southampton University to evaluate the side-effects of pesticides under semi-field conditions. The invertebrates tested using these techniques include members of the Carabidae, Staphylinidae, Coccinellidae, Lycosidae, Chrysopidae and Anthocoridae. The methods presented are not intended to serve as definitive guide-lines, but rather to provide points of reference that can be incorporated in the design and development of final methodologies. Details of the commonest problems encountered, with suggestions of how these may be solved, have been included, as have actual examples of data obtained using these methods. Some semi-field approaches are equivalent to tier I laboratory tests; special design features are needed if the test is to fulfil the requirements of a tier II test to give an estimate of risk.

1. INTRODUCTION

1.1 What is the semi-field approach ?

A 'semi-field' study is one in which the invertebrates under investigation are confined within treated areas in an outdoor crop environment. The crop should be appropriate to the intended use of the pesticide and treatments should be applied in such a way as to reproduce farm practices.

The confinement of the test organisms (usually within barriers or cages) ensures that they remain within the treated area and are thus exposed to pesticide residues throughout the study. The barriers or cages also prevent the recolonisation of plots by other, previously unexposed, invertebrates.

The use of the semi-field approach facilitates replication of treatments and the experimental data may therefore be subjected to statistical analysis. This enables comparisons of the toxicity of different pesticides or application rates and of the susceptibility of different organisms.

The measurement of effects from semi-field studies integrates the susceptibility of the test organism with realistic levels and routes of exposure to a test product. The fact that the test is undertaken in the field ensures that the pesticide is exposed to natural processes of degradation and these tests may therefore provide a realistic estimate of ecological risk. For some test organisms this approach can be used to answer specific experimental questions in a cost-effective and repeatable way. It also lends itself to quality control procedures and the requirements of Good Field Practices. To enhance repeatability between studies, acclimatised cultured organisms can be used and factors such as crop growth stage and density, pesticide application parameters and the degree of confinement of the test organism to specific parts of the crop can be controlled within the experimental design. By repeating tests in a single crop over a period of time, one can obtain detailed information on the direct effects of a product in the field, which will only vary as a result of differences in the prevailing environmental conditions.

1.2 The limitations of semi-field studies

Although a semi-field study can form a bridge between laboratory and full-scale field investigations, it does not fully replace either of them. For instance, the true ecological risk associated with the use of a product can only be determined if a test continues until no further toxic effects are discernable. Due to the problems involved with confining some organisms, it is often impractical to extend the duration of semi-field studies to such an endpoint and this limits their potential for use in tier II testing. In addition, if a risk index is obtained, it is highly specific to the conditions of the particular test system and it it not normally possible to make more general predictions which can be extended to other situations (i.e. other crop growth stages, application methods, etc.).

The confinement of test organisms also interferes with certain natural processes, such as colonisation and dispersal, and with interspecific interactions such as predation and competition. Because of this, the results of semi-field studies should not be used to make precise predictions of ecological hazard (as might be made from a true field study).

2. METHODOLOGIES

Semi-field methods that have been developed and exploited by the Ecotoxicology Research Group and Agrochemical Evaluation Unit at Southampton University, fall into three broad categories, each of which deals with specific problems in risk analysis and hazard evaluation.

2.1 Studies with released organisms to evaluate product toxicity and persistence on specific surfaces

There are a number of methods available for confining field-collected or laboratory-cultured invertebrates onto specific surfaces within a crop, such as the soil or foliage at different levels within the canopy. In some respects, such studies are an extension of laboratory tests rather than being true semi-field tests. For this reason, the numerous methods by which invertebrates can be confined to specific surfaces will not be discussed in detail here.

By exposing test organisms to localised parts of the crop, it is possible to provide an index of toxicity for individual types of surface (e.g. leaf and soil). The data generated can potentially be used to explain trends in product persistence on these surfaces and to estimate the risk posed to other species which inhabit similar localised areas in the same crop. The disadvantage of such *in situ* bioassays is that realism is sacrificed because organisms are confined to one type of surface and normally only for short periods.

An example of where an *in situ* bioassay has provided data for interpreting product effects is provided by Unal and Jepson (1991). In this study, cereal plots were treated with the aphicides deltamethrin, dimethoate and pirimicarb. Beetles were then confined to the soil or to individual leaves on the crop using polystyrene Petri dishes which were modified internally so as to limit the movement of the insects on the treated surface. Repeated 24 h bioassays with separate groups of field-collected insects allowed the decay of toxic product residues to be determined. For *Bembidion lampros* (Coleoptera, Carabidae), this demostrated low toxicity for pirimicarb (from which low risk could be inferred) but high toxicity with both deltamethrin and dimethoate over extended periods. However, for deltamethrin, the persistence of toxicity was significantly less when beetles were confined on soil than when confined on foliage. This may explain why this chemical has less effect on populations of ground beetles than organophosphate aphicides, when evaluated in open field studies.

In this study, insects that survived the initial 24 h exposure to the soil or foliage were confined to the treated soil for an extended period, until any further cumulative toxic effects had ceased. This provided a measurement of the maximum mortality that might occur in the crop and was thus closely related to true ecotoxicological risk. It also gave an indication of the time over which invertebrates recolonising the treated area might be in risk of a toxic effect.

2.2 Short-term studies of the toxicity of pesticides to released organisms, under realistic conditions of exposure

Studies in this category typically involve invertebrates being released into cages or barriers placed within the crop. The test organism, which may be field-collected or laboratory-cultured, can be introduced prior to product application, or at intervals afterwards if the persistence of toxic effects is being evaluated.

An important cautionary note is that these test methods will only yield a realistic prediction of risk (i.e. the probability of harm in the field) if the organism remains exposed to residues until an endpoint is reached. Where assessments cease after an arbitrary period (e.g. 48 h), a test may still provide a statistically valid measurement of the comparative toxicity of different treatments but this will only equate to true ecological risk if no further mortality would have occurred with additional exposure.

If a precise measurement of toxic effects is required over a fixed period, then the test organisms should ideally be removed from the plots and monitored until all the effects of the dose to which they have been exposed have occurred. This is most important for slow-acting products such as insects growth regulators and for chemicals such as pyrethroids which may have delayed effects.

Some of the bioassay methods suggested below were designed to compare the toxicity of candidate products with a toxic standard (typically dimethoate) and not strictly to estimate the ecological risk from those compounds. The results of such tests may therefore underestimate the full effects of field exposure and therefore might fall short of the requirements of a tier II test.

2.2.1 The use of barriers to confine arthropods in arable crops

Small portable barriers can be used to confine invertebrates in most types of seedling or low-canopy crop. Their use is most suited to studies with non-climbing, epigeal fauna, e.g. the carabid beetles *Poecilus cupreus*, *Pterostichus melanarius* and *Bembidion lampros*. With simple modifications, barriers have also been used for short-term studies with some Staphylinidae (e.g. *Tachyporus hypnorum*) and with wolf spiders (Araneae, Lycosidae).

Barrier design

Barriers can be constructed from sheets of galvanised steel. Metal barriers are preferable to wooden ones since they can be cleaned more easily. Suitable dimensions are 50 cm x 50 cm x 30 cm deep, but barriers of larger sizes can be used. As size increases, so does the area that has to be searched and probably the number of test organisms required.

The internal surfaces of the barriers can be coated with PTFE solution (Fluon) to reduce escape by climbing. If the barriers are too shallow larger Carabidae may still escape. It is therefore useful to build an inward-facing lip for the barrier: this should not however be in place when the barriers are sprayed. If the crop is lower than the barrier a fine-mesh netting cover may be fitted over the barrier to prevent the escape of insects which occasionally fly in favourable conditions.

Setting barriers

When in place, the barriers should be pushed/hammered into the soil to a depth of approximately 5-10 cm. Where possible, both the crop within the barrier and that immediately surrounding it should be disturbed as little as possible because changes to the crop canopy may influence the distribution of the applied spray. When studies are carried out in hot weather, it is advisable to avoid placing barriers where cracks have appeared in the soil. These will often provide deep refuges for beetles, which may not then venture onto the treated soil surface. It is sometimes useful to place inverted cards bearing freeze-killed prey such as pupae of *Musca domestica* in the arenas; predatory activity on the soil surface may then be demonstrated by the removal of eggs.

Collection of test organisms

Invertebrates may be collected using pitfall or gutter traps or by hand. If traps are used, they should be emptied as frequently as possible, ideally every 1-2 days, to prevent cannibalism or the general deterioration of the trapped arthropods. Invertebrates can be stored in single-species groups within ventillated boxes, under similar conditions to the field (e.g. 16°C, 16h daylength and moderate humidity). Most species can be fed with aphids collected from the field or cultured in the laboratory, or with a variety of products including moist, meat-

based pet biscuits. Field-collected organisms should be used in studies as soon as possible after collection and should not be stored for long periods.

To reduce confusion with individuals from natural populations already in the crop, test invertebrates should be marked prior to their release.

As a general principle, the species selected for testing should be active in the crop at the time of intended use of the pesticide. Population levels of test invertebrates may be higher than that normally encountered in the field; however, 10-20 carabid beetles in a barrier enclosing a 0.25 m^2 area is considered to be acceptable. Replication, with 4-5 barriers per treatment, is normally sufficient for statistical purposes.

Bioassay procedure

For each treatment, replicate barriers should be set up (2-3 m apart) within the relevant crop, which should itself be at the appropriate growth stage for the intended use of the test product. Treatments should be well separated (5-10 m) within the crop to avoid contamination with spray drift and in each test, the effects of the candidate compound should be compared with those of a proven toxic standard and with an unsprayed or water-treated control.

If invertebrates are placed inside the barriers prior to treatment, they should be given sufficient time (e.g. 1-2 h) to enable them to find refugia and assume a natural distribution before spraying. The plots should then be sprayed at the correct volume and mass application rates using suitable hand-held or tractor-mounted sprayers. Records of tank pressure, ground speed, nozzle types and flow rates should be kept, and temperatures and rainfall should be recorded throughout the assessment period. Factors such as crop growth stage, stem density, soil conditions, etc., should also be noted since these can help subsequent interpretation of data.

The duration of studies need not be standardised for all situations. However, confining beneficial arthropods to a treated area for 3-5 days will normally be sufficient to detect symptoms of any side-effects. If studies are extended beyond this, it may be necessary to provide the test organisms with supplementary food.

Assessments

When planning the timing and nature of assessments, factors such as the behaviour of the test species and the structure of the crop environment should be taken into consideration. For example, experience with large carabids such as *P. melanarius* and *Nebria brevicollis*, both of which are nocturnally active, has shown that they will often burrow into the soil during the day-time. This makes it almost impossible to recover the beetles without making a destructive assessment of the plot (i.e. by digging up the soil and plants). In this situation, a simple count of dead bodies found on the surface could be made after 24 and 48 h, and a more thorough destructive search delayed until a final assessment is made.

Assessments may be more straightforward for species that remain active during daylight, providing the crop is not too dense. The area inside each barrier should be searched for a standard time (e.g. 10-15 mins), with both live and dead (marked) insects removed using an

aspirator; the live insects can then be returned to the plots after counting. During preliminary searches, excessive disturbance of the soil surface and the crop canopy should be avoided because this will affect the distribution of available pesticide residues and the loosened soil will make it easier for insects to bury themselves. In the final assessment, plants and soil can be removed to recover as many insects as possible. Beetles and spiders should be recorded as being alive, dead or missing. Live insects may be further categorised as 'affected' or 'moribund'. Such categories need to be clearly defined in the test protocol.

When live invertebrates are removed from arenas during the final assessment, these should be returned to the laboratory and kept individually in Petri dishes, for 3 to 7 days, to permit any delayed effects to be recorded.

Healthy carabid beetles will often cannibalise dead beetles and it may not be possible to recover all of the original test insects at the end of a study. For this reason, any disembodied elytra or part-consumed bodies should be recorded and predatory insects that are not deliberately introduced should be removed from the barriers when they are seen.

Statistical analysis may be undertaken on survivorship data (the numbers and proportions of live organisms recovered). To determine the significance of any effects detected, one way ANOVA may be performed on arcsin-square root transformed percentages of original numbers recovered from the treated and control barriers.

Some examples of experimental data from small barrier studies are given below (Table 1) for a toxic standard chemical, dimethoate, applied to kale and mature winter barley crops. The analysis of these results indicated that dimethoate caused significant mortality to both species of carabid beetle tested. There were, however, varying degrees of success in terms of the retrieval of beetles (alive or dead). For *B. lampros* in particular, the numbers recovered in the dimethoate plots were initially low, although a larger proportion was detected in the final destructive assessment.

These and other data, have shown that, regardless of the care that is taken in setting up barrier studies and in thoroughly searching the soil at the end of each test, there will normally be some insects which are not recovered, typically between 5 and 30% of the test group. This percentage will vary with the species tested, the test crop, climatic conditions (losses increase in hot, dry weather) and the nature of the test chemical.

For some pesticides, soil conditions will significantly influence the effects that are detected e.g. where residues remain active for longer on sandy soils than on soils with a high organic content. However, a great deal of additional research will be required if recommendations on the choice of soil types are to be included in future guide-lines. For registration purposes, tests may be carried out on more than one soil type, representative of the area where the chemical is to be used.

Despite problems with recapture (which affect most semi-field techniques), the use of barriers is experimentally flexible, and the method has been used with a wide range of species and crop types. In addition to single-rate tests, field LD_{50}'s (expressed as multiples of field rate) can be obtained to demonstrate the margin of safety associated with existing application rates.

Table 1. Percentage recovery of carabid beetles from within-crop barriers treated with dimethoate EC (340 g a.i./200 l water/ha) or with water as a control. The percentage of insects alive in each treatment at each assessment time were compared using a one-way ANOVA (arcsine/square root-transformed data).

Time	Treatment	Alive	Missing	Dead	P {F1,8} **<0.01
B. lampros in kale at 3-4 leaf stage (June 1985)					
24 h	Control	86.7	13.3	0.0	**
	Dimethoate	10.1	69.7	20.2	
48 h	Control	75.5	23.5	1.0	**
	Dimethoate	10.0	57.0	33.0	
192 h	Control	77.6	17.3	5.1	**
	Dimethoate	0.0	43.4	56.6	
N. brevicollis in winter barley at G.S. 13 (October 1985)					
24 h	Control	82.4	17.6	0.0	**
	Dimethoate	12.0	4.8	73.6	
48 h	Control	80.8	18.4	0.8	**
	Dimethoate	8.0	0.8	91.2	
192 h	Control	78.4	17.6	4.0	**
	Dimethoate	2.4	1.6	95.2	

2.2.2 The use of large cages to confine plant-active invertebrates in treated arable crops

Cages constructed within the crop offer an alternative means of confinement for dispersive or flying invertebrates. Large cages may be used in a wide variety of crop types.

Cage design

A cubic cage construction, with sides of 2 m, is sufficiently tall to be placed over most arable crops. This type of cage is also large enough to permit assessments to be carried out by an observer within the cage, but small enough to allow the crop inside to be sprayed in a single pass using a hand-held sprayer fitted with an extended boom.

The framework can be assembled in the crop from steel scaffolding poles. This is then enclosed within purpose-made covers of coarse mesh netting. The covers used in studies at Southampton University are constructed from 'Tygan' netting (1 mm mesh size) by a commercial tent manufacturer; they have strengthened corners, flaps along the bottom edges to allow the base to be sealed by burying in the soil and zips up one side for access.

Releasing test organisms

To minimise contamination of the cage covers, they should not be in position when spraying takes place. Mobile species such as *Coccinella septempunctata* may be introduced to the crop immediately following spraying to permit access to fresh residues.

Ideally, the trial plots should contain food for the test organisms at the time of application. This will stimulate natural foraging behaviour. Where aphid-specific predators such as *C. septempunctata* are being studied, it may be necessary to provide supplementary feeding during the course of the study, partcularly if the original pest populations have been eradicated by the treatment.

An example of experimental data is given below (Table 2) from an experiment where winter wheat was treated at GS 65. The side-effects of dimethoate against *C. septempunctata* (25 per cage) were evaluated with five cages per treatment, spaced at least 2 m apart along the edge of the crop. Assessments were made after 48 h and 7 days. A pre-determined time (15 mins) was spent searching for coccinellids in each cage. The numbers recovered were, however, affected by the prevailing weather conditions. When sunny, many of the insects were active on the crop or the walls of the cage and were consequently easy to collect and count. When it was cool and overcast, the insects were often lower in the canopy or on the ground, and the numbers recovered were reduced.

Almost half of the insects were not relocated in the dimethoate treatment in particular by the end of this example study. These insects may have died as a result of the treatment but this could not be verified.

Table 2. Percentage recovery of *C. septempunctata* from within-crop cages treated with dimethoate EC (340 g a.i./200 l water/ha) or with water as a control. The percentages of test insects alive in each treatment at each assessment time were compared using one-way ANOVA (arcsine/square root-transformed data).

Time	Treatment	Alive	Missing	Dead	P {F1,8} **<0.01
48 h	Control	79.2	20.8	0.0	**
	Dimethoate	0.8	60.8	38.4	
7 d	Control	60.0	36.0	4.0	**
	Dimethoate	0.0	49.6	50.4	

Sotherton *et al.* (1988) studied the effects of dimethoate on *C. septempunctata* using smaller field cages and obtained higher recapture efficiencies than in the above example. The data from their investigation does not give as precise an estimate of risk however, because of the short period of assessments. For highly mobile invertebrates, the increased confinement to contaminated foliage in smaller cages may increase exposure and larger cages are preferable. The results of Sotherton *et al.* (1988) also give an indication of the variability in results that can occur between tests.

2.2.3 The use of 'sleeves' to confine plant-active invertebrates to treated foliage

A simple form of bioassasay cage that can be utilised in crops ranging from cereals to top fruit may be constructed from cylinders of fine-mesh, nylon netting. These 'sleeves' are pulled onto treated foliage, e.g. a branch of an apple tree or a cluster of 5-6 cereal tillers, and are then sealed at the base with plastic-coated wire. The test organisms (plus food if required) are introduced into the bag before it is sealed at the top.

This method of confinement can only be used in studies where the effects of residual exposure are being investigated, since the insects and net bags cannot be in place during spraying. It is particularly useful in studies where a measurement of the persistence of toxic side-effects is required. Such methods of confinement may enhance the level of exposure to treated foliage and results should be interpreted with caution. If there are prey items on the treated foliage (either present at spraying or provided afterwards), then the test organisms will be encouraged to forage over the sprayed crop.

The dimensions of the mesh cylinder should be adequate to enclose the foliage without crushing it (e.g. 50 cm wide x 80 cm long over apple branches). The bags are normally light enough not to require any additional support.

In bioassays at Southampton University, such cages have been used successfully to confine *C. septempunctata* on cereals and apple trees (in groups of 5-10 per bag) and *Anthocoris nemorum* (Heteroptera: Anthocoridae) on apple trees (in groups of 5 per bag). The method was less successful for larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae) when confined on apple trees (in groups of 5 per bag); this was because of high levels of cannibalism, despite the presence of alternative food.

Examples of experimental data are given in Tables 3 & 4 from a study in which *A. nemorum* was confined to the branches of small apple trees that had been treated with insecticide. The insects were enclosed in 80-cm-long nylon net sleeves, each containing five individuals. Untreated aphids were provided as food (eggs of moths such as *Ephestia* spp. are a suitable alternative) and an assessment of the numbers of anthocorids that were alive, affected, moribund or dead was made after 24 h. In this study the surviving insects were not retained after exposure to determine any latent effects. It is advisable however, that this procedure is included in test guide-lines to give a more accurate estimate of pesticide effects.

Two types of bioassay were carried out with *A. nemorum*. In the first, test insects were exposed to fresh (dry) residues on trees treated at full, half and quarter of normal field rate. The results (Table 3) indicated that some products are toxic to beneficial invertebrates, even at much reduced rates. In the second bioassay, insects were exposed to residues on trees treated at full field rate over a two-week period following treatment. The results (Table 4) indicated that the persistence of toxic effects varied considerably between pesticides.

Table 3. The percentage mortality of *A. nemorum* following 24 h confinement on branches of apple trees bearing fresh residues of EC formulations of fenitrothion, methyl parathion or cypermethrin. The products were applied at three rates, relative to their normal application rates of 55, 30 & 5 g a.i./ha, respectively. Data are corrected for control mortality using Abbott's formula.

	Application rate (as multiple of field rate)		
	0.25x	**0.5x**	**1x**
Fenitrothion	21	100	100
Methyl parathion	66	71	73
Cypermethrin	58	81	100

Table 4. The percentage mortality of *A. nemorum* after 24 h confinement on branches of apple trees treated with EC formulations of fenitrothion, methyl parathion or cypermethrin. Application rates were 55, 30 & 5 g a.i./ha respectively and the insects were confined at increasing intervals after product application over the following two weeks. Data are corrected for control mortality using Abbott's formula.

	Age of residue (days)				
	0	**1**	**4**	**7**	**14**
Fenitrothion	100	44	0	28	0
Methyl parathion	73	100	58	1	0
Cypermethrin	100	100	90	71	82

2.3 Medium-term studies to evaluate the toxicity of pesticides to confined natural populations

Barriered plots, confining natural populations have been proposed as a method of hazard analysis for registration purposes. The advantages of using barriered areas instead of open field plots are that it potentially reduces inter-plot interference and permits greater replication, thus increasing the ability of the technique to detect significant differences between test and toxic chemicals (Jepson, 1992). This method does however have substantial limitations. It only applies to epigeal invertebrates which are retrieved in the barriered area and 'trapping out' may artificially deplete control and treatment plots, reducing the period over which effects can be detected statistically.

An alternative application of this approach is as an aid to interpretation of full-scale field trials. In this case, barriered areas within plots may be used to quantify the extent of reinvasion by invertebrates from untreated areas within the same field. This technique is outlined by Jepson et.al. (1992) and may be the most appropriate use of this method.

Pullen (1990) reports the use of plastic barriers to measure the side-effects of summer-applied pyrethroids in cereals. These barriers enclosed plots of 10 m x 10 m in a winter wheat field. They were constructed from 60-cm-wide polythene sheeting ('Layflat' plastic tubing), buried to a depth of 15 cm using a modified plough. The polythene was suspended on nylon rope between large corner stakes and supported by smaller intermediate stakes. Each plot was separated from the next by 5 m. Such barriers involve a considerable investment in time and effort: this makes them unsuitable for short-term studies.

Epigeal invertebrates were sampled from the barriered plots using 6 pitfall traps in each; these were emptied every 3-6 days. The use of suction sampling methods was considered to be too disruptive in such a small area. The experiment came to an end 25 days after treatment when evidence for 'trapping out' was detected in the control plots.

In addition to trap sampling, Pullen determined the predatory capacity of beetles within each plot using *Drosophila* pupae as artificial prey. These were glued with a flour paste to cards (5 cm x 5 cm) which were placed face down and held just above the soil surface with pins. Four cards, each bearing 12 pupae were placed in each plot and these were replaced at the same time as the traps. Predation rate was determined by counting the numbers of prey removed and differences in predation potential were inferred from variation between treatments.

In this study, cypermethrin was applied to replicated plots at three rates (full, half and double of the standard 25 g a.i./ha). The results showed inconsistent trends in relation to the rates applied and suggested that, for both beetles and spiders, there was an interaction between effects on activity and survival, both of which will influence numbers of arthropods caught in pitfall traps. This type of study in which natural populations are confined in barriers may therefore raise interpretational difficulties. In particular, the confinement of the populations may artificially enhance capture rates where increases in activity result from sub-lethal effects of pesticides.

Certain questions require an answer before barrier techniques can be confidently applied to the quantification and analysis of longer-term effects on natural populations that are sampled by pitfall trapping.

2.4 Quality control for spray applications

To be of value for registration purposes, the semi-field approach should yield repeatable results for any given pesticide/invertebrate/crop combination. Beyond variation resulting from climatic conditions and soil type, the exposure of the organism should be controlled as far as possible by precision in the application and distribution of the product. This requires precise techniques and reliable methods of checking the results. Procedures which can be potentially included in bioassay design are as follows:

1. Details of the crop (e.g. growth stage and density) need to be recorded accurately and preferably in a standardised fashion. If this were possible, guidelines could include parameters for the crop conditions that would give realistic pesticide distribution and would help quantify the expected penetration of spray to the ground.

2. All application equipment (whether tractor-mounted or gas-powered, hand-held sprayers) needs to be calibrated and operated at the correct pressures and with the appropriate nozzles. Nozzle spacing and output rates should be recorded as well as operating pressures and ground speed. The nozzle type should be specified using a recognised code.

3. Spray distribution throughout the crop may be checked using simple coloured dye or fluorescent tracer techniques and compared with published information of spray distribution (Cilgi, Unal and Jepson, 1988; Cilgi and Jepson, 1992). Dead insects may also be placed in the crop at various levels to act as targets for collecting such tracer sprays.

4. Magnesium oxide-coated slides can be used to give an indication of spray drop size distribution, especially for CDA or ULV applications (Jepson et.al., 1987). For high volume sprays, these slides may only be effective at lower, sheltered positions in the crop canopy.

These techniques are all compatible with the requirements of Good Field Practices. They are necessary because it is possible to obtain inaccurate risk estimates if the crop density is not appropriate (i.e. high crop densities reduce ground contamination) or if the spray is incorrectly applied. This is especially the case with hand-held sprayers where wind gusting can, for example, divert the spray from the target area.

3. CONCLUSIONS

The questions being posed by a semi-field testing method need to be defined clearly. Some approaches are field-based equivalents of standard laboratory bioassays (tier 1 tests) and could be used to confirm safety as an alternative to laboratory tests. Thus, products which show no discernable effects may not require further testing. Tests of this type, which tend to be carried out over short periods, also have a role in the comparison of products.

To be eligible as a tier 2 test, the semi-field method must offer not only greater realism, but also an improved ability to estimate ecological risk. To enable this, organisms must be exposed to residues until a toxic endpoint is reached and they must be given access to natural refugia, which they might seek in the field. Care must therefore be taken if the distribution of the organism is excessively restricted by the test method. The opportunities for estimating hazard (i.e. the net ecological effect) from semi-field methods seem to be very limited and considerable ecological expertise is needed to interpret the effects detected in mesocosm studies which confine natural populations.

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GUIDELINE FOR THE EVALUATION OF SIDE-EFFECTS OF PLANT PROTECTION PRODUCT ON *TRICHOGRAMMA CACOECIAE*

S.A. HASSAN

Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Pest Control, Heinrichstr. 243, D-6100 Darmstadt, Federal Republic of Germany.

Parasites of the genus *Trichogramma* are world wide distributed and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops such as fruit, vegetable, cereal, corn, vine and forest. The use of *Trichogramma* in biological control has gained widespread interest in many countries. About 10 different *Trichogramma* spp. are being mass reared to control pests on corn, sugar-cane, rice, cotton, soybean, sugarbeet, vegetables, and pine in at least 13 countries.

Trichogramma cacoeciae Marchal (Hymenoptera, Trichogrammatidae) was chosen for the test but any other thelytokous *Trichogramma* species can be used.

Recognizing that no single test method would provide sufficient information to classify the side effects of pesticides on a beneficial organism, this guideline includes a sequential testing scheme that involves three laboratory and one field test method. The first laboratory test (exposure of adult *Trichogramma* to a fresh pesticide residue film on glass plates) allows products to be classified as harmless. It was shown by experience that no product classified as harmless in this test was ever found to be harmful in the field. The second laboratory test (direct spray of *Trichogramma* pupae within the host eggs) as well as the third laboratory test (persistence test - effect of aged residues on adults) provides further information on the toxicity of the product and helps to differentiate between harmful

preparations. Because field testing is time consuming and costly, the three laboratory tests may serve to classify many products as definitely harmless or harmful without having recourse to field tests. Finally, only products not classified by the laboratory tests are tested in the field, and compared with harmful and harmless reference products in order to decide on the final rating. This is further illustrated by the scheme in Fig. 1.

The tests were developed according to the standard guidelines (Hassan et al., 1985) of the working group "Pesticides and Beneficial Organisms" of the IOBC/WPRS (West Palaearctic Regional Section of the International Organization for Biological Control). An earlier version of this guideline was published by Hassan (1974, 1977, 1980), before passing through the EPPO guideline approval procedure.

Fig. 1.

I. First laboratory test: residual toxicity of products on adult *Trichogramma cacoeciae*

1. Experimental conditions

1.1 *Principle of the trial*

Trials are carried out in cages. Adults of *T. cacoeciae* (the stage of the life cycle which is most exposed to plant protection products) are exposed to residues of a product freshly applied on glass plates, and the survivors are offered, host eggs, *Sitotroga cerealella*. Both the mortality of *T. cacoeciae* and the percentage of parasitization of host eggs by *T. cacoeciae* are assessed.

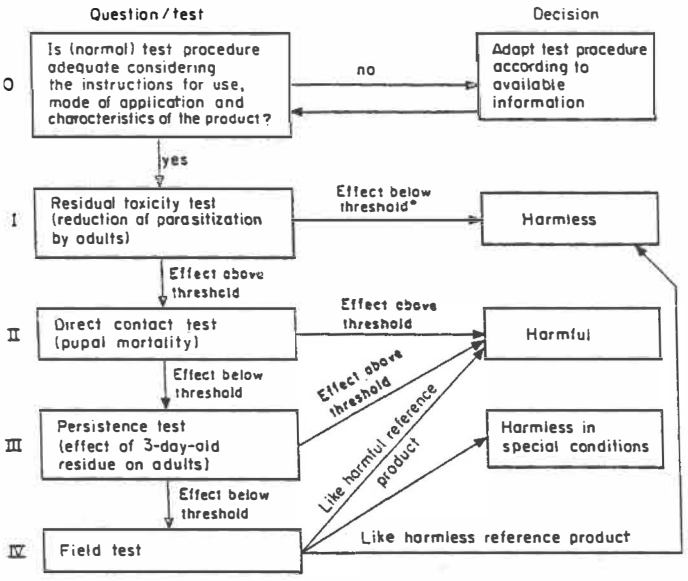


Fig. 1. Sequential testing scheme for evaluating side-effects of plant protection products on *Trichogramma cacoeciae*.

* The suggested threshold is in all cases 50%.

1.2 *Trial conditions*

The exposure cage consists of a frame and two square glass plates 13 cm long. Both glass plates are sprayed with the pesticide using a stationary laboratory sprayer, e.g. a "Potter Tower". The treated glass plates are fitted onto a square aluminium frame as floor and ceiling. The frame is 13 cm long, 1.5 cm high, 1 cm wide and is fitted with an adhesive tape with foam material (Tesamoll) to provide pads for the two glass plates. Each of three sides of the frame contains 6 ventilation holes 1 cm in diameter (Fig. 2a). The inside surface of the frame is coated with black, tight Helanca-batist material to cover the ventilation holes. The fourth side of the frame contains a slit opening in the middle (3.5 cm long and 1 cm high) that is used to introduce the host eggs on strips of paper as well as food for the parasites (honey/agar, see appendix 5). An additional hole 1 cm in diameter situated beside the slit opening is used for the introduction of the experimental parasites (Fig. 2b). Both openings are closed from the outside with black paper and adhesive tape. In order to deter the photopositive parasites from retreating to the untreated sides of the frame, the edges of the cage are darkened with a cover of black paper leaving a square area of 50 cm^2 uncovered in the centre of the glass plates. The cage is held together with two clamps. The tests are carried out in a climatic chamber under the same temperature and relative humidity conditions as for the *Trichogramma* rearing (cf. Appendix 2).

A continuous weak, diffused light is provided from above. Due to the continuous light, the parasites remain in the illuminated centre and avoid the untreated darkened sides of the cage. To prevent the possible accumulation of pesticide fumes in the cages, the air is continuously pumped out of the climatic chamber and replaced by fresh air. In addition to this, all the exposure cages are connected to sucking aquarium pumps through a tubal system. The pumps are adjusted so that the entire air in the cages is exchanged every 1 to 2 minutes.

Fig. 2a

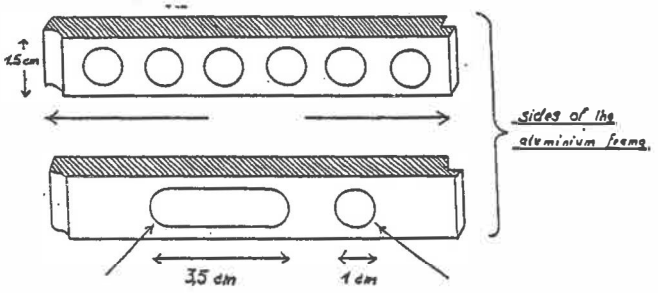


Fig. 2b

Fig. 3

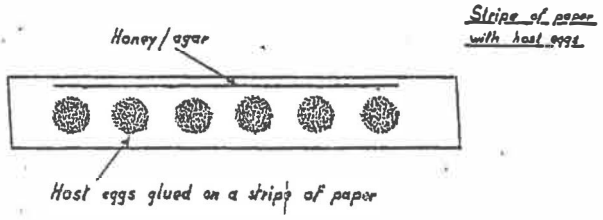
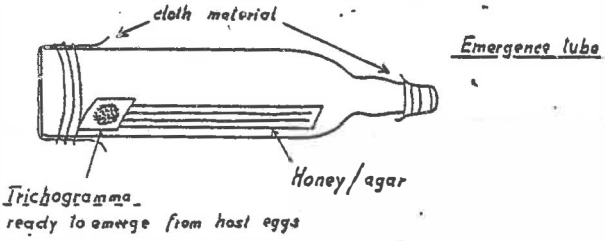


Fig. 4



1.3 *Preparation of the test arthropod*

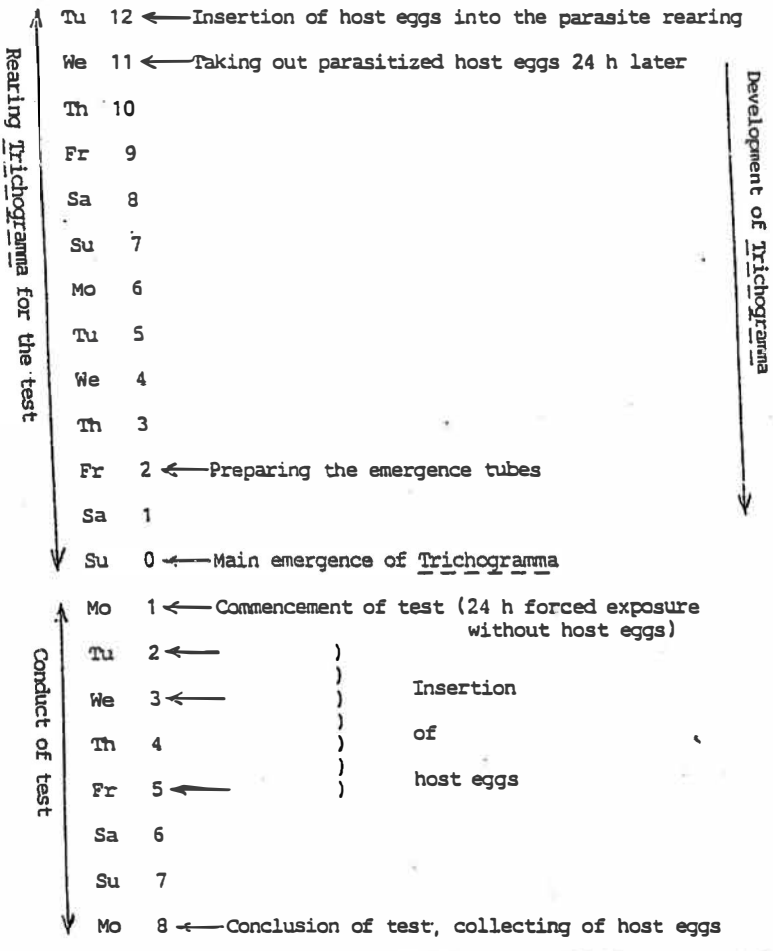
Since *T. cacoeciae* is parthenogenetic (thelytokous), only females exist. Therefore without the necessity of sexing the parasites, sexual homogeneity of test animals is assured. Parasites of uniform age are prepared for the experiments. Host eggs are parasitized by *Trichogramma* and are left until the parasites emerge. Fresh *Sitotroga*-eggs are glued with "Traganth" (see appendix 4) in circular pieces on strips of thin paper. Each disc is 1.2 cm in diameter and contains about 400 to 500 eggs. In order to obtain adult parasites of uniform age, the *Sitotroga*-egg-strips (egg-cards) are brought into the *Trichogramma* rearing units and taken out 24 h later. Parasites remaining on the egg-strips are blown away with cigarette smoke. The parisitized eggs are then kept in a climatic chamber, under the same conditions as indicated in appendix 1, to allow the parasite to develop. The parasite develops in about 12 days to adult (Table 1). Two days before emergence, the black eggs containing pupae of the parasites are kept in "emergence tubes" for the experiment (Fig. 4). Emergence tubes can be made of commercially available glass ampoules (12 cm long and 22 mm in diameter) with the tip of the pointed end cut off so that an opening of about 7 mm in diameter is left.
One disc of parasitized host eggs together with a strip of paper (6xl cm) with honey/agar is inserted in the tube and the latter is closed with white cloth material and rubber bands at both ends. The food should be concentrated and is applied in thin strips so that the *Trichogramma* do not get stuck on it. The adult parasites emerge and are left in the tube until they are 24 hours old.

1.4 *Preparation of the host*

As indicated above, *Trichogramma* is offered eggs of the host *S. cerealella* for parasitization. To facilitate handling, the eggs are glued on strips of thin white paper. The glue "Traganth" (based on the plant Tragacanth, see appendix 5) is evenly applied on paper discs using a brush and a circular plastic frame. The frame is carefully lifted and the *Sitotroga*-eggs are scattered through a fine sieve (0.5 mm mesh) on the glued areas (Fig. 3). Only fresh white yellowish (about 1 day old) and not the older reddish eggs or clusters of eggs are used for the experiment.

Table 1

Chronological test pattern



1.5. *Design and lay-out of the test*

Treatments: test product(s) and control treated with tap water.
Plot size: one cage
Replicates: at least 3.

Table 1

2. Application of chemicals/water

The product is applied, at the highest concentration registered, to the glass plates in order to ensure a standard uniform deposit (1 to 1.5 mg fluid cm^{-2}). This can be done in a laboratory spray tower. The droplets should cover about 80% of the glass surface. The glass plates of the untreated check are sprayed with tap-water. The plates are dried at room temperature for 3 h and then the cages are assembled (see 1.2). Only a square area of 10 x 10 cm in the centre of each glass plate is treated with the pesticide. To avoid the contamination of the Tesamoll pads of the exposure cage, the margin on the glass plates is left untreated by covering it with a 14 x 14 cm plastic frame with a central square area of 10 x 10 cm during spraying.

3. Conduct of trial

The *Trichogramma* adults are introduced into the exposure cage by opening the emergence tube at the pointed end, darkening it with a cover of black paper and connecting it for one hour to the cage. The *Trichogramma*, attracted by light, will walk out of the darkened emergence tube into the illuminated cage; this prevents late emerging adults from entering the cage and will keep the exposure time of the wasps on the treated surface constant. In order to calculate the number of parasites that entered the cage (see next chapter), the emergence tube is closed again and kept for another 4 days in the climatic chamber to allow the remaining *Trichogramma* to emerge.

The parasites in the test cages are supplied with honey/agar that is offered on a narrow strip of paper 10 x 0.5 cm. The test starts with a 24 h period of forced exposure without host eggs, so that any possible toxic effects of a pesticide will appear before the wasps start to parasitize. Including the forced exposure period, the experiment continues for 7 days (Tab. 1).
If total mortality occurs during the initial 24 hours of preliminary exposure the test is stopped. Otherwise about 5000 *Sitotroga*-eggs are offered per cage on the 2nd, 3rd, and 5th day of the experiment. These eggs are given on strips of paper with 6 egg-discs 1.45 cm in diameter each. Honey/agar is applied beside the eggs, 3 to 5 mm apart. Two paper strips each with 3 egg-discs are brought into each cage. The paper strips with the host eggs are inserted through the slit opening of the cage using a pair of tweezers. New egg-discs are placed over old ones. It is important that all egg-discs are brought into the illuminated part of the cage. The number of eggs offered during the test is adjusted to suit the maximum parasitization capacity of the wasps. At the end of the experiment (8th day), the cages are dismounted and the *Sitotroga*-egg-strips are kept in the climatic chamber until they are evaluated. The honey/agar on the paper strips should be removed by cutting the paper so that the egg-cards do not stick to one another.

4. Mode of assessment

The number of wasps that entered the cage is calculated by examining the emergence tube. Because some parasites emerge in the tube <u>after</u> the beginning of the experiment, the emergence tube should be examined not earlier than 4 days after the beginning of the test. All the parasitized *Sitotroga*-eggs on the 1.2. cm diameter egg-discs (black in colour) are counted and the number of adult *Trichogramma* stuck on the food strips or remaining in the tube is subtracted.

The number of host eggs parasitized during the course of the experiment can be counted on the egg-strips 8 to 9 days after the introduction of the particular egg-strip. At this time, parasitized eggs have turned black and are easily recognized. After the emergence of the *Sitotroga*-larvae, unparasitized eggs remain yellowish. If the parasitized eggs are not counted until the 11th day

after parasitization, the eggs should be heated to 70°C for about 30 minutes. This will stop the *Trichogramma* from emerging without affecting the black colouring of the parasitized eggs, after which the strips can be stored in a refrigerator for counting later.

5. Results

The number of parasitized eggs is counted for each cage. The percentage parasitism (mean per cage per egg strip per female present at the start of the test) is calculated. For each test product, the mean reduction in parasitism is calculated as a percentage of the control.
Products reducing the capacity of parasitism by less than the accepted threshold value can be classified as harmless, and need not to be tested further. However, if they have an unusual mode of action (growth regulator, specific ovicide), they should undergo an additional laboratory test measuring the specific action (the results being interpreted as above). Products reducing the capacity of parasitism by more than the accepted threshold value should pass on the next test in the scheme.

II. Second laboratory test - direct contact toxicity of products on pupae of *Trichogramma cacoecia*.

1. Experimental conditions

1.1 *Principle of the trial*

Mature pupae of *T. cacoeciae* within *Sitotroga*-eggs (the stage of the life cycle which is least exposed to plant protection products) are directly sprayed by the product. Parasitizition capacity of emerging adults is compared to those of the water treated control.

1.2 *Trial conditions*

About 1 day old *Sitotroga*-eggs are glued in discs ca. 1.2 cm in diameter (= 400-500 eggs) on strips of paper and are parasitized by *Trichogramma* for 24 hours (see I.1.3 and I.1.4). The parasitized eggs are left for 7 days to develop at: 16 h light, 28°C, 50-70% RH - 8 h darkness, 18°C, 80-90% RH.

1.3 *Design and lay-out of the trial*

Treatments: test product(s) and control treated with water.
Plot size: one egg-disc.
Replicates: three (discs).

2. Application of treatments

The treatment is carried out at the 7th day by spraying three egg-discs (black eggs containing pupae) with each pesticide to be tested. In each experiment, 3 discs are sprayed with water for control. The spraying procedure, concentration and rate of application are given under chapter I.2. The untreated control is sprayed with tapwater.

3. Conduct of trial and mode of assessment

After spraying, the treated eggs are left for 3 hours to dry, transfered to new containers and then kept to develop under the standard experimental conditions. Two days before emergence, the sprayed black eggs containing *Trichogramma* pupae are transfered into "emergence tubes" (see I.1.3). The emerging parasites, if any, are tested for their capacity to parasitize host eggs. The same experimental cage as described in I.1.2 but with untreated glass plates and the same method used in the first laboratory test (see 1.3) are used. Assessment of parasitism is carried out as given in I.4.

4. Results

Products causing a reduction of parisitism above the accepted threshold are classified as harmful and need not be tested further. Products causing mortality below the accepted threshold should pass to the next test in the scheme (persistence test).

III. Third laboratory test - persistence toxicity of products on adults of *Trichogramma cacoeciae*

1. Experimental conditions

1.1 *Principle of the trial*

To assess the duration of harmful activity, the product is applied to potted vine plants. After drying and ageing of the residue under field or field simulated conditions for a given period, adults of *T. cacoeciae* are exposed to the residue on leaves in test cages and the parasitization capacity is estimated.

1.2 *Trial conditions*

The treated plants are placed either in the field, or in a climatic chamber under field simulated summer day conditions. Experiments have shown that a close correlation exists between these two weathering conditions.

The plants kept in the field are maintained under transparant polyethylene rain cover about 50 to 100 cm high. To expose the plants to direct sun, the rain cover is removed for 3 hours once every weak.

In a climatic chamber (0.40 m^3), the following conditions are suitable for simulating field conditions: 13 h at 27°C, 65% RH - 11 h at 17°C, 95% RH and 15 h light - 9 h darkness. The light in the climatic chamber may be supplied by 7 OSRAM-L-tubes (2 White-Universal 30 W/25-2, 2 Fluora 30 W/77-2, 2 Warmtone de Luxe 30 W/32-2 and 1 Fluorescence-Black-Light 20 W/73), that provide a total of 2000 Lux at a distance of 30 cm from the lamps with a large spectrum of light wave lengths. In addition to 2 ventilators that circulate the air inside the chamber, a Helios R 10 air pump (Schwenningen, GFR) with the capacity of 95 m^3/h is suitable to renew the air. These conditions can also be simulated in a suitable greenhouse. The use of artificial weathering conditions allow to conduct experiments in all seasons of the year.

1.3 *Design and lay-out of the trial*

Treatments: test product(s) and control treated with water.
Plot size: one cage with a treated leaves.
Replicates: at least 3.

2. Application of chemicals/water

Vine plants (variety Müller-Thurgau) are grown in 15 cm diameter, 16 cm high pots under field or greenhouse conditions. The plants should be about 30-40 cm high with ca. 10 leaves. After sprouting, the plants need at least 6 weeks to reach the height of 40 cm, higher plants are cut back. Each plant is used only once for testing.
The product is applied to test plants until run off, with currently used equipment. It is tested at the highest concentration registered. After spraying, the plants are left for 3 hours in a well ventilated area to dry.

3. Conduct of trial and mode of assessment

Exposure tests are carried out 3, 10, 17, 24 and 31 days after treatment of the vine plants. Leaves with residue are picked and spread inside the exposure cage (the same cage described under I.1.2. but with untreated glass) to cover the entire lower surface. Laboratory mass reared *Trichogramma* is introduced into these exposure cages. The cages with leaves are then placed in the climatic chamber. Effects on parasitization capacity is assessed in the same way as in the first laboratory trial (see I.4.).

4. Results

The number of parasitized eggs is counted for each cage. The percentage parasitism (mean per cage per female) is calculated. For each test product, the mean reduction in parasitism is calculated as a percentage of the control.

The reduction in parasitisation capacity calculated for each exposure test at the different time-intervals after application, is plotted on a probit scale against time. The "duration of harmful activity" 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. If 15-day old residues on leaves cause mortality above the accepted threshold in *T. cacoeciae* adults, the product is classified as harmful. The period during which a product remains harmful may be mentioned on the (proposed) label, valuable information. Products causing mortality below the accepted threshold should pass on to the field test.

IV. Field experiments to test the side-effects of products on *Trichogramma cacoeciae*

All products not classified according to the earlier tests should be subjected to a field test.

1. Experimental conditions

1.1 *Selection of crop and cultivar, test organisms*

Trials can be carried out on any fruit (i.e. apple, prune) or field crop (i.e. wheat). Any cultivar may be used. Test organism: Any relevant *Trichogramma* sp. can be used.

1.2 *Trial conditions*

The experiments are carried out in fruit orchards or in fields that has not recently been treated with any plant protection product. *Trichogramma* is released in the field by distributing cardboard boxes that contain parasitized *Sitotroga* eggs, shortly before emergence. Growth and cultural conditions, temperature and humidity should be homogeneous for all plots.

1.3 *Design and lay-out of the trial*

Treatment: test product(s), reference product(s) and water treated control, arranged where possible in a randomized design. Plot size (net): at least 3 large apple trees, the equivalent of pillar trees or 100 m2 of a field crop. The distance between the plots should be at least 10 m. Replicates: at least 3.

2. Application of treatments

2.1 *Test product(s)*

The named formulated product under investigation.

2.2 *Reference product(s)*

Use products registered for use on fruit or field crops and which have proved satisfactory in practice. At least one should be recognized to be harmless and another recognized to be harmful to *T. cacoeciae*. In general, formulation type and mode of action should be close to those of the test product.

2.3 *Mode of application*

Applications should comply with good agricultural practice.

2.3.1 *Type of application*

According to the instructions of the (proposed) label. Normally a spray.

2.3.2 *Type of equipment used*

Application with currently used equipment which should provide an even distribution of product on the whole plot. Factors which may affect action on *T. cacoeciae* (such as operating pressure, nozzle type) should be recorded together with any deviations in dosage of more than 10%. Special attention should be paid to avoiding drift.

2.3.3 *Time of application*

The treatment should be carried out one day after the main wave of *Trichogramma* has emerged.

2.3.4 *Doses and volumes used*

The product should normally be applied at the highest dosage recommended on the (proposed) label for use in the relevant crop. This will normally be expressed in kg (or litre) of formulated products per ha. It may also be useful to record the dose in g of active ingredient per ha. For sprays, data on concentration (%) and volume (liter ha^{-1}) should also be given.

2.3.5 *Data on chemicals used against other pests*

Interference with other chemicals should be avoided.

3. Mode of assessment, recording and measurements

3.1 *Meteorological data*

Record temperature and humidity during the whole trial period.

3.2 *Type, time and frequency of assessment*

3.1.2 *Type*

Assessment is carried out by randomly distributing 60 baiting cards (Sitotroga eggs glued on strips of green paper) in each plot. The egg cards, 2 x 2 cm large, are fastened to the under surface of the leaves using clamps. After 1 or 2 days the cards are collected and taken to the laboratory. They are kept at 25°C until they are examined. The number of parasitized host eggs (which have turned black) are counted at least 5 days after the exposure in the field. The reduction in parasitism, as compared with control (treated with water) is used to measure the effect of the chemical.

3.2.2 *Time and frequency*

The first baiting cards are distributed 24 hours after the application of the plant protection product and are changed at least three times at an interval of 1 to 2 days.

3.2.3 *Disturbance by weather and by predators in the field*

Rain reduces the concentration of the product and can damage the baiting cards. The experiments should be stopped if it rains during the first two days of the experiment. Cold weather reduces the activity of *Trichogramma* in the plots resulting in lower parasitism, making assessment difficult. If so, monitoring could be extended for a second week. Predators may feed on the baiting cards and disturb assessment. These losses can be met by increasing the number of baiting cards and changing them at shorter intervals.

3.3 *Direct effects on the crop*

Direct effects on the crop should already have been evaluated in the trials on product efficacy (see relevant EPPO guidelines), but any particular effects observed should be recorded.

4. Results

Effects on *T. cacoeciae* comparable to those caused by the harmful reference product are classified as harmful; effects consistently comparable to those caused by the harmless reference product are classified as harmless. Intermediate results are classified as harmless in special conditions (end of sequential testing scheme).

V. Presentation of results

At all stages in the scheme, the results should be reported in a systematic form and the report should include an analysis and evaluation. Original (raw) data should be available. Statistical analysis should be used, where appropriate, by methods which should be indicated.

Appendix 1. *Biology of the test arthropod (Trichogramma cacoeciae)*

The adult females attack and parasitize Lepidoptera eggs. The parasite eggs (ca. 0.1 mm in length) are inserted into the host and the hatching larvea feed in the vitelline mass of embryo of the host. There are three larval instars, all sacciform. These are followed by a prepupa, when the adult characters form, and a pupa. At the beginning of the third larval instar, the host eggs turn black due to the deposition of black granules at the inner surface of the chorion.

In the experiments, eggs of the angoumois grain moth (*Sitotroga cerealella*) are used as a host. At the laboratory conditions (16 h light, 28°C, 50-70% relative humidity - 8 h darkness, 18°C, 80-90% relative humidity) the longevity of the adults is about 14 days, the development time is about 13 days and egg production is about 30 per female.

Appendix 2. *Rearing of the test arthropod Trichogramma cacoeciae*

Only healthy *Trichogramma* should be used for test, their mortality rate should not exceed 10% during development. The fertility should remain constantly high; this can be constantly checked in the untreated units of the test experiments.

In order to promptly discover any genetic changes that might occur in the continued laboratory rearing of the parasite, a comparison with field insects should regularly be carried out.

It is advantageous to rear *Trichogramma* in the laboratory under variable conditions) i.e. 16 h light, 28°C, 50-70% RH - 8 h darkness, 18°C, 80-90% RH). These field simulated conditions were shown to be more favourable than constant conditions. The parasites can be reared in cylindrical glass cages (about 25 cm long and 10 cm in diameter). Food (see appendix) and host eggs are replaced three times per week. 1/3 of the parasitized host eggs should be returned in each cage to allow a continuous rearing.

Appendix 3. *Rearing of the host, the angoumois grain moth (Sitotroga cerealella)*

Insecticide-free wheat is mixed with water and heated in a cabinet at 70°C for about 6 hours. This procedure helps to disinfect and soften the grains. The wheat is spread on trays, infested with *Sitotroga*-eggs and placed in an incubator for about three weeks at 27 ± 2°C and 70 ± 10% RH to allow the *Sitotroga* larvae to invade the grains, develop and pupate. The trays with the infested grains are placed in cages and the emerging moths are collected.

The eggs are collected by placing the adults in smaller cages that contain stainless steel screen. No water or food is given to the adult moths. For further informations see HASSAN (1981).

Appendix 5. *Preparing the glue (Traganth)*

The fine powdered glue "Traganth", a product of the company Merck, DAB 7, article No. 8405 which is completely harmless to *Trichogramma* is used. A half tea spoon of "Traganth" powder is stirred in distilled water to make 50 ml of the mixture. 24 hours later, the mixture will become white in colour and free of clumps. The glue is then ready for use and should be kept in a glass with a wide opening in the refrigerator at about 5 to 7°C. New glue should be made at least every 3 weeks.

Appendix 6. *Preparing the honey/agar food*

120 g honey and 50 cm^3 agar solution are needed for preparing the food. To avoid fungus growth in the food, 0.13 mg Nipagin M is dissolved in 100 ml distilled water. 2 g agar powder are added to the Nipagin solution and heated in a boiling water bath until fully dissolved. 50 ml of this solution are added to the above noted amount of honey and the mixture is heated until it is homogenous. The food is applied on strips of paper using an injection syringe. The filled syringe is kept in a container with the needle being continuously immersed in water, this will prevent the food from hardening and blocking the needle.

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RICHTLINIE ZUR ERMITTLUNG DER NEBENWIRKUNGEN VON PFLANZENSCHUTZMITTELN AUF DIE SCHLUPFWESPE *COCCYGOMIMUS TURIONELLAE*

Hermann Bogenschütz

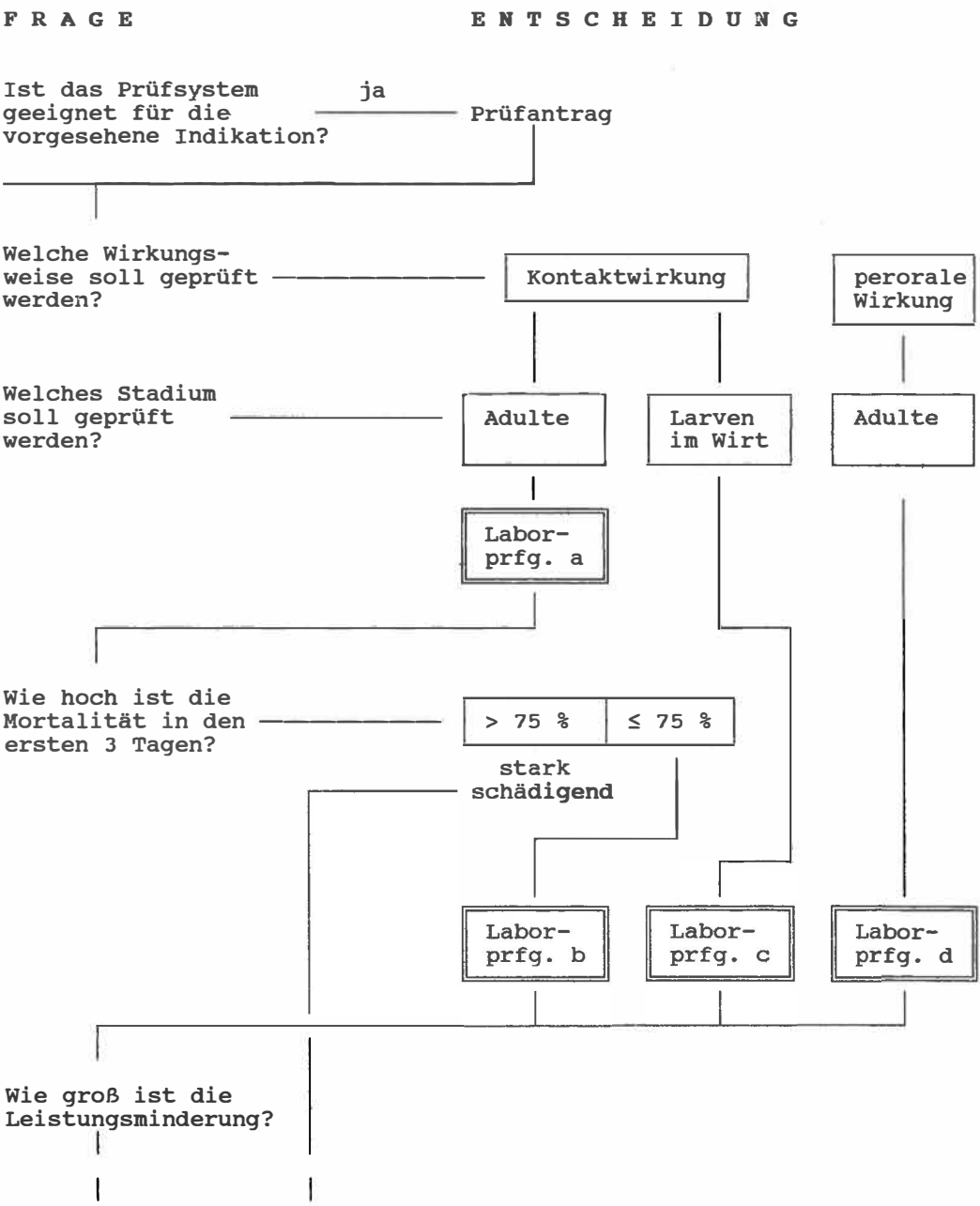
Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Postfach 708, D-7800 Freiburg

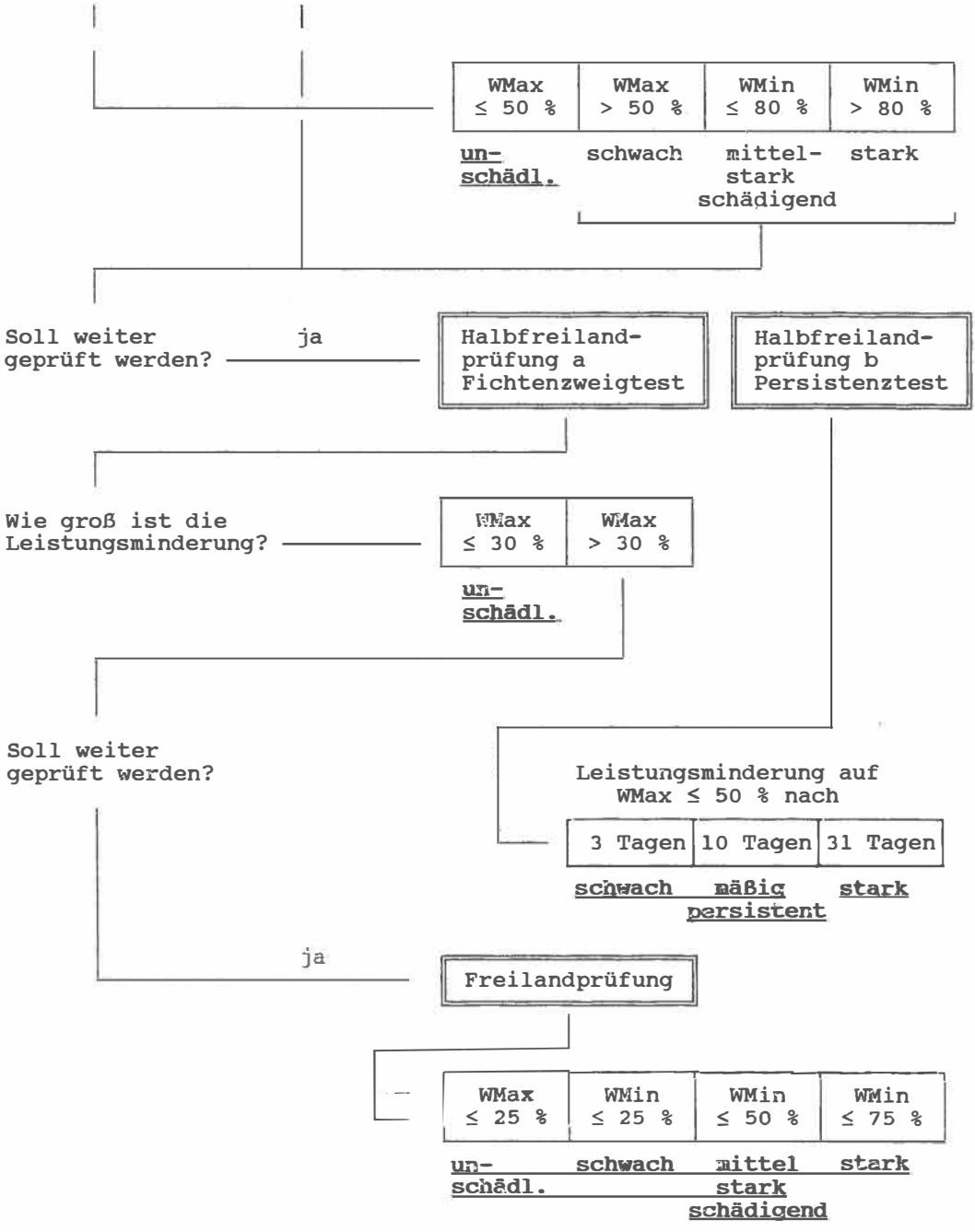
Die Kenntnis des Einflusses von Pflanzenschutzmittel-Einsätzen auf Nicht-Zielorganismem, insbesondere auf Nützlinge, gewinnt sowohl im Bereich der Zulassung als auch im Rahmen des integrierten Pflanzenschutzes immer mehr an Bedeutung. Um diese Kenntnis zu erhalten, werden Untersuchungen mit Arten durchgeführt, die bestimmte ökologische Gruppen repräsentieren und in land- bzw. forstwirtschaftlichen Kulturen, in der die Zielart vorkommt, eine beachtliche ökologische und möglichst auch ökonomisch sich auswirkende Rolle spielen. Ein solchermaßen ausgewählter Bioindikator sollte leicht züchtbar sein und das ganze Jahr über zur Verfügung stehen, um standardisierte Prüfungen jederzeit und mit akzeptablem finanziellem Aufwand durchführen zu können.

Der polyphage Puppen-Endoparasitoid *Coccygomimus turionellae* (L.) (Hymenoptera, Ichneumonidae) ist ein typischer Vertreter "großer Schlupfwespen", die in Wäldern und vielen landwirtschaftlichen Kulturen zur Dezimierung wichtiger Schadlepidopteren beitragen, und stellt somit ein repräsentatives Nutzinsekt für Kulturen im Gemüse-, Obst- und Weinbau sowie für den Forst dar (BRASSE 1990).

Pflanzenschutzmittel können durch direkten Kontakt, über die Luft und durch Aufnahme mit der Nahrung wirksam werden. Von größter Bedeutung ist die Kontaktwirkung, die deshalb vorrangig untersucht wird. Nicht völlig auszuschließen ist die Wirkung über die Gasphase, die jedoch bei den gängigen Versuchsanstellungen durch Zwangsventilation weitgehend reduziert wird. Die Ermittlung der Fraßgiftwirkung erfordert spezielle Untersuchungen, da bei der Prüfung der Kontaktwirkung nicht-kontaminiertes Futter angeboten wird.

Aus Kostengründen ist es erforderlich, vor Beginn der Prüfung eines Mittels die Geeignetheit des Prüfsystems festzustellen. Danach folgt der Einsatz verschiedener Prüfverfahren in festgelegter Reihenfolge nach folgendem Schema, in dem das endgültige Urteil durch **Fettdruck** hervorgehoben ist:


F R A G E
E N T S C H E I D U N G
Ist das Prüfsystem geeignet für die vorgesehene Indikation?
ja
Prüfantrag
Welche Wirkungsweise soll geprüft werden?
Kontaktwirkung
perorale Wirkung
Welches Stadium soll geprüft werden?
Adulte
Larven im Wirt
Adulte
Labor-prfg. a
Wie hoch ist die Mortalität in den ersten 3 Tagen?
> 75 %
≤ 75 %
stark schädigend
Labor-prfg. b
Labor-prfg. c
Labor-prfg. d
Wie groß ist die Leistungsminderung?


WMax ≤ 50 %
WMax > 50 %
WMin ≤ 80 %
WMin > 80 %
unschädl.
schwach
mittelstark schädigend
stark
Soll weiter geprüft werden?
ja
Halbfreilandprüfung a Fichtenzweigtest
Halbfreilandprüfung b Persistenztest
Wie groß ist die Leistungsminderung?
WMax ≤ 30 %
WMax > 30 %
unschädl.
Soll weiter geprüft werden?
Leistungsminderung auf WMax ≤ 50 % nach
3 Tagen
10 Tagen
31 Tagen
schwach persistent
mäßig
stark
ja
Freilandprüfung
WMax ≤ 25 %
WMin ≤ 25 %
WMin ≤ 50 %
WMin ≤ 75 %
unschädl.
schwach
mittel stark schädigend
stark

Die Prüfverfahren wurden Anfang der 70er Jahre entwickelt, seither in vielen Punkten verbessert und mehrfach in Veröffentlichungen dargestellt (ALBERT und BOGENSCHÜTZ 1986, BOGENSCHÜTZ 1975, 1984, 1988, BOGENSCHÜTZ et al. 1986). Einige Verfahren werden in der vorliegenden Arbeit zum ersten Mal beschrieben, nicht alle sind bereits mit einer ausreichenden Anzahl Prüfungen erprobt.

Die Methoden der Zucht von *C. turionellae* und seines Wirtes *Galleria mellonella* (Große Wachsmotte) wurden zuletzt 1989 veröffentlicht (SAMSØE-PETERSEN et al. 1989).

Am 20.6.1991 wurde vom Ministerium für Umwelt Baden-Württemberg die GLP-Bescheinigung "Prüfkategorie ökotoxikologischer Eigenschaften: Auswirkungen von Pflanzenschutzmitteln auf *Coccygomimus turionellae* (L.) im Laboratorium nach BBA-Richtlinie Teil IV, 23-2.1.3 in der geltenden, neuesten Fassung" ausgestellt.

Die Prüfungen werden in Versuchsreihen in Käfigen durchgeführt. Jede Versuchsreihe besteht aus folgenden Gliedern (in der Regel mit 6 Käfigen):

- mit Leitungswasser behandelt (0-Kontrolle)
- mit Prüfmittel behandelt
- mit Vergeichsmittel (Standardchemikalie) behandelt (nicht bei jeder Prüfung erforderlich)

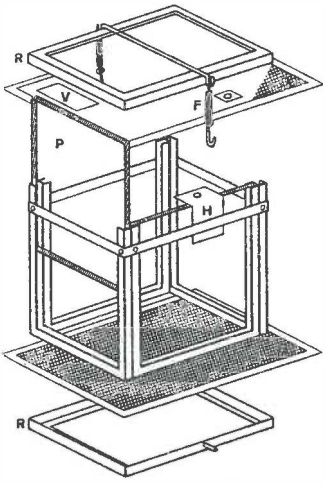
In jeden Käfig werden, falls nicht anders erwähnt, 5 Weibchen eingesetzt. Insgesamt wird folglich die Reaktion von mindestens 30 Weibchen ermittelt. Die Prüfung muß in der Zeit der größten Legeaktivität (zwischen dem 15. und 30. Lebenstag der Imagines) erfolgen.

Bei den Laborprüfungen bestehen die Käfige aus vier quadratischen Glasscheiben (P) von 16 cm Kantenlänge, die in ein Gestell aus Leichtmetallschienen eingeschoben werden, und die Seitenwände bilden (Abb. 1a). Über die offene Boden- und Deckenfläche wird Glasfasergewebe (G) gespannt, das durch Leichtmetallrahmen (R) mit Schraubenfedern (F) festgehalten wird, und eine gute Durchlüftung der Käfige ermöglicht. Durch die Deckenfläche ragt eine an einem Träger (H) befestigte Tränke mit Leitungswasser in den Käfig. Im Glasfasergewebe der Deckenfläche befindet sich ein Schlitz, durch den die Versuchstiere in den Käfig eingesetzt werden, und der danach mit einem Stück Klebeband (V) verschlossen wird. Auf der Bodenfläche liegt ein Kartonstreifen mit Futter-Agar-Tropfen. Die Wirtspuppen werden in einer mit Wellpappe ausgelegten Petrischale unter der Bodenfläche angeboten. Das Glasfasergewebe behindert die Weibchen beim Stechen nicht.

Die Käfige für Halbfreilandprüfungen (Abb. 1b) entsprechen den Laborprüfkäfigen. Anstelle des Gestells mit den Glasplatten wird jedoch ein nur 5 cm hoher Rahmen aus Aluminium verwendet. Auf diese Weise wird der Bewegungsraum für die Versuchstiere eingeschränkt und somit der Kontakt mit den behandelten Kulturpflanzen erhöht.

Um eine konstante und reproduzierbare Durchlüftung zu gewährleisten, werden die Prüfkäfige auf die Drahtgitterfläche eines Entlüftungskasten gestellt. Die Umdrehungszahl seines Ventilators ist so zu wählen, daß etwa alle 2 Minuten ein völliger Luftaustausch in den Prüfkäfigen erfolgt. Diese werden täglich nach einem festgelegten System umgestellt, so daß ein Einfluß von möglicherweise auftretenden Differenzen in der Geschwindigkeit des Luftstromes in den Prüfkäfigen eliminiert wird.

a



b

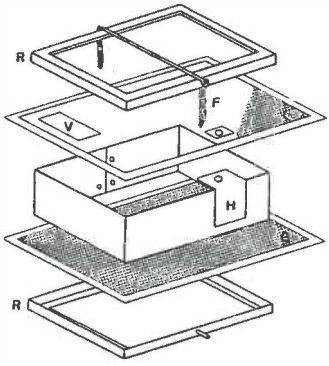


Abb. 1: Prüfkäfig für Glasplatten- (a) und Persistenz test (b)

Alle Labor- und Halbfreilandprüfungen werden unter den gleichbleibenden Bedingungen in Klimakammern durchlaufen: 15 Stunden Tag bei 23°C; 7 Stunden Nacht bei 15°C; 2 x 1 Stunde Dämmerung, während der sich Licht und Temperatur linear ändern; relative Luftfeuchtigkeit konstant 65%.

Der Käfig für die Freilandprüfungen wird in Kap. VII. bei den Versuchsbedingungen beschrieben.

Zur Festlegung der Bewertungsklassen sind weitere Diskussionen in der IOBC/WPRS Arbeitsgruppe "Pflanzenschutzmittel und Nutzorganismen" erforderlich.

I. Laborprüfung a (Mortalitätstest):

Wirkung des Kontakts mit frisch ausgebrachten Pflanzenschutzmittelbelägen auf Glas auf die Lebensdauer der Imagines.

Zweck der Prüfung
Unter standardisierten Laborbedingungen soll die toxische Wirkung von frisch angetrockneten Pflanzenschutzmittelbelägen auf Glas auf die Lebensdauer adulter Weibchen bei Dauerkontakt ermittelt werden.

Versuchsbedingungen
Je zwischen 18 und 24 Tage alte Weibchen werden in behandelte Prüfkäfige (Abb. 1a) eingesetzt und diese auf einen Entlüftungskasten gestellt. Die Prüfdauer beträgt 3 Tage.

Behandlung
Die zu prüfenden Pflanzenschutzmittel werden in der festgelegten Konzentration mit einer Laborspritzanlage (Laufkatzenprinzip; Hersteller: Norddeutsche Laborbau G.m.b.H., Kaltenkirchen) auf Glasscheiben und Glasfasergewebe ausgebracht. Die O-Kontrolle wird mit Leitungswasser behandelt. Die Aufwandmenge beträgt 1,5 g/cm² und wird durch Änderung der Laufgeschwindigkeit oder des Spritzdrucks reguliert. Die Düse ist so zu wählen, daß der mittlere Volumendurchmesser des Tröpfchenspektrums etwa 200 µm beträgt. Nach dem Antrocknen des Belages werden die Käfige zusammengebaut und frühestens 2 Stunden nach der Applikation die Versuchstiere eingesetzt.

Ergebnisse
Die Anzahl der toten Weibchen wird täglich gezählt und nach Ende des Versuches die Gesamtmortalität (M) berechnet.

M = Anz. überlebender Weibchen/Anz. aller Weibchen x 100

Der Versuch wird nur dann gewertet, wenn in der O-Kontrolle kein Weibchen stirbt.

Bewertung
Beträgt die Mortalität über 75 %, ist das Pflanzenschutzmittel stark schädigend (Weiterprüfung: Halbfreilandprüfung). Bei allen anderen Mitteln folgt II. Laborprüfung b.

II. Laborprüfung b (Glasplattentest):

Wirkung des Kontaks mit frisch ausgebrachten Pflanzenschutzmittelbelägen auf Glas auf die Eilegeleistung der Weibchen.

Zweck der Prüfung
Unter standardisierten Laborbedingungen soll die toxische Wirkung von frisch angetrockneten Pflanzenschutzmittelbelägen auf Glas auf die Natalität adulter Weibchen bei Dauerkontakt ermittelt werden.

Versuchsbedingungen
Die Abhängigkeit der Eilegeleistung vom täglichen Wirtsangebot fordert die strenge Einhaltung des folgenden Versuchsablaufs in drei Phasen (Abb. 2):

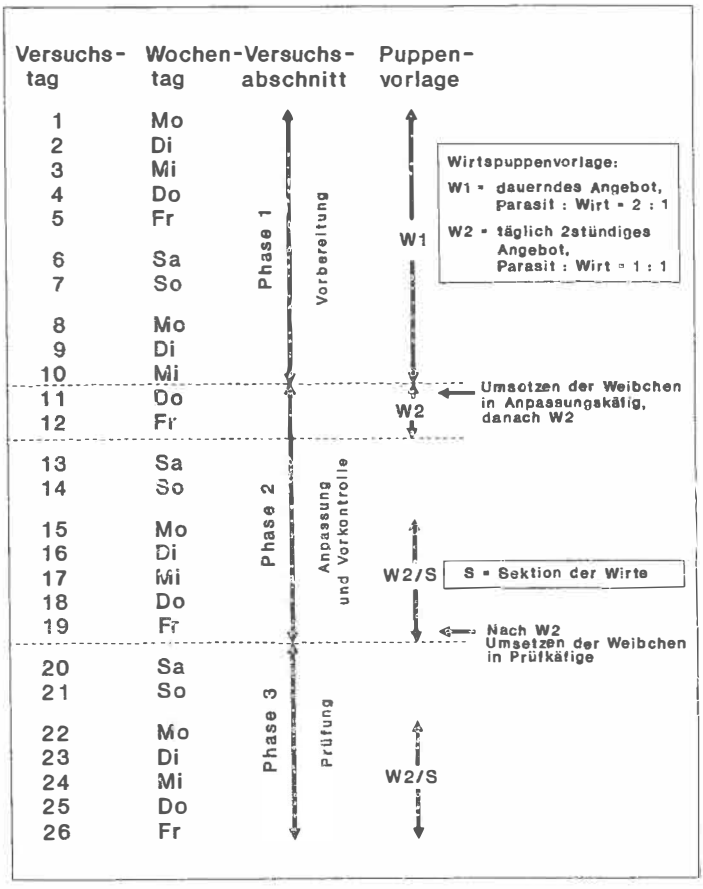


Abb. 2: Versuchsablauf

In der 1. Phase befinden sich je Versuchsvariante 40, über das Wochenende geschlüpfte Weibchen in 30 cm x 30 cm x 60 cm großen Zuchtkäfigen und erhalten ein täglich zu erneuerndes Wirtsangebot, damit die Stech- und Legeaktivität in Gang kommt.

Zu Beginn der **2. Phase** (Anpassungsphase) werden die Versuchstiere auf die Versuchsglieder verteilt. Zur Gewöhnung an die Versuchsbedingungen werden sie zu je 5 in 30 cm x 30 cm x 30 cm großen Käfigen gehalten, deren Holzboden einen Ausschnitt von 9 cm x 9 cm Größe besitzt. Diese Öffnung ist mit Glasfasergewebe verschlossen, unter dem täglich 5 Wirtspuppen auf einer Petrischale 2 Stunden lang vorgelegt werden.

Während der **3. Phase**, der eigentlichen Prüfung, befinden sich die Versuchstiere in den Prüfkäfigen (Abb. 1a), die bis zum Versuchsende nicht mehr geöffnet werden. Die Wirtsvorlage erfolgt wie in der 2. Phase.

Behandlung
Wie bei I. Laborprüfung a.

Ergebnisse
Der Parameter für die zu ermittelnde Leistung ist die Anzahl der täglich von den 5 Weibchen eines Käfigs abgelegten Eier. Ein Versuch wird nicht gewertet, wenn in den Kontrollkäfigen während der 2. und 3. Phase mehr als ein Weibchen stirbt.

Da unter den Versuchsbedingungen die Weibchen von *C. turionellae* nicht zwischen bereits parasitierten und nichtparasitierten Wirten unterscheiden können, d.h. Superparasitierungen der Wirte die Regel sind, müssen die Puppen vom 15. bis 19. Tag und 22. bis 26. Tag bei 6- bis 10-facher Vergrößerung unter dem Stereomikroskop geöffnet und die Anzahl der abgelegten Eier ausgezählt werden.

Können aus arbeitstechnischen Gründen nicht alle Wirte am Tag der Parasitierung bearbeitet werden, sind sie nach Käfigen getrennt in kleinen, mit Wasser gefüllten Dosen (z.B. Salbentöpfchen) bis zur Sektion tiefgekühlt zu lagern.

Im Rahmen umfangreicher Voruntersuchungen wurde festgestellt, daß große Unterschiede in der Anzahl abgelegter Eier bereits im unbehandelten Zustand bestehen und das Umsetzen der Weibchen von den Käfigen der Anpassungsphase in die Prüfkäfige der 3. Phase eine Leistungsänderung bewirken kann (Umsetzungseffekt U).

Wegen der Leistungsunterschiede zwischen den Käfigen wird zur Berechnung des Wirkungsgrades ein statistisches Verfahren gewählt, in dem die durch die Behandlung bewirkte Änderung der Eizahl in jedem Käfig individuell bestimmt wird. Der Umsetzungseffekt wird aufgrund der Ergebnisse in den Käfigen der 0-Kontrolle wie folgt berechnet:

$$U = \sum_{j=1}^{NK} NEK3_j \Big/ \sum_{j=1}^{NK} NEK2_j,$$

wobei

NK — Anzahl der Kontrollkäfige

$NEK2_j$ — Anzahl abgelegter Eier im j-ten Käfig der 0-Kontrolle in der 2. Versuchsphase

$NEK3_j$ Anzahl abgelegter Eier im j-ten Käfig der 0-Kontrolle in der 3. Versuchsphase

Entsprechend sei

$NEP2_j$ Anzahl abgelegter Eier im j-ten zu behandelnden Käfig in der 2. Versuchsphase

$NEP3_j$ Anzahl abgelegter Eier im j-ten behandelten Käfig in der 3. Versuchsphase.

Die um den Umsetzungseffekt bereinigte Eizahl - d.h. diejenige Eizahl, die in der 3. Phase allein aufgrund des Umsetzungseffektes zu erwarten wäre, wenn kein Pflanzenschutzmittel zugefügt worden wäre bzw. dieses keinen Einfluß hätte - wird als $NEP3Kor_j$ bezeichnet:

$$NEP3Kor_j = U \times NEP2_j$$

Der Einfluß des Pflanzenschutzmittels wird als Wirkungsgrad (WG) berechnet:

$$WG_j = 100 \times (1 - NEP3_j/NEP3Kor_j)$$

Die durch das Experiment ermittelten Wirkungsgrade unterliegen Schwankungen, die von unberücksichten Störfaktoren bzw. von Meßfehlern herrühren. Sie werden daher modellmäßig als Realisierungen von Zufallsgrößen aufgefaßt.

Die Wirkung eines Mittels wird durch das Zentrum (Median) der Verteilung der Wirkungsgrade charakterisiert. Sie wird als Untergrenze (WMin) bzw. Obergrenze (WMax) eines einseitigen Vertrauensbereiches für den Median mit zugelassener Fehlerwahrscheinlichkeit 1. Art (FW 1) von 0,1 (10%) definiert, d.h. das Zentrum der Verteilung liegt mit einer Sicherheit von mindestens 90% oberhalb bzw. unterhalb des jeweilig zu berücksichtigenden Grenzwertes WMin bzw. WMax.

Die im Rahmen von Voruntersuchungen durchgeführten Analysen ergaben keine Hinweise auf Abhängigkeit oder Unsymmetrie in der Verteilung der Wirkungsgrade. Unter der Annahme, die Wirkungsgrade sind Realisierungen unabhängiger und stetig verteilter Zufallsgrößen mit der gleichen bezüglich des Medians symmetrischen Verteilung (in diesem Fall stimmen Median und Mittelwert der Verteilung überein), wird WMin bzw. WMax aus den NP(NP+1)/2 Durchschnitten:

$$(WG_j + WG_k)/2,\ k \leq j,\ j=1,2,\ldots NP$$

NP Anzahl der Käfige (in der Regel 6)

mit Hilfe des Quantils des WILCOXON-Vorzeichenrangtests bei zulässiger FW 1 von 10% und einem Stichprobenumfang NP [QWVRT(0,1,NP)] ermittelt.

Werden die Durchschnitte nach zunehmender Größe geordnet, entspricht

WMin dem Wert, der an der Stelle QWVRT(0,1,NP)+1 steht, WMax demjenigen an der Stelle NP(NP+1)/2 - QWVRT(0,1,NP) (vergl. LIENERT 1978, Seite 90 ff.).

Die Werte für QWVRT betragen bei 6 Prüfeinheiten

QWVRT(0,1, 6) = 3,

d.h. WMin liegt an der Stelle 4, WMax an der Stelle 18.

Bewertung
Die Bewertung der Ergebnisse erfolgt nach Tab. 1.

Tab. 1. Bewertung der Prüfungsergebnisse des Glasplattentests

Wertziffer	Grenzwerte der Leistungsminderung	Bewertungsklasse
1	WMax ≤ 50 %	**unschädlich**
2	WMax > 50 %	schwach schädigend
3	WMin ≤ 80 %	mittelstark schädigend
4	WMin > 80 %	stark schädigend

Unschädliche Pflanzenschutzmittel werden nicht weiter geprüft. Bei den anderen Mitteln folgt die Halbfreilandprüfung.

III. Laborprüfung c (Puppentest):

Wirkung auf Wirtspuppen ausgebrachter Pflanzenschutzmittelbeläge auf die Entwicklung des Parasitoiden.

Zweck der Prüfung
Unter standardisierten Laborbedingungen soll die toxische Wirkung von Pflanzenschutzmitteln auf die Schlüpfrate ermittelt werden, wenn diese direkt auf parasitierte Wirtspuppen ausgebracht werden.

Versuchsbedingungen
Je 50 Wirtspuppen, die vor 7 bzw. 14 Tagen parasitiert worden waren, werden auf Wellpappe ausgelegt und behandelt. Nach dem Antrocknen des Belages werden die Puppen auf frische Wellpappe in Petrischalen übertragen und in Prüfkäfigen bis 3 Wochen nach der Behandlung gehalten.

Behandlung
Wie bei I. Laborprüfung a.

Ergebnisse
Schlüpfdatum und Anzahl der Imagines (I) werden registriert und die Schlüpfrate (SR) berechnet.

$$SR = I/50$$

Der Wirkungsgrad (WG) eines Pflanzenschutzmittels beträgt

$$WG = (SR_o - SR_b)/SR_o$$

wobei SR_o die Schlüpfrate der 0-Kontrolle
SR_b die Schlüpfrate der behandelten Puppen
bedeutet.

Nach Versuchsende werden die noch geschlossenen Puppen unter dem Stereomikroskop geöffnet, um festzustellen, ob und in welchem Stadium der Parasit einging.

Bewertung
Grenzen für die Wertziffern 1 bis 4 wurden noch nicht festgelegt.

IV. Laborprüfung d (Nahrungstest):

Wirkung mit der Nahrung aufgenommener Pflanzenschutzmittel auf die Eilegeleistung der Weibchen.

Zweck der Prüfung
Unter standardisierten Laborbedingungen soll die toxische Wirkung von peroral aufgenommenen Pflanzenschutzmitteln auf die Natalität adulter Weibchen ermittelt werden.

Versuchsbedingungen
Die Versuchsbedingungen entsprechen denen von II. Laborprüfung b. In der 3. Phase werden die Versuchstiere jedoch nicht in behandelte Prüfkäfige eingesetzt, sondern es wird ihnen in unbehandelten Käfigen behandeltes Futter angeboten.

Behandlung
Das übliche Agargemisch wird in die Bohrungen eines Futterträgers (Abb. 3) getropft und dieser wie bei I. Laborprüfung a behandelt. Um den Kontakt der Versuchstiere mit Pflanzenschutzmittelbelägen möglichst gering zu halten, wird vor der Behandlung der Futterträger mit einer Schablone so abgedeckt, daß ausschließlich die Futtertropfen von der Spritzflüssigkeit getroffen werden. Die Schablone wird mit einem Klebestreifen am Futterträger fixiert und sofort nach der Behandlung wieder entfernt. Nach dem Antrocknen der Beläge werden die Futterträger in die vorbereiteten Prüfkäfige eingebracht.

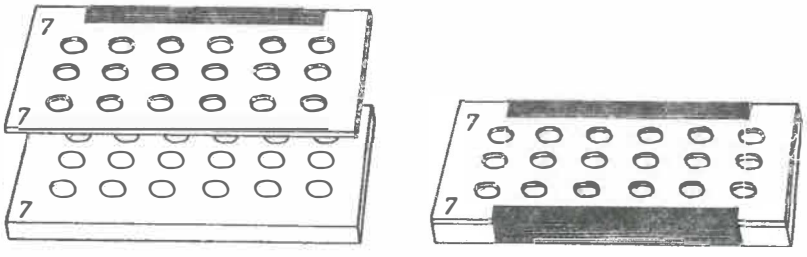


Abb. 3: Futterträger

Ergebnisse
Wie bei II. Laborprüfung b wird die Anzahl der täglich abgelegten Eier ermittelt.

Um sicherzustellen, daß nicht Repellenz die Nahrungsaufnahme verhindert, wird während der Prüfung 2x täglich 10 Minuten lang außerhalb der Wirtsvorlagezeit beobachtet, ob und in welchem Käfig Weibchen an den Tropfen fressen.

Die Berechnung des Wirkungsgrades erfolgt wie bei II. Laborprüfung b.

Bewertung
Wie bei II. Laborprüfung b.

V. Halbfreilandprüfung a (Fichtenzweigtest):

Wirkung des Kontakts mit frisch ausgebrachten Pflanzenschutzmittelbelägen auf Kulturpflanzen auf die Eilegeleistung der Weibchen.

Zweck der Prüfung
Entsprechend I. Laborprüfung a soll die toxische Wirkung von frisch angetrockneten Pflanzenschutzmittelbelägen auf der Kulturpflanze ermittelt werden.

Versuchsbedingungen
Die Versuchsbedingungen entsprechen denen von II. Laborprüfung b. In der 3. Phase werden die Versuchstiere jedoch nicht in behandelte Prüfkäfige eingesetzt, sondern mit behandelten Fichtenzweigen zusammengebracht. Der Prüfkäfig ist entsprechend Abb. 2b abgeändert.

Behandlung
Die Behandlung erfolgt mit einer Hochdruckrückenspritze. Möglichst dicht benadelte Fichtenzweige werden flach ausgelegt und bis zum Zusammenfließen der Spritzflüssigkeitstropfen behandelt. Nach dem Antrocknen des Belags werden die Zweige umgedreht und noch einmal bespritzt. Wenn auch dieser 2. Belag angetrocknet ist, werden in jeden Käfig Triebe von 60 cm Gesamtlänge gelegt und die Versuchstiere eingesetzt. Während der 8tägigen Versuchszeit fallen die Nadeln nicht ab.

Ergebnisse
Wie bei II. Laborprüfung b wird die Anzahl der täglich abgelegten Eier ermittelt. Der Wirkungsgrad eines Prüfmittels wird wie dort berechnet.

Bewertung
Ein Mittel wird als **unschädlich** eingestuft, falls WMax $\leq$ 30 %. Schädigende Mittel werden nach VII. Freilandprüfung weitergeprüft.

VI. Halbfreilandprüfung b (Persistenztest):

Wirkung des Kontakts mit verschieden alten, auf Kulturpflanzen ausgebrachter Pflanzenschutzmittelbeläge auf die Eilegeleistung der Weibchen.

Zweck der Prüfung
Entsprechend der Halbfreilandprüfung b soll die Dauer des schädigenden Einflusses von Pflanzenschutzmittelbelägen auf der Kulturpflanze (Schadwirkungsdauer) ermittelt werden.

Versuchsbedingungen
Die Versuchsbedingungen entsprechen denen von V. Halbfreilandprüfung a. Die Beläge auf den Fichtenzweigen sind jedoch 3, 10 bzw. 31 Tage gealtert. Der Versuchsablauf entspricht Abb. 2.

Die behandelten Zweige werden in Wasserkultur bis zum Beginn der Prüfphase in Lichtthermostaten unter folgenden Bedingungen gehalten: 16 Stunden Licht (Leuchtstoffröhren eines Beleuchtungskastens über dem Thermostaten, zusätzlich eine UV-Leuchtstoffröhre im Innenraum) und 26°C; 8 Stunden Dunkelheit und 17°C; relative Luftfeuchtigkeit unter 40%. Aus dem Innenraum wird ständig Luft abgesaugt, so daß etwa alle 2 Minuten die gesamte Luft erneuert wird.

Behandlung
Die Fichtenzweige werden wie bei V. Halbfreilandprüfung a behandelt.

Ergebnisse
Wie bei II. Laborprüfung b wird die Anzahl der täglich abgelegten Eier ermittelt. Wie dort wird auch der Wirkungsgrad berechnet.

Bewertung
Ausschlaggebend für die Bewertung ist eine Senkung von WMax auf 50 % oder darunter aufgrund des Wirkstoffaubbaus durch Alterung der Pflanzenschutzmittelbeläge. Die Bewertungsklassen sind Tab. 2 zu entnehmen.

Tab. 2. Bewertung der Prüfergebnisse des Persistenztests

Wertziffer	Schadwirkungsdauer	Bewertungsklasse
1	≤ 3 Tage	**kurz wirksam**
2	≤ 10 Tage	**schwach persistent**
3	≤ 31 Tage	**mäßig persistent**
4	> 31 Tage	**stark persistent**

VII. Freilandprüfung:

Wirkung unter praxisnahen Bedingungen ausgebrachter Pflanzenschutzmittelbeläge auf die Eilegeleistung der Weibchen.

Zweck der Prüfung
Unter möglichst praxisnahen, aber kontrollierten Freilandbedingungen soll die Wirkung frisch angetrockneter Pflanzenschutzmittelbeläge auf die ökologische Nutzleistung der wirtesuchenden Weibchen ermittelt werden.

Versuchsbedingungen
Wegen der geringen natürlichen Populationsdichte und der großen Mobilität von *C. turionellae* muß die Freilandprüfung mit gezüchteten Versuchstieren in Käfigen erfolgen. In die Käfige werden praxisüblich behandelte Fichten eingestellt. Aus Gründen einer besseren Reproduzierbarkeit der Prüfergebnisse werden die Versuchskäfige vor Niederschlägen geschützt aufgestellt. Auf Wunsch kann Regen simuliert werden.

Der Käfig besteht aus einem würfelförmigen Rahmen aus gelochten Stahlprofilen (Kantenlänge 1 m), über den die Käfighülle (Seitenwände und Decke) aus SARAN-Gewebe gestülpt wird. Bogenförmige Öffnungsschlitze sind durch Reißverschlüsse zu verschließen. Am unteren Rand der 4 Seitenwände besitzt die Hülle eine 10 cm breite Schürze. Diese liegt einer Tischplatte aus wasserfestverleimten Sperrholz (1,09 m x 1,09 m, 19 mm stark) flach auf und wird durch einen knapp über den Käfig passenden Holzrahmen mit Hilfe von 12 Schraubzwingen fest angepreßt.

Die 80 cm großen Versuchsbäume sollen eine volle, dicht benadelte Krone besitzen, d.h. möglichst einer Weitverbandpflanzung entstammen. Es ist sicherzustellen, daß Pflanzenschutzmaßnahmen zuvor nicht zur Anwendung kamen. Unmittelbar vor der Behandlung werden die Fichten abgeschnitten und zur Entfernung eventuell vorhandener räuberischer Arthropoden abgeklopft. Nach der Behandlung werden sie in den Bohrungen (3 cm Durchmesser) einer Holzplatte (gleichseitiges Dreieck mit 60 cm Seitenlänge) mit 14 cm hohen Füßen verkeilt.

Beim Zusammenbau der Käfige ist streng darauf zu achten, daß mit einem Pflanzenschutzmittel behandelte Fichten nicht mit den Käfigwänden in Berührung kommen.

Je Veruchsvariante werden 3 Wiederholungen durchgeführt.

Der Versorgung der Versuchstiere mit Wasser dient eine Vogeltränke, die mit einem Klebeband an eine Fichte befestigt wird. Eine Karte mit Schlupfwespenfutter wird mit einer Wäscheklammer an einen Zweig geklemmt. Die Wirtspuppen werden auf zwei Petrischalen mit Wellpappe-Einlage exponiert. Eine Schale befindet sich zwischen den Fichten auf dem Holzdreieck, die andere auf einem Ständer in 68 cm Höhe (von der Tischplatte aus gemessen). Auch diese Schale wird möglichst nah an die Zweige der Fichten gerückt. Futter, Wasser und Wirte werden nicht behandelt.

Die Käfige werden unter einem Schutzdach auf einer 2,5 m hohen, allseitig offenen Balkenkonstruktion aufgestellt. Das transparente Dach aus Polyester (Lichtdurchlässigkeit 70 bis 80 %) ist 3 m breit, so daß die Käfigwände auch von schräg fallendem Regen nicht erreicht werden. Ein Thermohygrograph registriert den Verlauf der Temperatur und relativen Luftfeuchtigkeit.

Der Versuchsablauf entspricht II. Laborprüfung b mit dem Unterschied, daß nicht 5 sondern 10 Weibchen in die Adaptationskäfige der 2. Phase eingesetzt werden, und in den Käfigen der 3. Phase die Anzahl der Versuchstiere noch einmal verdoppelt wird. Die Weibchen werden auf die obere bzw. untere Petrischale mit je 5 Wirtspuppen aufgesetzt, um die Wirtsfindung unter den ungewohnten Bedingungen zu erleichtern. Wegen der erschwerten Wirtsfindung werden zudem die Puppen 24 Stunden lang exponiert. Die Verlängerung der Expositionszeit und die Vervierfachung der Anzahl Versuchstiere pro Käfig ist notwendig, um eine für die Auswertung ausreichende Eizahl zu erhalten.

Behandlung
Die Fichten werden mit einer Hochdruckrückenspritze tropfnaß behandelt. Der Spritzflüssigkeitsbedarf wird ermittelt.

Ergebnisse
Die Anzahl der täglich abgelegten Eier wird wie bei II. Laborprüfung b ermittelt. Wie dort wird auch der Wirkungsgrad berechnet.

Bewertung
Die Bewertung der Ergebnisse erfolgt nach Tab. 3.

Tab. 3. Bewertung der Prüfergebnisse der Freilandprüfung

Wertziffer	Grenzwerte der Leistungsminderung	Bewertungsklasse
1	WMax ≤ 25 %	unschädlich
2	WMin ≤ 25 %	schwach schädigend
3	WMin ≤ 50 %	mittelstark schädigend
4	WMin ≤ 75 %	stark schädigend

Berichterstattung

Werden Prüfungen im Auftrag durchgeführt, wird nach Abschluß der Untersuchungen ein Bericht in systematischer Form mit allen Rohdaten, den statistischen Berechnungen und der abschließenden Bewertung der Ergebnisse dem Antragsteller zugestellt.

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Pesticides toxicity over pupal of Cales noacki Howard (Hym.: Aphelinidae).

A. GARRIDO
Plant Protection Department
Entomology Section
Instituto Valenciano de Investigaciones Agrarias (IVIA)
Moncada (Valencia) SPAIN

ABSTRACT

We are reporting the results and conclusions obtained in the laboratory tests conducted with some pesticides on pupal of Cales noacki How.

The methods used in the tests are described and were presented at the meeting of this working group held in Copenhagen in September 1982.

KEY WORD: Cales noacki, Pesticides toxicity.

INTRODUCTION

Cales noacki Howard is an important aphelinid parasite of Aleurothrixus floccosus Maskell occasionally maintaining this pest below the economic threshold.

However, it is often necessary to use chemical pesticides against whitefly, and against other pests and disease which occur simultaneously, and it is vital to the success of integrated control that the products used be safe to beneficial predators and parasites. This compatibility between chemical and biological materials is the concern of an OILB work group on "PESTICIDES AND BENEFICIAL ORGANISMS".

In relation to C. noacki, useful publications have appeared from CARRERO (1979), GARRIDO et al. (1982), and SANTABALLA et al. (1980). All these references provide information on the direct effect of several pesticides on the parasite in question.

The purpose of this paper is to present the results of studies which investigated the pesticides toxicity over nymphal stages of C. noacki.

MATERIALS AND METHODS

The living material of beneficial insects was obtained from captivity rearing made for this purpose in a breeding room at 20ºC., 65+5% relative humidity, and 15 hours light. This enabled to have homogeneous biological material available -as what evolutive stage concerns- for each insect under study, and for each pesticide tested.

Considering that the pupal aged 6 days, with cherry coloured eyes of the insect can develop into adult, and not being necessary that the plant support that feeds them remains attached to the mother plant, the pesticides were applied on pieces of leaves containing pupal of the beneficial insect. Orange tree leaves were used for the tests.

Pesticides applications was made with a manual sprayer type "Sprahgerat-spray-gun-pulverisateur", allowing a continuous and constant flow of the broth prepared and a variable amount of the commercial formulation, according to the rate of the brot to be used (see table I).

Rates used for each pesticide are shown in table II.

In all trials a control was used, having at least 150 individuals, and three replications of about 50 individuals for each product; the results from each replication were accumulated; the percentage of final mortality was found by substracting the controls mortality values from mortality obtained in the accumulated data.

To assess the possible insect mortality, the following technique was adopted from Garrido (1982):

1st) Sufficient insects for each treatment were collected, counting them with the aid of a stereoscopic microscope, and transferred to the individual bean and orange leaves.

2nd) The leaves infested with the pupal stages were attached to pieces of synthetic material such as polyvinyl or cork using pins to keep the leaves flat, thereby facilitating assessment.

3rd) Following this, the leaves were sprayed with pesticide or water depending on the treatment.

4th) The material was then placed on a tray and left until dry.

5th) The treated and untreated samples were kept in a room with controlled temperature and humidity.

6th) Every two days the emergence of adults was assessed and puparia with holes were discarded. This was continued for one month after which the study was terminated. However, if emergence of adults in untreated samples was less than 95%, the experiment was repeated.

RESULTS AND CONCLUSIONS

Table II shows the results obtained.

Sometimes, carbaryl did not produce immediate death in *C. noacki*, and even these can operculate and then die from ingestion.

As indicated in table II, from the pesticides group, the

insecticides were the most harmful products for the insect under study; even though among these, there are hamless products.

Concerning acaricides, fungicides, herbicides and growth regulators, non harmful substances were found for Cales, except for Carbaryl, that although considered in the present work as a growth regulator it is known to be used as an insecticide. This product was already evaluated as harmful for Cales in studies Carried out by GARRIDO et al (1982), the present results confirm the previous ones.

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TABLE I

Rates of comercial products in mgr./cm2, applied with a manual sprayer type "spruhgerat-spray-gun-pulverisateur", obtaining a good surface coverage from application of 71.98 mm3/cm2.

Rate of broth in		Comercial product applied in mgr/cm2
%	p.p.m.	
0.0125	125	0.0089
0.0250	250	0.0179
0.0300	300	0.025
0.0500	500	0.0359
0.0600	600	0.0431
0.0700	700	0.0503
0.1000	1.000	0.0719
0.1500	1.500	0.1079
0.1870	1.870	0.1346
0.2000	2.000	0.1439
0.2500	2.500	0.1799
0.3000	3.000	0.2159
0.3750	3.750	0.2699
0.4000	4.000	0.2879
0.6000	6.000	0.4318
0.7000	7.000	0.5038
1.0000	10.000	0.7198
1.5000	15.000	1.0797
1.7000	17.000	1.2236
3.0000	30.000	2.1594
8.0000	80.000	5.7584

TABLE II

Pesticides toxicity on nymphal stages of Cales noacki How

CLASS	Pesticides tested				
	Active ingredient	Commercial preparation (Form.)	Applied rate %	Mortality %	Ation
INSECTI-CIDES ACARICIDES	Acephate	Orthen WP 50 %	0.15	23.60	1
	Amitraz	Maitac EC 20%	0.30	86.27	3
	Azinphos-methyl	Gusation WP 50 %	0.20	76.77	2
	Deltamethrine	Decis EC 25 %	0.06	95.43	3
	Fenitrothion	Folithion EC 55 %	0.10	84.37	3
	Ethiophencarb	Croneton EC 50%	0.10	38.69	1
	Heptanophos	Hostaquick EC 560 g/l	0.10	42.88	1
	Etrimfos	Ekamet EC 50%	0.20	97.94	3
	Tetradifon	Tedion V 18 EC 8%	0.20	39.51	1
	Vamidothion	Kilval EC 40%	0.125	22.08	1
FUNGICIDES	Chlorotalonil	Dacomil 500 EC 5%	0.3	45.33	1
	Chinomethionate	Morestan WP 25%	0.1	1.39	1
	Prochloraz	Sportak EC 40%	0.187	65.10	2
	Thiram	Pomarsol forte WP 80 %	0.20	-	-
	Mancoceb	Dithane ultra WP 80 %	0.10	0.72	1
HERBICIDES	Amitrol+Diuron	Ustinexpa WP 30% + WP 50 %	1.0	-	-
	Bromacil	Hyvarse WP 80 %	0.2	1.34	1
	Bromafenoxim	Faneron WP 50 %	1.7	-	-
	Simazine	Gesatop 50 WP 50 %	0.375	-	-
GROWTH	Chlormequat	Cycocel extra EC 41.2% + EC 28.8%	0.7	1.25	1
	Carbaryl	Prosevor 85 WP 85 %	0.125	86.61	3
REGULATORS	1 Naphthylacetic Acid.	Rhodofise WP 1 %	0.15	0.68	1

Toxicity (Rate evaluation):
1 = 50 % = harmless
2 = 50-79 % = slightly harmful
3 = 80-99 % = moderately harmful
4 = 99 % = harmful

SIDE-EFFECT TESTS FOR PHYTOSEIIDS AND THEIR REARING METHODS

Frank Bakker[1], André Grove[1], Sylvia Blümel[2], Johan Calis[1], Pieter Oomen[3]

1) University of Amsterdam; Department Pure and Applied Ecology; Section Population Biology; MITOX; Kruislaan 302; 1098 SM Amsterdam The Netherlands.

2) Bundesanstalt für Pflanzenschutz; Trunnerstraße 1-5; A 1020 Wien Austria.

3) Plantenziektekundige Dienst; P.O.Box 9102; 6700 HC Wageningen
The Netherlands

Preamble

Along with the increasing importance of phytoseiids as biological pesticides (e.g. *P. persimilis* against spider mites, *A. cucumeris* and *A. barkeri* against thrips) or as natural regulators in integrated control schemes (e.g. *T. pyri, A. andersoni, A. finlandicus, T. occidentalis* and *A. fallacis* in orchards), the number of (lab-) tests designed to assess the compatibility of chemical pesticides with these natural enemies also increased. Examples of such tests can be found in van de Vrie (1962), van Zon & Wysoki (1978), Alianazee & Cranham (1980), Streibert (1981), Overmeer & van Zon (1981,1982), Hassan (1982), Samsøe-Petersen (1983, 1985), Croft & Alianazee (1983), Overmeer (1985, for a review), Oomen 1988, Bakker & Calis (1989), Baier & Karg (1990) and EPPO (1990). In earlier communications the IOBC Working Group "Pesticides and Beneficial Arthropods" has repeatedly pointed at the need for standardization of testing methods and guidelines have been proposed accordingly (e.g. Hassan 1985, Samsøe-Petersen 1990).
Although the primary task of the Working Group has been extension to growers, the methodology developed has also been incorporated in testing procedures used for registration purposes (*e.g.* EPPO, 1990). The methodology proposed here is intended for both purposes. Essentially the compatibility of pesticides with biological control agents is assessed according to a sequential decision making scheme such as those described by Hassan (1985), Oomen (1988) and Samsøe-Petersen (1990). These schemes are comprised of a sequence of toxicity tests done in increasingly complex environments. A flow chart for sequential testing procedures developed specifically for phytoseiids is presented in section 2 of this paper.
The first section of this paper will be devoted to a description of yet another testing method for laboratory assessment of pesticide side- effects on phytoseiid mites. This is because the lab tests currently used by the Working Group have been developed for particular species of phytoseiids and not for the family as a whole. Consequently a test used for species 1 cannot be used for species 2 and so on. As will be clear from the next section, the test described here is appropiate for all species of phytoseiids that are of current economic interest and presumably also for those that are of future interest.

Section 1) Laboratory testing

According to the Working Group's recommendations lab and extended lab experiments represent 'worst case' situations. Hence, experiments are conducted with the most susceptible stage (protonymphs in this case), while exposure to the pesticide is both standardized and maximized. All other trial conditions should be such that they are optimal for the beneficial (cf. Hassan ,1985 and Samsøe- Petersen 1990).
Existing testing methods for phytoseiids do not necessarely comply with the Working Group's recommendations regarding exposure. It can also be argued that environmental circumstances too are difficult to standardize and optimize with existing methods. In a series of dose-response studies to evaluate the various methods Bakker *et al.* (1991) demonstrated that both the detached leaf test currently used for *P. persimilis* (cf. Oomen 1988, and Samsøe-Petersen 1983, 1985) and the inert substrate test described by Overmeer & van Zon (1981,1982) for the orchard mites *T. pyri* and *A. andersoni* (= *A. potentillae*) provide refuges. This leads to an underestimation of the effect. This was shown by comparison with a method without such refuges, *viz.* the 'coffin cell' method (Bakker & Calis, 1989) where phytoseiids are exposed to pesticide residue in ventilated glass cages. The underestimation was quantified by three parameters: 1) higher LC-50 values, 2) less steep slope of the dose-response curve and 3) delayed mortality. It was also found that testing in glass cages led to more homogeneous results and thus facilitated statistical interpretation of the data. These findings will be published elsewhere (Bakker et al, in prep).
Thus the lab test described in this guideline (and in Bakker and Calis 1989) has several advantages over existing methods. These are:

a) It can be used for all species of phytoseiids currently tested in the Working Group's joint testing programme and can presumably also be used for any new species that might become relevant for testing. In other words, it is a 'standard' method.

b) It represents a 'worse case' situation since refuges are absent and added effects of irritancy (sensu Lockwood et al 1984) do not bias testing results.

c) It is highly standardizable with respect to environmental circumstances. Temperature and humidity can be specified within a very narrow range, which makes the test especially appropiate for GLP purposes.

d) It complies with all requirements for a lab (a) test put forward by the Working Group, including forced ventilation.

The only disadvantage seems to be that the test is not suitable for products that produce an opaque residue (e.g. captan). However, the advantages were recognized by the Working Group and at the Technical Meeting in Southampton (september 1991) it was agreed that the method should be adopted for lab tests on *T. pyri*, *A. andersoni*, *P. persimilis* and *A. cucumeris* from the 7th joint testing programme onwards.

Procedure:

1. Experimental conditions.

1.1 *Principle of the trial*: Mites are exposed to pesticide residue in dismantable glass cages. All parts of the cage have been sprayed in horizontal position under the Potter precision spray tower at the (highest) recommended field concentration at a residue density of 1 mg/cm^2. Effect on juvenile survival and on reproduction are studied separately and combined to a single measure of effect. Both a negative and a positive control are included in the test.

1.2. *Description of the cages:*

1.2.1. *Components.*

Glass plates thickness 0.3 cm; glass plates thickness 0.15 cm; glue Stabilit Ultra®; soft silicon rubber tubing outer diameter=0.6 cm/inner diameter 0.4 cm, gaflon tubing outer/inner diameter 0.4/0.2 cm, PVC tubing inner diameter 0.6 cm, gauze mesh size 80µm, cotton wool, diluted vaseline (10 parts vaseline, 1 part linseed oil), rubber bands, plexiglas tube 90cm (ø=4cm), 2 water baths (90x4x4 cm), air pump.

1.2.2 *Preparation.*

Each cage (coffin cell) consists of one bottom glass plate (10x5x0.3 cm.) one top glass plate (10x5x0.15 cm.; thinner glass facilitates observation), two long sides (7.6x1.2x0.3 cm.) and two short sides (5x1.2x 0.3 cm.) (see fig. 1). The long sides are glued lengthwise on a glass plate (10x0.75x0.3 cm.), such that a T-shape ensues (see fig. 1a). This is for two reasons: first, the 'foot' enables horizontal spraying and second, it ensures the ribs will stay in position during the experiment. In the bottom plate three holes are drilled (ø=0.6 cm) (fig 1). The two outer holes serve the air circulation system (1 inlet, 1 outlet), the central hole is to provide the mites with a source of drinking water. The latter is achieved by plugging the hole with a cotton taper covered with tissue paper and nylon gauze (mesh size 80 µm).
The taper is inserted in the silicon rubber tubelett (length 2 cm), such that it just reaches one end, whereas from the other end a long free stretch of cotton should come out that can reach a water basin below the cell. A small piece of tissue paper and a small piece of gauze (ca. 1x1 cm) are put on top of the tubelett, which is then pushed through the wider polythene tube (length= 1 cm.), such that a rim of 0.3 cm of the inner tube with nicely stretched gauze protrudes (see fig. 2b). The inlet tube is constructed by glueing a similar piece of gauze onto the gaflon tube (length ca. 2 cm) and subsequently pushing this through a piece of silicon rubber tubing (slightly shorter than the inner tube) until tubes and gauze form a flat surface (fig. 1a). The outlet tube is identical except that the lower part is provided with a socket of polythene tubing that allows for connection with the central circulation system (see below, fig. 1c).

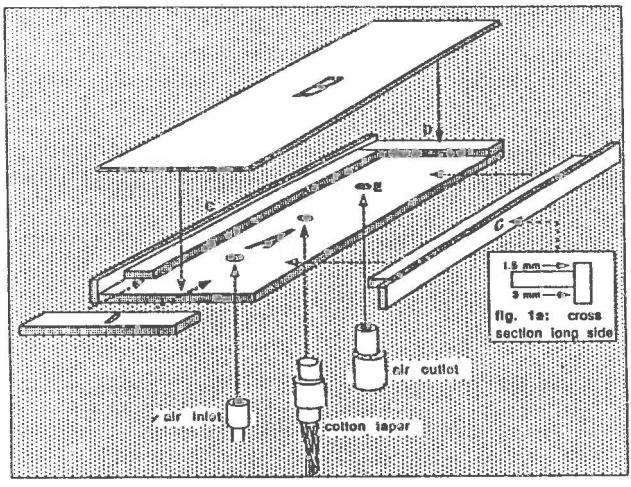

Figure 1) Construction of the glass cage (coffin cell). A: bottom glass plate; B: top glass plate; C: long ribs; D: short ribs; E: holes in bottom glass plates.

1.3 *Spraying the test units.*

Homogeneous residues are obtained by using a Potter Precision Spray Tower. Before spraying the test units the amount of solution in the Potter Tower is calibrated such that it repeatedly produces 1 mg spray fluid/cm2. The concentration used should be the highest recommended

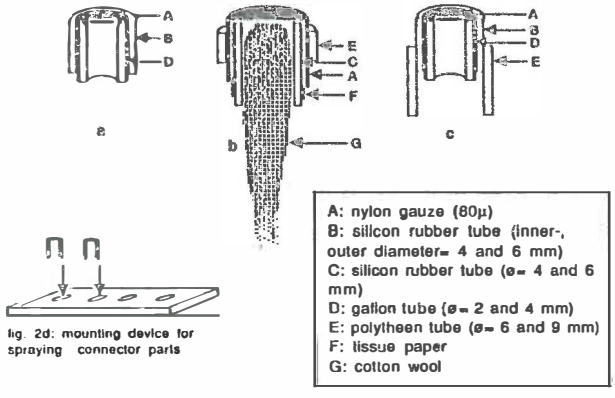

Figure 2) Connecting tubes used for the coffin cells. The outer tubes serve as air inlet (a) and air outlet (b). The middle tube serves as a water supply.

field concentration. Potter Tower settings are: table distance 1.7 cm, air pressure 15 lb/in2 (ca. 1.1 kg/cm2). When sample tube is empty wait 5 seconds before turning off airpressure. All parts are sprayed in horizontal position on a dry, clean tissue paper. This requires 2 sprayings per unit, one with top and bottom plates, and one with ribs and air circulation connector parts. The latter are held

in vertical position by mounting them on a piece of rubber with 4 sockets that can hold the connector parts (fig 2d). The small ribs are kept upright between the larger ones that stand on the perpendicular platelett. Two cells are needed per replicate, one for assessment of the effect on juvenile survival and one for the effect on reproduction. These two cells can be sprayed in three bouts. The cotton taper is <u>not</u> sprayed. Before spraying check for fingerprints or other contaminants. Remove with ethanol 96%. Every other spraying clean bottom of Potter tube to avoid falling droplets.

1.4 *Assembling test units.*

Immediately after drying of the residue (ca. 0.5 hour) the cages are assembled. Air tightness (and mite proofness) of the cells is secured by applying diluted vaseline on the outermost edges of all side ribs, such that a thin film spreads between glass plates and ribs after assemblage. Contact surface between long and short ribs is also treated with vaseline. A soft brush is used to apply vaseline. After cell assemblage (hold bottom, add ribs and subsequently top plate) two rubber bands are used to tighten the device. Bands are put lenghtwise around the cells (fig. 3). Finally the two air circulation connector parts are inserted in the two outermost holes such that (sprayed) gauze and glass plate are at the same level. The cotton taper covered with gauze is similarly inserted in the central hole.

1.5 *Installing test units and trial conditions.*

The experiment is carried out in a climate box that allows for precise regulation of humidity and temperature. If only temperature can be precisely regulated, regulation of humidity can be achieved by leading watersaturated air into the box. This is done by leading compressed air through a series of water flasks before entering the box. Tuning is achieved by mixing with dry air. Experimental conditions should be 25 ± 1 °C and 85 ± 5 %RH and monitored throughout the experiment.
In this box a plexiglass tube is placed (length 90 cm, ø ca. 4 cm). The tube is closed on both ends and has 4 outlet and 30 (15 on each side) inlet connector parts (fig. 3). The outlets are equidistant on top of the tube and by using silicon rubber tubing combined into a single outlet tube (polythene or PVC, ø=1 cm) that is connected with a pump (e.g. Leybold Heraeus MINI A). Just before the pump the combined outlet tube has a T-junction. The side tube here connected is closed with a screw tubing clamp that can be opened to regulate the amount of 'false air' entering the pump (fig. 3a). This enables regulation of wind speed in the cages.

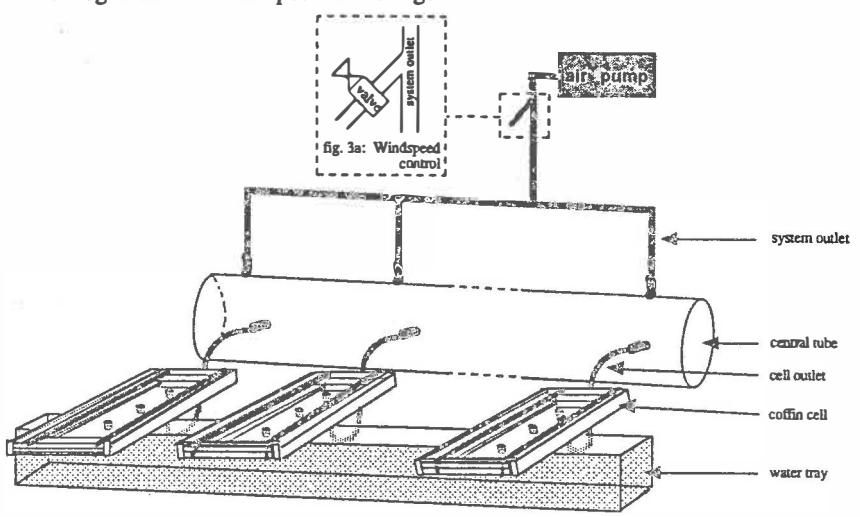

Figure 3) Complete set-up of 'coffin cell' test showing assembled glass cages, collector tube, water tray, pump and tubing.

Parallel to the central tube two water baths are placed (90x4x4 cm). After assemblage the cages are put on top of this water bath, such that the taper dangles in the (deionized) water. Inlet connector part should be free in the box. The cage outlet is connected with one of the central tube inlets using silicon tubing. Inlets on the central tube that are not used should be cross connected in order to close the system. After all cages have been mounted the pump is turned on and windspeed is adjusted to 0.1 m/s by using the outlet near the pump (fig. 3a). Windspeed is measured within the cells using an anemometer. This requires a cell with a central hole in the upper glass plate made to fit the anemometer probe. This measuring cell can be placed instead of one of the testing cages.
After all cages have been connected to the central tube and windspeed within the cages has been adjusted, the cages are disconnected one by one and provided with predators and their food (see below). To provide the cell with food one of the outer tubes is removed and food is added using a soft brush; the tube is then connected again. The middle cotton taper is now removed and the cell is provided with 20 individuals of the species to be tested. The cotton taper is reconnected and the cell is again connected to the central tube.
Preferably all cell parts should be used once and discarded after each experiment. If this is not feasible they are thoroughly cleaned.

1.6. *Preparation of the predators*

The test will be done with the most susceptible life stage, *i.e.* protonymphs (larvae are too fragile to be used). To obtain a cohort of nymphs the following procedure is followed. Gravid females are collected from the rearing (see appendix 1) and transferred to an arena appropiate for the species to be tested, *i.e.* a detached leaf or a PVC tile. The objective is to obtain a cohort of eggs less than 24 hrs old. Therefore enough females should be collected to get the required amount of eggs, *i.e.* roughly 0.5-0.7 times the amount of eggs. The number of eggs needed will be 1.5 - 2 times the number of individuals needed for the test. Food should be amply supplied and of the same type as used in the rearings (appendix 1). The next day either all females are removed or all eggs are transferred to a new arena. The remaining egg cohort is left undisturbed until protonymphs appear, usually 2 or 3 days later depending on the species. These are then used for the test.

1.7 *Design of the trial.*

Treatments should consist of spraying with 1) test product(s), 2) control with deionized water (same as used for dilutions) and 3) reference product of known toxicity, preferably at a concentration near the LC50. If the tests are done with a resistant strain, resistance should be documented and regularly checked.
Test units are glass cages treated as described above. Each cage receives 20 protonymphs.
Replicates should be at least 5 per treatment.Two cells should be prepared per replicate. One to be used in the first period for assessment of juvenile mortality, the other in the second period for assessing reproduction.

1.8 *Conduct of the trial and mode of assessment.*

Before the trial is started developmental time from protonymph to egg laying female is assessed. This defines the time span of the experiment. The experiment is partitioned into measuring effect on juvenile survival and effect on reproduction. Effect on reproduction is assessed in fresh cells that are sprayed simultaneously with cells in which effect on survival is measured. This is done with the females originating from the batch of treated juveniles. Both experimental periods should be of equal length and predetermined. (For example with *P. persimilis* the experiments last 2x4 days, for *T. pyri* 2x7 days).
- Day 0: After assembling the entire test unit, cells are disconnected one by one and receive food first and then predators (as described under 1.5). In some cases (*P.persimilis*) food will consist of spider mite eggs, collected as described in appendix 3, in others of (broad bean) pollen, collected as described in appendix 1. Not too much food should be added as this will lead to contamination with fungi. Before reconnecting the cage a final check on predator number is made. Predators are best introduced into the cells with cages placed on a black background using a binocular microscope.

After the introduction of each predator the entrance hole is temporarely closed with a microscope cover slide to avoid escapes. The climate box is tightly closed.
- Day 1: The box is opened and a check is made on ambient conditions.
- Day 3: The box is opened and a check is made on ambient conditions. Cells are disconnected one by one and the numbers of dead and surviving predators are recorded.

- Reproductive day 0: This is the predetermined moment where final juvenile survival is assessed and the predators are transferred to fresh cells. First it is determined whether the juveniles have indeed reached the adult stage and oviposition has begun. If so, the number of surviving males and females should be assessed and the adults should be transferred to the fresh cells. Sex ratio in the cells is calculated. It should resemble sex ratio in the cultures. If not, males from the culture (or preferably from a spare test unit) are added. The number of eggs present are also recorded. Food is added to all test units, while making sure no mites escape. If no eggs are found at this stage cells are checked daily until they appear. The moment of first oviposition is treated as day 0 for the subsequent phase of the test, that is assessment of the effect on reproduction. It must be stressed that if onset of oviposition is observed in one of the treatments only, this moment will be denoted as day 0 for the other treatments as well, *i.e.* even when no eggs are observed. This is to incorporate possible effects as delayed development c.q. oviposition in the outcome of the trial.
- Reproductive day 0+1: Number of eggs, juveniles and surviving females are recorded. Add males if necessary.
- Reproductive day 0+3: Number of eggs, juveniles and surviving females are recorded. Add males if necessary. Check food, add if necessary.
- Reproductive day 0+5: Number of eggs, juveniles and surviving females are recorded. Check food, add if necessary.
- Final day of the experiment: The moment where the second period equals the first period. Number of eggs and surviving females and juveniles are recorded. Then cells are dismantled and number of empty egg shells is determined if number of eggs could not be assessed accurately during the experiment (*e.g.* because they were too clumped).

1.9 *Results.*

1.9.1 *Processing of the data.*

For all cells mortality until reproductive day 0 (*i.e.* juvenile mortality) is calculated as follows:

$$M_j = (d/(a+d)) \times 100\%$$

Where: M_j = Juvenile mortality
d = Number of individuals found dead
a = Number of individuals found alive

For calculation of mortality ali cells where (a+d)<15 are neglected. If this leads to less than 3 cells per treatment the experiment is repeated. Subsequently numbers for all treatments are pooled and control mortality is calculated. If control mortality exceeds 20% the experiment must be repeated.

1.9.2 *Calculation of effect.*

First correct mortality recorded in the treated groups for control mortality according to Abbott (1925) with the following formula:

$$M_a = (M_t - M_c)/(100 - M_c) \times 100\%$$

Where: M_a = Mortality corrected according to Abbott
M_t = Mortality in treatment
M_c = Mortality in control

Subsequently reproduction for all treatments is calculated. In principle this is done by summing all eggs found on reproductive day 0, 3 and 5 and final day and then dividing by the summed number of females found on the same days (thus $R=\Sigma eggs/\Sigma females$). However, a possible ovicidal effect is checked first by comparing juvenile : egg ratio in treated and control group (*e.g.* using a binomial test for differences of proportions). If there is a significant difference the fraction of viable eggs is determined experimentally by exposing eggs to aged residue. In this case reproduction is calculated by summing the number of viable eggs and dividing this by the number of females alive.
If, for any reason the number of eggs cannot be assessed accurately (*e.g.* because they are too clumped) the number of empty egg shells counted afterwards can be used instead.
Effect on reproduction is determined with:

$$E_r=R_t/R_c$$

where: E_r= Effect on reproduction
R_t= Reproduction in treated group
R_c= Reproduction in control

Subsequently effect on survival and effect on reproduction are combined using the following formula:

$$E=100\%-(100\%-M_a) \times E_r$$

1.10 *Rating of results and decision making.*

In the Working Group's joint pesticide testing programme the following classes are used:

class 1: E< 30%	(harmless)
class 2: 30<E<80	(slightly harmful)
class 3: 80<E<99	(moderately harmful)
class 4: E> 99%	(harmful)

However, in the sequential scheme presented in the next section only two class boundaries are of importance, *viz.* E<50% is harmless, E>99% is harmful. Other values mean continue testing. The reasons for and validation of this deviation from the Working Group's standard procedures are presented in thenext section.

Section 2) Sequential decision making

The primary aim of screening pesticides for side-effects on beneficial organisms is to assess the compatibility of the two. The intention is not only to advise growers involved in IPM, but also to provide guidelines for those working in the field of pesticide regulation. Given the vast number of pesticides to be screened it will be clear that testing procedures should be quick, inexpensive, efficient and reliable. It is therefore not desirable that all products are screened under field conditions as this would be a very time and labour consuming activity. This notion led to the concept of sequential testing, the idea being that in certain cases field experiments are not necessary provided they can be preceded by tests that will reliably predict side-effects of a product under field conditions. Unfortunately, not all outcomes of laboratory tests are conclusive and in certain cases the feasibilty of using a compound in integrated pest management can only be assessed after doing a field experiment. However, there appears to be disagreement over which outcomes necessitate further testing and which ones do not. Given that each subsequent level of testing tends to be more complex and time-consuming than the previous one, it is obviously important that the lab trials and especially the decision rules to interpret them are devised such that they reduce the amount of field (and semi-field) trials to the necessary minimum. But there is a trade-off because the number of erroneous decisions should also be minimized.

Two types of erroneous decisions are possible. In the first one harmlessness is concluded at an early stage while the compound proves to be harmful under field conditions. The second type is that harmfulness is decided where a compound turns out to be harmless. In the Working Group it has repeatedly been stated that a harmless classification implies that further testing is not necessary, argueing that practical experience has shown that exceptions are rare (*cf.* Hassan 1985, Samsøe-Petersen 1990). On the other hand harmful classifications are considered to be inconclusive and thus necessitate further testing. The line of reasoning behind this is that, because the nature of a lab test is such that it represents a 'worst case' situtation (maximum exposure/dosage compared to optimal control), it is not conceivable that if a compound turns out to be harmless (read: not toxic) under these conditions it will be harmful under the presumably less severe field conditions. While this is undoubtedly true as far as direct toxic effects are concerned, it denies the possibility of indirect side effects, *e.g.* the elimination of crucial alternative food sources. Moreover, slight reductions in predator density may have profound effects on population dynamic phenomena, *e.g.* when a population can attain two or more alternative steady states (Jansen, *in press*). And, as we shall see later (table 1), compounds that appeared to be harmless initially may indeed appear harmful in the field. From the point of view of IPM advisory services this unpredicted harmfulness is much more serious than erroneously deciding upon harmfulness.
It is therefore surprising that an advisory group such as the Working Group so reluctantly accepts harmfulness, whereas the other extreme, harmlessness, can be assigned rather easily. However, it is equally defendable to set criteria to laboratory test results for deciding about harmfulness provided they are validated by field data in the same way criteria for harmlessness are validated, *i.e.* such that exceptions are rare. Oomen (1988) recognized this and composed a sequential decision making scheme where harmfulness could be assigned at each stage of testing whereas the harmless classification was more difficult to achieve. Again, one can argue that it is unjust to classify a product as harmful when this is only assessed under the rigorous laboratory conditions. However, it does not suffice to remain with these verbal arguments. Any sequential decision making scheme ought to be validated with experimental data and not only with logical arguments.
Recently the BART-group proposed a beneficial arthropod testing framework for regulatory studies. We feel that their flowchart is also appropiate for advisory studies and have adopted their approach. However, in the BART proposal decision criteria are lacking and we have taken things further by giving these criteria and subsequently validating the testing scheme with data obtained in the IOBC joint pesticide testing rounds 3, 4, 5 and 6. (Samsøe-Petersen 1990, Hassan *et al.* 1988, Hassan *et al.* 1991, Hassan *et al.* in prep.) and with data from Oomen *et al.* (1991). The scheme we propose is given in fig. 4. It should be stressed that this schedule was developed especially for phytoseiid mites and takes into account peculiarities of their biology, such as short life span, small radius of action and absence of least exposed stages.

Using the sequential scheme

As can be seen in fig. 4 the test comprises three levels of decision making. To classify a pesticide, one proceeds step by step through the levels of the sequential decision making scheme. Depending on how test results match the criteria in the schedule a hazard criterion can be decided upon, or one proceeds to the next level. Use and special aspects of each level are discussed below.

Tier 0

The first level (tier 0) is to make an *a priori* assessment of the adequacy of the envisioned testing procedure. Information about unusual use patterns, mode of application or characteristics of the compounds (*e.g.* dusting application, root application of systemic pesticides, space treatments) may render the routine decision-making scheme (based on assessing residual toxicity) inadequate. The scheme should be adapted after consulting the advisory service on biological/integrated control and be based on information on the chemical provided by the chemical company.

Tier 1

This is the test described in this paper. Summarized: juvenile predators are exposed to a fresh pesticide residue applied on glass substrate in the laboratory. The density of the residue tested is

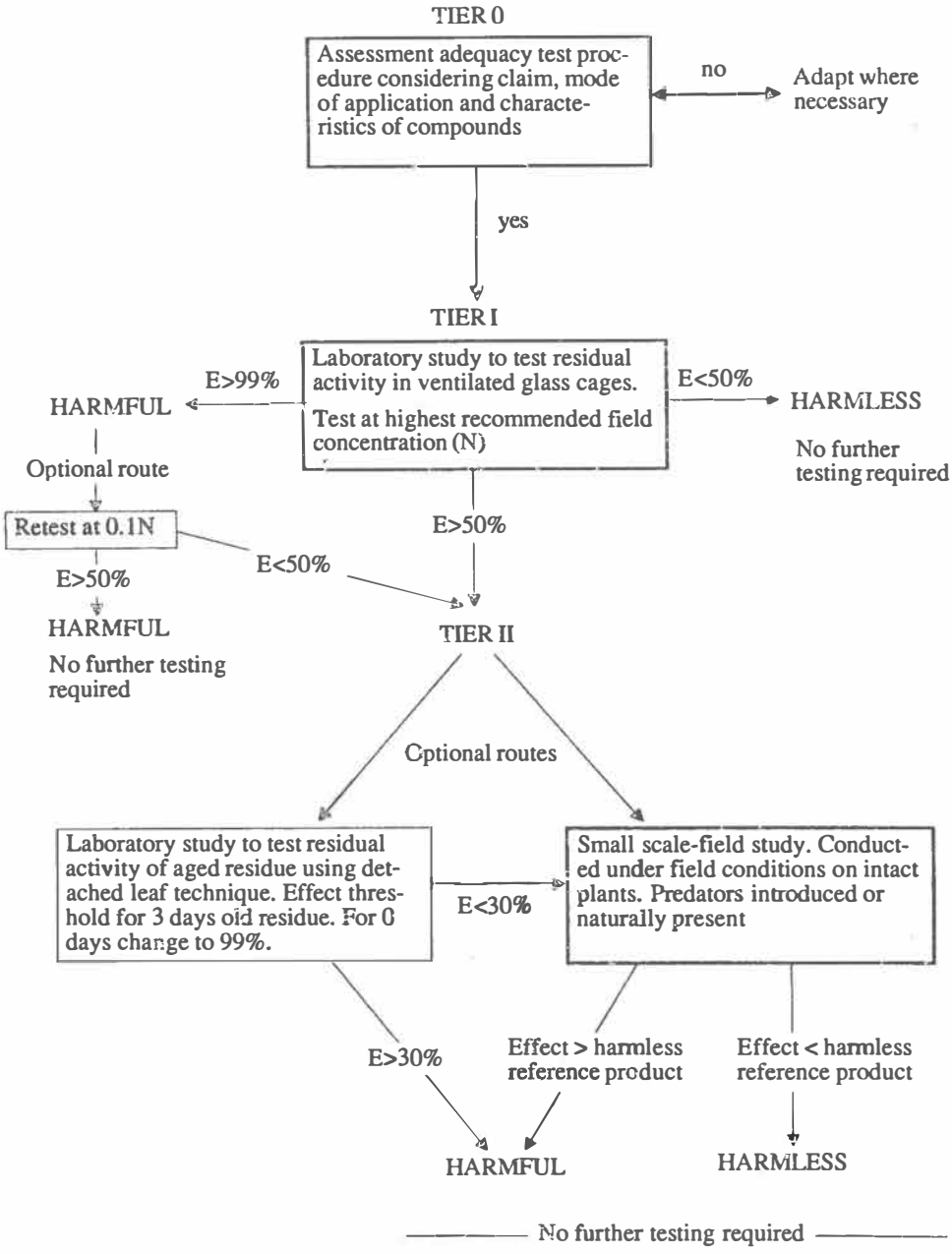

Figure 4) Sequential decision making scheme to evaluate the side-effects of pesticides to phytoseiid mites. Criteria to evaluate test results are given as percentage effect on the beneficial capacity of phytoseiids assessed according to IOBC recommendations. See text for further explanation.

related to the density to be expected on leaves under field conditions. Effect on juvenile survival and

adult reproduction are measured using the same cohort of test animals. Pesticides can be classed as definitely harmless (effect<50%) or as harmful. Although during the past 4 testing rounds of the working group harmfulness has always been correctly decided upon using the >99% effect criterion (see below: validation of the testing criteria) we have incorporated into the scheme an optional route where the product is retested at 0.1 times the highest recommended field concentration (N). If the test then still yields an effect of >50% a product is classified as definitely harmful. However these criteria are tentative since test results for validation are absent (one might *e.g.* argue testing at 0.5N and setting the decision criterion at >99%). We have included this optional route for those who are reluctant to assign a harmful classication already after the first lab test. Based on our validation, it is presumably more important for regulatory services than it is for advisory ones. The interesting feature of this second lab test is that may add a dose-response aspect (be it very rudimentary) to the test. However, if the lab test yields an effect between 50% and 99% one proceeds to the next stage (tier 2).

Tier 2

At this stage products that could not be definitively classified after the first test are retested under field conditions. Again, an optional route has been incorporated. If (based on experience or the outcome of level 1 tests) a product is likely to be harmful in the field one can avoid the field experiment by performing a persistence test. The persistence test exposes juveniles in the laboratory to a pesticide residue on natural substrate (leaf). The residue is aged under field conditions. Measurement variables and interpretation of data are as in test 1. The persistence test makes it possible to classify products, without further testing, as definitely harmful if after 3 days of ageing under field conditions they still cause >30% effect (or alternatively if they cause >99% effect after 0 days of ageing, which is equivalent to performing a detached leaf test). In all other cases proceed to the next test, *i.e.* the small-scale field test. According to our experience harmlessness cannot be determined with the persistence test (see below: validation of testing criteria).
Field tests serve to class all remaining pesticides. These tests should include a recognized harmful reference product (harmless to the prey) and a harmless reference product. The use of a harmful reference product (*e.g.* cypermethrin) ensures that the phytoseiids were at risk under the environmental conditions of the test. The use of a harmless reference (*i.e.* having acceptable, though not negligable, side-effects on biological control by phytoseiids, *e.g.* pirimicarb) is necessary in order to enable evaluation of the effects of the compound tested. Effects equal to or less than those caused by the harmless reference lead to the classification harmless; effects greater than those caused by the harmless reference lead to the classification harmful. Examples for the design of field trials can be found in IOBC (1988), *viz.* Oomen (for *P. persimilis*), Boller *et al.* (*T. pyri*) and Sterk and Vanwetswinkel (*A. finlandicus*). We have only mentioned small-scale field experiments in the sequential schedule because the small radius of action of phytoseiids does not seem to justify the effort involved in large-scale experiments. However, if data are available from field experiments or from actual programmes of integrated pest control this may of course replace the field testing as envisioned here.

Validation of test criteria

With the criteria given in the sequential decision making scheme (fig.4) it is possible to assign a final classification (harmless or harmful) at each stage of testing, *i.e.* even before the final and conclusive field experiment. The obvious question is whether this is legitimate. Of course this hinges on the accuracy with which, given the decision rules involved, the lab test can predict the effect of a product in the field. To test this premise the criteria given here have been validated with results obtained in the Working Group's previous four testing rounds (3, 4, 5 and 6) mentioned earlier. This was possible because during these testing rounds most field tests were performed regardless of the outcome of the lab-assay. As a result both products that appeared harmless in the lab and products that appeared harmful have been retested independantly in the field. For the validation of our criteria we could therefore select from these testing rounds all cases where both a lab and a field test were performed with the same compound and organism. This resulted in the following dataset: 31 pairs of tests with an OP-resistant *T. pyri* strain; 28 such pairs for a susceptible *T. pyri* strain; and 6 for *A. andersoni* (=*A. potentillae*). We also had at our disposal 66 field experiments with *A. finlandicus*. Because this species was never tested in the lab we have compared the outcomes of these field trials with the corresponding laboratory test for *A. andersoni*, assuming their toxicology would not be too different. Given the ecological similarity between these two species this seemed otherwise a legitimate comparison. From

table 1 it appears that this was indeed the case.
Tests performed within the frame of IOBC's joint pesticide testing programme may lead to four hazard classifications at each level of testing (*cf.* Hassan 1985 for class boundaries). However, the tests described here can only lead to two hazard classifications (harmless and harmful) and, at the first level of testing, to the decision that further testing is required. Hence, for the purpose of validation, some of the IOBC classes had to be pooled such that they would match our criteria. For the lab test (tier I) the hazard classes were harmonized by pooling IOBC-classes 2 and 3. This resulted in three hazard classes separated by class boundaries that corresponded with the criteria given here (*viz.* E=50% and E=99%). For tests performed at tier II the final classification is determined by the effect of a reference compound. For this validation exercise this effect is set at two values, *viz.* 25% and 50%. At the former level the IOBC dataset could be used by pooling classes 2,3 and 4 to obtain one harmful class for effects >25%, whereas the latter threshold could be obtained by pooling classes 1+2 and 2+3.
Because the field test can only have two outcomes and the lab test three, it is possible to assess the predictive value of conclusions obtained in the lab by plotting the classifications resulting from lab and field tests in a 2x3 matrix as shown in table 1. If no datapoints arise at the intersection of the harmless row with the harmful column and *v.v.* the accuracy is maximal. Using the 131 pairs of tests described above the following result is obtained:
At a field effect threshold of 25 %, the laboratory test quite accurately predicts field outcomes. Harmfulness was never erroneously decided upon in the lab when the 99% criterion was used (this illustrates why the optional route on tier 1 is in fact optional). This result remains unchanged if we set the field effect level to 50%. However, in 10 out of 51 cases a product was incorrectly classified as harmless after the lab test. If again the field threshold is raised to 50% this becomes 4 out of 51 cases. Hence, the number of erroneous classifications is 7.6% at a field effect threshold of 25%, and 3% at a threshold of 50%. However, all these cases apply to comparisons involving *A. andersoni* - *A. andersoni* (3x) or *A. andersoni* - *A. finlandicus* (7x). Moreover, none of these incorrect classifications involved insecticides/acaricides. Seven out of 10 concern a fungicide, 1 was a herbicide and 2 were plant growth regulators. The most likely explanation for these deviations is not that these products were more toxic in the field than they were in the lab, but that their side-effect was indirect. In this respect the most conceivable cause is the elimination of alternative food sources. Recent investigations on phytoseiid ecology have repeatedly shown that these may be crucial for the persistence of phytoseiid populations. Mildews for example have been shown to be such an alternative food source. This may help to explain the negative effect of fungicides that were harmless in the lab.

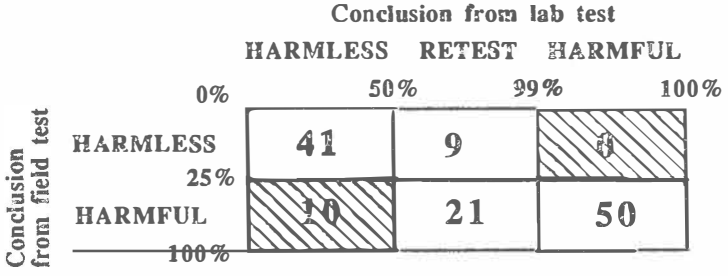

Table 1. Results of comparing 131 field trials with their corresponding lab tests.
Hatched area represents erroneous decision. Percentages refer to class boundaries.

When applied to *T. pyri,* the decision criteria at level 1 given in fig 4 never lead to erroneous conclusions (the matrix for *T. pyri* would read from left to right: 19; 2; 0 and 0; 17; 21). This also holds when the field effect threshold is set at 50%.
With respect to the efficiency of the sequential decision making scheme given here it is significant that

the lab test proved to be a powerful tool for reducing the number of field tests required. In only 30 out of the 131 examples the lab test produced inconclusive results making it necessary to retest in the field. This retesting led in 21 cases to a harmful classification (raising the field effect threshold to 50% will still lead to 20 harmful classifications). Oomen *et al.* (1991) reached a similar conclusion for their sequential decision making scheme deviced for *P. persimilis*. In their case out of 139 tests, 83% could already be classified after the laboratory test alone, which compares well with the 78% given here. In their case the lab data were not validated with field experiments but with field experience.
Finally it remains to be discussed why the persistence test cannot be used to decide upon harmlessness. This will be done using data from Oomen *et al.* (1991). In 4 cases a persistence test was actually followed by a field experiment. The effect levels measured in the persistence test were resp. 19, 0, 7 and 0, but only in the latter case (dinocap) was the product harmless under field conditions. Clearly the latter clasification cannot be determined with a persistence test. Whether harmfulness can be correctly judged using the criterion given here remains to be established. Therefore the persistence test remains optional. When validating this test it may appear that it is not a very efficient tool for reducing testing efforts. Oomen *et al.* performed 9 persistence tests, of which only two resulted in a definitive classification.
It can be concluded that, based on the validation given here, the criteria given in fig. 4 can be safely used to classify products either as harmless or as harmfull after the first laboratory test. How robust this conclusion is was demonstrated both by the insensitivity of the validation to varying field effect thresholds and to the species used for the validation. With respect to the former this means in practice that the harmless reference product can be chosen such that the effect lies between 25% and 50%. The latter observation is also interesting. Because it appeared that, with the afore mentioned few exceptions of *A. andersoni - A. finlandicus*, the lab classifications correctly predict the outcome of field trials *regardless of the species tested*, it might be argued that it will suffice to consider only a single phytoseiid species as an indicator of the hazardousness of pesticides. The fact that no differences were found between a susceptible and a resistant strain of *T. pyri* corroborates this argument. Moreover, the validation given here also seems to apply to *P.persimilis*. Apparently the differences between harmless and harmful are much larger than interspecific differences in susceptibility. Clearly, choosing only one species as an indicator would largely increase the efficiency of phytoseiid side-effect testing procedures. Nonetheless, for each individual species the use of the criteria given here will already reduce the amount of field tests with ca. 80% (given that all products would otherwise be tested in the field). This is an improvement in efficiency of 40% when compared to a decision making scheme where all products initially classified as harmful are retested in the field (*cf.* Hassan 1985, Samsøe-Petersen 1990). This improvement in efficiency is not offset by a loss in accuracy with respect to the final classification. The testing schedule given here thus complies with the objectives of procedures having to be quick, inexpensive, efficient and reliable.

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APPENDIX I) **Rearing protocol for phytoseiids**

At present two main ways of rearing phytoseiid mites are used. The first method of rearing uses an anorganic material (such as polythene or glass) as substrate for the predators, whereas the second group of rearing methods uses a variety of organic substates, such as detached leaves or wheat bran. In the following descriptions of either method will be given.

Anorganic substate rearing method:

Since this method was first published by McMurtry & Scriven (1965) it is mostly referred to as the McMurtry-Scriven method. The protocol given here serves only as guideline and is open for amendments and tuning to locally available materials. It should be pointed out that the advantage of this method over the organic substrate methods lies in the ease with which cultures can be handled and the low chance of contamination with other species than the desired one. Moreover, in those cases where the phytoseiids to be reared do not necessarely rely on prey material, but can be fed with *e.g.* pollen this method enables the rearing of phytoseiids without the labour intensive concurrent rearing of prey material. The disadvantage of the method is that not all species of phytoseiid mites can be reared on anorganic substrates; a good example are mites of the genus *Phytoseiulus*. However, where the method can be applied successfully it should be preferred over any other method, at least as long as the objective is to establish a maintainance culture. This method is appropiate for o.a. *T. pyri, A. andersoni, A. cucumeris* and *A. barkeri*. For the establishment of mass-rearings other methods may be more appropiate, though not necessarely.

Description of the McMurtry-Scriven method:

Material requirements for 1 unit:

-1 plastic tray
-1 sponge
-1 black tile (*e.g.* polythene, but rubber is an alternative option)
-4 tissue papers (Kleenex or equivalent)
-small piece of transparant plastic sheet (1 cm x 1 cm) used for overhead projection
-tanglefoot caterpillar glue
-small piece of cotton wool (optional)

Procedure for assembling:

I have not given sizes of tray, sponge an tile since the size of the rearing depends on the number of predators initially introduced and ultimately wanted. For our maintainance cultures in Amsterdam the size of the tile is approximately 8 x 15 cm. What is important is that between the sides of the tray and the tile a barrier of free water exists of at least 2 cm. The size of the sponge should be similar to that of the tile. Setting up the rearing unit is at follows:

1) Fill the tray halfway with water
2) Put the sponge in the middle and soak it until saturation
3) Adjust water level such that is is not higher than the sponge
4) Put the tile on the sponge
5) Cover the edges of the tile (ca. 2cm) with tissue paper wich is folded 3 times, soaked with water and then folded one more time the moment the tissue is put on the tile. The free end of the tissue should be dangling in the water surrounding the sponge. The result should be a tissue barrier of ca. 2 mm along all edges of the tile. A water film between tile and tissue paper should be seen. The function of the tissue is to prevent escapes and to provide a drinking source for the predators (especially important when they are fed with pollen only).

5) Make a tanglefoot barrier along the outermost edges of tile and tissue using a syringe filled with the glue.
7) Fold the small piece of plastic slightly such that is gets a V-shape and put it on the arena
8) Provide food first and then put on predators.

Food provisioning:

With this method of rearing two food sources are important, *viz.* pollen and spider mite eggs. The use of other stages than eggs (e.g. by brushing mites from a leaf onto the arena) should be avoided since they produce web and make the culture messy). Some colleagues find that the use of small amounts of honey (diluted with water and applied with a very small amount of cotton wool) increases rearing success.

a. Pollen:
Not all species of pollen are equally suitable as a food source for phytoseiids, moreover a kind of pollen that is adequate for one species may not be for another. The species of pollen to be used should therefore be determined experimentally. Collection of pollen is as follows: Collect the flowers and cut out the anthers. Put anthers in a petri-dish and store overnight in a stove at 40°C for drying and softening. Next day try to separate as much as possible pollen from anthers. Put pollen in a small vial and store refrigerated. Remains fresh for at least one year. Apply using a brush. When feeding the cultures with pollen it is advisable to wear a dust mask since allergies easily arise (this applies in general to working with living mites). When feeding the cultures try to use the smallest amount of pollen possible, since surplusses get mouldy and allow rapid fungal growth. This leads to deterioration of the culture. Mites require less food than one expects, for a good colony containing, say, 200 females a small tip of a fine brush is more than sufficient. Try to develop a feeling for this and adjust the amount of food to age of the colony and the number of mites present.

b. Spider mite eggs:
The method for collecting spider mite eggs is again due to McMurtry. Needed are a spider mite culture using potted plants (see protocol on rearing tetranychids, app II) and a so-called mite washing apparatus. A detailed description of this apparatus is given in appendix 3.

Maintainance protocol:

Assuming that predator cultures have been established maintaining them is as follows:

Feeding: supply the cultures with food every two days except on the week-end (Mon/Wed/Fri). Also adjust water level during the feeding bouts. As said, try to adjust food supply to colony demand. Remove old pollen and eggs in case of decay. Be careful, however, since often eggs will be deposited in this fungal mess.

Renewing: colonies should not be kept for too long because the tiles will inevitably get contaminated with fungi. As a general rule part of the colony should be transferred to new arenas at least once a week and the motherculture should not be kept longer than 5 weeks. Always label the colonies with species name, date of initialization and number of individuals originally transferred. Now, starting 'subcultures' from a mother culture can be done in two ways. First, it can be done by transferring all eggs present on the motherculture arena (or all arenas that contain eggs) to a newly prepared arena. This means that every colony is started with a cohort of eggs. The advantage of this is that it reduces cannibalism tremendously (females tend to feed on larvae, especially when the only food source is pollen). The total output of a given colony in terms of number of females produces will therefore increase significantly if eggs are regularly removed and used to start a new colony. The more frequent the eggs are collected, the faster overall population growth will be. In case a colony needs to be boosted (e.g. when you need mites for an experiment) remove the eggs every two days. The mite keeper can thus manipulate the rate of colony growth 1) by the amount of food added (though this will usually be a surplus) and 2) by the frequency with which eggs are transferred to new arenas.

However, collecting eggs can sometimes be a tedious job, especially in those cases where eggs are randomly distributed over the arena or deposited in the pollen mass. This leads us to the function of the small V-shaped shelters: they are put on the arena because many phytoseiids tend to deposit their eggs under such a shelter. This of course facilitates their collection. If this doesn't work for the species you want to culture there is another trick, i.e. put some (very very few) threads of cotton wool on the arena (e.g. under the shelter). The mites will deposit their eggs on the threads. If this trick also doesn't produce the desired result (i.e. the aggregation of eggs) you have to reside to the second way of renewing cultures. i.e. by transferring adult females. Once a week a random subset of 50 females should be taken from the mother culture to start a subculture. Although much less laborious this method is also much less productive than working with egg cohorts.

General: Always try to keep good track of the development of your colony. Monitoring should be once a week and numbers of females and eggs should be recorded and filed. By doing so you will be able to diagnose problems at an early stage. One special warning regards a disease that frequently occurs in rearings of mites; microsporidiosis. This disease is caused by a protozoan (microsporidium) and may lead to the extinction of your colony. It can easily be diagnosed because the microsporidium changes the appearance of the mites into pale whitish slow moving apparantly sick creatures. Try to remove as soon as possible the healthy looking individuals and keep watching those. Burn the others.

In case you are culturing more than one species at the same time utmost care should be taken with respect to contamination. Try to have one brush for each colony, also have one brush for each food source. Store the brushes in water, or surrounded by water (to handle pollen you need a dry brush). When feeding never touch the arena with your brush, drop food on from above. Try to isolate different cultures as much as possible. Our cultures are standing in water basins in which water is continuously running, so that escaping mites will be washed away. Try to make a cover for each colony (e.g. aquarium or insect cage upside down). Don't make it too low, for your humidity will be too high. At regular intervals make identity checks for your cultures.

Conditions of the rearing room: Many phytoseiid mites are extremely sensitive to drought. Species are known of which eggs no longer hatch at humidities lower than 75%. Therefore humidity in the rearing chamber (or at least at the level of the rearing arena) should be 80%. Too high humidities will make your cultures deteriorate too rapidly. A convenient temperature for rearing is 25°C. However, this is not as critical as RH, let the temperature setting depend on what you can easily obtain locally.
As said, arena's should be well separated and preferably be in a water basin (some acarologists use cages on legs standing in oil). Do not cram too many arena's in one room Have a microscope in the rearing chamber. Try to avoid walking with cultures. If you have to, put them in a well closed cage so that no wind will blow over them.

Organic substrate rearing method

In case all attempts to use the above described method fail because the predators keep on running from the arena into the glue barrier you will have to use the so-called detached leaf cultures. As will be clear from the below this in fact is an extremely simple way of rearing predacious mites and one might ask why we should not rear all phytoseiids in this way. There are two imperative reasons to avoid using detached leaf cultures. First: manual contact with the colony is unavoidable. Second: contamination risk (both with and of other species) is very high and contaminants are dificult to detect at an early stage. However, for those cases where it is unavoidable (e.g. *Phytoseiulus*) the following method is appropiate.

The brick method

Material requirements:

- A large tray
- a brick

- a metal grid
- a cover cage that fits into the tray, tanglefoot

Procedure:

Fill the tray with water, place the water-saturated brick (or flower pot) in the middle and put on the metal grid. Cover with the cage. Put some spider mite infested leaves on the grid (they should not touch the wet brick) and introduce predators.

Maintainance:

Add well infested leaves every other day (ca. 4 large bean leaves). Drop them on the culture without touching the older leaves. Continue doing so until the pile gets too high. In this case take off the youngest leaves with a forceps and put them on a new rearing unit. Add fresh leaves. Immediately wash the forceps. Store in water. Similarly the culture can be augmented by regularly taking off a few leaves to start new (sub)colonies.

The leaves to be chosen when using this method should be vigorous succulent leaves that do not deteriorate too quick. We use bean leaves (*Phaseolus vulgari*) var. Arena.

Appendix II) Rearing protocol *Tetranychus urticae*

The following protocol describes a rearing method for the spider mite *Tetranychus urticae* and is especially suitable for rearing these mites as a food source for predatory mites reared according to McMurtry & Scriven (Arena method) or/and according to the "brick method". The tetranychid mites are reared on bean plants (*Phaseolus vulgaris*; variety 'Arena'). All stages of the rearing from sown seeds to well-infested plants are placed in a single room or greenhouse in order of age. When the oldest plants are removed they are replaced with pots containing freshly sown seeds. This prevents moving- or walking with the plants and thus infesting too young plants. Rearing conditions in the growth chamber are 25°C and ca. 80% RH.

The following procedure is for a harvest of ca. 70 well infested leaves three times a week and in addition to that for feeding about 1000-1500 individuals three times a week according to the Mc. Murtry & Scriven method by using the mite washer. When other amounts are required the number of plants can be adapted to this.

- Fill about 24 flower-pots (12X12X12 cm) for 2/3 with soil. Sow in each pot 12 seeds of *Phaseolus vulgaris* (variety 'Arena'). Cover them with 1/2-1 cm soil. The pots are placed in a square, close to each other. The space between the pots should be about 3 cm or less to ensure a closed canopy that enables the mites to walk from one plant to another. After sowing some water is put on top of the soil. Sowing is on Tuesday and Friday.

- After ca. two weeks, when the top leaves (true leaves) appear, the plants can be infested with *T. urticae*. True leaves are recognized by their trifoliate shape (in contrast to the single-, heart shaped cotyledon leaves). Infest the plants with leaves from the oldest 'well infested but still a little green' plants. Spread about 20 of such leaves homogenous over the new plants. The exact amount of infestation should be determined experimentally. From three days after infestation the plants can be used for harvesting leaves for the phytoseiid rearings (brick method). These leaves should be well infested but still mainly green. The cotyledon leaves are usually not well infested.

- During the rearing routine the plants should be regularly pruned to obtain a more or less homogeneous canopy. Only well developed leaves are suitable for rearing with detached leaves. The new shoots that are cut off during pruning can be used for collection of eggs in the 'mite-washer'.

- When the plants are not used anymore for harvesting leaves they can be cut and used in the mite-washer in order to collect the eggs. These plants should still be at least a little green and should be

cut a few centimeters above the soil. The plants can be collected in one or more plastic bags and either immediately used or stored (at most one week) at 4°C.

- Water all the plants three times a week; Monday, Wednesday and Friday. Apply this water on the soil and not on the plants because this will wash the mites from the leaves. The amount of water given must be determined experimentally. Both too much water and not enough water will cause plant stress.

It is very important that the rearing is checked regularly (at least once a week) for contaminations, especially predators of the tetranychid mites. The check can be done by taking leave samples randomly from all parts of the rearing and verification with a microscope. Clearly as many precautions as possible should be taken to prevent contamination of the rearing. The best precaution is to give one and only one person access to the spider mite rearing. This person should never enter the predator rearings or other evil places.

Appendix III) Collecting spider mite eggs

To obtain a more or less purified egg mass a so-called mite washing apparatus is used. In short the apparatus does no more than rinsing mite-infested leaves and pouring the waste water over a series of sieves that separates as much as possible eggs from other stages. In figure 5 a visual description of the washing apparatus is given. It should be emphasized that this apparatus is only one of the possible forms such a device may take. For example, the central oulet tube (collector tube) may also be peripheral. This is much easier to accomplish. In Amsterdam compressed air is used to achieve agitation, however, if compressed air is not available reasonable results may also be obtained using a strong jet stream of water. As will be clear from the description hereunder we use 10 sieves to obtain a rather pure egg mass. Clearly this is quite an investment. The result will already be quite good with 4 or 5 sieves (250 µm, 125 µm, 106 µm and 90 µm). As with the rearing protocols, this mite washing protocol is open for amendments and tuning to local circumstances and materials. A final remark is that the method as it is described here was developed for separating eggs of *T.urticae*. Although the procedure will remain unchanged for other mite species, the material requirements may differ slightly. Since not all spider eggs have equal dimensions especially sieve mesh size must be tuned to egg size of the spider mite species that is going to be worked with.

Procedure:

1) Collect a (shopping) bag with spider mite infested leaves, preferably young leaves where relatively (compared to the other stages) more eggs will be found.
2) Check whether sieve order is correct. It should be from top to bottom: 500-; 350-; 250-; 180-; 125-; and 90 microns.
3) Fill the cylinder for about 2/3 with water. Optionally a very low concentration of detergent may be added (such as Tween or Triton).
4) Put the leaves in the tank of the mite washer. Use an amount that still allows agitation of the water and circulation of the leaves. You may add to the latter by stirring with a stick.
5) Agitate for ca. 1 hour thereby ensuring a continuous overflow of water through the sieves via the collector tube. Water flow should not be too strong, and adjusted such that (almost) no leaf material goes through the collector tube.
6) Check regularly whether sieves are overflowing. If so, decrease or even stop water flow.
7) After one hour stop agitation and water flow. Empty the contents of the cylinder through the sieves using the water outlet valve.
8) Remove sieves for further handling. The intermediate result should be as follows:

500 microns	: Large particles of plant material
350	: Smaller plant debris
250	: Adult females
180 and 125	: Mixture of all mite stages
90	: Eggs, fecal pellets

9) Further isolation of the eggs by repeated rinsing of the sieves:

a) Rinse the sieves under the water tap in order of decreasing mesh size. Remove each sieve after rinsing.

b) Separate sieves 125 and 90 and rinse their contents through a new stack of sieves consisting of a 125-; a 106-; and a 90 micron sieve. Do this by putting them upside-down on the new stack under the tap until all material is removed.

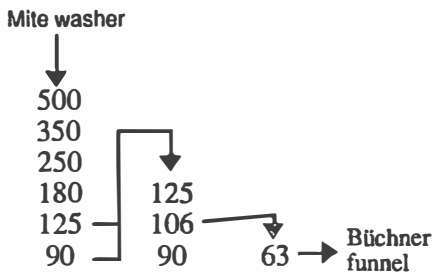
c) Rinse sieve 125 of the <u>new</u> stack thoroughly (though gentle) until most eggs are removed. Check this with a microscope.

d) Rinse sieve 106 shortly and very gentle. This sieve should contain the pure egg mass. Check this with the microscope.

e) Check the contents of sieve 90 with the microscope. If 106 was rinsed too thorough many eggs will be in this sieve. The contents will ,however, be polluted with debris, such as fecal pellets. In this case re-sieve through 106 and 90 microns, until most eggs are in 106.

f) Transfer the contents of sieve 106 (and optionally 90) to a 63 micron sieve by strongly flushing it.

Schematically the whole procedure is as follows:



10) Transfer the contents of sieve 63 to a Büchner funnel using plenty of water. Connect the vacuum pump and dry for ca. 1/2 hour. The optimal drying time should be determined experimentally by regular microscope checks. The eggs should remain round and shiny, but not wet. Instead of using the vacuum pump, the eggs can also be dried with a fan above the funnel.

11) Remove eggs from filter paper by scratching with a scalpel . Store in small vial with lid in the refrigerator. Eggs will remain fresh for at most 1 week.

12) Put the eggs on the arena using a brush. Before doing so it may be wise to loosen the egg mass with a needle.

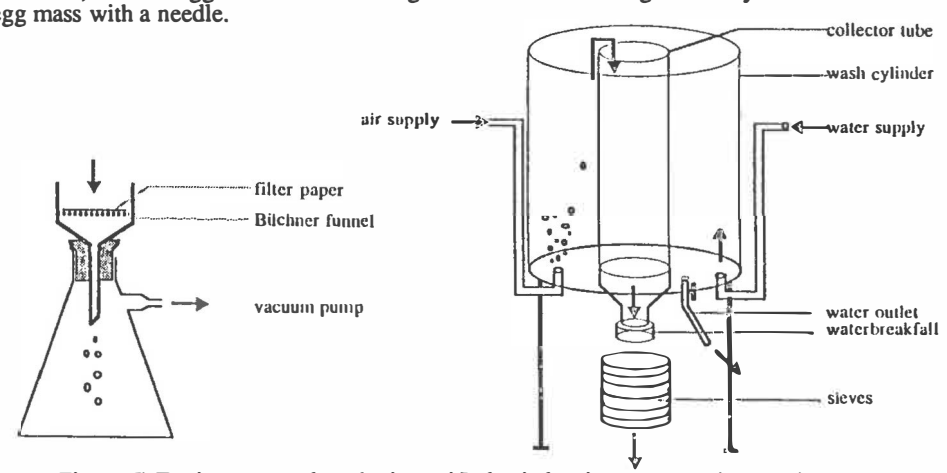


Figure 5) Equipment used to obtain purified spiedr mite egg mass (see text)

LABORATORY METHOD FOR TESTING SIDE-EFFECTS OF PESTICIDES ON THE ROVE BEETLE *ALEOCHARA BILINEATA* - ADULTS

LISE SAMSØE-PETERSEN

Research Centre for Plant Protection, LYNGBY, Denmark

The rove beetle *Aleochara bilineata* (Gyll.) is known to be an important natural enemy of onion flies, cabbage root flies, turnip root flies and others (e.g. Read 1962, Wishart et al. 1956, Andersen 1983).

The ***adults*** are polyphagous predators living in and on the soil near cabbage, onion, beet, bean and lupin plants as pupae of flies living on these plants are the hosts for the larvae. Their lifespan may be as long as three months.

Eggs are deposited in the soil near fly-infested plants, and the newly hatched ***larvae*** (carrying resources for about three days of activity) move around until they encounter a host pupa. The pupa is examined by the larva and, in case of acceptance, the larva gnaws hole, enters the pupa, and closes the hole with an intestinal secretion. Thereafter, development and pupation take place inside the host puparium from which the adult emerges about six weeks later.

REARING OF THE INSECTS

The rearing of *A. bilineata* and larvae of the onion fly, *Delia antiqua* (Mieg.), took place in controlled environment cabinets at 22±0.7°C, 90±10% RH and a 16 L:8 D photoperiod (50-75 LUX). Adult onion flies were kept in a room with controlled environment: 22±2°C, RH = 65-85% and strong light (approx. 2000 LUX).

For rove beetles the procedure followed the method of Hertveldt et al. (1984), while the rearing of onion flies was based on the description by Ticheler (1971).

A detailed account of rearing procedures is given in Samsøe-Petersen et al. 1989.

PREPARATIONS FOR THE TEST

EXPERIMENTAL CONDITIONS

All experiments were carried out in controlled environment cabinets at 22±0.7°C, 90±10% RH and a 16 L:8 D photoperiod (50-75 LUX). These cabinets were isolated from the ones used for the rearing.

To ensure that no residues of previously used pesticides interfered with the tests, the sand was discarded after each experiment, and glass cells were washed in a detergent (Deconex 11 R). Residual analysis had proved that this cleaning was sufficient for removing residues from glass surfaces (detection limit: 0.06 μg/10 cm^2). The plastic tubes for ventilation were washed in 70% alcohol. They were always used in the same places: the clean ones at the air inlet and the others, possibly contaminated, at the outlet.

PREPARATION OF THE PREDATOR/PARASITE

Adult females of *A. bilineata*, 7-14 days old, were chosen as suitable objects for this test. They were mated and matured and therefore egg production was maximal. Three days before the test the rove beetles were offered fly eggs to let them adapt to this diet.

The sexes could not be distinguished morphologically unless the beetles were immobilised under the microscope. As anaesthesia might have influenced the susceptibility, sexing was done by observation of matings: Immediately before the test, the rove beetles were placed in a large petri dish with moist filter paper and food. Here they were observed, and the females were picked up when copulation took place. The males often tried to copulate with other males. In this case the act was interrupted after a few seconds whereas a proper mating usually took 10-30 seconds.

With a little practice, this selection of females was very reliable, though a quicker method would be desirable. Among the first 176 beetles so selected 8 did not lay eggs. Among the next 184 only 2 did not lay eggs. The egg-laying ability of untreated females was the relevant feature in the test, and so this method seems preferable to sexing by examination of genitalia.

PREPARATION OF FOOD FOR THE PREDATORS

Adults in experiments were fed with house fly eggs, killed by freezing. Small pieces of filter paper (8 mm diameter) were moistened and provided with at least 80 fly eggs each. One piece of paper was given per adult every day during the test.

PERFORMANCE OF THE TEST

EXPERIMENTAL PROCEDURE

The test took place in glass cells with washed, ovendried, moistened sand as substrate and with forced ventilation changing the air about once every minute - a rate that did not disturb the beetles.

Each glass cell measured 5 × 10 cm and consisted of three layers.

1. The bottom was a 1-cm-thick glass plate with a circular hole in the centre (3.5 cm diam.) to which a 3 mm glass plate was glued with an inert glue.
2. The middle layer was another 1 cm glass plate with a 3.5 cm central hole and with two 5 mm holes at right angles to the central one.
3. The top was a 3 mm glass plate closing the cell.

The three parts of the cell were kept together by means of two rubber bands.

In each test nine cells were treated with water and nine with pesticide.

At the start of the test the bottom part was filled with dried sand (0.9-2.0 mm) which was moistened and sprayed, the plastic tubes for ventilation - covered by a fine gauze (100 μ) - were placed in the small holes in the middle part, the test animals were put into the cells, which were closed (sealing the space between layers with a drop of water) and connected to a vacuum pump for ventilation.

On the first day the glass cells were set up. Dried sand was filled into the bottom part of the cell, moistened with 3 ml of demineralised water (20% w/w), sprayed, provided with fly eggs as food, and one adult female was placed in each cell.

On day 2 to 4 survival was recorded, and the old filter paper with fly eggs was exchanged for a new one with at least 80 fresh eggs.

On the 5th day the test was ended and survival was recorded. The sand was washed in a 475 μ mesh sieve, through which eggs of the predator were washed onto a fine mesh (200 μ). They were then washed onto a filter paper, counted and returned

to the controlled cabinet. After 10 days, hatching was recorded by counting the unhatched eggs left on the petri dish.

The following data were now available from each cell (each individual):

1. Survival, 2. Egg production, 3. Hatch of eggs.

APPLICATION OF THE CHEMICALS

A Potter Precision Spray Tower was used with a pressure of 0.68 atm and 6 ml. of solution per spraying, giving a deposit of 6.0 $\pm$0.2 μl/cm^2. (6 μl/cm^2 is equivalent to 600 l/ha.). The concentration of the pesticide was the maximum recommended for practical use. Formulated pesticides were dissolved in demineralised water when possible.

Pure active ingredients that could not be dissolved in water were dissolved in acetone. This was done by making for instance a 1% solution in acetone, 5 ml of which were dissolved in 95 ml of water (0.05% active ingredient in the aqueous solution). This required 18 rove beetles for control - nine treated with water and nine with acetone in water in the same concentration as in the test solution. With magnetic stirring while samples were taken, the pesticide remained evenly distributed in the water. This was documented by residual analyses with chlorfenvinphos, but should be checked for other chemicals dissolved in acetone (see also p. 6).

Only the bottom part of the cells (filled with moist sand) were sprayed. To avoid unnecessary application of pesticides on the glass plates, the cell was covered with a filter paper mask with a 3.5 cm hole over the sand during spraying.

The pesticide solution was applied to the vertical walls of the cell by a fine brush. The whole cell was assembled after drying. This means that the only untreated areas in the cell were the ceiling and the mesh covering the ventilation holes. The rove beetles could not enter the ceiling, and usually they did not sit on the mesh. No chemicals with a repellent effect were used, but if a pesticide should be repellent enough to keep the rove beetles on the mesh, they would neither eat nor lay eggs. Floun coating of the walls was tried, but the rove beetles were stressed by this and spent all the time trying to climb up the smooth surface.

ASSESSMENT AND EVALUATION

The egg production (and hatch) gave an overall picture of the secondary effects on the rove beetles and revealed reduction in fecundity by the pesticides. Furthermore,

as a linear relationship between egg production and predation was demonstrated, the first may reflect the latter. The results from 71 treatments representing 828 individuals were pooled. The mean predation and egg production were calculated for each treatment, yielding the following regression:

Number of eggs laid = 0.244 x number of fly eggs eaten ÷ 7.832
and an $r^2 = 0.85$.

INTERPRETATION OF RESULTS

The results were interpreted according to the standard used by the IOBC working group "Pesticides and Beneficial Arthropods". The reduction in beneficial capacity was calculated as percentage reduction in treated compared to untreated cells. The pesticides were then categorised in the classes 1-4.

The difference between ***egg production*** in the control and in the treated cells (with the concentration recommended) was tested by an analysis of variance. If the difference was significant at the 5% level, the reduction caused by the pesticide was calculated. The ***hatch of eggs*** was tested with a Chi-square-test (hatched/unhatched eggs in treated/ untreated). If reduction was significant at the 5% level, it was estimated from the mean of the fraction of eggs hatched from the 9 untreated and the 9 treated cells respectively.

DISCUSSION

The number of replicates

The test was performed on batches of nine individuals for the control and nine for treated. Based on 57 untreated rove beetles (with a mean egg production of 52.75) 90% confidence-limits were calculated as 37.52-67.98 (n = 9), or the mean value ±29%. Obtaining a 90% confidence interval of only ±20% of the mean would require 25 individuals per treatment, which is far too laborious (and expensive) to be justified by the increase in precision.

In standard use of the test it should be checked that the mean egg production is about 50, and that the standard error does not to any considerable degree exceed 8. This should ensure that all the test insects are females in a good condition.

Reproducibility of the test

Survival in this test was high. Survival of the 649 untreated individuals used during the development of the method was 95%.

Egg production was examined for the 57 untreated females offered 80 fly eggs per day. If the method was reproducible the results from these six different experiments (one including 12 untreated individuals) should be similar since they were drawn from the same population. To investigate this the results were tested for normality ($p > 0.15$) and an analysis of variance was performed ($P = 0.81$) indicating homogeneity of the results. Hatch of eggs was examined in a similar way. The test of normality yielded $P > 0.15$ and the analysis of variance $P = 0.40$.

Thus egg production as well as hatch of the eggs laid are reproducible in untreated beetles.

Homogeneity of results from water- and acetone-treated controls

As acetone has to be used for dissolving certain active ingredients, results from 4 tests with water-treated as well as acetone-treated controls were analyzed as described above. The results show that acetone has no effect *on egg production or hatch of eggs.*

Reproducibility of the pesticides exposure

Four identical tests were performed with 0.05% chlorfenvinphos dissolved in 5% acetone in water as described above (p.4)

In these experiments most of the rove beetles (67%) survived, but in poor condition, and the egg production was low.

A Kruskal-Wallis test was used to test for homogeneity of the numbers of eggs laid and a Chi-square test to examine whether the numbers of hatched/unhatched eggs differed. In both cases, differences between experiments were insignificant ($P = 0.41$ and $P = 0.2$).

Conclusion

The test based on nine individuals per treatment is considered reliable and reproducible as far as the basic setup is concerned and also as regards the exposure and reaction of the rove beetles and the hatching percentage of their eggs to the pesticide treatments.

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LABORATORY METHODS FOR TESTING SIDE-EFFECTS OF PESTICIDES ON THE PREDATORY BUG ORIUS NIGER WOLFF.

ir. M. Van de Veire
University of Ghent
Faculty of Agricultural Sciences
Laboratory of Agrozoology
Coupure Links 653 B-9000 Ghent Belgium

Summary.

Orius spp. appear most promising for the control of the western flower thrips, **Frankliniella occidentalis** in glasshouse sweet peppers and cucumbers.

We have found **O. niger**, a native predatory bug, in glasshouse peppers in the fall of 1990, and were able to rear the insect in appropriate amounts for laboratory purposes (development of test methods to study the side-effects of pesticides).

At first, we have exposed adults and nymphs to spray residues on glass plates (lab a and b tests) for 4 days followed by a check of the mortality, egg deposition on pepper plants, egg hatch, and nymphal viability (initial contact toxicity test).

In an extended laboratory test, we have sprayed plants, containing eggs which hatched soon after treatment. The test was meant to evaluate the effect of a pesticide treatment on the young nymphs, the most sensitive stages. The latter was done by checking the number of surviving nymphs/adults. These tests allow to classify products according to their harmlessness.

Finally a lab test for persistence was developed to determine the duration of the harmful activity of pesticides which were toxic in the lab a and b tests. Plants are sprayed with appropriate concentrations of pesticides. At different times, adult **O. niger** are put on the plants, and assessment of mortality, fertility and fecundity is done afterwards.

1. Introduction.

Orius niger Wolff, a native predatory bug in Belgium, looks very promising for the control of the western flower thrips, **Frankliniella occidentalis** Pergande, in glasshouse vegetables (sweet pepper, cucumber, etc.).

The insect is a predator not only of thrips, but also aphids, mites, small caterpillars, and other arthropods.

It appears as if **Orius** will replace the predatory mite **Amblyseius cucumeris** for thrips control in the near future. This is mainly due to its polyphageous character and its excellent predatory capacity. Also the fact that predatory mites do not kill adult thrips while **Orius** does, makes the bug interesting for biocontrol.

We found native **O.niger** adults in a sweet pepper greenhouse near Ghent in the autumn of 1990 (1)(2). We reared the insect, as a test organism for testing the side effects of pesticides. All tests were laboratory tests. They may serve to classify pesticides as harmless or harmful.

2. Rearing of O. niger.

Adult **O. niger** (10 males and 10 females) are put in containers, containing a small sweet pepper plant, and fed eggs of **Mamestra brassicae**. Sterility of the eggs is not obligatory as **O. niger** feeds on small larvae up to the third larval stage.

The containers are placed in a plastic support at D/N 24°-20°C in the laboratory, under a light regime of L/D : 16/8. Each support contained 10 cages with the roots of the small plants in water.

The females are kept in the containers for 2 days, to obtain a homogeneous nymphal population. They insert their eggs in the tissue of the plant veins or petiole. After 2 days the insects are removed from the containers, and may be used again for rearing purposes.

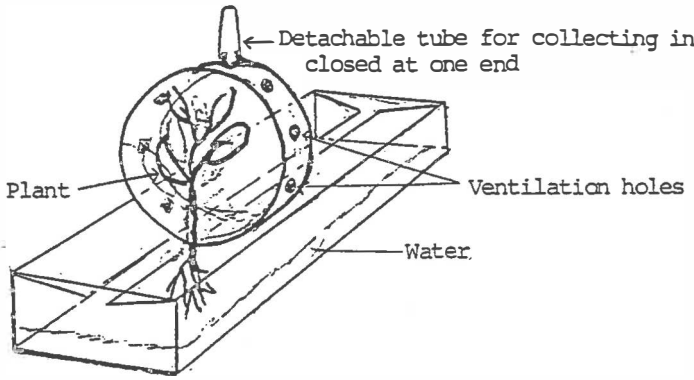
Eggs start to hatch after 4 days, and the young nymphs search for food. Again, **M. brassicae** eggs are offered every other day. Small pieces of kitchen paper are put in the containers, which serve as a hiding place for the nymphs, to avoid cannibalism.

After 17 days, adult **O.niger** can be collected from the containers.

Breeding of the plant material is done as follows: spanish peppers are sown in soil, at 20°C and 90% RH; L/D: 16/8. After 2 weeks, young seedlings with 4-5 leaves, are placed in containers, which consist of a plexi ring, with ventilation holes, covered with 2 glass plates (diam:9 cm). (Fig. 1).

Fig. 1.

Test cage for testing toxicity of pesticides on adult **O. niger.**



The plant roots are positioned out of the container and placed in a support filled with water.

Plants are kept at D/N 24°-20°C; 75 % RH; L/D: 16/8. Growth of the plants is slow, which is an advantage, as they have to be kept healthy for about 3-4 weeks. Fast growing plants are less interesting as testing materials because there is only a limited amount of space available.

3. Test procedures.

3.1. Laboratory test: initial contact toxicity of pesticides on **O. niger** adults. (Lab b test).

3.1.1. Principle of the experiment.

Adults of **O.niger**, which are the less sensitive stage as the majority of them hide in the flowers during the day, are exposed to fresh spray deposits of a pesticide on glass plates for 4 days and survivors are then placed on seedlings of spanish peppers in a closed container. The mortality, the number of deposited eggs and fertility is checked.

3.1.2. Materials and methods.

Ten male and 10 female **O. niger** adults (age:0-1 day) are put in test cages (Fig.1.) for 4 days, together with **M. brassicae** eggs, deposited on paper strips.

The cage consists of a plexi glass frame (diameter: 9 cm; height: 3.5 cm), and two round glass plates of the same diameter. After spraying the pesticide on the glass plates, they are fastened on the frame with adhesive tape. During the experimental period, the cages are kept in a climatic chamber (D/N: 24°-20°C; 75% RH; L/D: 16/8). They are placed in horizontal positions during the 4 day exposure period. The cages are ventilated through a gauze covered ventilation hole connected with a tube to an suction air pump.

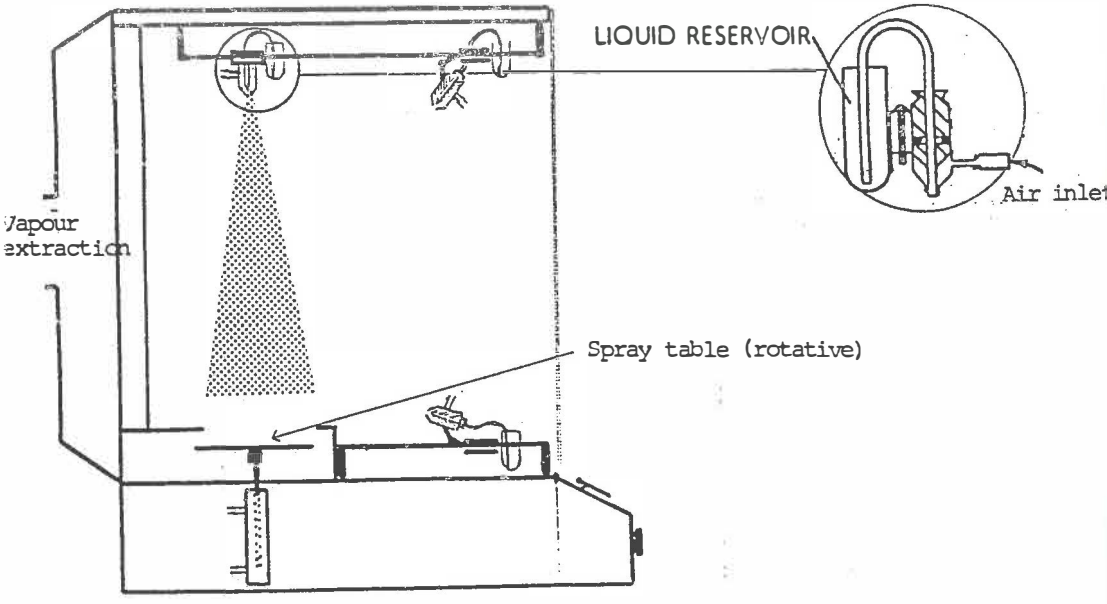
The products are applied at the maximum recommended dosage, to one side of the glass plates, by using a spray chamber. (Fig. 2). This home made spray chamber is superior to the Potter tower.

A very homogeneous spray deposit (1 mg fluid/cm2) is obtained by spraying (2.5 sec) 0.5 ml of pesticide solution (at a pressure of 1 bar) on a glass plate (diameter: 9 cm). A high reproducibility is obtained (s/x . 100 = 6% ; n=10). The plates are then dried and assembled to form test cages.

With the closed spray chamber (Fig. 2), very low volumes of even extremely toxic chemicals can be applied, in contrast with the Potter tower. However, a pull type ventilator is necessary to remove excessive spray mist during operation.

Fig. 2.

Laboratory spray chamber with accesories.



Each treatment was replicated 3 times.

On the 4th day the number of **O. niger** adults surviving the exposure is counted. They are collected and put in another identical cage, which is covered with untreated glass plates, and contains a spanish pepper plant, wherein, the females can deposit eggs. The insects remain in the cage for 3 days. These cages are placed in vertical positions. Eggs hatch from the 4th day after egg deposition. The number of eggs/female and emergence rate is determined 2-3 days later.

3.1.3. Evaluation of results.

The mortality and the mean number of deposited eggs/female and hatching rate in the treatment and the control is determined. The reduction in hatching rate due to the product is calculated as a percentage of the control.

The product is classified according to the 4 evaluation categories agreed by the IOBC-Working Group "Pesticides and beneficials organisms". 1 = harmless (<30%); 2 = slightly harmful (31-79%); 3 = moderately harmful (80-99%); 4 = harmful (>99%).

3.2. Laboratory test: initial contact toxicity of pesticides on **O. niger** nymphs. (Lab a test).

3.2.1. Principle of the experiment.

Nymphs of **O.niger**, which are the most sensitive stage as the majority of them do not hide in the sweet pepper flowers during the day, are exposed to fresh spray deposits of a pesticide on glass plates for 4 days and survivors are then placed on seedlings of spanish peppers in a closed container. The mortality, the number of deposited eggs and fertility are checked.

3.2.2. Materials and methods.

Twenty **O. niger** nymphs (4th nymphal stage) are put in test cages (Fig.1) for 4 days, together with **M. brasicae** eggs, deposited on paper strips. Cage design, methods of application, treatment replicates are identical as described in 3.1.2.

On the 4th day the number of **O. niger** surviving the exposure is counted. They are collected and put in another identical cage, which is covered with untreated glass plates, and contains a spanish pepper plant, wherein, the females can deposit eggs. The insects remain in the cage for 5-6 days. These cages are placed in vertical positions. The number of eggs/female and the emergence rate is determined 6-7 days later.

3.2.3. Evaluation of results.

The mortality of the insects and the mean number of deposited eggs/female and hatching rate in the treatment and the control is determined. The reduction in hatching rate due to the product is calculated as a percentage of the control. The product is classified according to the 4 evaluation categories described in 3.1.3.

3.3. Extended laboratory test for studying the initial contact toxicity of pesticides on **O. niger** nymphs.

3.3.1. Principle of the experiment.

O. niger nymphs, the less protected stage of the predator, soon after hatching, are exposed to pesticide spray deposits on spanish pepper plants, for two weeks and checked on the development to the adult stage. In this test, young nymphs are exposed to pesticides, whereas in the lab a test older nymphs are exposed.

3.3.2. Materials and methods.

The cage design, described under 3.1.2. is used. Previously mated adult male (10) and egg laying female (10) **O.niger** are put in a cage, containing a spanish pepper plant. Three days later (i.e. one day before the hatch of the first deposited eggs) the plant is sprayed thoroughly with the pesticide, in a lab spray chamber, at the maximum recommended rate. Each treatment is replicated 3 times. Water treated plants serve as a control.

The cages are kept in a climatic chamber (D/N 24°-20°C; 75% RH; L/D: 16/8) and placed in vertical positions. Assessment of the pesticide effect is done by counting the number of **O.niger** adults which developed from the egg stage.

3.3.3. Evaluation of results.

The effect (E) of the pesticide is given by the following formula:

$$E = 100 - \frac{Nn \cdot Ne}{N'e \cdot N'n} \cdot 100$$

E = effect of the pesticide (%)
Nn = no. of live adults/no.hatched eggs in the treatment
N'n= no. of live adults/no.hatched eggs in the control
Ne = no. of hatched eggs/female in the treatment
N'e= no. of hatched eggs/female in the control

Classification of the pesticide is done according to the evaluation categories: 1=harmless (< 25%); 2=slightly harmful (25-50%); 3=moderately harmful (51-75%); 4=harmful (> 75%).

3.4. Persistence test on **O. niger** adults.

3.4.1. Principles of the experiment.

The pesticide is applied on spanish pepper seedlings. At different times, (up to 30 days) **O. niger** adults are put on the plants and exposed to the spray residues.

3.4.2. Materials and methods.

Spanish pepper plants are sprayed thoroughly (i.e. upper and underside of the leaves), in the lab spray chamber, with the by the manufacture maximum recommended dosage of the pesticide. Water treated plants serve as a control.

Five, 15, and 30 days post-treatments, the plants are put in a cage (see 1.2) together with adult **O. niger** (10 male and 10 female) of one day old. The insects are kept in the cage for 7days. Each treatment is carried out 3 times.

The cages are kept in a climatic chamber (24°-20°C; 75% RH; L/D: 16/8). Mortality, egg deposition and hatching rate are determined.

3.4.3. Evaluation of results.

Assessment of the pesticide effect is done by comparing the mortality of those **Orius** "exposed" to the pesticide with the untreated control, followed by regular checks of egg deposition, egg hatching and viability of the nymphs.

Classification of the pesticide is done according to the evaluation categories for duration of harmful activity: 1 = short lived (<5days); 2 = slightly persistent (5-15 days); 3 = moderately persistent (16-30 days); 4 = persistent (>30 days).

Aknowledgement.

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GUIDELINE FOR THE LABORATORY EVALUATION OF PESTICIDES AGAINST THE APHID PREDATORY MIDGE *APHIDOLETES APHIDIMYZA* (RONDANI) (DIPTERA: CECIDOMYIIDAE)

by NEIL HELYER
Horticulture Research International, Worthing Rd., Littlehampton, West Sussex, BN17 6LP, UK

Introduction

Aphidoletes aphidimyza (Rondani, 1847) is a non-specific aphid predator with a wide host range, which is introduced to many greenhouse crops where it may become established. The midge also occurs naturally on many field and orchard crops throughout the northern hemisphere. A review of the biology of *A. aphidimyza* can be found in Nijveldt (1988). Adult midges are nocturnal, hiding on the underside of leaves or other dark crevices during the day. The larva feeds voraciously on aphid body fluids and drops off the leaf to form a cocoon on the ground in which it pupates.

Adult midges are exposed to fresh dry pesticide residue. Adult mortality, the number and the viability of eggs is then assessed with time after exposure. Larval mortality is assessed in a separate test. Persistence is determined by treating whole plants and removing leaves at various times after treatment.

Test apparatus

The cages recommended are manufactured by Picador of England (see References) as electric motor mounting blocks which consist of a cast aluminium frame 15 cm x 15 cm 3 cm high and 1 cm material thickness. On the side of each face are two milled slots 1 x 4.8 cm, which in all but one case are covered internally with cotton lawn material (Figs 1 & 2). The last slot is left open to allow insects to be passed into the cage and is sealed externally with sticky tape. To prevent insects from sticking to the tape, a second, slightly smaller piece is stuck over the tape left internally exposed. The top and bottom edges of the cage are fitted with soft EPDM (ethylene propylene dimer) draught excluding strip on to which two glass plates (15 cm x 15 cm) are placed. EPDM strip is soft and makes an excellent filler between the glass and the frame. It is also non-absorbent to many solvents found in pesticide formulations and can therefore be reused several times. The glass plates are held in position by spring clips, thus making an insect proof cage. On one face an additional hole is drilled for connection to an air line. Compressed air is blown at low pressure through each cage via a manifold system connected to an air line. The air is compressed outside the building thus eliminating any chance of circulating contaminated air. As compressed air is very dry, it is humidified by passing it through a sintered glass aerator submerged in distilled water. If compressed air from an external source is not available air can be drawn through the test apparatus under vacuum and then piped out to atmosphere.

1. Test procedure for *A. aphidimyza* adults

1.1 Treatments

Test product prepared and applied at maximum rate for any particular crop.

1.2 Replicates

At least 4 per pesticide plus water treated controls.

1.3 Pesticide application procedure

Pre-cleaned glass plates sprayed under a Potter tower (Potter, 1952) with 1 ml of pesticide. To prevent pesticide contamination of the EPDM rubber strip a perspex template is placed over the glass during spraying, allowing an area of 10.5 cm x 10.5 cm to be treated. Under these conditions, about 160 mg of the spray solution is applied to the glass plate; this is equivalent to 1.45 mg fluid/cm^2 or 320 mg per cage. Treated plates are left to dry at 20°C for 30 minutes before the test cages are assembled.

1.4 Assembly of the test cage

As each cage is assembled, an adult feeding unit (Fig 3) is placed onto the base plate. Adult feeding units are made from two polythene lids (glass-tube stoppers), one of which fitted inside the other. The smaller lid (11 mm internal diameter) is filled with a tissue wick (4 x 1 cm) allowing approximately 1 cm to overhang. The wick is then soaked with 10% sucrose solution. A larger lid (14 mm internal diameter) is pushed over the base lid thus sandwiching the wick between the two lids. The wick is trimmed to leave 2 mm overhanging from which the adults could feed without getting trapped (Fig 3).

1.5 Preparation of adult midges

Test insects are reared in a small insectary (Samsøe-Petersen et al., 1989) where midge cocoons are formed in white capillary matting which is placed in inflated and sealed polythene bags. Bags containing emergent adults are opened and placed in a perspex rearing cage (90 x 40 x 50 cm) on the evening before the test. Adults of between 12 and 24 h old are collected from the perspex cage and introduced to the test cage (15-20 per cage).

1.6 Test conditions

The assembled cages are maintained at 16:8 light:dark and 20-22°C. During adult tests a sheet of white paper is placed over the top of the cages to give a more diffuse light. A count of initial 'handling' mortality is made 30 minutes later.

1.7 Adult fecundity

After the 24 h assessment a section of *Myzus persicae* infested Chinese cabbage leaf is prepared and inserted into the cage. The leaf is mounted, abaxial surface uppermost, on agar (1.2%; Technical N° 3; Oxoid Ltd.) which is poured into a perspex frame (75 x 40 x 5 mm) recessed to a depth of 4 mm (Fig 1).

1.8 Assessments

Mortality is assessed at 24 and 48 h. After the 48 h assessment, the perspex frame with leaf is removed and the number of eggs counted to record adult fecundity. The slides are then transferred to 9 cm plastic Petri dishes with the adaxial leaf surface uppermost ie. normal on-plant position. To prevent the insects being crushed the slide is placed over two pieces of wooden dowelling. Egg incubation at 20-22°C takes 2 days, subsequent egg hatch and larval survival is therefore assessed 5 days later. Any control mortalities are used to adjust experimental mortality by Abbott's correction formula (Abbott, 1925).

1.9 Pesticide persistence

This is determined by spraying large Chinese cabbage plants (1000 l/ha) which are maintained in a glasshouse chamber at 22°C. Leaf material is removed and insects enclosed over the leaf at 1, 3, 7, 14 and 21 day intervals as above.

2. Test procedure for *A. aphidimyza* larvae

2.1 Treatments

Test product prepared at maximum rate for any particular crop.

2.2 Replicates

At least 4 plus water treated controls.

2.3 Preparation of larval test cage

A perspex template 15 x 15 x 0.6 cm with the centre milled out to 10.5 cm x 10.5 cm is placed on to a clean glass plate. Molten agar (technical N° 3) is then poured into the template on the glass (Fig 2). When the agar is at the point of setting, a section of clean, flat Chinese cabbage leaf is placed, abaxial surface uppermost on the agar.

2.4 Pesticide application procedure

The pesticide is applied directly to the leaf in the same way as for the adult tests. When dry, approximately 50 *M. persicae* and 15 first instar *A. aphidimyza* larvae are placed on the leaf.

2.5 Assembly of test cage and test conditions

The cages are assembled with the treated glass plate uppermost and a clean plate at the bottom. The finished cages are connected to the air line and maintained at 20-22°C and 16:8 light:dark throughout the trial.

2.6 Assessments

The number of live and dead larvae are assessed 24, 48 and 72 h after treatment. Any control mortalities are used to adjust experimental mortality by Abbott's correction formula.

2.7 Pesticide persistence

This is determined by spraying large Chinese cabbage plants (1000 l/ha) which are maintained in a glasshouse chamber at 22°C. Leaf material is removed at 1, 3, 7, 14 and 21 day intervals. The leaves are then mounted in agar and either adults or larvae caged over the leaf as above.

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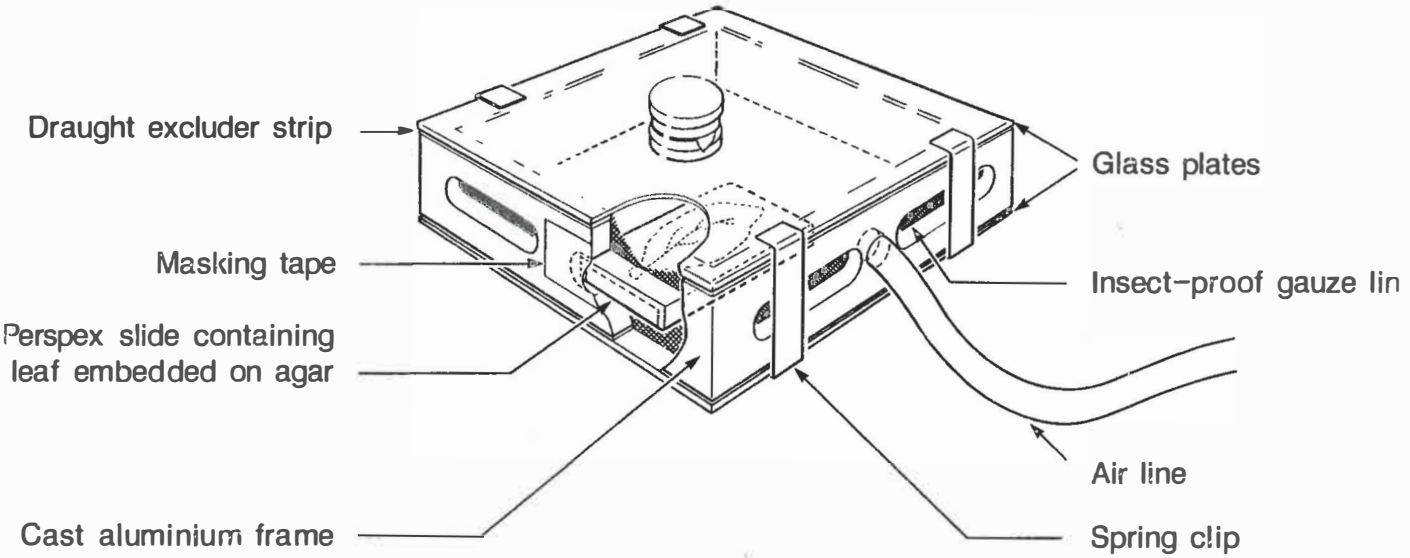
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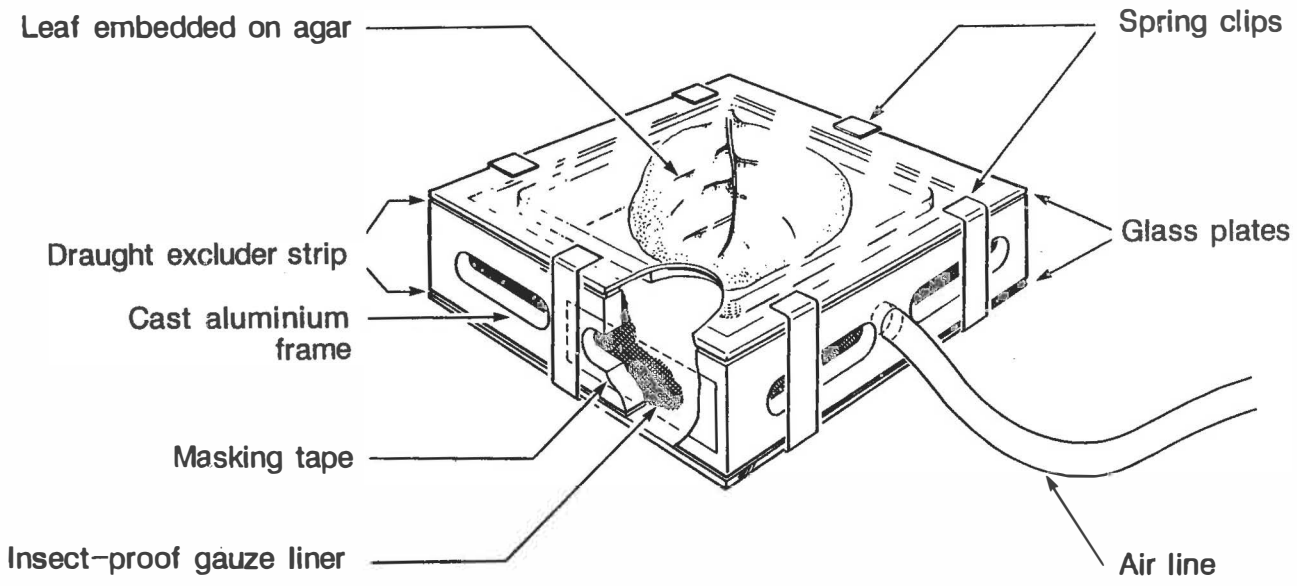
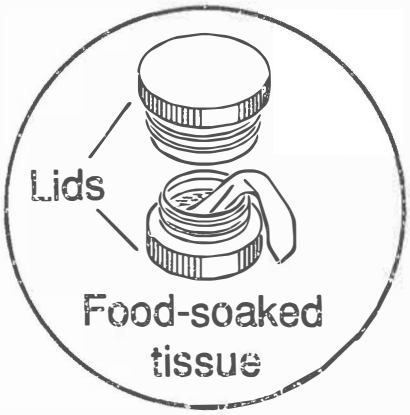
Glass plates
Insect-proof gauze lin
Air line
Spring clip


Leaf embedded on agar
Spring clips
Draught excluder strip
Glass plates
Cast aluminium frame
Masking tape
Insect-proof gauze liner
Air line

Food container/
dispenser



Laboratory method to test effects of pesticides on *Poecilus cupreus* (Coleoptera, Carabidae)

Udo Heimbach
Federal Biological Research Centre for Agriculture and Forestry,
Institute for Plant Protection in Field Crops and Grassland,
Messeweg 11/12, 3300 Braunschweig, Germany

Abstract

A laboratory test is described, measuring effects of pesticides on the predatory ground beetle *Poecilus cupreus* L.. 30 beetles per treatment are sprayed in boxes with sand as substrate. Effect assessment is done at least during 14 days; evaluation parameters are mortality, food consumption and behavioural changes, compared with a water treated control and a toxic standard. The classification of the data is based on IOBC-guidelines.

Introduction

Many methods of testing effects of pesticides on natural enemies of plant insect pests (Hassan et al., 1985) including some concerning carabid beetles (Edwards et al., 1984, Chiverton, 1988) have been developed. Since the new law of plant protection is in force in Germany (1986) some more guidelines have been developed in Germany. The intention of the laboratory tests is, to find out whether a pesticide is harmless. If it is harmful in the laboratory test, further testing in semi-field or field experiments has to be carried out (Brasse, 1990).

Carabid beetles are known as important predators of pest insects. Therefore a laboratory test was designed (Heimbach & Brasse, 1991), to test effects of pesticides on the ground beetle *Poecilus cupreus* L.. Today the breeding of this carabid species and the test described is done by several testing institutions in Germany.

Material and Methods

Test species

As test species *Poecilus cupreus* was selected. It is encountered frequently in agricultural sites, and can be bred easily for many generations (Heimbach, 1989, Heimbach & Brasse, 1991). *Poecilus cupreus* is a predatory ground beetle which is diurnal and univoltine like most carabid beetles and needs to hibernate as adult to reach maturity.

Before oviposition the beetles stay for at least 2 months in a short day cycle (8/16 h) and cold conditions ($\leq$ 10 °C). About 2-3 weeks after changing to long day conditions (16 h light) and 20 °C they start laying eggs. For this purpose 5 males and 5 females live in vessels with wet expanded clay granules and a gauze at the bottom, so that the eggs can be washed out with water. Eggs are placed separately in cluster plates with wet filter-cards to prevent cannibalism. The beetles lay between 75 and 250 eggs per female. Hatching of larvae is controled daily. The hatched larvae are isolated in glass tubes (19 ml) filled with wet peat where they stay until the adult beetles hatch. Larvae and adults are fed twice a week with cut pupae of *Calliphora* sp. or larvae of *Tenebrio molitor*, which had been usually deep-frozen. At 20 °C the time of development from the freshly laid egg to the adult is about 50 days. On an average about 50 % of the eggs develop to adult beetles. The sex ratio of the hatched beetles is about 1:1. Males and females are easily distinguished by their tarsae of the forelegs (figure 1).

Only reared beetles are used for the tests, which are at least 2 weeks old and not older than 10 weeks. Before being used in tests the newly hatched beetles are kept together in groups of about 16 at long day conditions (16 h light, 8 h dark, 20 °C) in boxes of about 19 x 19 cm filled with humid peat.

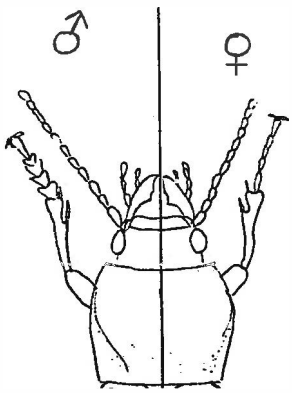


figure 1. Tarsus of the forelegs of a male and female ***Poecilus cupreus***

Test substrate and test-boxes

As test substrate pure quartz-sand (particle size 0,1 - 0,4 mm, 99,7 % Silicium dioxide) is used. 250 g of dry sand is put into plastic boxes (Bellaplast) of 1 l content (dimensions 18.3 x 13.6 x 6 cm height, surface area about 175 cm²). They are closed with a plastic lid, that is cut out up to 1 cm to the edges and closed again with gauze of about 2 mm mesh size.

The upper part of the box walls are painted with "Fluon" (Polytetrafluoroethylene, ICI), preventing the beetles to escape. The sand is moistened to 70 % of its maximum water holding capacity (about 45 g water for 250 g of sand).

One box contains 6 beetles (3 males and 3 females). Each treatment is replicated five times.

Test substances

The control is sprayed with water (equivalent to 400 l water/ha). For the toxic standard usually 1 l/ha Afugan (294 g a.i/l pyrazophos) or another toxic pesticide is used. The negative standard should cause 70 $\pm$ 30 % mortality of the beetles. All tested pesticides should be used in the highest recommended amount for the use in the field. All substances are diluted to a spraying suspension equivalent to 400 l water/ha. Substances which are sprayed with a higher amount of water e.g. in orchards, are sprayed in the tests

with 400 l/ha too, but the concentration of the mixture is increased accordingly.

Application of the pesticide

A qualified spraying equipment is used which resembles field application. Nozzle type, pressure and speed should ensure a realistic particle distribution and particle size. The application has to be done at temperatures between 10 °C and 25 °C in a well ventilated laboratory or outdoor, if there is no wind and no direct sunshine.

Test procedure

The last three days before the application the beetles are kept without food. Right before spraying all beetles are moved to the surface of the sand, missing or injured individuals are replaced, the sand is moistened again to 70% of its water holding capacity and 1 *Musca*-pupa (the integuments of the pupae are punctured with a needle) per beetle is inserted. The walls of the box are protected with an inlet (the same boxes without bottom are suitable for this purpose). Immediately afterwards the boxes are sprayed. Consequently the beetles do not have time to dig themselves into the sand. Thus the application is done onto the beetles, the sand surface and the food.

After the application the wall protection is removed and the boxes are covered with the lid and kept for 2 hours at a well ventilated place. Until the end of the testing period the boxes are kept in a ventilated room (20 °C, light/dark ratio of 16/8 hours, 500 - 1500 lux). A high air humidity of about 85 % is favourable. After 4, 7 and 10 days the water content of the sand has to be checked and water added to 70 % of the maximum water holding capacity.

Assessments

Direct toxicity

2, 6 and 24 hours after the application the boxes are investigated (without digging the sand) for dead and damaged beetles. In case of symptoms in the first evaluation an additionally assessment is carried out after 4 hours. At the 6 hours and at all following assessments knocked down beetles (lying on their backs) are placed

in a distinct corner of the box. If they did not move from this place until the next evaluation interval and they still lay on their back, they are removed from the box and imputed to the dead. Assessments are made also 2, 4, 7, 10 and 14 days after the treatment. Any symptoms occuring (knock down, behavioural changes etc.) and all beetles alive, dead or digged into the sand are recorded. Dead beetles are removed from the boxes. If more than 2 dead beetles are found in a treatment during the second week of the testing period, the experiment for this treatment will be prolonged by 14 days with 2 checks per week. All observations except that of the last assessment are made without breaking the sand, for usually the beetles die on the surface of the sand.

Food consumption

Besides the observation on the beetles another parameter regarding the food consumption is evaluated. The fly pupae are collected 2, 4, 7, 10 and 14 days after the application and recorded as consumed (partly or completely eaten) or untouched. Missing pupae are denoted "consumed". Besides the food given just before the application (6 per box), a fresh pupa is added on day 2, 4, 7 and 10, but only one for each surviving beetle. At the end of the experiment the sand is searched for untouched pupae not discovered. If the food consumption in a treatment is 50 % below the control (in relation to the consumption of the surviving individuals, not the total consumption of all beetles of a treatment), the experiment will be prolonged for 14 days with two checks per week.

Proposal for a time frame

Friday	preparing of boxes and inserting the beetles without food
Monday	recording number and condition of the beetles and adding water and food, treatment application assessments after 2, (4) and 6 hours
Tuesday	Assessment
Wednesday	Assessment, exchanging of food

Friday	Assessment, exchanging of food, adding water if necessary
Monday	" " " " "
Thursday or Friday	" " " " "
Monday	Assessment (if the final one, searching of the substrate for missing beetles and food)

If the experiment has to be prolonged, checking, feeding and watering twice a week for further 14 days.

Evaluation

A test is valid only if the mortality in the control (water treated) is not more than 10 % and if the effects in the negative standard are as usual.

Two criteria are used for the IOBC classification, the assessments of the affected beetles and the average food uptake of the surviving beetles. If the IOBC values for these two criteria differ, the worst value of these two classifications should be used to decide whether semi-field or field tests should be carried out or not.

The number of dead males and females, of sublethal affected beetles and the average food consumption per living beetle of a treatment should be listed per box for each treatment and assessment. If the number of dead and/or damaged (e.g. abnormal behaviour, uncoordinated movements, lying on the back) beetles of a treatment at any assessment carried out 24 hours after the application of the pesticide or later exceeds 29 % (more than 8 beetles of one treatment), it is classified according to IOBC class 2, from 80 to 99 % class 3 and more than 99 % class 4.

The average food uptake of the beetles treated with a pesticide is compared with the food consumption of the water treated beetles. The average food uptake is calculated by adding the five values for each of the 5 dates of food assessment (e.g. 0.5 pupa per beetle for the second food assessment if 14 pupae were consumed by 28 beetles alive at day 2 after application) and dividing it by 5. If it is $\geq$ 30 % less than in the control, it has to be classified like shown above and semi-field or field tests should be carried out in order to reach final classification.

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A laboratory test method for assessing the toxicity of pesticides to the ground beetle Pterostichus melanarius (L.) (Carabidae, Coloeoptera).

G.B.LEWIS, R.A.BROWN, P.J.EDWARDS & L.C.CANNING. ICI Jealotts Hill Research Station, Bracknell, Berks RG12 6EY (United Kingdom)

A method is described for assessing the acute and residual toxicity of pesticides to the carabid beetle, Pterostichus melanarius, a polyphagous predator found on arable farmland. It is designed to be a severe, laboratory-based test, fulfilling the requirements of the first tier of a stepwise risk assessment scheme. The pesticide is applied, with a spray resembling a field application, at maximum field rate to beetles caged individually. Effects are assessed in terms of mortality, sub-lethal effects (e.g. behavioural) and feeding activity.

1. Introduction

Native natural enemies (predatory and parasitic insects) are beneficial organisms that have been shown to be potentially important in limiting the numbers of their prey/host populations (Wratten, 1987). It is therefore desirable to assess pesticides for their safety to those natural enemies which are likely to be exposed to the compounds following recommended field use. In particular, such data are now of increasing importance for use in designing integrated control programmes (Graham-Bryce, 1987).

The method described here is designed to look at the acute and residual toxicity of pesticides to the carabid beetle, Pterostichus melanarius. The toxicity is assessed in terms of mortality, sub-lethal effects (e.g. behavioural) and feeding activity. This is a Tier I test in a stepwise approach to risk assessment. Its objectives are to (1) provide a severe test of a chemical so that those showing no significant toxicity can be confidently classed as harmless with no further testing required, (2) provide information which can be useful in interpreting Tier II and III tests.

2. Materials and methods

2.1 Test species

Carabid beetles are important polyphagous predators which have been shown to be capable of exerting an important influence on the numbers of cereal aphids (Edwards et al, 1979). P. melanarius in particular has been found to feed over a wide range of aphid densities (Sunderland & Vickerman, 1980). It has also been highly ranked amongst polyphagous predators as an aphid predator particularly later in the season when its numbers have built up (as the new generation of adults emerge) (Sunderland et al, 1987).

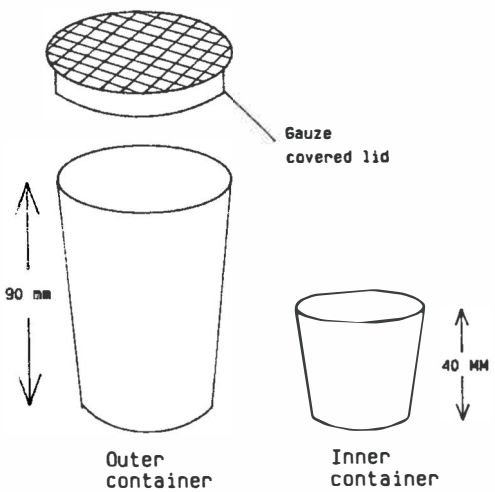
P. melanarius is a relatively large predatory ground beetle 13-17 mm in length (Trautner & Geigenmuller, 1987). It is active on the soil surface and is encountered frequently on arable farmland. It has a univoltine life-cycle: it overwinters both as adults and larvae, the latter emerging from March onwards with adult numbers reaching a peak in late summer and breeding occurring in the autumn. They can be caught in large numbers from about May through to August, particularly on uncultivated land (e.g. grassland) where no pesticide treatments have been made in the past year, and they survive well in the laboratory.

The beetles are collected from the field using pitfall traps and held in large plastic containers (about 37 x 53 x 33 cm) containing moist peat. They are held for at least two weeks before testing in order to acclimatise them to laboratory test conditions: 20°C ± 2°C; 50-70% RH; 16 hour day. The beetles are fed with dead blowfly larvae (Caliphora spp.) during this period (these can be stored frozen and defrosted prior to feeding). Alternatively, laboratory reared animals may be used.

2.2 Test system

The test cage is shown in Figure 1. The outer container consists of a plastic cup, the top and bottom diameter being 7 cm and 4.5 cm respectively and with a height of 9 cm. The lid comprises a plastic ring, covered with gauze, which just fits into the top of the outer container, so sealing it. The inner container is another plastic cup with the top cut off so as to reduce its height to 4 cm. Both containers have a hole made in the centre of the base for drainage (about 2 mm diameter).

Figure 1: Holding Cage



The inner container is filled to the brim with a sandy soil (sandy loam/loamy sand). This should be within the following specifications: clay - 5-15%; sand - 50-85%; silt - 10-40%; organic matter < 3%; pH - 5.5-7.5. The soil is first sieved with a 2 mm sieve. It can be stored in sealed bags at 4°C until required. The short cups (the inner containers) are then placed inside the taller cups (the outer containers) and with the lid in place this creates a cage for holding a single beetle.

2.3 Test substances

Control - deionised water.

Toxic standard - dimethoate (an emulsifiable concentrate formulation), applied at a rates of 700 g ai ha^{-1}. This is used to check reproducibility of method.

Test compound - applied at the maximum field rate.

The test chemicals are diluted to a spraying suspension to give the maximum field rate at an application volume equivalent to 200 l ha^{-1}. This is three times the concentration recommended by the International Organisation for Biological Control (IOBC) for glass plate tests at the same spray volume of 200 l ha^{-1}. According to the guidelines of the IOBC this provides a deposit on soil equivalent to that on a glass plate (the surface area of a soil is about three times that of a glass plate).

2.4 Application method

Applications are made using a hydraulic nozzle which ensures that the spray resembles a field application with a realistic droplet size and distribution. An appropriate applicator would be a hydraulic track sprayer fitted with a single stainless steel jet (Teejet 8001 EVS). The spray nozzle is set to travel at a constant speed, spray pressure (40 psi) and swath height above the target (20 cm) to produce a spray volume of 200 l ha^{-1} $\pm$ 10 %.

Calibration checks are made before and after the treatment application. Two filter papers, 5.5 cm in diameter are clipped together with a paper clip and placed in petri dishes which are in turn placed in 4 positions along the spray swath and sprayed with deionised water. The second 5.5 cm filter paper ensures any excess moisture from the first paper is absorbed. The 5.5 cm filter papers rest on a larger filter paper which absorbs any excess moisture caught in the petri dish. The two 5.5 cm papers are weighed before and immediately after spraying and the weight of the deposit compared to the nominal value thus verifying the application rate.

2.5 Test Procedure

On the day of the test the beetles are removed from the holding cages and placed in plastic pots (diameter 9 cm) lined with filter paper, which hold groups of 10. The filter paper soaks up any excess moisture during treatment. The inner containers of the beetle cages, filled with soil, are placed on wet paper towels, soaked to excess, until water appears on the soil surface of each one. They are then removed and allowed to stand to allow any excess water to drain so leaving them at field capacity.

Both the soil surface and the beetles are sprayed. The replicate number for each treatment is 30. Three plastic pots containing 10 beetles each and 30 inner containers filled with soil are placed in the spray cabinet, aligned along the centre of the spray path. The controls (deionised water) are sprayed first followed by the test compound and the toxic standard (dimethoate). The spray jet is flushed through between applications, first with deionised water and acetone (50:50) and then with deionised water only. Once sprayed, the beetles, and soil filled cups are removed from the spray cabinet, the inner containers are placed in the tall cups and a single beetle added to each and the lids put on.

The sprayed soil is kept moist throughout the test period by watering with deionised water, from the base, on days 3 and 5. This is done by placing the test cages in trays containing wet paper towel and leaving them to stand on this for between 0.75 and 1 hour. During the test the beetles are held at 20°C $\pm$ 2°C; 50-70% RH; 16 hour day / 8 hour night.

2.6 Assessment of test animals

Mortality and behavioural assessments are carried out at 3 hours, 1 day, 2 days and 6 days after treatment. Only animals on the soil surface are assessed: any beetles that have buried into the soil prior to an assessment are left undisturbed and recorded as not visible. Buried beetles are removed from the soil at the 6 day assessment and their condition recorded. The assessment categories are:

H : alive and active, normal behaviour compared to the control.
B : moderate knockdown, poor coordination of movement.
C : lying on back with twitching legs, does not walk when blown on.
D : dead, no sign of movement when blown on.
NV: not visible.

Feeding activity is monitored by feeding carabids on days 0 and 4 and assessing after 48 hours (days 2 and 6 respectively). Each beetle is presented with one dead Drosophila melanogaster adult, stuck to a card using a "Fastik" glue pen to facilitate assessment of predation.

Feeding activity is scored as follows:

0 - no evidence of Drosophila having been eaten.
1 - Drosophila at least partly eaten.

The percentage mortality, percentage affected (B + C + D) and percentage feeding is calculated for the treated and control group. If the control mortality is >10% the test is invalidated and must be rerun. Mortality in the two groups can be compared using the Fisher's Exact Test, being the most appropriate analysis for this method (a t-test would be confounded because the control mortality will be too small to allow a normal approximation to the binomial distribution). The percentage mortality of the treated groups at 6 days is used to determine the IOBC effects category for each chemical tested (Hassan, 1975; revised 1990).

IOBC Effects Categories

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

Feeding activity is expressed as an index I:

$$I = \frac{\text{Treated (No. Drosophila eaten / No. surviving beetles)}}{\text{Control (No. Drosophila eaten / No. surviving beetles)}} \times 100$$

Where I = 100, feeding activity is not different from the control. The feeding data can also be analysed statistically in the same way as the mortality data.

3. Discussion

A study has been conducted in which a range of tests were conducted on P. melanarius to compare a number of laboratory test methods using several compounds with known profiles in the field (Brown et al, 1990). The tests were ranked in the severity of the effects they produced. Most severe was the track-sprayer (beetle contact + soil residual) test, as described here, followed by track sprayer (beetle contact only), field spray, track-sprayer (soil residual) and lastly microapplicator (contact) tests.

In this test method the beetles are held singly which has a number of advantages. It prevents cannabalism which can occur if the beetles are confined together thus obscuring the effects of the chemical (the basic information required from a tier I study). Additionally, it maximises the number of independent samples for a given number of beetles so making the most efficient statistical use of resources. The method is also a convenient one for ease of experimental

procedure. The cages are cheap and quick to make and easy to assemble. Disturbance of the beetles is kept to a minimum. Also treating the soil and the beetles seperately maximises exposure: the soil surface is not sheltered by the beetles or cage walls (only the inner container is sprayed). The beetles and the soil surface are also not disturbed by the watering which is from below (this also avoids the risk of washing the chemical through the soil).

The assessment of effects provides the mortality data which is required as the trigger determining the need for a Tier II study. In addition this method provides other information which can be useful in assessing the possible effects of pesticides in the field e.g. reversible 'knockdown' effects and changes in feeding activity.

A test method needs to be repeatable and representative of a worst case in the field to be of value as a Tier I test method. This test method for assessing the toxicity of pesticides to P. melanarius, is both rigorous in its assessment of toxicity and of a clear, straightforward design. It is thus eminently suitable for this requirement.

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METHODE DE LABORATOIREPOUR EVALUER L'EFFET DES PESTICIDES SUR *FORFICULA AURICULARIA* L. (DERMAPTERA : FORFICULIDAE).

B. SAUPHANOR (1), P. BLAISINGER (2) & F. SUREAU (1)
(1) INRA, Station de Zoologie et d'Apidologie, Domaine St Paul, 84140 MONTFAVET
(2) INRA, Station de Zoologie, 28 rue de Herrlisheim, 68000 COLMAR

INTRODUCTION

Comptant parmi les effets non intentionnels des traitements phytosanitaires, la destruction partielle des auxiliaires naturels des cultures, prédateurs, parasitoïdes ou entomopathogènes, a souvent pour conséquence l'introduction de déséquilibres, l'accroissement à moyen terme des populations du ravageur cible ou des changements de dominance.

La mise en place de stratégies de protection intégrée inclut donc l'étude de la toxicité des pesticides sur les auxiliaires. Elle est réalisée selon différentes approches, sur la faune globale d'une culture ou, sur une espèce déterminée, selon un processus de test séquentiel développé par le groupe de travail OILB/SROP "Pesticides et organismes utiles" (HASSAN *et al*, 1985) : l'expérimentation au champ, coûteuse et laborieuse, n'est mise en oeuvre que pour des spécialités s'étant révélées toxiques lors de tests rigoureux de laboratoire puis de semi plein air.

La méthodologie décrite concerne le test de laboratoire pour *Forficula auricularia*, sur le stade sensible et le stade robuste. Cette espèce, très polyphage, peut être nuisible sur certaines cultures florales, maraîchères ou fruitières (pêcher, abricotier),mais son rôle comme prédateur de pucerons notamment est souvent prépondérant. Il constitue par ailleurs un bon exemple d'effet secondaire néfaste d'utilisation de pesticides, puisqu'il a été récemment mis en évidence que son éradication de vergers de pomacées, par des traitements visant *Cydia pomonella* L., était fréquemment suivie de pullulations de pucerons dont principalement *Eriosoma lanigerum* Haussmann (RAVENSBERG 1981). La connaissance de la toxicité de différentes matières actives sur *F. auricularia* sera donc précieuse pour les cultures où le contrôle des aphidiens paraît difficile, mais également lorsque l'on souhaite obtenir un effet freinant sur cette espèce.

MATERIEL BIOLOGIQUE

Obtention de la souche

Les insectes utilisés pour l'expérimentation sont issus de pontes obtenues en élevage.

Des adultes en cours de maturation sexuelle sont à cet effet collectés dans la nature au moyen de bandes de carton ondulé fixées à la base des troncs d'arbres fruitiers et relevées au début de l'automne. Ils sont alors isolés par couple dans des pondoirs constitués d'une boite de pétri à demi remplie de sable humide, et alimentés avec des grains de pollen.

L'espèce étant univoltine, les premières pontes sont obtenues en conditions naturelles de photopériode, de novembre à février. L'incubation dure 15 à 20 jours à 18°C.

A l'issue du premier stade, les larves sont séparées de leur mère et regroupées dans des cages de 1 litre, à raison de 50 par cage, et peuvent être utilisées pour l'expérimentation. Elles sont alimentées avec du milieu artificiel gélosé à base de pollen. Des températures d'élevage comprises entre 9 et 25°C peuvent être utilisées pour ralentir ou accélérer l'incubation ou le développement larvaire selon les besoins de l'expérimentation.

Il faut compter en moyenne cinquante larves par femelle pour la première ponte. Chaque femelle dépose une seconde ponte 35 à 45 jours après la première éclosion, et parfois une 3ème ponte.

Insectes testés

Les tests portent à la fois sur le stade le plus sensible de l'espèce (test a) et sur le stade "robuste" (test b).

Le stade le plus sensible, le 1er stade larvaire, n'étant pas exposé aux produits antiparasitaires (il reste confiné dans le terrier maternel), l'expérimentation est réalisée sur le 2ème stade larvaire de l'espèce, qui en compte 4. Les résultats incluent la mortalité et la réduction de consommation dues au traitement.

Le stade robuste est ici l'adulte. Il est soumis aux tests quelques mois après l'émergence, en cours de maturation sexuelle. Les insectes proviennent également d'éclosions en élevage. Ils sont conditionnés en photopériode intermédiaire (12h / 12h) pour l'initiation de la vitellogenèse, puis en photopériode courte (10h / 14h) pour la ponte. Les résultats incluent la mortalité et la réduction de fertilité dues au traitement. Cet effet n'étant pas pris en compte pour le stade sensible, tous les produits doivent être testés sur adultes (contrairement à la procédure séquentielle, qui exclut du test sur stade robuste les traitements non toxiques pour le stade sensible).

MODALITES D'EXPOSITION

Les produits sont appliqués à la plus forte concentration autorisée sur un support en verre, constitué par un couvercle de boite de pétri de 10 cm de diamètre. La pulvérisation, à la dose de 1.7 mg/cm2, est réalisée au moyen d'une tour de POTTER-BURGERJON, dont l'embase rotative assure une couverture homogène du support (BURGERJON, 1956).

Après séchage du produit pendant 2 heures à température ambiante, les cages sont assemblées autour du support traité et les insectes introduits.

le fond de la cage est constitué par le couvercle de boite de pétri et les parois par une boite en polystyrène de 8 cm de diamètre et 5 cm de haut, retournée, et dont le fond (représentant ici le plafond de la cage) est percé d'un orifice de 3 cm de diamètre. Les parois sont recouvertes intérieurement de fluon (Polytetra-fluoréthylène Dispersion ICI) pour prévenir les évasions, et la cohésion de l'ensemble maintenue par un collier élastique.

Un morceau de carton noir posé sur le fond des enceintes offre aux insectes une protection contre la lumière, les maintenant ainsi en contact permanent avec le résidus.

DISPOSITIF

Cinq couvercles de boites de pétri sont traités simultanément dans la tour de pulvérisation, correspondant aux cinq répétitions du traitement. Chaque enceinte reçoit 10 insectes du stade étudié (10 larves pour le stade sensible, 5 couples pour le stade robuste). Ils sont déposés avec une pince souple, successivement dans tous les traitements d'une même répétition pour limiter la variabilité liée à l'échantillon. L'effectif est donc de 50 larves ou 25 couples par traitement.

L'alimentation, renouvelée tous les deux jours et disposée sur le carton noir, n'est pas en contact avec le produit.

Pour chaque test, un témoin traité à l'eau est réalisé, ainsi qu'une référence moyennement toxique (deltamethrine 25g/l EC, à la concentration de 0.07% de produit formulé) pour prendre partiellement en compte les éventuelles variations de sensibilité des souches étudiées.

Les enceintes sont ensuite placées dans une étuve régulée aux conditions suivantes :

Température constante de 18°C ± 1

Photopériode 16h jour/8h nuit pour le stade sensible et 12h jour/12h nuit pour le stade robuste

Humidité relative 70% ± 5. Cette condition est obtenue par le greffage sur la partie arrière de l'étuve, où sont situées les arrivées d'air, d'un caisson où débouche un conduit d'air humide alimenté par un humidificateur.

L'étuve est enfin munie d'un extracteur d'air pour éviter l'intoxication des insectes par inhalation et les interférences entre produits.

Ce dispositif permet de tester simultanément 18 traitements, incluant le témoin eau et la référence moyennement toxique.

OBSERVATIONS

Mortalité

Les insectes sont exposés aux traitements pendant quinze jours. La mortalité est relevée au bout de 24h, puis tous les deux jours pendant la durée de l'exposition, à l'issue de laquelle les insectes sont placés par traitement et par répétition dans de nouvelles cages, non traitées.

La mortalité est évaluée sur l'ensemble du développement préimaginal pour le stade sensible, et jusqu'à l'éclosion des oeufs après mise en condition de ponte pour le stade robuste.

Alimentation

Pour le stade sensible, on note dans chaque enceinte la quantité d'aliment consommée pendant la période de test. Le milieu artificiel, qui présente une vitesse de dessication modérée, permet de limiter à 2 par semaine la fréquence des renouvellements de nourriture. Un témoin d'humidité est réalisé, et le rapport de consommation entre les objets traités et le témoin eau est évalué à partir des poids secs, obtenus après séjour 24 h dans une étuve à 70°C du milieu non consommé.

Reproduction

Pour le stade robuste, le insectes survivants après exposition sont placés par couple en pondoirs (décrits plus haut), afin de suivre l'effet de l'exposition aux produits sur la fécondité et sur le taux d'éclosion des oeufs. Les pondoirs sont constitués un mois après le début de séjour des insectes en photopériode courte. Le mâle est retiré du pondoir dès que la ponte est constatée. Les oeufs sont dénombrés sous binoculaire, un à deux jours après le début de la ponte, en écartant momentanément la femelle. L'expérimentation s'achève par le comptage des larves, deux à trois jours après le début des éclosions.

EVALUATION ET ANALYSE DES RESULTATS

L'effet global du traitement s'évalue par une combinaison de la mortalité et de la réduction de fertilité pour le stade robuste (OVERMEER & VAN ZON 1982), de la mortalité et de la réduction de la consommation pour le stade sensible.

Effet traitement sur le stade sensible :

$$E = 100\% - (100\% - Mt / 100\% - Mnt) \times Ct / Cnt$$

Mt : mortalité insectes traités, Mnt : mortalité témoin eau.
Ct : consommation insectes traités, Cnt : consommation témoin eau.

Effet traitement sur le stade robuste :

$$E = 100\% - (100\% - Mt / 100\% - Mnt) \times Ft / Fnt$$

Mt : mortalité insectes traités, Mnt : mortalité témoin eau.
Ft : fertilité par femelle traitée, Fnt : fertilité témoin eau.

La mortalité "naturelle" en conditions d'élevage s'établit aux environs de 10% pour l'achèvement du développement larvaire. Les résultats de l'expérimentation ne doivent être considérés que si la mortalité dans le témoin eau n'excède pas 20% pendant la durée du test : mortalité préimaginale pour le stade sensible, et jusqu'à l'éclosion des oeufs pour le stade robuste. Les cages utilisées pour l'exposition, puis pour le suivi du développement préimaginal pour le stade sensible, ne permettent pas les évasions. Les insectes morts peuvent être consommés entièrement par les survivants, et les disparus sont dans ce cas comptabilisés comme morts. Quelques évasions des pondoirs sont observées pour le stade robuste, mais restent dans nos conditions à un niveau acceptable. L'effectif pour l'évaluation de la fécondité est réduit du nombre de femelles évadées.

Les résultats sont soumis à une analyse statistique. Nous procédons à une analyse de variance sur l'effet des traitements après transformation angulaire de Bliss (Arcsin $\sqrt{x}\%$). Après vérification de l'hypothèse de normalité des résidus et de la valeur du coefficient de variation, les moyennes son comparées par le test de Newman-Keuls à 5%.

Si le test est valide, les traitements sont classés conventionnellement selon l'échelle de toxicité suivante :

Effet traitement de 0 à 30% : Classe 1 "non toxique"
31 à 79% : Classe 2 "peu toxique"
80 à 99% : Classe 3 "moyennement toxique"
99 à 100% : Classe 4 " très toxique"

Cette convention d'écriture, commune aux expérimentateurs du groupe "pesticides et organismes utiles", n'exclut pas que des produits de deux classes de toxicité différentes soient statistiquement non différents. Il est bon pour cela de considérer également les données expérimentales lorsque l'on s'intéresse à un organisme particulier.

La conduite de ces tests fournit d'autre part des résultats, non pris en compte dans la formule globale, mais qui peuvent orienter utilement les investigations sur le mode d'action des traitements : pour les larves le délai de mortalité et la durée de développement préimaginal, pour les adultes la consommation, l'accouplement, le délai de ponte, le comportement de fouissage puis de soin aux oeufs (protection, léchage, transport, consommation éventuelle), durée d'incubation.

Il convient de vérifier, avant la mise en place de l'expérimentation, que le dispositif et le mode d'exposition soient bien compatibles avec le mode d'action des formulations à évaluer. Dans le cas contraire (produits actifs uniquement par inhalation, entomopathogènes dont l'ingestion par l'insecte conditionne l'efficacité, certains Régulateur de Croissance des Insectes), les conditions expérimentales sont à adapter en conséquence.

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Entwurf einer Vorläufigen Richtlinie zur Prüfung der Wirkung von Pflanzenbehandlungsmitteln

auf *Isotoma tigrina* NICOLET (*Collembola*)

Pa HUANG

Niedersächsische Forstliche Versuchsanstalt,
Grätzelstraße 2, D-3400 Göttingen

Übersicht

1 VORBEDINGUNGEN FÜR DIE PRÜFUNG

1.1 Vorbemerkung

Es mangelt in der forstlichen Praxis an standardisierten Verfahren zum Nachweis der subletalen Wirkungen von Schadstoffen (z.B. Pflanzenbehandlungsmitteln) besonders auf Bodentiere. Informationen dieser Art sind Vorbedingung für die gezielte Anwenderberatung, für die Entwicklung alternativer Verfahren und ggf. für die Empfehlung, auf bestimmte Maßnahmen ganz zu verzichten. Dabei liegen Erfahrungen sowohl im Bereich der sog. Nützlingsprüfungen (HASSAN et al. 1991 u.a.) als auch im Bereich der Toxizitätstests an Bodenorganismen (IGLISCH 1986 u.a.) vor. Es fehlte bislang an einer Zusammenführung der vorliegenden Kenntnisse und Techniken zu standardisierten Test-Verfahren, die zu reproduzierbaren Ergebnissen führen. Aus technischen Gründen muß der Ansatz von einem Mono-Spezies-Test ausgehen. Die Frage nach der Repräsentanz des gewählten Objekts stellt sich zunächst nicht: Es dürfte relativ leicht sein, verwandte Arten, z.B. *Folsomia candida* oder *Onychiurus spec.*, für die Zuchtverfahren und z.T. auch schon Vorschläge für Labor-Prüfungen vorliegen, in das hier vorgestellte Prüfverfahren an *Isotoma tigrina* einzubeziehen.

Die Collembole *Isotoma tigrina* wird seit 1983 bei der Abt.Waldschutz, NFV Göttingen, im Labor ganzjährig in Massenzucht gehalten. Der Zuchtstamm von *I.tigrina* stammt von einer Ausgangspopulation von mehreren hundert Tieren aus Buchenwald im Solling. Er läßt sich durch Neufänge jederzeit auffrischen. Die Art gilt als stabil und gut geeignet für Zucht und Experiment.

Bei der Prüfung handelt es sich um Laborprüfungen auf künstlichem Medium (Quarzsand), hier als Initialtoxizitätsprüfung eingesetzt, und auf natürlichem Material (Bodenstreu), das im Freiland behandelt wurde, hier als Persistenzprüfung eingesetzt. Beide Prüfungen wurden nach dem Vorbild der Nützlingstests der internationalen Arbeitsgruppe "Pesticides and Beneficial Organisms" und ihren Standards bzw. der entsprechenden BBA-Richtlinien entwickelt.

1.2 Biologie des Prüftieres (*Isotoma tigrina*)

I.tigrina NICOLET (= *I.olivacea* TULLBERG; GISIN 1960, FJELLBERG 1979) ist 1.0 - 1.5 mm groß, hell- bis dunkelgrau, behaart und sprungfähig. Die Art ist weltweit verbreitet und häufig in der Streuschicht von Laub- und Nadelwäldern zu finden (FJELLBERG 1979). Im Freiland ernährt sich *I.tigrina* von abgestorbenen Pflanzenteilen und Pilzhyphen. Die Weibchen beginnen ca. vier Wochen nach dem Schlüpfen aus dem Ei bei Temperaturen von 20°C ihre Eier abzulegen. Die Embryonalentwicklung dauert 10 Tage (20°C). Häutungen erfolgen alle 3 - 4 Tage während der gesamten Lebenszeit. Das Geschlechterverhältnis beträgt etwa 1:1.

1.3 Zucht der Prüftiere (*Isotoma tigrina*)

Die Tiere werden nach einer von GOTO (1960) beschriebenen Methode in Glasbechern (φ = 80 mm, h = 50 mm) auf einem Gemisch von Gips (90 %) und Aktivkohle (10 %) gehalten. Der Boden muß stets feucht gehalten werden. Das Gefäß wird mit einem Korken verschlossen. Als Nahrung wird handelsübliche Trockenhefe auf einem Deckglas geboten und alle 2 Tage gewechselt, um Verpilzung zu verhindern. Die Zuchtgefäße werden in Thermostatenschränken bei 20°C aufbewahrt. Der Tag-Nacht-Rhythmus richtet sich nach dem sommerlichen Langtag (16h/8h). Durch den Korken wird der Effekt einer Beschattung erzielt. Nach der Eiablage werden Imagines - täglich bis wöchentlich je nach dem Zweck - in ein neues Zuchtgefäß mit Hilfe eines Schlauchs (vorsichtiges Ausblasen der Tiere aus dem Gefäß) umgesetzt. Die Eier bleiben im alten Gefäß, so daß für Experimente Tiere in definiertem Alter zur Verfügung stehen.

2 VORBEREITUNG DER PRÜFUNG

2.1 Technische Versuchsbedingungen

2.1.1 Initialtoxizitätsprüfung

In kleine Bechergläser (φ = 50 mm, h = 50 mm) wird Quarzsand, vorher durch Hitze desinfiziert, ca. 5 mm hoch gefüllt (Abb.1a). Der Quarzsand wird anschließend (vorsichtig vom Gefäßrand her) mit entionisiertem Wasser befeuchtet.

2.1.2 Persistenzprüfung

Zur Persistenzprüfung - unter naturnahen Bedingungen - werden als Prüfbehälter Glaszylinder (φ = 63 mm, h = 200 mm) verwendet. Die untere Seite des Glaszylinders ist scharf angeschliffen, so daß mit ihnen unmittelbar Streuproben ohne Veränderung des natürlichen Bodenprofils entnommen werden können. Diese untere Öffnung des Zylinders wird nach der Probenahme mit einem Plastikbeutel verschlossen (Abb.1b). Die Proben (ca. 70 - 80 mm hoch) werden über Nacht einer Temperatur von 40°C ausgesetzt (zur Abtötung der Bodentiere, vor allem der räuberischen Tiere) und danach befeuchtet.

2.1.3 Belüftung der Versuchsbehälter

Die Prüfbehälter beider Typen stehen in einem größeren und höheren Glaszylinder (Abb.1a) bzw. Glaskasten (Abb.1b) auf Drahtgitter (Maschenweite 1.7 mm) über einer Belüftungsanlage (Abb.1c), mit deren Hilfe sie durch einen von oben nach unten durchströmenden Luftstrom gleichmäßig belüftet werden. Die durch den Belüftungskasten gesaugte Luft wird durch ein Rohr (φ = 12 cm) und einen Fenster-Auslaß ins Freie geleitet. In dem Belüftungskasten befindet sich ein Kleinraumventilator (MAICO, ECA 10-2), der stufenlos regelbar ist. Seine maximale Drehzahl beträgt 2.600 U/min, sein maximales Fördervolumen 138 m^3/h. Die Dreh-zahl wird so geregelt, daß ein Luftaustausch in der gesamten Anlage in etwa 2 Minuten erfolgt.

Die Prüfungen erfolgen im Prüfraum bei 20°C und einer Luftfeuchtigkeit von 70 - 80 %.

2.2 Vorbereitung der Prüftiere

Da *I.tigrina* im Alter von 4 Wochen mit der Eiablage beginnt, werden 5 Wochen alte Tiere zum Versuch verwendet. (Fünf Wochen vor dem Versuch werden die neugeschlüpften Tiere in einem besonderen Zuchtgefäß gesammelt.)

3 DURCHFÜHRUNG DER PRÜFUNG

3.1 Umfang der Versuche

Jeder Versuch umfaßt in der Regel 6 behandelte und 6 unbehandelte Versuchsgefäße. In jedes Versuchsgefäß - bei der Initialtoxizitäts- wie bei der Persistenzprüfung - werden 100 *I.tigrina* eingezählt. Dazu werden die Tiere aus dem Zuchtgefäß in kleinen Gruppen auf eine helle Unterlage geschüttet oder geblasen, gezählt und in das Versuchsgefäß gegeben.

3.2 Applikation der Präparate

3.2.1 Initialtoxizitätsprüfung

Die vorbereiteten Testgefäße, mit Quarzsand gefüllt und mit Wasser befeuchtet, werden auf einer 1-m²-großen Fläche aufgestellt. Mit Hilfe einer Laborsprühanlage werden die Pflanzenbehandlungsmittel in praxisüblicher Dosis senkrecht von oben über die Fläche gleichmäßig verteilt.

Technische Daten der Laborsprühanlage sind nicht Gegenstand dieses Richtlinien-Entwurfs.
In Ermangelung einer Laborsprühanlage kann notfalls der gleiche Effekt mit einem anderen Gerät, z.B. mit einem Hand-Spritzgerät des Typs B-20 L, erreicht werden.

3.2.2 Persistenzprüfung

Über eine 2 m x 5 m große Fläche auf Waldboden werden die Pflanzenbehandlungsmittel in praxisüblicher Dosis mit Rückenspritze oder Motorrückensprühgerät, notfalls auch mit dem Hand-Spritzgerät des Typs B-20L gleichmäßig ausgebracht. Die behandelten Flächen werden eingezäunt.

3.2.3 Kontrollen

Für die unbehandelten Kontrollen [UB] werden entsprechend vorbereitete Gefäße bzw. Flächen in gleicher Weise wie bei der Behandlung mit reinem Wasser gespritzt, die Flächen anschließend - wie die behandelten Parzellen - gegattert.

3.3 Versuchsdurchführung

3.3.1 Initialtoxizitätsprüfung

Die behandelten Testgefäße werden 3 Stunden an der Luft bei Zimmertemperatur getrocknet. Danach wird ein kleines Deckglas (7 mm x 7 mm) auf den Quarzsand gesetzt, darauf wird Trockenhefe gegeben. Anschließend werden die Collembolen eingezählt. Die Gefäße werden mit feiner Gaze (0.3 mm Maschenweite) geschlossen und auf den Lüftungskasten im Prüfraum gestellt (s.2.1.3). Die Tiere werden täglich mit Hefe gefüttert, der Quarzsand wird mit Wasser vorsichtig von der Glaswand her befeuchtet. Die toten Tiere werden jeden Tag herausgenommen und gezählt. Der Versuch dauert zwei Wochen.

3.3.2 Persistenzprüfung

In den angeschliffenen Glaszylindern werden jeweils am 5., 15. und 30. Tag nach dem Spritzen aus der behandelten Probefläche Streuproben entnommen (s.2.1.2). Die Proben werden sterilisiert, befeuchtet (2.1.2) und danach mit je 100 fünf Wochen alten *I.tigrina* besetzt. Hefe wird als Futter auf die Streu gegeben. Die Zylinder werden oben mit feiner Gaze verschlossen und auf den Lüftungskasten im Prüfraum gestellt (s.2.1.3). Die Tiere werden täglich mit Hefe gefüttert und die Streuproben mit Wasser vorsichtig von der Glaswand her befeuchtet. Der Versuch dauert zwei Wochen.

3.3.3 Dekontaminierung

Testgefäße und Testzylinder werden nach dem Versuch in einer Labor-Spülmaschine gereinigt und anschließend in einem Trockenofen zwei Stunden lang einer Temperatur von 200°C ausgesetzt.

Bei einfacheren Prüfgefäßen (*Drino*-Prüfung) ist es sicherlich richtig und machbar, einmal behandelte Glasteile überhaupt nicht wieder zu verwenden. Bei unseren Zylindergläsern verbietet sich das schon aus Kostengründen. Das vorgeschlagene Reinigungsverfahren hat sich in der Praxis bewährt: Die Resultate werden durch die ständige Kontrolle von Nullproben überprüft.

3.4 Versuchsbonitierung und Auswertung

Als Parameter für die Schädlichkeit der geprüften Pflanzenschutzmittel wird die Verminderung der Fertilität, hier der Zahl der im Versuchszeitraum geschlüpften Jungtiere, herangezogen.

3.4.1 Feststellung der Fertilität

3.4.1.1 Initialtoxizitätsprüfung

Am Ende des Versuchs werden die aus abgelegten Eiern im Versuchszeitraum geschlüpften Jungtiere gezählt. Dazu werden

die Tiere zunächst durch vorsichtiges Anfeuchten aus dem Sand ausgetrieben, dann von der Oberfläche in ein Zählgefäß geblasen (s.1.3), in Alkohol abgetötet und unter dem Binokular gezählt.

3.4.1.2 Persistenzprüfung

Mit Hilfe von KEMPSON-MACFADYEN-Extraktoren werden die Collembolen in den Streuproben vorsichtig ausgetrieben. Die Streu-/Bodenproben werden aus den Glaszylindern so in die Austreibegefäße (Kästen 20 cm x 20 cm, Kunststoff) gedrückt, daß die natürliche Schichtung im wesentlichen erhalten bleibt. Die Austreibung erfolgt mittels Rotlicht (150 W) durch grobe Gaze hindurch in einen gekühlten Auffangbehälter. Die Tiere werden trocken aufgefangen (Pikrinsäure-Fixierung ist zu aufwendig). Die Austreibe-Temperaturen werden schrittweise (um jeweils etwa 2°C) von anfangs 20° auf (am 7.Tag) 32°C erhöht. Es dauert etwa eine Woche, bis alle Tiere ausgetrieben sind. Die Anzahl der überlebenden Collembolen und der aus den Eiern geschlüpften Jungtiere wird anschließend ermittelt.

3.4.2 Berechnung der Fertilität

Die Minderung der Fertilität (d.h. der Anzahl der Jungtiere) - in Prozent, bezogen auf die unbehandelte Kontrolle, - wird nach Feststellung der Zahlen der geschlüpften Jungtiere wie folgt berechnet:

$$\frac{LUB - LB}{LUB} * 100 \quad [\%]$$

Wobei
LUB : Gesamtzahl der geschlüpften Jungtiere im unbehandelten Vergleich,
LB : Gesamtzahl der geschlüpften Jungtiere in der behandelten Probe.

4 BEWERTUNG DER ERGEBNISSE

4.1 Initialtoxizitätsprüfung

Als Prüfungs-Ergebnis liegt die mittlere Fertilitätsminderung (3.4.2) gegen UB aus 6 Versuchswiederholungen vor. Eine Bewertung kann darüber hinaus - verabredungsgemäß - nach Wertstufen vorgenommen werden, indem z.B. eine Minderung der Fertilität von <30 % als "unschädlich" akzeptiert wird.

4.2 Persistenzprüfung

Persistenzprüfungen werden an allen Mitteln durchgeführt, die in der Initialtoxizitätsprüfung als "schädigend" eingestuft wurden.

Tritt bei der jeweiligen Wiederholung (am 5., 15. und 30. Tag nach der Behandlung) eine Minderung der Fertilität von <30

% auf, so wird das Pflanzenbehandlungsmittel in die jeweilige Persistenz-Wertstufe eingestuft:

Tag nach der Behandlung	Minderung der Fertilität	Wertstufen
5. Tag	< 30 %	nicht persistent
15. Tag	< 30 %	schwach persistent
30. Tag	< 30 %	mäßig persistent
	> 30 %	stark persistent

4.3 Statistik

Eine statistische Auswertung der Ergebnisse ist mit dem U-Test von WILCOXON, MANN und WHITNEY (SACHS 1978) möglich. Für den vorgesehenen Stichprobenumfang von m = 6, n = 6 sind die kritischen Werte von U gerade noch tabelliert. Die Statistik besagt lediglich, daß die Mittelwerte der 6 behandelten und der 6 unbehandelten Gruppen signifikant (mit einer Irrtumswahrscheinlichkeit von P %) verschieden sind.

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5 ANMERKUNGEN

5.1 zu 3.1: Umfang und Dauer der Versuche

Bei der Entwicklung der Tests wurde auf einen Umfang von je 6 Wiederholungen abgezielt, der sich bei anderer Gelegenheit (*Drino inconspicua*; HUANG 1986) als praktikabel erwiesen hatte. Bei Initialtoxizitätstests auf Quarzsand reicht dieser Versuchsumfang aus, um deutliche und sicherbare Ergebnisse zu erhalten (Abb.2,3, Tab.1). Die Versuchsdauer wurde aufgrund der Daten von Abb.2 von ursprünglich 21 Tagen auf 14 Tage reduziert.

Bei Initialtoxizitätstests auf natürlichem Medium (Streuauflage auf Quarzsand) scheint der Versuchsumfang von 6 Wiederholungen nur knapp zu genügen (Tab.2). Hier müssen die Ergebnisse weiterer Testserien abgewartet werden.

Die als Persistenztests durchgeführten Versuche mit natürlichem Boden und Freilandbehandlung

(In den eigenen Tests wurden i.d.R. die Proben nach einem Tag bzw. nach 7 Tagen entnommen und untersucht.)

5.2 zu 3.2: Applikation der Präparate

Technische Daten der Laborsprühanlage sind nicht Gegenstand dieses Richtlinien-Entwurfs.
In Ermangelung einer Laborsprühanlage kann notfalls der gleiche Effekt mit einem anderen Gerät, z.B. mit einem Hand-Spritzgerät des Typs B-20 L, erreicht werden.

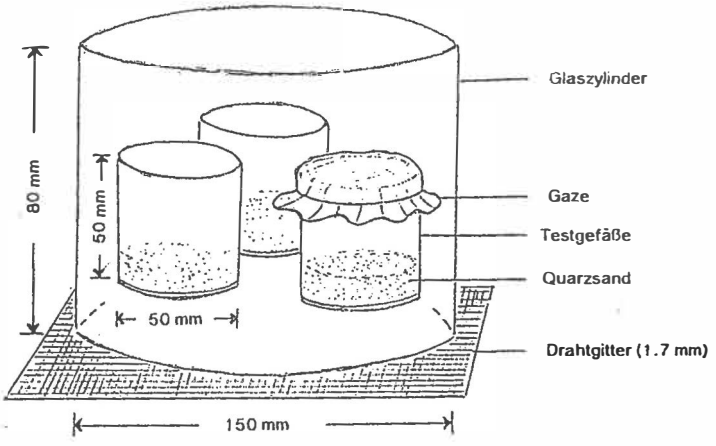
Bei der "praxisüblichen Dosis" ist zu berücksichtigen, daß z.B. bei Einsatz von Luftfahrzeugen nur ein Bruchteil der über dem Kronendach ausgebrachten Mittel-Mengen den Boden erreicht. Bei unseren Tests gingen wir von einem Anteil von 1% aus.

5.3 zu 3.3.3: Dekontaminierung

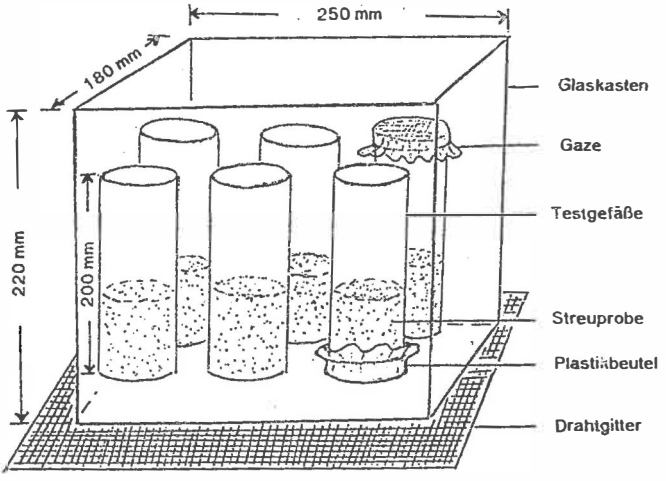
Bei einfacheren Prüfgefäßen (*Drino*-Prüfung) ist es sicherlich richtig und machbar, einmal behandelte Glasteile überhaupt nicht wieder zu verwenden. Bei unseren Zylindergläsern verbietet sich das schon aus Kostengründen. Das vorgeschlagene Reinigungsverfahren hat sich in der Praxis bewährt: Die Resultate werden ja durch die ständige Kontrolle von Nullproben überprüft.

5.4 zu 3.4.1.2: Austreibeverfahren

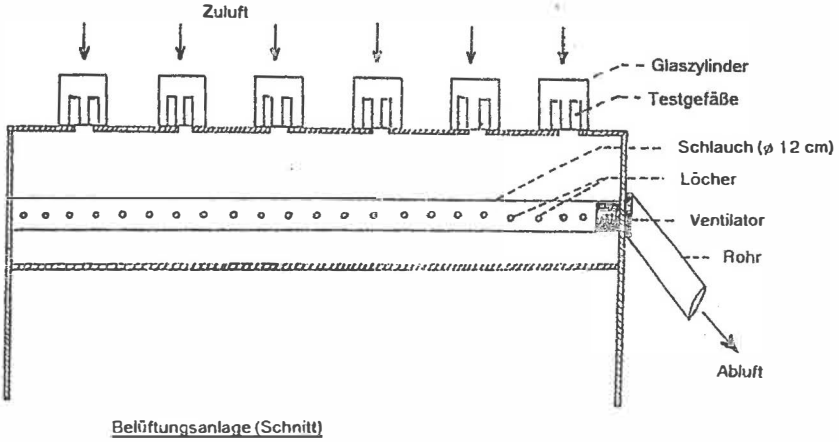
Die Tiere wurden in unseren Versuchen trocken aufgefangen, nicht fixiert. Die vielfach gebräuchliche Pikrinsäure-Fixierung ist zu aufwendig. Ergebnisse wiederholter Kontrollen dieses vorsichtigen Austreibens sind in Tab.3 zusammengestellt.



Prüfbehälter für Initialtoxizitätsprüfung



Prüfbehälter für Persistenzprüfung



Belüftungsanlage (Schnitt)

Abb.1
Laboreinrichtungen:
(a) Initialtoxizitätsprüfung
(b) Persistenzprüfung
(c) Belüftungsanlage

Guideline for testing the effects of pesticides on Folsomia candida Willem /Collembola/: laboratory tests

I. Kiss and G. Bakonyi
Department of Zoology and Ecology. University of Agricultural Sciences, H-2103 Gödöllő /Hungary/

1. Introduction

Springtails /Collembola/ are wide-spread all over the world. Most of the species play an important role in the decomposition of plant residues that get into soil as well as in the mineralization of nutrients.
That is why the pesticide side-effect on collembolan populations can influence the nutrient supply of the crop plants, too.

There are some ways in which springtails may come into contact with pesticides. Among others, a drop of spray falls on the animal or the animal passes through the pesticide that has got into the soil-water-capillaries. Mention must be made of the effect of the gases accumulating in the recesses of the soil, which gases are the compound complexes evaporating from the pesticides.

Several Collembola species were tried as test animals with different kinds of laboratory methods but no usually accepted test method exists /e.g.: Scops-Lichtenstein, 1967; Sanocka-Woloszyn-Woloszyn, 1970; Thompson-Gorf, 1972; Thompson, 1973; Eijsackers, 1978; Ulber, 1978/79; Subagja-Snider, 1981; Mola et al., 1987/.

For evaluating the side-effects of pesticides on

collembolan the species Folsomia candida is very suitable, because it is easy to rear and relatively much information exists about their biology in the literature. But the other two collembolan species used in our tests Heteromurus nitidus and Sinella coeca may be tested in the same way. Our tests were developed according to the standard principles of the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms" /Hassan, 1985/.

1.1. Biology of test animal /Folsomia candida Willem, 1902/

Folsomia candida is one of the most frequent beneficial species among springtails. It is a white animal without eyes. The adult's length is between 1.5 and 3 mm. Its springtail is long and powerful. Folsomia candida is wide-spread all-over Europe where the soil of arable lands, orchards and glasshouses is rich in organic matters.

Under laboratory conditions it multiplies parthenogenetically and it is easy to rear /Törne, 1964, 1966/.

1.2. Rearing of test animals

Plastic boxes with lids are used as rearing dishes. A thin layer of sterilized river-sand is placed in the bottom of the boxes. The sand has to be kept wet all the time. A perfect nourishment for the animals is the food the ingredients of which are suggested by Törne: 100 portions of water, 30 portions of oatmeal, 30 portions of agar and

333 portions of milk. There is just one deviation from the original recipe, namely, milk is not added to the other ingredients. The food enriched with milk goes bad and becomes mouldy more easily and quickly. Under the above-described breeding and nourishing conditions springtails multiply at a very fast rate. This species puts up with the changes of the temperature, the accidental lack of food and the transfer unavoidable during tests.
This species has been successfully kept alive in laboratory breeds for more than five years.

2. Experimental conditions and test procedures

2.1. General aspects

In the course of the tests 10 cm long open test-tubes with a diameter of 1.5 cm are used as containes. Ten tubes are used for each pesticide to be tested. In each test type ten control tubes are used which are treated with water. In order to achieve a continuous change of air in the tubes, they are installed in a ventilation box.

The average temperature in the ventilation box is 20 ± 2^{o} C and the speed of the air motion is so high that the air column in the test-tube is exchanged in 10 minutes.

All products are applied at the highest recommended concentration registered for practical spraying use.

The introduction and the exact counting of the animals are carried out using a vacuum inhaling equipment suitable for the pick up and transfer of the collembolan without

causing harm to them.

Fifteen adult animals are put in the tubes containing the appropriate concentration of the chemicals.

The number of dead animals is counted 72 hours after starting the treatment. The collembolan is dead when it lies without any move even if pricked with a needle.

2.2. Special aspects: direct contact test

Two milliliters of pesticide solution or water /in the case of the control/ are filled into the test tubes. The animals are put on the surface of the liquid after measuring the appropriate concentration of the chemicals /fig.1./.

2.3. Special aspects: the effect of the evaporating gas of the pesticides

To measure the toxic effect of the evaporating gas of the pesticide a small piece of a glass-tube /about 2 cm in length and 1 cm in diameter/ is prepared. The 15 adult animals are put into the small glass tubes. In order to keep a high relative humidity /about 80-90 rel.%/, a piece of wet filter paper is put into these small tubes. The filter paper must be kept wet all day by some drops of water. The small glass-tube is closed with a tull-net at both of ends, and it is hung into the test tube as deep as 2 cm above the surface of the pesticide solution. The experimental design is shown in Fig.2.

2.4. Special aspects: tests using natural substrats

In the case of these testing methods sterile sand and, respectively, brown forest soil is put into the tubes before measuring the appropriate concentration of the chemical.

From the sterile sand 3.5 g is put into the test tubes and then it is wetted with o.5 ml of pesticide solution /Fig.3./.

The forest soil must be prepared for the test. The soil is sieved with a 2 mm mesh and then dried out at 80°C. After rewetting to 25 % moisture content it is incubated for 7 days at 25°C.

The pesticide solution is filled cautionously with a pipette on the inside wall of the test tubes to avoid the destruction of the soil texture. So the animals can move on the soil surface and in the holes of the soil, too. 0.5 ml of pesticide solution is filled into the tubes /Fig.4./.

3. Evaluation of results

The average mortality per treatment is calculated and is corrected using the result of untreated control.

Normally the mortality percentage in the control tubes cannot be higher than 2-3 %.

The effect of the pesticides in the four types of test methods is expressed in categories as stated by the IOBC/WPRS Working Group /Hassan, 1985/.

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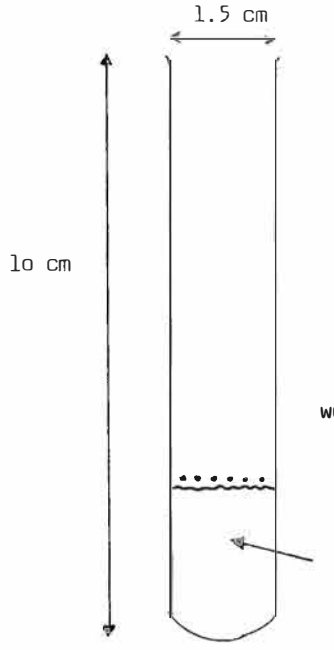

Fig. 1.

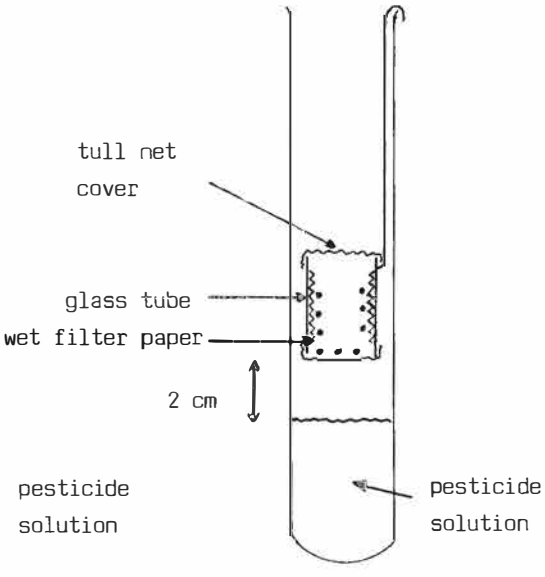

Fig. 2.

= test animals

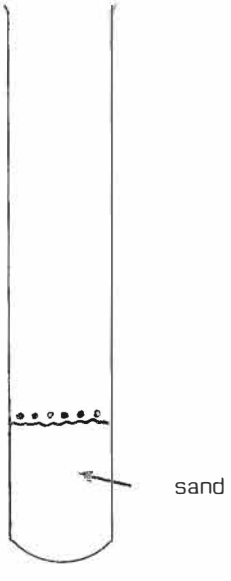

Fig. 3.

Fig. 4.

Fig. 1-4.: Prepared test tubes used for the various test types

IOBC Working group "Pesticides and Beneficial Organisms"

Guidelines for the Daniel funnel test - a laboratory test to measure side effects of pesticides on the earthworm *Lumbricus terrestris* L.

M. Bieri
Bahnhofstrasse 30, CH-8803 Rüschlikon
Switzerland

Purpose of the test

With this test side effects of different pesticides on the earthworm *Lumbricus terrestris* L. can be measured in the laboratory (BIERI *et al.* 1989). The test is considered to be used in a test sequence to evaluate the pesticide hazard to earthworm populations (BIERI and AMMON, 1990).

Special characteristics of the test

The test allows to simulate a) the distribution of the pesticides on and in the soil, and b) the actual exposure of the earthworm to the pesticide as it occurs in the field (e. g. when granules are worked into the soil, slug pellets spread on the soil surface, herbicides sprayed on the soil before the culture emerges or pesticide residues on or in plant debris). The test is designed for recording behavioral changes, individual weight losses and mortality in *L. terrestris*. It is highly sensitive in measuring behavioral reactions.

The importance of the test organism *Lumbricus terrestris* L. in agroecosystems

Investigations such as the long term studies of STEINER *et al.* (1985) and HÄNI (1990) on different soil tilling systems show that farmland with large earthworm populations produces more stable, long-term yields.

The earthworm species *Lumbricus terrestris* L., common in Western Europe, the East of North America and in New Zealand, is often the dominant species in agricultural ecosystems. Its almost vertical burrows BOUCHE (1977) can extend to a depth of more than 1 meter (SATCHELL, 1955). *L. terrestris* draws litter from the soil surface into its burrow (DARWIN, 1881) and mixes it with the mineral components of the soil. RAW (1962), EDWARDS and HEATH (1963) and LAING *et al.* (1985) found *L. terrestris* to be primarily responsible for working litter into the soil. The rapid incorporation of litter and the intensive mingling of organic matter and mineral soil components by earthworms result in a more efficient recycling of plant nutrients and in increased carbon content of the topsoil (HOOGERKAMP *et al.*, 1983). The steady production of earthworm casts is of great importance for the supply of plant nutrients normally bound to the soil. Earthworm casts are rich in easily exchangeable, and thus bio-available, ions (GRAFF, 1971 a; GRAFF and MAKESCHIN, 1980; HILDEBRAND, 1988). In general, only about a third of the burrows in any given soil are inhabited. The burrows abandonnend by *L. terrestris* are ideal channels for plant roots (GRAFF, 1971 b). In addition, the presence of earthworms promotes root development (VAN RHEE, 1977; EDWARDS and LOFTY, 1980; GRAFF and MAKESCHIN, 1980; HOOGERKAMP *et al.*1983) and leads to a higher water infiltration capacity (EHLERS, 1975; HOOGERKAMP *et al.*, 1983; EDWARDS *et al.* 1988). Precipitation enters the soil instead of running off and causing erosion. Plants on such soils suffer less from water stress in summer when water supply is usually at a minimum. Efficient recycling of nutrients and proper root development, as well as optimal gas and water conditions in the soil are prerequisites for unimpaired plant growth and are important elements in integrated production management. Certain soil cultivation methods, particularly tilling, can drastically reduce earthworm populations (ZICSI, 1967; EDWARDS and LOFTY, 1969; TEBRÜGGE, 1987). It is therefore important that earthworms still present in the soil are not harmed or weakened by any additional interference, especially pesticide applications.

The following method is a further development of the method described by BIERI *et al.* (1989).

Method

The animals and their preconditioning for the test

Due to the abundance and importance in agricultural soils *L. terrestris* fulfils the criteria of a representative species of the soil fauna being influenced by the pesticides (BOSTRÖM and LOFS - HOLMIN, 1982). The use of wild animals is recommended as rearing of this species in the laboratory is difficult. To ensure an optimal test group (least amount of inhomogeneity) the following points should be considered:

1) All animals should originate from the same area. The soil should be free of heavy metal charges, PCB, pesticides and sewage sludge. Abundant and stable *L. terrestris* populations are mainly found along riversides, in meadows and in woods where hornbeam (*Carpinus betulus*), maple (*Acer* spp.) or ash-trees (*Fraxinus excelsior*) are dominant.

2) Since some of the methods used to collect earthworms harm the animals, the test worms should be collected by handsorting, extraction with a highly diluted chloroacetophenone (50 ppm) solution or the "Oktett" method (THIELEMANN, 1986, CUENDET *et al.* 1991).

3) Only juveniles with a live biomass of higher than 1.5 g should be used in the test.

4) The animals must be conditioned for 3 days at 12°C in clean, unchlorinated and aerated tap water, before they can be used in the test. During this period all dead animals are removed and counted. If mortality clearly exceeds 5%, the whole group must be rejected.

5) All tests should be carried out with a negative standard, 30 mg/l chloroacetamid solution.

The test containers (funnels)

High density polyethylene funnels with an upper diameter of 12 cm and vertical borders on top are used (e.g. manufacturer: Stefan Plast, Castegnero, Italy). A lid consisting of a ring covered by plastic or fine wire mesh allows light and air to penetrate and prevents the worm from escaping (see figure 1). The kaolin sand mixture (or soil) will be filled to the upper rim of the funnel. After the application of the test substance the vial is covered with the lid described above. At the lower end of the funnel a 30 cm long silicone tube, sealed with a rubber plug, is attached. The base of the funnel and the silicone tube are protected by a removable black polyethylene tube to assure that the earthworm remains in the dark.

The soil used in the funnel

A mixture of 30% kaolin (china clay) and 70% quartz sand (10% corn size: < 0.1 mm; 40% corn size: 0.1 to 0.7 mm; 20% corn size: < 0.7 to 1,5 mm) moistened to 100% field capacity is used (For special purposes, also normal soil from A_p horizons can be used). The funnel is filled to the top and the soil gently compacted by hand. With a nail or a screw driver a vertical canal is drilled into the centre creating a continuous burrow from the soil surface to the lower end of the funnel into which the earthworm is introduced.

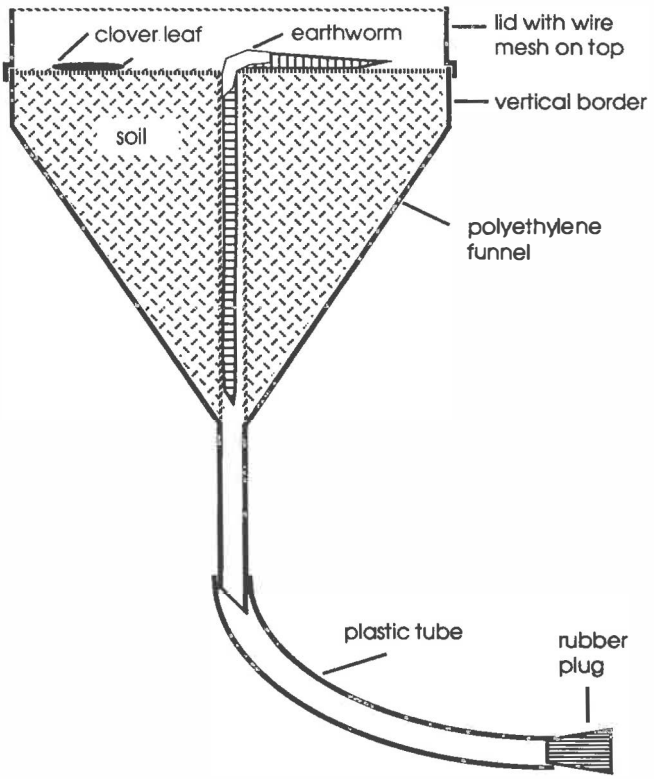

Figure 1: Section through a test vial.

Test conditions

Treatments

Three treatments per test are recommended: 1) a control, 2) the recommended field dosage and 3) a negative standard at normal dose. As negative standard 30 mg/l chloroacetamid in water is recommended.

The substance to be tested must be applied as it would be under field conditions and at the recommended doses.

- pellets or granules are scattered on the soil surface or incorporated into the upper soil layer as recommended by the manufacturer.
- the liquids are sprayed in equivalent doses per surface area over the soil surface and over the leaves on the soil. Exact doses can be obtained with the help of an air brush. While spraying the edges of the soil surface must be protected with a ring template to prevent percolation of liquid along the funnel walls into the plastic tube at the lower end of the funnel. Alternatively sprayed leaves are placed on the soil surface, e.g. when apple fungicides are to be tested.

For the establishment of a dosis-activity response, dosages in a logarithmic sequence are to be used (see next page).

Number of replicates per treatment

Per test (control: 20, field dosage: 20, negative standard: 20) 60 funnels in total with one *L. terrestris* each should be used as minimum.

Food

Per animal and funnel 10 white clover leaves (*Trifolium repens*) are placed on the soil surface. All leaves must be dried before use in the experiments; 24 h before the test they should be remoistened.

Duration of the test

The duration of the test is at least 14 days but it can be extended to 21 days.

Climatic conditions

After application of the pesticide the funnels with the animals are placed into a fully dark climate control chamber with a constant temperature of 14° C and maximum relative humidity. During the whole experiment the soil surface must remain moist.

Supervision of the experiment

The funnels should be checked daily, or at least every second day and water loss in the funnels should be replaced, by spraying a water mist on the surface.

The parameters measured

Withdrawn leaves and surface applied granules

At each inspection the numbers of withdrawn leaves and granules per funnel are recorded. A leaf or granule can be considered as "withdrawn" as soon as one end of it is incorporated in the earthworm burrow. Every 7 days the withdrawn leaves are replaced but pesticide granules are not replaced.

Weight

At the beginning and at the end of the test the live weight (gut emptied) of each individual is taken. At the end of the test the animals have to be kept for 24 hours at 14° C in clean, unchlorinated and aerated tap water before weighing. The live weight must be determined in a vial containing water.

Mortality

At each inspection the condition of the animals is checked. Dead animals are recorded and removed with their funnels.

Final report

The final report contains the mortality of the earthworms, the pattern of activity characterised by the number of withdrawn leaves over this period and differences in body weight of the worms in each treatment compared with the control. Unusual observations as well as results of residue analyses must be reported. In an appendix a copy of the original data sheets should be added.

Assessment of LC 50 acute toxicity

Facultatively, it is possible to screen for LC50 acute toxicity with the described test-device. For this purpose the use of 6 different concentrations in logarithmic intervals (e.g. 1, 10, 100 ...) of the test substance with 10 replicates each (and one *L. terrestris* per funnel) is recommended.

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Guideline for laboratory testing of the side-effects of pesticides on entomophagous nematodes *Steinernema* spp.

Aana Vainio
Agricultural Research Centre of Finland
Institute of Plant Protection
SF-31600 Jokioinen
FINLAND

This paper provides a guideline for a standardized evaluation of the side-effects of pesticides on entomoparasitic *Steinernema* spp. nematodes in the laboratory. The use of nematodes alone or with decreased amount of pesticides is a potential method of integrated pest management. Entomopathogenic nematodes often occur naturally in cultivated soils, but they are also used as biopesticides. To know some facts about the influence of pesticides on these nematodes facilitates the choise of pesticides which are compatible or less harmful to this important group of natural enemies.

Most authors have tested the toxicity of chemicals on nematodes in a water solution (Prakasa Rao et al. 1975, Hara & Kaya 1983, Das & Divakar 1987, Heungens & Buysse 1987, Rovesti 1989, Rovesti et al. 1989, Zimmerman & Cranshaw 1990). Some studies have been made with Petri dishes containing a thin layer of agar with pesticide (Fedorko et al. 1977abc, Hara & Kaya 1982). For studying the initial toxicity the water solution - method appears as the most feasible way.

Preparations for the test

In the test one unit consists of five glass test tubes (volume 4 ml). The tested pesticides are mixed with distilled water, which is used for control as well. To investigate changes in the beneficial capacity additional fifteen tubes are needed. Altogether twenty tubes per treatment are needed for the laboratory test.

During the incubation time the tubes are kept in dark and at room temperature (22-23 ^{o}C) (Das & Divakar 1987). The temperature is quite significant because Heungens and Buysse (1987) found that especially at lower temperatures the toxicity of a pesticide depends more on the temperature of the solution than on the concentration of the active ingredient.

Infective juveniles (J3) of the nematodes in water solution are used for the test. They can be mass produced in *Tenebrio molitor* larvae under laboratory conditions and may be stored in a refrigerator (5-8 ^{o}C) if necessary, but they should not be stored longer than one week for the test.

Laboratory-reared *T. molitor* larvae are used to test the maintenance of beneficial capacity of the nematodes.

The effect of pesticides is measured by using test tubes with 2000-2500 J3-nematodes in 1 ml of distilled water. 1 ml of the pesticide at twice the highest recommended concentration of active incredient is added to the nematode suspension. Final dilution of the pesticide becomes thus the same as the highest recommended concentration. Distilled water is used in the control. All the tubes are stirred well after the pesticide application and at every measuring time.

Performance and the procedure of the test

a) Initial toxicity, viability

Viability of the nematodes is recorded after 2, 5 and 7 days (48, 120 and 168 hours) from the same five tubes per treatment every time. The suspension containing the J3 nematodes and the pesticide is stirred well, and 50-100 μl (approximately one hundred nematodes) is examined under a stereomicroscope.

The living and the dead individuals are counted from the randomly sampled 100 nematodes. Those moving actively or responding to probing with a needle are considered alive, and those not responding are recorded as dead (Hara & Kaya 1983).

b) Initial toxicity, beneficial capacity

The infectivity of the nematodes is checked after the same periods as viability. After every period five tubes per treatment are used to test the infectivity. The tubes are stirred and filled with water. Nematodes are allowed to settle for about half an hour in a refrigerator. The superposed (~3 ml) liquid is discarded. This washing procedure is repeted three times (Rovesti et al. 1989). After the third time 200 μl is taken from the bottom of each tube and pipetted onto a filter paper disk (~20 cm^2) in a Petri dish.

One *T. molitor* larva is placed on the disk. The dishes are closed and kept in dark at room temperature for five days. The disks are kept moist all the time. Dead larvae are moved to clean Petri dishes with moist filter paper and kept in dark for three more days, after which they are dissected to check the presence or absence of the nematodes.

Assesment and evaluation

a) Viability

The results of the counts of dead nematodes are expressed as percentages. The results from the last recording are used primarily. The period of the recording should be mentioned if it differs from the standard (seventh day after application).

The % mortality of the treated series are corrected with the % mortality of the untreated (Abbot 1925):

$$M = \frac{t\,\% - c\,\%}{100\,\% - c\,\%} * 100\,\%$$

M = corrected mortality
t = mean of the mortality of exposed nematodes
c = mean of the mortality of control nematodes

b) Beneficial capacity

Any decrease in infectivity compared with the control is expressed as percentages. If the time of exposing differs from seven days, it should be mentioned and motivated.

Interpretation of the results

The results of the tests are evaluated according to the categories as selected by the IOBC Working Group "Pesticides and Beneficial Arthropods":

Reduction in viability or infectivity in the laboratory (initial toxicity):

1) < 50 % = harmless
2) > 50 % = requires further testing under semi-field and/or field conditions. (harmful in the laboratory)

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BIOASSAY OF THE SIDE-EFFECTS OF PESTICIDES ON *Beauveria bassiana* AND *Metarhizium anisopliae*: STANDARDIZED SEQUENTIAL TESTING PROCEDURE

H. M. T. HOKKANEN & R. KOTILUOTO

Agricultural Research Centre of Finland
Institute of Plant Protection
SF-31600 Jokioinen, FINLAND

1. Introduction

An IOBC/WPRS guideline for testing the side-effects of pesticides on entomopathogenic fungi exists so far only for *Verticillium lecanii*, for assessing the initial toxicity in the laboratory (Tuset, 1985). Other species of entomofungi may differ in their reaction, and need to be included in the test protocols. Also, species operating mainly in the soil environment, such as *Beauveria bassiana* and *Metarhizium anisopliae*, are likely to differ in response to pesticide treatments from species infecting pest populations mainly in the field layer.

Practically all pesticides sooner or later enter the soil environment, either unchanged or as breakdown products, and sometimes these breakdown products may be even more toxic to the beneficial fungal species than the original pesticide (see Vänninen and Hokkanen, 1988). Therefore all pesticides should be tested for their side-effects on beneficial organisms inhabiting the soil. It is also necessary to incorporate tests of the breakdown products into the evaluation procedures, as outlined in Hassan (1989).

2. Laboratory tests, initial toxicity

a) *General*

A multitude of procedures have been used to test the effect of various pesticides on the growth or viability of entomopathogenic fungi *in vitro*. Most commonly the pesticide is mixed into the growth medium before inoculation, and either the growth in biomass or the growth of colonies of the test species is measured after some time (e.g. Zimmermann 1975, Bajan and Kmitowa 1977, Tedders 1981, Clark et al. 1982, Gardner and Storey 1985). Another method allows first the fungus to grow normally on the agar, after which the chemical is applied as a spray (Vänninen and Hokkanen 1988) or on a circular piece of filter paper in the middle of the petri dish (Tedders 1981).

Since there is a standard test method for *Verticillium lecanii* already (Tuset 1985), the same principles should be followed also in the case of *Beauveria bassiana* and *Metarhizium anisopliae*.

b) *Test procedure*

The pesticide to be tested is mixed to potato-dextrose agar (PDA) when the medium is at a temperature of 50 °C, at the highest concentration recommended for use. The fungus to be tested is transferred to the centre of the test agar in a piece of agar (5 mm diameter) containing mycelium from a fresh culture of the fungus growing on pure PDA.

Three replicates of five petri dishes (9 cm diameter) each (15 altogether) are used for the test. In each replicate, one untreated control is included. The inoculated petri dishes are incubated at 22-24 °C in the dark. Fungal development is examined 5, 10 and 15 days after inoculation.

c) *Evaluation of results*

Mean colony diameter (linear growth) is measured in all petri dishes. This is used to calculate the total surface area of the colony. The initial result is obtained by comparing the colony area in the control to the colony area in the pesticide treated petri dishes.

This evaluation method alone, as used in Tuset (1985), however, does not very well assess the "reduction in beneficial capacity", but should be complemented by a bioassay of the infectivity of the material. Vänninen and Hokkanen (1988) noted several types of reactions, such as decreased or delayed sporulation, which despite abundant growth may greatly reduce the beneficial capacity of the entomofungi. Therefore for those pesticides, which appear harmless in the initial evaluation, an additional assessment should be made before the final ranking.

This assessment should have two steps:

1. Determination of sporulation intensity and the viability of the spores

This should be done for each replicate separately 15 days after inoculation (after the measurement of linear growth). Remove the spores from the agar plates and make a spore suspension using Tween 20 (0.05%) as detergent. Determine the number of spores under a microscope using a blood cell counter (e.g. Bürker), and with the serial dilution technique determine the viability of the spores either on PDA agar.

2. Bioassay on insects

Use part of the suspension from the previous step for a bioassay on a living host, ***Tenebrio molitor***. Place 5 last instar larvae on a 9 cm petri dish and spray, prefereably with the Potter tower, 4 ml of the spore suspension (10^6 spores/ml) on the petri dish. Use three replicates if possible. Allow the moisture to dry and assess the mortality of the test insects after 5 and 10 days. Verify the regrowth of mycelium from the cadaver after about 15 days of treatment. Water treated controls serve as a reference.

Final evaluation

The reduction in beneficial capacity is evaluated based on the total production of viable, infective spores in each treatment, in comparison to the reference. If reduction in beneficial capacity is less than 50%, the pesticide is classified as harmless (Hassan 1989). If the reduction is greater, the product needs to be tested under semi-field conditions.

3. Semi-field, initial toxicity

a) *General*

Only a few studies have dealt with the effect of individual pesticides on entomofungi under semi-field or field conditions (exceptions e.g. Clark et al. 1982, Loria et al. 1983). No publications describing practical methods for a general assessment are known to the authors at present.

Since the testing of the initial toxicity in the laboratory is rather crude, we consider that the next step in the testing sequence for entomopathogenic fungi should be conducted under simulated field conditions in the greenhouse.

b) *Test procedure:*

1. Prepare a standard soil containing 1/3 peat, 1/3 black humus soil, and 1/3 fine sand. Uniformly inoculate the soil with spores of the beneficial species concerned to reach a concentration of 10^6 spores/cm^3.

2. Place 5 dl of the mixture into a 1,5 l pot and moisten moderately. Keep the pots throughout the test at 22-24 °C in a greenhouse.

3. Spray with the recommended rate / ha of the pesticide to be tested, or apply the pesticide to the soil according to the label instructions. Use four replicates for each of the pesticides, and water as a control.

4. One day later, water the pots using a small watering can with approximately 0.3 dl of water to simulate rain.

5. One day later, i.e. two days after spraying with the pesticide, take from each pot one soil sample of approximately 20 g. Use a sampling borer (diameter about 20 mm) and take the sample all the way through the soil column in the pot. Homogenize the sample well and divide into two 10 g parts. Mix one part into 100 ml of sterilized water, which also contains a drop of Tween 20. Keep the mixture in a shaker for 20 minutes. Let stand still for 5 minutes, and prepare a series of dilutions of the sample. Five concentrations should be obtained: 10^{-1}, 10^{-2}, ..., 10^{-5} of the original stock.

From each concentration take 1 ml and place on 9 cm petri dishes. Pour onto each petri dish 10 ml of OAES-agar containing additionally 0.5 ml of 4% cycloheximide and 0.5 ml of 1% chloramphenicol per 100 ml of agar to inhibit bacterial growth (Tyni-Juslin 1990). These chemicals should be added into the autoclaved agar at 45 °C. Gently mix the agar and the sample solution and incubate at room temperature for two weeks. To obtain the number of viable spores, count the number of colonies on each petri dish. Use five replicates for each concentration.

Weigh the other 10 g of the soil and oven dry at 104 °C for 8 h. After cooling weigh the sample again to obtain the dry weight of the sample. The final number of viable spores should be expressed as spores/g of dry soil.

c) *Evaluation of results*

Compare the number of viable spores in the treated and untreated pots, and use the evaluation categories of Hassan (1989) for the final evaluation.

4. <u>Semi-field, duration of harmful activity</u>

The experiment described in part 3 above should be continued up to one month, or until the spore concentrations in the controls have strongly declined due to natural mortality, to assess the duration of harmful activity, and/or the toxicity of the breakdown products. Keep the soil in the pots moderately moist throughout the experiment. Two additional assessments are usually adequate: with *Beauveria bassiana* 10 and 17 days after treatment, and with *Metarhizium anisopliae* 10, 17, and 24 days after treatment.

5. Field

Field plots with crop cover (e.g. wheat or rape at an early stage of development, plants being about 10 cm tall) should be used for this evaluation. First the entomofungus species has to be applied as a spore suspension at the rate of 10^{14} spores/ha to the field. After one day parts of the inoculated area are sprayed with the pesticide to be tested. No other pesticide treatments should have been done on that plot during the test season. An assay should be done as described in part 3 above (semi-field test). Evaluation should be done as in part 3, described above.

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Richtlinie zur Prüfung der Nebenwirkung von Pflanzenschutzmitteln auf Aleochara bilineata Gyll. (Col., Staphylinidae) (erweiterter Laborversuch)

L. MORETH und E. NATON

Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, München

Einleitung

Aleochara bilineata ist aufgrund seiner prädatorischen und parasitischen Lebensweise ein Nützling von beachtlicher Bedeutung. Die adulten Käfer ernähren sich räuberisch von Eiern und Junglarven verschiedener Insekten, die sie im Boden oder auf der Bodenoberfläche erbeuten. Die Larven entwickeln sich parasitisch in den Puparien verschiedener Dipterenarten, besonders der als Schädlinge bekannten Delia- und Pegomyia-Arten (Kohlfliege, Zwiebelfliege, Rübenfliege u.a.).

Die Zucht der Versuchs- und Wirtstiere

Die Käfer und ihre Larven werden in einem eigenen Zuchtraum (Labor) herangezogen, in dem nicht mit Pflanzenschutzmitteln gearbeitet werden darf. Klimatische Anforderungen bestehen nur hinsichtlich der Temperatur (18°C). Höhere Temperaturen verkürzen die Lebenszeit der Käfer außerordentlich. Die Imagines werden in Vollglasaquarien (30 x 22; h = 15 cm) gehalten, die mit einer Glasscheibe abgedeckt sind (Unterlegung eines Kartonstreifens = feiner Lüftungsschlitz). Der Boden der Aquarien ist etwa 4 cm hoch mit feuchtem Sand bedeckt (Feuchtigkeitsgrad = lufttrockener Sand : Wasser = 10 vol : 1 vol.). In jedem Aquarium sollten nicht mehr als 1.500 Käfer gehalten werden. Fünfmal pro Woche werden die Käfer gefüttert (Chironomuslarven, im Handel erhältlich unter dem Namen "gefrorene Mückenlarven", die in kleinen Häufchen auf der Sandoberfläche ausgelegt werden). Zur Dämpfung des Lichts werden über die Zuchtgefäße große Hauben aus Styropor gestülpt, denen die Rückwand fehlt.

Da die Käfer ihre Eier in den Sand legen und die Eilarven die Wirtspuparien, in welche sie sich hineinbohren, aktiv aufsuchen, erhalten die Zuchtgefäße jeweils eine Woche nach dem Ansatz die erste Portion Wirtspuparien Delia antiqua Meig.) ($\sim$ 2.500 Stück = ca. 60 cm³; sorgfältig und unter Schonung der Käfer in den Sand einarbeiten, da sie von den

Eilarven nicht parasitiert werden, wenn sie nur an der Oberfläche liegen). Nach einer weiteren Woche, also 2 Wochen nach Ansatz der Zuchtschale, werden alle Käfer als dem Sand herausgesucht, gezählt und in einer neuen Zuchtschale (Aquarium) mit neuem Sand angesetzt. Gleichzeitig wird der Verlust (= tote Käfer) durch Jungkäfer ergänzt. Dieser zweiwöchentliche Rhythmus des Käferzählens und des Neuansatzes gewährleistet einen guten Überblick über die Populationsstärke der Zucht und verhindert außerdem, daß Jungkäfer unkontrolliert in den Aquarien schlüpfen (Protokoll!). Bei dieser suboptimalen Haltung sterben innerhalb der ersten zwei Wochen etwa 10 % der Jungkäfer und nach vier Wochen sind noch etwa 40 % der ursprünglich eingesetzten Käfer am Leben.

Der alte, gebrauchte Sand enthält nach dem Entfernen der alten Käfer nur noch Wirtspuparien sowie Eier und Eilarven dieser Käfer. Diesem Sand wird nun eine weitere Portion Puparien hinzugegeben; der Sand wird noch eine Woche in einer geschlossenen Glasdose bei Zimmertemperatur (20-22°C) aufbewahrt, damit er feucht bleibt und die Eilarven die zuletzt hinzugegebenen Puparien parasitieren können. Nach einer weiteren Woche Trocknungszeit werden die Puparien abgesiebt und trocken aufbewahrt. Es ist wichtig, diese Trockenperiode sorgfältig einzuhalten, da sonst die Jungkäfer vermilben. Etwa drei Wochen danach beginnt der Schlupf der Jungkäfer, die während der nächsten zwei bis drei Wochen täglich von den Puparien gewonnen werden können (Puparien in ein weitmaschiges Sieb geben, durch dessen Maschen die Käfer herausfallen).

Eine wichtige Voraussetzung für eine gut funktionierende Aleochara bilineata-Zucht ist eine entsprechend große Zucht des Wirtstieres, und der relativ günstigen Handhabung wegen wurde hierfür Delia antiqua Meig. (Zwiebelfliege) ausgewählt.

Imagines und Larven müssen aus Gründen der Zuchthygiene in getrennten Räumen gehalten werden. Die Imagines leben in geräumigen Zuchtkäfigen (1,20 x 0,85 x 0,55 m), die aus Kunststoffgaze (ca. 6 Maschen/cm) und Kunststoffrahmen bestehen. Unter der Decke hängt längsgespannt, hängemattenartig eine etwa 15 cm breit Polyethylenfolie (ca. 0,2 mm stark), die an ihrer Oberseite mit Honigfutter (streichbares Gemisch aus Honig mit Sojamehl + Trockenhefe (5 : 1) bestrichen ist. Am Boden stehen zwei große Petrischalen (Ø 12 - 15 cm) mit Trockenfutter (Zucker, Milcheiweißpulver, Sojamehl und Trockenhefe: 10 : 10: 1 : 1). Gleichgroße,

aber etwas höhere Petrischalen, gefüllt mit ständig nasser Watte, dienen als Tränke (wöchentlich erneuern). Auf dem Käfig liegen zwei große Petrischalen, deren Außenseite mit Hefeextrakt bestrichen ist und der Käfigdecke dicht anliegt (wichtige Eiweiß- und Vitaminquelle). Die Fliegen saugen sie durch die Käfigmaschen hindurch auf. Das Futter reicht für die Lebenszeit einer Fliegengeneration im Käfer (4 - 6 Wochen). Zur Darbietung von Zwiebelsaft und zur Eiablage enthält jeder Käfig noch zwei Schalen (18 x 12 x 2,5 cm), die bis zum Rand mit ständig feuchtem Sand gefüllt und mit zwei Zwiebelhälften (Schnittfläche nach oben) oder entsprechend dicken Zwiebelscheiben versehen sind (~2 x/Woche wechseln!). Nach vier bis sechs Wochen Betriebsdauer werden die Käfige mit Wasser gewaschen (Dampfstrahlgebläse, Chloraminzusatz zur Desinfektion).

Der Inhalt einer Eiablageschale wird in einer großen Aufzuchtschale (z. B. 32 x 32 x 14 cm), die bereits etwa zu einem Drittel mit längs halbierten Zwiebeln auf feuchtem Sand gefüllt ist (Zwiebelhälften auf der Wurzelpartie stehend, nicht liegend!), einigermaßen gleichmäßig verteilt (Eiablagezwiebeln dazu zerlegen!) und wird mit angefeuchtetem Sand abgedeckt. Vier bis fünf Kontrollen pro Wochen geben darüber Aufschluß, ob und wann noch längs halbierte Zwiebeln nachgelegt werden müssen (auf der Schnittfläche liegend, mit Sand abgedeckt). Grundsätzlich wird der erste Nachschub eine Woche nach dem Ansatz gegeben; weitere, wenn die Zwiebeln stark zerfressen sind. Die Kontrollen zeigen auch, ob die Kultur zu feucht oder zu trocken ist (optimal ist der Feuchtigkeitsgrad guter, normal feuchter Gartenerde). Dementsprechend muß trockener Sand oder Wasser hinzugefügt werden. Bei Zimmertemperatur dauert die Entwickung etwa drei Wochen. Durch Auswaschen mit Leitungswasser (20°C) werden danach die Puparien gewonnen und zum Abtrocknen auf Filterpapier ausgebreitet. Während die Imagines bei einer etwas erhöhten Raumtemperatur von 20-22°C und Langtagebeleuchtung (16 Stunden Licht; Leuchtstoffröhren, Osram L 40 W/25; 8 Stunden Dunkelheit) gehalten werden, lassen sich die Larven ohne künstliche Beleuchtung, aber bei ebenfalls etwas erhöhter Raumtemperatur (20-22°C) aufziehen.

Durchführung der Prüfung

Die Prüfungskäfige bestehen aus Duran-Glaszylindern (Ø = 14 cm; h = 10 cm; Wandstärke = 5 mm; Kanten geschliffen), die auf einer Glasscheibe stehen

und mit einer Deckscheibe (mit 5 cm großem Loch, gegen ein Entweichen der Käfer mit feinmaschiger Kunststoffgaze (20 Fäden/cm) gesichert) versehen sind. Im unteren Teil des Glaszylinders befindet sich eine gut passende Scheibe aus Styropor (~ 2 cm dick), aus der zwei Plastikscheiben (Ø = 5 cm) mittels zweier Nägel, die 1 - 2 cm nach unten aus der Scheibe ragen, befestigt sind. Auf diesem Styropor-Bodenstück befindet sich eine ca. 4 cm hohe Sandschicht mit dem Feuchtigkeitsgrad des Sandes der Zuchtaquarien (= Mischung von 300 cm^3 lufttrockenem Sand und 30 cm^3 Wasser je Käfig). Entsprechend den Praxis- (= Freiland-) Verhältnissen ist eine Zwangsbelüftung nicht vorgesehen.

Die Versorgung der Käfer erfolgt 5 x pro Woche; das Futter wird auf der Sandoberfläche ausgelegt.

Für jeden Prüfungskäfig werden 10 Käferpärchen im Alter von ca. 3 Tagen benötigt. Da Aleochara bilineata über keine leicht sichtbaren sekundären Geschlechtsmerkmale verfügt, ist eine Geschlechtsbestimmung zur Auszählung der gewünschten Pärchen nur durch Beobachtung der Käfer möglich. Hierzu werden 100 - 200 ein bis zwei Tage alte Tiere auf 10 - 15 Petrischalen verteilt und beobachtet. Kopulierende Pärchen werden separiert. Jeweils 10 Pärchen werden bis zur Prüfung in kleinen Glasdosen mit nur minimalem Sandbelag, aber gut versorgt, aufbewahrt.

Für jedes Testpräparat werden 3 Prüfkäfige verwendet; für jede Prüfung sind insgesamt 3 Kontrollkäfige vorzusehen.

Die Prüfung wird entweder unter einem lichtdurchlässigen Regenschutzdach im Freien durchführt oder im Labor (Ventilator-Zwangsbelüftung des Raumes).

Für Versuche mit Aufwandmengen über 600 l/ha werden die Styropor-Bodenstücke in den Zylindern so weit hochgeschoben, daß die Oberfläche des eingefüllten Sandes und der Oberrand des Zylinders in einer Ebene liegen. Dadurch wird verhindert, daß der Spritzstrahl die Innenseite des Zylinders trifft und durch Herablaufen der Tröpfchen Konzentrationserhöhungen im anliegenden Sand eintreten. Für Versuchs mit Aufwandmengen bis 600 l/ha wird der Sand so eingefüllt, daß die Sandoberfläche 3 - 4 cm unterhalb des Zylinderoberrandes liegt. Während der Applikation schützt ein Pappstreifen die Glaswände vor Kontamination. Er wird nach der Behandlung entfernt. Unmittelbar vor der Spritzung wird in den Sand ein etwa

3 cm tiefes Loch gegraben, in welches die Käfer geschüttet werden und das dann sofort wieder verschlossen wird. Bei den Versuchen mit Aufwandmengen über 600 l/ha werden sofort nach der Spritzung die Styropor-Bodenstücke an den Nägeln 3 - 4 cm nach unten gezogen. Die Bodenglasscheiben und die Deckel werden nicht mitbehandelt.

Die Pflanzenschutzmittel werden in der höchsten zugelassenen Konzentration bzw. Aufwandmenge mit Hilfe eines Laborspritzgerätes auf fahrbarem Gestänge gespritzt, wobei durch Einhaltung der technischen Betriebswerte (Flachstrahldüsen 110-2, Druck 4,5 atü, Fahrgeschwindigkeit 0,33 m/sek. und Entfernung von Düse zur Applikationsfläche = 0,7 m) die Reproduzierbarkeit der Behandlung gewährleistet ist. Das Spritzgerät wird zweimal über die ausgelegten Käfige gefahren. Bei einer Aufwandmenge von 600 l/ha beträgt die ausgebrachte Spritzbrühmenge 6 mg/cm².

Bei der Prüfung von Granulaten, die in den Sand eingearbeitet werden, bleiben die Styropor-Bodenstücke unten; die Käfer werden nach der Einarbeitung des Granulates analog dem Ablauf der Prüfung eines spritzbaren Präparates in einem Loch eingegraben (vergl. oben).

Die Spritzapparatur ist unmittelbar nach dem Gebrauch zu entleeren und öfters gründlich zu spülen (Düsen 2-3 Stunden lang); nach einer mehrere Tage langen "Einweichzeit" (Aktivkohle) wird sie dann endgültig gereinigt.

Die erste Fütterung der Käfer erfolgt einige Stunden nach dem Versuchsbeginn.

Danach werden während der gesamten Prüfungsdauer die Käfer 5 x/Woche gefüttert. Am 8., 15. und 22. Prüfungstag erhält jeder Käfig ca. 500 (= 12 cm³) Zwiebelfliegenpuparien (sorgfältig und unter Schonung der Käfer in den Sand einarbeiten!). Da es nicht möglich ist, eine frühzeitige völlige Mortalität der Testtiere festzustellen, muß die Prüfung und damit auch die regelmäßige Puparienzugabe bis zum vorgesehenen Abschluß durchgeführt werden, da ausgetrockneter Sand die Käfer, Eier und Eilarven schädigen würde. Am 29. Tag, also vier Wochen nach Prüfungsbeginn, werden die Deckel entfernt; der Sand soll nun austrocknen. Eine Woche später können die Puparien ausgesiebt und weiterhin getrocknet werden, bis die letzten Käfer geschlüpft sind.

Die Glaszylinder, Glasbodenplatten und Glasdeckel werden in der Laborspülmaschine gewaschen, die Styroporböden werden nicht weiter benützt.

Als Maßstab für eine Nebenwirkung der Pflanzenschutzmittel gegenüber Aleochara bilineata dient die Fertilität der Testtiere bzw. die Parasitierungsleistung ihrer Nachkommen im Vergleich zur Leistung der unter den gleichen Bedingungen lebenden Kontrolltiere. Da die Käfer ihre Eier frei in den Sand ablegen, können die Pflanzenschutzmittel an zwei Stellen wirksam werden: Sie können die Käfer abtöten, dann gibt es keine Eier und keine Nachkommen; sie können aber auch den Käfern gegenüber wirkungslos bleiben, jedoch die Eier oder die Eilarven beeinträchtigen. Auf welchen von diesen beiden Möglichkeiten eine u. U. festgestellte Leistungsminderung beruht, ist für die Bewertung der Prüfung unerheblich, da es letztlich nur auf die Parasitierungsleistung der F_1-Generation ankommt.

Nach dem Schlüpfen der letzten Käfer (~ 6 - 8 Wochen nach dem Absieben der Puparien) werden die von Käfern verlassenen Puparien - kenntlich an den großen Schlupföffnungen - gezählt. Die auf diese Weise ermittelte Zahl der lebenstüchtigen Nachkommen wird als Prozentwert (= Lebendparasitierungsrate) aus der Gesamtzahl der angebotenen Puparien dem Prozentwert der Nachkommenschaft der Kontrolltiere gegenübergestellt. Zum Vergleich zur Kontrolle (= 100 %) wird dann die Parasitierungsleistung der Testtiere in Prozent umgerechnet und als Leistungsminderung oder -mehrung angegeben.

$\frac{P \times 100}{K}$ = % Parasitierungsleistung (Minderung oder Mehrung).

P = Prozentwert der Parasitierung der Puparien in den Prüfkäfigen
K = Prozentwert der Parasitierung der Puparien in den Kontrollkäfigen.

Zur Ermittlung der Gesamtparasitierung (lebende Käfer und in den Wirtspuparien abgestorbene Entwicklungsstadien von Al. bilineata) werden alle Puparien, aus denen weder ein Käfer noch eine Fliege geschlüpft ist, unter dem Binokular aufpräpariert. Man findet, sofern eine Parasitierung stattgefunden hat, tote Käferpuppen oder tote, schlüpfunfähige Käfer. Die Berechnung der Leistungsminderung oder -mehrung erfolgt nach dem gleichen Modus wie oben angegeben.

Da die Präparation der geschlossenen Puppen ausgesprochen zeit- und arbeitsaufwendig ist, kann die Ermittlung der Gesamtparasitierung wegfallen, wenn die Lebendparasitierung zur Bewertung 1 oder 2 geführt hat.

Die Bewertung der Ergebnisse erfolgt nach 4 Wertstufen:

1	= schonend	= 0 - 25 %	Minderung der Parasitierung
2	= schwach schädigend	= 26 - 50 %	Minderung der Parasitierung
3	= mittelstark schädigend	= 51 - 75 %	Minderung der Parasitierung
4	= stark schädigend	= 76 - 100 %	Minderung der Parasitierung

Für die statistische Verrechnung wird davon ausgegangen, daß die beiden durchschnittlichen Parasitierungsraten (Versuch und Kontrolle) zwei Stichprobenwerte, bei Stichprobenumfang n = 3, aus normal verteilten Grundgesamtheiten darstellen. Die Mittelwertsunterschiede werden mittels dem "t-Test nach Student für unabhängige Stichproben" auf ihre Signifikanz überprüft (Freiheitsgrad FG = 2). Bei Ablehnung der Nullhypothese muß das Signifikanzniveau angegeben werden.

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A semi-field method close to field conditions to test effects of pesticides on *Poecilus cupreus* L. (Coleoptera, Carabidae)

Udo Heimbach, Wolfgang Büchs & Christian Abel

Federal Biological Research Centre for Agriculture and Forestry,
Institute for Plant Protection in Field Crops and Grassland,
Messeweg 11/12, 3300 Braunschweig, Germany

Abstract

A semi-field test method close to field conditions designed for the carabid beetle *Poecilus (Pterostichus) cupreus* L. is described. The aim is to be as close as possible to field conditions. The test equipment is metal frames of 0,25m² up to 1m² size which are sunk into the soil of the field, so that the soil structure and type of the field as well as the crop plants are included and all abiotic factors (rain, wind, sun etc.) are able to act nearly unaffected. Ten individuals of *Poecilus cupreus* L., reared in the laboratory, are released in each frame. The assessments are done at least for 21 days and include especially the registration of mortality, behavioural changes and food consumption.
The results regarding the test substance are compared with a water treated control and a toxic standard. Evaluation is done according to IOBC criteria.

Introduction

Up to now members of the IOBC-working group "Pesticides and Beneficial Organisms" developed a number of test methods for a wide range of test organisms, but mainly for laboratory purposes (SAMSOE-PETERSON 1990). Following HASSAN (1989) there is still a lack of semi-field and field methods.
The method described here has shown its suitability under various conditions (BÜCHS et al. 1989, 1991, HEIMBACH & ABEL 1991 and other authors) and for different carabid species (e.g. *Poecilus cupreus* L., *Pterostichus melanarius* (Ill.), *Carabus granulatus* L., *Bembidion lampros* (Hbst.)). There is a well-based knowledge about advantages and limits of this method which facilitates the interpretation of the data recorded.
It is assumed that the semi-field test will in many cases be the last step suitable for routine testing. This is due to the high costs of real field experiments, the need for a high number of replications over a longer period of time and by the difficulties regarding their interpretation (many influencing factors and great variations in natural occuring populations).
The described test represents a section of the field fenced off, only introducing laboratory reared species. Thus, quite natural conditions are given by including crop plants and the "natural soil". Abiotic factors are left nearly unaffected. This method, very close to real field conditions, enables to test the effects of pesticides by using a variety of application techniques, for example spraying, in furrow application, banded application, seed dressings and granular pesticides.

Some problems in handling the test might occur on using it when the crop plants have grown up and some restrictions are given by the dependence on the soil type and by the relative high amount of work necessary for the recapture of the beetles. Therefore, especially if a more "worst case" situation with many observations about sublethal effects during trial run shall be regarded, the more standardized method described by ABEL & HEIMBACH (1992) might be used.

Basic requirements for the test

The test described represents one step in a sequential testing scheme, therefore some basic principles of the laboratory test (HEIMBACH & BRASSE 1991) are applied here as well.
The test should contain at least three basic treatments: a treatment with the test substance, a toxic standard and an untreated control.
If the application is done by spraying, the untreated control should be sprayed with the same amount of water as the treated plots, according to the recommendations of the IOBC.
Additional informations about the test conditions are provided by using a toxic standard as third basic treatment. According to experiences from many field tests dimethoate (400 g a.i/ha) is recommended.
Each treatment has to be carried out in at least four replications.

Test species

Although there is a background of experiences with other carabid species, the test described here is designed for *Poecilus cupreus* L. Its suitability for other carabid species has to be proved finally. *Poecilus cupreus* L. is a carabid species which is widespread in agricultural crops. Its ecological preferences are described as eurytope, hygrophile and campicole (KOCH 1989). Copulation period is spring, eggs are positioned near surface level into the soil or under stones. Embryonic development takes about 12 days. Pupation happens until the end of summer. The hibernation stage is that of the imago (BURMEISTER 1939). In comparison to other species mass rearing is possible and comparatively easy (HEIMBACH, 1992).
Only laboratory reared organisms should be used in the test. For the rearing procedure see HEIMBACH (1992). Because most applications are done in spring or summer, hibernated individuals should be used for the test. Only if applications are carried out later than july, it might be possible to use newly metamorphosed adults of equal age of between two and ten weeks like in the laboratory test. The beetles should have the opportunity to recover from hibernation for at least two weeks before being used in tests.

Test equipment

For the caging of the released beetles square metal frames, made of zinced (or V2A steel) metal sheets, at least 100 mm high, enclosing an area of 0,25 m^2 to 1 m^2 are used. To prevent the escaping of the beetles by climbing, the upper inner walls of the

metal frames have to be coated with a gliding substance like "Fluon" (ICI), and the upper edge of the frame should be bent inwards for about 10 mm. As *Poecilus cupreus* L. is able to fly it is necessary to stick the elytra together with a drop of liquified wax (ABEL, in preparation).

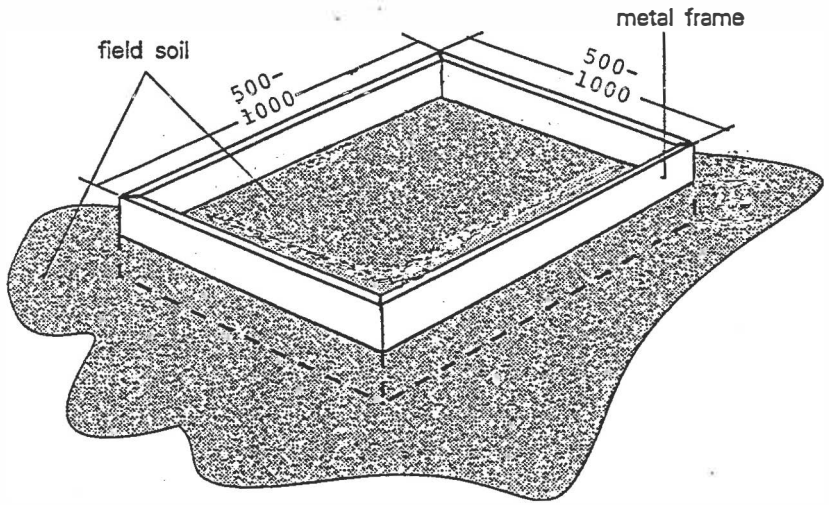

fig. 1: Test equipment used in the semi-field test (see text; linear measures in mm)

Crop plants inside the frame should not touch the walls of the frame or other plants outside the frame, so that the test organisms are unable to climb out.
If the test is endangered by birds picking up the beetles, especially when the test is carried out in early stages of the crop plants wire screens of 10 to 20 mm mesh size should be used to protect the frames.

Location of the test

As location for the test a field should be chosen which is integrated in a usual crop rotation. The soil of the experimental site should be characteristic for the crop in which the test will be performed. The test should be carried out with different soil types, crops and at different seasons.
It is assumed that pesticides act more agressively if the percentage of clay and organic carbon (ABEL et al., 1992) is lower. If it is intended to do the test in a more "worst case" situation a location with sandy soil should be selected if it is not unsuitable for the crop in which the test has to be carried out.
If spraying equipment allows, the replications of the different treatments should be placed randomly because of variations within the field (HEIMBACH et al., 1992). Plot design should avoid the risk of contamination of adjacent plots by drift and trial plots should not be situated at the edges of the field.

Application of the test substance

For the application a modern professional spraying equipment should be used which is currently used in agricultural practice. Timing of application should be adapted to the period and conditions (e.g. temperature, humidity) indicated on the label and in correspondence with good agricultural practice.
The handling of the test procedure is easier if the pesticide application is conducted in the earliest recommended stage of crop plant development. Thus the probability to discover dead or live carabid beetles when checking mortality is higher. The application should be carried out in the morning at temperatures exceeding 10 °C if possible.
During the testing period no other pesticides or fertilizers should be used.

Survey of additional data

Amongst climatic factors at least air temperature and rainfall should be measured three days before the beginning until the end of the test period. Additional data on the temperature in the crop layer and on the soil surface are as well useful as data of relative humidity, soil temperature in a depth of 5 cm, soil moisture, wind speed and hours of sunshine.
Basic soil characteristics should be recorded, e.g.: grain size, organic matter content, water capacity and pH.
Furthermore should be registered: crop plants/m², weeds/m², drilled seeds/m², stage of development of the crop plant, plant height, covering rate (separately for crop plants, weeds and organic litter). The proportion of organic litter should be estimated. The structure of the soil and the soil surface (e.g.: straw cover) has to be described.
For further information samples of the soil and of the crop plant might be taken before and at the end of the test. Additionally it is possible to take samples at any time of the test period to determine pesticide residues and metabolites. For this purpose additional frames should be sprayed.

Test procedure

The metal frames are sunk into the soil for at least 5 cm. (These are minimum measurements which give sufficient results. Anyway it would be favourable, to use higher frames, sunk into the soil more deeply, but this will cause more work) The frames should not overtop the soil surface more than 5 cm to avoid a reduced evaporation of pesticides (HEIMBACH et al., 1992). When setting up the frames neither the soil structure and surface nor the crop plants should be damaged. Therefore the setting up of the frames should be done at an early stage of crop plant development.
To get the frames into the soil more easily, the soil is pricked with a spade along the outer edges of the metal frames. Afterwards the frames are pressed into the ground. This operation should be

repeated until the frames are dug in deep enough. It is possible to accelerate this procedure by using a kind of sledge hammer with a round elastic or a nylon head, but before it is necessary to cover the metal frame with a board made of hardwood to avoid damages of the metal frames.
Although there is natural occuring prey in the area enclosed by the metal frames, additional feeding of the beetles is recommended. The beetles are kept without food for three days before starting the test. Before the application one *Musca*-pupae per beetle is placed in an defined area in the frame. Feeding with one fly pupae per beetle is repeated every three or four days.
At least two hours before the application of the test substance five males and five females of *Poecilus cupreus* L. are released in each frame. Just before the application the additional food is placed in each frame.
6 hours after the application flat stones should be placed in the corners of the frames where the beetles can hide. This gives a better chance to find some beetles when checking.
For the checking of mortality and behavioural changes the stones in the corners of the frames are removed carefully and the soil surface is searched visually without destroying the soil structure and crop plants inside the frames.
The following time schedule for checks after the application is proposed: at 2h, 4h, 6h, 24h, 2d, 3d, 4d, 7d and twice a week afterwards at least for 3 weeks alltogether. The fly pupae are collected every third or fourth day and replaced by fresh ones. The number of untouched and eaten pupae has to be recorded. It should be given attention to the way the pupae are opened, for often mice or birds feed on the pupae which results in a different view of the left integuments of pupae. Mice will open the pupae at one end, while carabids will open them along the long side.
Beetles which are hurt, but still alive (e.g. lying on their backs and twitching their legs when stimulated) should be collected in a defined place of the frame and observed whether they will recover or not. They might be used to prove the content of residues of the test substance. Sublethal effects like behavioural changes and also the food consumption should be noted as described below.
The time schedule of checks should be adapted to the way of acting of the test substance. A test period of at least 21 days is recommended. Depending on the test course it might be necessary in some cases to prolong the test period up to 6 weeks: if effects still occur (in week 3: an average mortality of more than 10 % or more than 20 % of the observed beetles with sublethal effects) or if due to climatic conditions less than 20 % of the inserted carabids can be observed in the average of the checks of the first three weeks.
At the end of the main test period the stones and plants (incl. the roots) and the soil surface are searched for dead or living beetles and for fly pupae. Untouched fly pupae which have not been recorded before are also registered.
The soil within the frame is removed down to the lower edge of the frame and is sifted by using a sieve of about 4 mm mesh width. It is obvious that this process can be stopped at any time when 100% of the released beetles are recaptured. If not all beetles are found the frames can be flooded to force the remaining beetles to

come up. All other arthropods found when sieving the soil or watering should be recorded.

Reporting of the results

The results of the treatment with the test substance are compared with those of the water treated control and the toxic standard. Each result should be shown as the average of its replications. The standard deviation of the averages and also the extreme values have to be mentioned. The chronological development of a parameter might be shown as table or a graph. A table should number the beetles found dead or alive or with sublethal damages and the total recapture rate.
The number of fly pupae consumed should be recorded at any time when new fly pupae are placed into the frames. The pupae have to be registered as "consumed" or "untouched". Pupae which are not found are evaluated as "consumed" as well as the pupae which are partly or completely eaten. The results are described as number of pupae consumed per frame separately for each treatment considering the number of pupae which are found while sifting the soil at the end of the trial.
Rate and type of behavioural changes should be noted, when symptoms are observed as follows:
- obstructions in coordination of the legs like
  - paralyzation of hind legs or
  - staggering movements.
- continuous cleaning of antenna and mouthparts
- non-rhythmic movements of mandibulae
- regurgitation
- hyperactivity
- lethargy

Beetles showing these symptoms are rated as "affected". If beetles are laying on their back and twitch their legs when being touched they are recorded as "badly affected" and collected in a defined area of the frame.
Other insects found when sieving and watering the soil should be recorded for each treatment.

Evaluation of the test

The results are only valid if the mortality in the untreated control is lower than 20% and if the recapture rate of the released beetles is at least 70% in the untreated control.
Beetles, still lying on their backs at the following assessment date starting with the check 24 h after the application are evaluated as dead.
A statistical survey of the results concerning the mortality and food uptake is recommended by using for example a chi-square-test. A significance level of at least $p < 0,10$ should be selected.
The effects of the pesticides are calculated by taking into account the mortality in the untreated control. The grade of effectiveness is calculated according to Abbott (1925) and evaluation is done according to the IOBC-categories.
1 = harmless (< 25%), 2 = slightly harmful (> 25% - 50%)
3 = moderatly harmful (> 50% - 75%), 4 = harmful (> 75%)

Special incidents like lightning, storm, heavy rain, longer drought etc. shortly before or during the test have to be recorded and considered in the interpretation of the effects of the test substance.

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A semi-field testing method for the evaluation of pesticides with the hoverfly *Episyrphus balteatus* (Dipt., Syrphidae)

I.TORNIER & W. DRESCHER
Institut für Landwirtschaftliche Zoologie und Bienenkunde
Melbweg 42, D-5300 Bonn 1, GFR

1.Introduction

To strengthen the basis for integrated pest control, the testing of beneficial insects proceeds, comparable to the testing of the bee-toxicity of pesticides, in three steps: laboratory - semi-field - field (HASSAN et.al., 1989). Concerning syrphids a laboratory testing method has been developed for the larval stages of the syrphids (RIECKMANN, 1989).

A semifield testing method for the verification and completion of the laboratory data has been developed at the "Institut für Landwirtschaftliche Zoologie und Bienenkunde".

Principle of the test:

This method includes the evaluation of the oral and contact toxicity-effects on imaginal syrphid flies of *Episyrphus balteatus* Deg. and also on their fecundity.

2.Materials and methods

2.1. Biology of test organism

Only second to the hymenoptera,the syrphids, a Diptera family rich in species, are one of the most important pollinators. Adults of all known species feed on pollen, nectar and honeydew of homoptera. The larvae of approximately 40% of the palaearctic species are zoophagous and thus of economic interest for biological pest control. *Episyrphus balteatus*, an abundant syrphid species, is polyvoltine with four generations per year. It can be observed throughout the entire vegetation period, the adult flies frequently visiting the flowers of the order umbelliflorae. Like in most syrphid species, the larvae are polyphagous. NINOMIYA (1957) found them feeding on 37 different aphid species.

2.2. Mass rearing of syrphids

For testing the side-effects of pesticides on beneficial insects, an efficient mass-rearing method of the predator satisfying the qualitative and quantitative requirements is necessary.

The adults of *Episyrphus balteatus* are kept in gauze-covered wooden cages (75x60x50cm) in environmentally controlled greenhouse-cabins with a photoperiod of L:D = 16:8. The animals are feed with:

- cubes of cristallized sugar
- solution of 80% honey and 20% water, offered on yellow sponges
- water in petridishes with cotton-swaps
- freshly ground pollen, collected by honeybees

The food should be replaced once a week, otherwise fungi will grow in the honey solution and the pollen becomes too moist.

Oviposition generally starts with 8-10 days old females. For this purpose aphid infested bean-shoots are placed into the cages early in the morning for two hours. 20 female hoverflies per cage deposit about 400 eggs within this period.

To produce a high number of fertile eggs, the following conditions must be fulfilled:

- cages must be large enough, since mating of *E. balteatus* occurs only during flight
- light intensity must be at least 7000 lux
- bean-shoots (*Vicia faba*), added for oviposition, must be heavily infested with aphids.

Shortly before the hatching of the larvae the eggs (200-250) are distributed on trays with freshly germinated beans under laboratory conditions (20^0 C and rel. humidity of 50-60 %). The larvea are fed with aphids. In our rearing system a mixed population of *Aphis fabae, Acyrtosiphon pisum* and *Megoura viceae* proved to be the most useful.

This mass rearing technique can also be applied to other aphidophagous syrphid species, for instance *Sphaerophoria scripta* as tested by S. NENGEL & W. DRESCHER (1990).

2.3. Requirements for the experiments

2.3.1. Testplant

The testplant must meet the following requirements:

- it should belong to cultivated plant species
- the anthers and nectar must be easily accessible for syrphids
- the plants should be easy to cultivate, with a relatively short vegetative phase but a long flowering period
- the leaf area should have a sufficient size for pesticide application.

Experiments proved that buckwheat (*Fagopyrum esculentum*) is especially suitable. Buckwheat is quite easy to grow in crates. The plant will reach the budding stage about 6-8 weeks after planting. It is also very frequently visited by hoverflies in the field. For the pesticide application the size of the leaves is sufficient.

2.3.2. Experimental cages

Each experimental phase requires four cages (two for pesticides, two for control) measuring 1 x 1 x 1m. The cage-frames are completely covered with white plastic screen. To facilitate the daily control the cages are placed 50cm above the ground. The cage-floor is also covered with gauze in order to prevent the formation of puddles deriving from rain or plant watering. Due to a zipper at the front side, the inside of each cage can easily be managed during experiments. To prevent the escape of syrphids while working in the cages a transportable gauze-tunnel is used as a sluise. Through this the con-trolling person can enter the cage with head and arms.

2.3.3. Pesticide application

A newly designed device facilitates the simulation of spraying under field conditions (BÜCHLER & DRESCHER, 1989) This apparatus proved to be suitable for spraying buckwheat in crates, too.

A spraying nozzle, which can be adjusted in height and drop size, is fastened above guiding rails. An electrically driven sledge, moving back and forth with two different speeds, is placed on these rails underneath the spraying nozzle. Crates, containing 12-15 plants in the budding stage are put on the sledge and sprayed with concentrations used under normal field conditions. The plants in the two control cages are treated with water. If there are a few open blossoms they are removed before.

2.4. Performance of the pesticide screening

2.4.1. Initial toxicity test

As soon as the sprayed pesticide film has dried up, one crate with buckwheat plants is placed in the middle of each of the two test cages. A container with aphid infested beans is added at about blossom level. Then 16 about four to six-day-old imaginal hoverflies (8 females, 8 males) are released in each cage. In order to guarantee uniform material required for the evaluation these animals should be reared under laboratory conditions (same age, similar fitness).

The following parameters are registered daily:

- number of dead animals (The dead animals are removed from the cages with forceps and the sex is determined)
- behavior of the syrphids (abnormal behavior, flight-activity, flower visits, visit of the aphid infested beans)
- oviposition.

2.4.2. Residual effects of the pesticide

Eight days after pesticide application the buckwheat plants are in full bloom. At this stage another 16 adult hoverflies per cage of known age and sex are released. First, animals which survived the initial toxicity test are removed from the cages and the aphid

infested beans are replaced. The control parameters of this test are equal to those of the initial toxicity test of the same pesticide. If eggs have been deposited on the beans, these are replaced by new ones (3 times during the experiment). Bean leaves, on which eggs have been laid, are removed from the plant and kept in petri-dishes, so the larvae can hatch under laboratory conditions. Their number is counted after two days. If the eggs are placed in water (20°C) without leaves cannibalism among the hatching larvae can be avoided and counting is easier.

2.4.3. Meteorological data

Temperature and humidity are recorded during the entire test period.

3. Number of replications

For statistical reasons the experiment is repeated at least with two experimental cages and two control cages each time within the same vegetation period. Analogous to the bee-toxicity test, a parallel evaluation at other laboratories would be desirable.

3.1. Recording of mortality

The number of dead animals is registered daily and summed up at the end of the experiment. The test for initial toxicity ends four days after spraying; the residual test starts on the eighth day of the test procedure and lasts for six days. For the calculation of the pesticide-effect the SCHNEIDER-ORELLI formula is used.

3.2. Recording of the fertility.

In the residual test the average fertility of treated females is compared with that of the control females. The values are corrected by the ABBOTT-formula.

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Testing effects of pesticides on Poecilus cupreus (Coleoptera, Carabidae) in a standardized semi-field test

Christian Abel & Udo Heimbach

Federal Biological Research Centre for Agriculture and Forestry,
Institute for Plant Protection in Field Crops and Grassland,
Messeweg 11/12, 3300 Braunschweig, Germany

Abstract

The described semi-field test is designed as a mono species test, representing the second step in a sequential testing scheme, whose laboratory test is already established. The test is conducted with a fixed number of individuals of the predatory ground beetle *Poecilus (Pterostichus) cupreus* L. reared in laboratory. It is carried out in the field with the beetles being kept in a separate box, using standardized soil. All climatic factors can act uninfluenced. Effect assessment is done for at least 14 days following the application of pesticides. It is based on the registration of mortality, food consumption and behavioural changes and is compared with a water treated control and a toxic standard. The classification of the data is based on IOBC-guidelines.

Introduction

A number of methods for testing the effects of pesticides on beneficial arthropods have been developed until now for a variety of testing organisms by members of the IOBC working group "Pesticides and Beneficial Organisms" (Samsoe-Petersen, 1990). Most of them are laboratory testing methods, amongst them some concerning carabid beetles (Chiverton, 1988; Heimbach & Brasse, 1991). The majority of them use laboratory reared testing organisms. In contrast, testing the effects of pesticides on carabid beetles in the field has mostly been carried out on field-collected species, with only a few exceptions (Förster, 1991; Büchs et al., 1991; Heimbach et al., 1992). Having established a mass rearing of *Poecilus cupreus* (Heimbach, 1989) for laboratory testing purposes, we found it useful to also do the second step in the sequential testing scheme with laboratory-bred animals, having a large amount of data and experiences as a background for the interpretation of data obtained from semi-field testing. Other features of the described semi-field test were influenced by the fact, that this would normally be the last step in testing the effects of pesticides due to increasing costs when continuing testing. Therefore the test should represent a "worst case" field situation. If the aim is to be as close as possible to field conditions and if special application techniques shall be tested the method described by Heimbach et al. (1992) might be taken into consideration.

Methods and Materials

Test species

Imagines of the carabid beetle *Poecilus (Pterostichus) cupreus* from a laboratory breeding stock are used as test organisms. *Poecilus cupreus* is a predatory ground beetle, encoun-

tered frequently on agricultural sites. It is diurnal, univoltine like most carabid beetles and belongs to the group of spring breeders with obligatory diapause. Breeding is done as described by Heimbach (1989) and Heimbach & Brasse (1991). In deviation from the laboratory test (Heimbach & Brasse, 1991), overwintered beetles are used in the semi-field test, kept between 2 and 4 months under short-day conditions and at temperatures below 10 °C. Thus the beetles have reached maturity when they are used in the test.

Test containers and test substrate

The test containers are made of zinced sheet metal. They measure 26 cm to 23.5 cm and are about 12 cm high (figure 1). The bottom of each container consists of a fine mesh gauze supported in the middle by a small metal profile. The bottom is fixed to the walls using silicone adhesive. The upper part of the inner surface of the container is lined with a self-adhesive plastic film. On the day before application and before filling in the soil this plastic film is coated with "Fluon" (ICI, polytetrafluoroethylene) a gliding substance

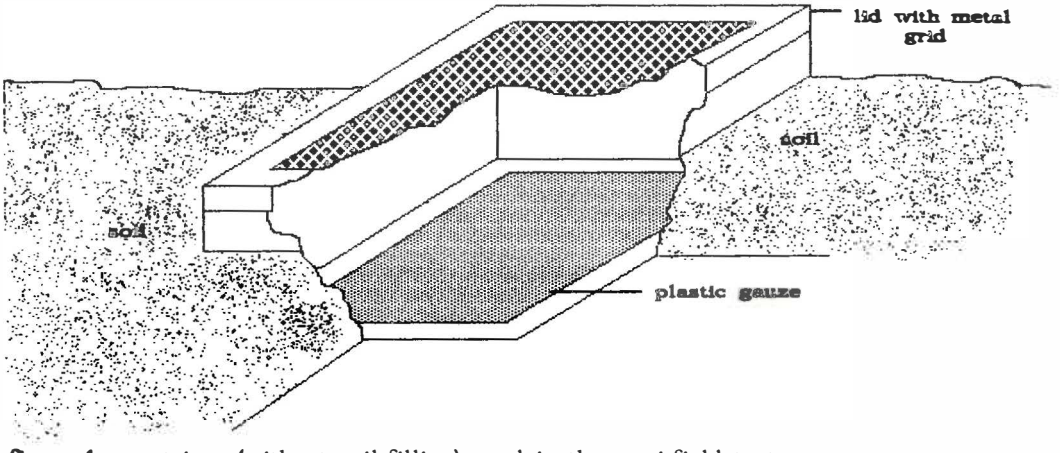

figure 1 : container (without soil-filling) used in the semi-field test

preventing beetles from leaving the container. The whole container is covered by a metal frame, holding a metal-grating with a mesh-size of about 1 cm. On the one hand this shall prevent birds from picking out the beetles and on the other hand the surrounding crop from looming into the container. The containers are brought to the field at an early development stage of the crop to keep damage of the surrounding crop as low as possible. They are sunk into the soil, so that the substrate, filled in on the day before application will be at the same level as the surface of the surrounding soil. As test substrate a silty sand (for example 54.1 % sand, 39 % silt, 6.9 % clay, 0.95 % $C_{org.}$) is used. Before filling it into the boxes, the air-dried soil is strained through a sieve of 2 mm mesh-size. Water content and maximum water holding capacity is determined. 4 kg soil, containing an amount of 60% of the maximum water holding capacity is filled into the test containers on the day before application and moistened on the surface immediately before application if necessary, so that the soil surface is wet at the time of application. Each treatment consists of six replicates containing 6 beetles each with an equal sex ratio.

Test substances

The control is sprayed with water (equivalent to 400 l/ha). As toxic standard parathion is used (105 g a.i./ha) or another pesticide toxic to *Poecilus cupreus*. The toxic standard should cause 70 ± 30 % mortality. All tested pesticides should be used with the highest dose recommended for field use. All substances are sprayed with an amount of water equivalent to 400 l/ha. If deviating amounts of water are in compliance with good agricultural practice (for example orchards) substances should be sprayed with this amount of water.

Application of the pesticide

The equipment used for application should be of a type in current use. Nozzle type, pressure and speed should ensure a realistic particle distribution and particle size. The time of application must be during the recommended period (i.e. according to proposed label timing). Application should take place within the recommended period but when ground cover of the crop is as low as possible, at temperatures exceeding a daily mean of 10 °C on the day of application if possible. Testing should be done at different times of the year or in different regions.

Test procedure

The beetles are kept without feeding under climatic conditions similar to field conditions for the last 3 days before application. Immediately before spraying the soil is moistened if necessary, then food is given in excess (at least 10 *Musca* spec. pupae per container, fixed number in all containers) and finally all beetles are introduced. Then the application is made. Thus beetles, soil and food are exposed to the pesticide. While spraying the cover of the containers is removed and reinstalled afterwards.

Proposal for a time frame

T_{-1} Preparation of containers (coating of inner walls, filling in the soil)

T_{0-1h} Moistening of soil, introduction of food and test organisms

T_0 Application

T_{0+2h} Assessment (behaviour)

T_{0+4h} "

T_{0+6h} "

T_{+1} "

T_{+2} Assessment (control of behaviour/removal of pupae and insertion of fresh food/ removal of dead individuals)
same procedure every second day

T_{+14} End of trial if consistent condition of animals between last two assessments and reduction in individual feeding rate below 25 %, otherwise prolongation for another 14 days

$T_{+14} - T_{+28}$
Assessments as described above two times a week

T_{+14} or T_{+28}
End of trial with searching for missing beetles and missing pupae

Assessments

Direct toxicity

After the application the impact of the pesticides on the behaviour of the test organisms is assessed after 2, 6 and 24 hours and every second following day until day 14. An additional assessment after 4 hours is done if first symptoms occur after 2 hours already. Only those individuals are rated which can be seen without digging the soil. Rating is done as follows: individuals with no unusual behaviour are rated "normal", those with abnormal behaviour such as starking with the hind legs, uncoordinated movement, unusual rhythmic extension of mandibles, intensive cleaning of antenna and dragging of hind legs are rated as "ill" with description of the type of damage. If an individual is lying on its back, this is to be recorded separately, for often this is a sign of an irreversible damage. If a beetle doesn' t move even after tactile stimulus, it is rated as "dead" and taken away from the test container. If no stable condition has been reached between the last two assessments of the two week testing period, the test is prolonged for 14 further days, with two assessments a week. At the end of the trial, the containers are taken to the laboratory and searched for surviving beetles. This is done by flooding the containers with water from the bottom side, so that beetles get up to the surface and can be collected. If individuals are still missing, the whole content of the container is passed through a sieve of 2 mm mesh size using a jet of water.

Food consumption

Every second day following application fly pupae are removed from the containers and controlled for feeding marks. If partly or completely eaten, they are rated as consumed, otherwise as untouched. Missing pupae are denoted "consumed". A fixed number of fresh pupae (higher than effective consumption) is introduced to the containers. If the average individual feeding rate within a treatment shows a reduction of 25 % compared with the control (taking into account the number of surviving individuals) the trial will be prolonged for 14 further days with two assessments per week. At the end of the trial the soil is searched for not discovered untouched pupae.

Evaluation

A test is valid if the mortality in the control does not exceed 10 % and if the effects caused by the pesticide used as toxic standard are within a normal range. Two criteria are used for the IOBC classification. The number of affected beetles and the average food intake of the living beetles of a treatment. If the IOBC values for these two criteria differ, the higher value of the two should be used to decide whether further testing is necessary. The number of dead males and females, of affected beetles on a sublethal level and the average food consumption per living beetle of a treatment should be listed per box and for each treatment and assessment. If the number of dead and/or affected beetles (e.g. abnormal behaviour, uncoordinated movement, lying on the back) of a treatment at any assessment carried out 24 hours after the application of the pesticide or later exceeds 24 %, it should be classified according to IOBC class 2, from 51-75 % class 3 and with more than 75 % class 4. The average food intake of the beetles

treated with a pesticide is compared to the food consumption of the water sprayed beetles. The average food intake is calculated by adding the 6 values for each date of food assessment and by calculating the mean. If it differs at least 25 % from the control, it has to be classified as shown above and further testing should be carried out. Several tests should be carried out during the year under different climatic regimes to gain more information on the range of action of a pesticide.

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A field method for testing effects of pesticides on larvae of the green lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae)

Heidrun VOGT[1], Silke RUMPF[1], Carmen WETZEL[1] and Sherif HASSAN[2]

Federal Biological Research Centre for Agriculture and Forestry

1) Institute for Plant Protection in Fruit Crops, Dossenheim

2) Institute for Biological Pest Control, Darmstadt

1. General remarks

Laboratory and semi-field methods to test the side effects of pesticides on *Chrysoperla carnea* have recently been published by BIGLER (1988) and by BIGLER and WALDENBURGER (1988). The method used for mass rearing of the green lacewing was described by HASSAN (1975). In this work, a field method to test the side effects of pesticides on this predator is described, which completes the sequential testing scheme.

2. Materials

2.1 Test plants

The tests are carried out under field conditions on young dwarf apple trees (M 9) with a tree-crown of approximately 1 m in diameter. The distance between the trees should be at least 2.50 m. The trees should be homogeneous in growth, vigour and health status and should be free of phytophagous insects and mites. For each pesticide tested as well as for the water treated control 10 apple trees are needed. Each tree serves as a replicate. - Previous experiments have shown that the tests can also be carried out on crops such as sugar beet (HASSAN et al. 1985), strawberries, red or black current (WETZEL 1988, WETZEL et al. 1990) and wheat (HASSAN, unpublished results).

2.2 Test-insects

Laboratory reared *C. carnea* larvae uniform in age are used for the test. In order to prepare rationally the large number of the predators needed for the test, the following proceeding has been developed: Cotton cloth pieces with about 1000 *C. carnea* eggs on each of it, deposited within one day in the mass rearing, are placed in flat containers (40 x 15 x 4 cm). Upon emergence, the young larvae are fed with eggs of the Angoumois grain moth *Sitotroga cerealella* (Oliv.). The containers are kept at 25° C, 50-70 % relative humidity and 16 h light until the larvae have reached the beginning of the second instar. This will require about 3 days after the beginning of hatching. The containers

used fit into each other when piled. An adhesive tape of foam material (Tesamoll padding) along the outer walls provides sealed compartments when the containers are piled. - 400 larvae are needed for each tree. L2 larvae are recommended when testing "classical compounds", e.g. compounds which operate neurotoxically and kill the organisms rather quickly. Larval stage "end of L2" to " beginning of L3" is recommended when testing juvenoids, which have no initial toxicity but prevent the larvae from pupating.

2.3 Baitcards

Baitcards are used for the recapture of surviving larvae and for the observation of pupation, if juvenoids are tested. The baitcards contain *S. cerealella* eggs as food and they offer shelter. Different types of baitcards have to be used, depending on their position in the tree-crown or on the ground and depending on the compounds tested.

A) For testing "classical compounds":
Black cardboard pieces of 5 x 7 cm are folded three times, fan-shaped (fig. 1). The baitcards are fixed to the tree with pegs.

B) For testing juvenoids:
Black velvet cardboard (one side of the cardboard is coated with a velvet material) is cut into pieces 4 x 5 cm, folded three times, fan-shaped like type A. As a hiding place for pupation the larvae prefer the velvet material to the cardboard. The folded velvet cardboard pieces are placed into small plastic tubes, which have grooves. These tubes are 3 cm in length, cut off from black electric insulating pipes, with an inner diameter of about 17 mm and an outer diameter of about 20 mm. The tubes are then fixed to the branches of the trees with pegs, fig. 3 (RUMPF 1990, RUMPF et al. 1990).

C) Baitcards used for recapture on the ground (for testing "classical compounds" and juvenoids):
Black cardboard pieces as used for type A are folded only once (like a tent, fig. 2). Pegs, fixed to the baitcards, serve as weights.

With a brush a special glue (Planatol HW 7, manufactured by Fa. Planatolwerk W. Hesselmann, Postfach 280, D-8200 Rosenheim), slightly thinned with water, is applied to a small area of about 1 cm in diameter on the inner surface of the baitcards and eggs of *Sitotroga cerealella* are strewn over it. The baitcards

have to be stored at a low temperature (about 5° C).

3. Test procedure

Day 1

400 *C. carnea* larvae are put on each tree. In order to facilitate the distribution of the larvae, they are tansferred into open vessels, the upper part of their walls being coated with fluon (Polytetraflouroethylene) in order to prevent the insects from escaping. Fan-shaped folded paper strips in the vessels offer the larvae shelter. From these paper strips the larvae are brushed off carefully onto the leaves counting them exactly. In order to obtain an equal distribution of the larvae and to reduce errors when several persons are involved in the experiment, it is recommended to distribute the larvae according to a "rotating system": each person puts 50 larvae on a tree, notes this on a label fixed to the tree, changes to the next tree, puts 50 larvae on it and so on. - According to our experience the distribution of the larvae on 20 trees (i.e. 8000 insects) takes about 3 to 4 hours for 3 persons.

Pesticide treatment

After the distribution of the larvae has been finished, the plants are treated with the pesticides at the highest recommended concentration to the point of run off using a knap-sack handsprayer. The untreated control plants are sprayed with water.

Day 2

24 hrs after the treatment of the trees 15 baitcards of type A or B are attached to each tree and between 10 to 15 baitcards of type C are put on the ground around the tree trunk. These cards allow the recapture of surviving larvae. The baitcards of type B additionally allow the observation of pupation.

Day 3

In the morning of the third day the first evaluation of recaptured larvae is started. The larvae found under the baitcards are counted and put into a vessel containing *S. cerealella* eggs. Baitcards, on which the eggs have been sucked out, are replaced by new ones. When all the baitcards from one tree have been assessed, the larvae are put back onto the tree using a fine brush. Assessment of one tree takes between 20 to 30 minutes. Control trees and those treated with the test product should be checked alternately so that possible influences on the activity of the larvae by rising temperatures and sunshine are the

same for both variants.- The baitcards have to be assessed very carefully as the larvae are not only found on the cardboard but also on the pegs, especially in the metal whorl. In the baitcards of type B they like to hide in the grooves of the tubes. For pupation the same places are used as mentioned above. Therefore, when looking for cocoons, these places again have to be checked very carefully.

Day 4 and following days

Evaluations as described above are continued. When testing "classical compounds" daily monitoring is necessary, because of the rather quick effects. When testing juvenoids, the baitcards are checked daily for about 2-3 days and then only every second day in order to reduce disturbance of the pupating larvae. According to our experience about half of the baitcards have to be replaced when monitoring daily and all baitcards have to be replaced when monitoring every second day. Sufficient food supply is essential for obtaining high recapture rates, because otherwise the larvae leave the plants searching for food. Good recapture rates in the tree crown (control trees) amount to 10 or 15 % for several days. In the baitcards on the ground less larvae are to be found: usually < 5 %.

End of the test

With "classical compounds" the final evaluation is carried out when the recapture of larvae gets very low, usually after 3 to 5 days. With juvenoids the test ends when the number of cocoons found in the baitcards gets very low. A test with juvenoids can last 14 days.

4. Statistical analysis

If only one pesticide has been tested, the t-test is used (for data values of normal distribution) and the Wilcoxon, Mann, Whitney U-test, respectively, if the data values are not normally distributed. - If more than one pesticide has been tested, the mean values are examined with an analysis of variance and in the following the Scheffé-test is used in order to find out which mean values differ significantly.

5. Classification of the pesticides

The results are calculated using the formula of ABBOTT (1925) as follows:

5.1 "Classical pesticides":

$$\text{Effectiveness \%} = \frac{\text{larvae/tree WT - larvae/tree PT}}{\text{larvae/tree WT}} \times 100$$

(WT = water treated; PT = pesticide treated)

5.1 Juvenoids:

$$\text{Effectiveness \%} = \frac{\text{cocoons/tree Wt - cocoons/tree PT}}{\text{cocoons/tree Wt}} \times 100$$

The classification the pesticide's harmfulness follows the guidelines of the IOBC working group "Pesticides and Beneficial Organisms" (HASSAN 1985):

Class 1 = harmless:	< 25 %	mortality or reduction of cocoons
Class 2 = slightly harmful:	25-50 %	" "
Class 3 = moderately harmful:	51-75 %	" "
Class 4 = harmful:	> 75 %	" "

6. Final remarks

Up to now different types of pesticides have been examined with the method described here. The method is appropriate for the compounds mentioned above and it worked well with a fungicide (Triforin, common name = Saprol). First experiences exist with the testing of a chitin synthesis inhibitor (Diflubenzuron, common name = Dimilin). Using the type B baitcards the method seems to be suited for testing these products, too.

As to the test plants, strawberry plants as well as red and black currant bushes probably can be used. With currant bushes one plant serves as a replicate, with stawberries a testing unit consists of several plants. With these fruit crops experiments were carried out releasing about 1000 *C. carnea* larvae per replicate. This number was not counted exactly but only estimated. The recapture rates were satisfactory. But as the estimation of the number of released larvae was not exact enough, further tests have to be carried out with the release of less larvae, which can be counted exactly without taking too much time (e. g. 400 as mentioned for the apple trees).

Developing the method we also tested the effect of a barrier on the ground

around the tree trunk, encircling an area the diameter of the tree crown. As it did not in all cases raise the number of recaptured larvae and as digging in these barriers is very laborious we do not recommend the use of barriers.

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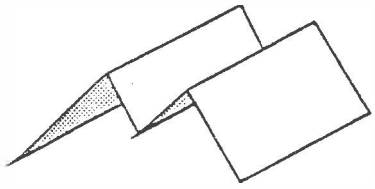

Fig. 1 Baitcard type A

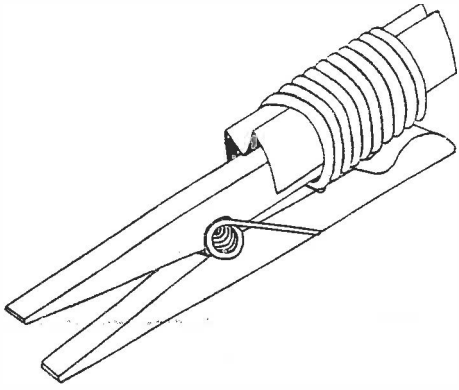

Fig. 2 Baitcard type B

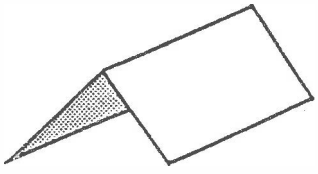

Fig. 3 Baitcard type C

THE FIELD APPROACH TO PESTICIDE ECOTOXICITY TESTING AGAINST BENEFICIAL INVERTEBRATES IN CEREALS

Paul Jepson and Mike Mead-Briggs

Agrochemical Evaluation Unit
Department of Biology
School of Biological Sciences
Bassett Crescent East
Southampton
SO9 3TU
United Kingdom

1. CURRENT FIELD-BASED TESTS

One field-based hazard evaluation method now has the status of an official registration requirement (Working Document 7/7: Guide-line to study the within-season effects of insecticides on beneficial arthropods in cereals in summer: MAFF, Pesticide Safety Division, Rothamsted Experimental Station, Harpenden, AL5 2BD, United Kingdom).

Spray application takes place during the recommended period for aphid control in a winter-wheat crop. This follows monitoring of natural enemy population levels within the crop to ensure that populations are increasing after the spring hiatus between the overwintering and summer generations of many species (Jepson, 1989). The experiment quantifies any decrease caused by the test chemical in relation to untreated areas in open field plots, or small barriered areas. The data are acceptable for consideration, if it has been demonstrated that a positive control (a toxic broad-spectrum pesticide) has had an effect. Further comparison with a negative control (a relatively non-toxic compound) are also recommended. Four-fold replication of each treatment is required, for statistical reasons, in a randomised block design and experimental data from at least two seasons are necessary.

Two alternative experimental configurations are possible: open field plots of >1ha or small, barriered areas of 100 m^2. The former design is thought to have a high level of realism, with no risk of oversampling, limited reinvasion and the possibility of wide taxonomic breadth in the evaluation. The barrier design is much more compact and may permit greater replication or the selection of several sites within a year and some reinvasion by epigeal species is prevented. Risks are associated with each design for example, fields are often not uniform in terms of invertebrate distribution and large experimental designs may suffer from excessive variance in within-treatment means. Barriered areas may be reinvaded by flying insects and are subject to oversampling and unpredictable effects (Jepson and Mead-Briggs, this volume). The taxa to be studied are divided into categories that take into account variation between sites and between years. Representatives of highly-ranked beneficial species from within the Carabidae, Staphylinidae and Linyphiidae are required in densities that permit statistical analysis. In addition, aphid specific predators (Coccinellidae, Syrphidae etc), parasitoids and the chick-food species of game-birds are also sampled and some representation by species in these groups is required to qualify an experiment for consideration by the registration authorities. If important groups are not represented then laboratory or semi-field data may be acceptable.

Pitfall traps (nine traps in each 1 ha plot) are used to sample epigeal invertebrates on a series of occasions, twice before application, then on the day of spraying and two and four days later. They are open for 48 h on each occation. Sampling then takes place at weekly intervals for seven weeks. Trapping with three traps per plot in the barriers, takes the same form unless populations are significantly depleted by trapping in the control plots. Nocturnal sweep netting has been selected as the method of sampling plant-borne insects on the night before spraying and at 5, 10, 15 and 30 days following application, although an insect suction sampler may be used. No attempt has been made to validate or calibrate these methods. In addition, emergence traps may be used for syrphids and parasitoids and the latter group may be collected as immature stages within aphids and stored until emergence takes place.

Following identification of the sampled invertebrates to species, data for abundant taxa are analysed by two-way analysis of variance (blocks and treatments) carried out for each sampling occasion. If a species is present throughout the study it may be appropriate to use an analysis of co-variance with the pre-treatment data as a co-variate.

If species are largely unaffected by treatment then they will assumed not to be at risk. If however, significant depletions occur, further semi-field enclosure or barrier studies may be specified to quantify the hazard posed more precisely.

2. EXPERIMENTAL CONSTRAINTS AND LIMITATIONS

2.1 Trapping methods

Pitfall trap capture rate is activity dependent (Southwood, 1978; Topping and Sunderland, 1992) and does not provide an absoloute estimate of population density. Sub-lethal effects on activity may therefore cancel out the effects of numerical depletion. Pitfall traps do however sample continuously and from a large area of the plot so, despite variation in capture efficiency between species, they are currently a favoured method (Sotherton *et al.*, 1988).

2.2 Population recovery by diffusion from untreated areas

An important constraint in the use of within-field experiments to determine pesticide hazard is the area of land available for a well replicated experiment (Sotherton *et al.*, 1988). Jepson and Thacker (1990) and Thomas *et al.*, (1990) have demonstrated that treated plots in cereal fields are progressively reinvaded by the invertebrates which colonise untreated control plots in the same field. This leads to a progressive levelling in invertebrate densities within the field and an underestimate of the duration of side-effects. The only practical way to correct for this at present is to limit the duration of sampling, to ensure that plots are as large as possible, to make sure that they are square and that sampling takes place in the centre.

2.3 The importance of field boundaries

To understand the processes by which beneficial invertebrate populations recover following application of toxic pesticides, the degree to which field boundaries limit movement requires investigation. For example, our observations have demonstrated that linyphiid spiders do not actively move through field boundaries. In within-field experiments which include untreated

control areas, the rate at which spider populations recover in treated plots may therefore be unrealistically rapid. In the real world, where whole fields are sprayed, long-range aerial dispersal between fields may be the predominant mode of recolonisation. The latent period for population recovery would be greater therefore. This is supported by the findings from large scale investigations, where significant depletions of certain invertebrate populations (including spiders) have occured between seasons when whole fields are treated (Burn, 1992). Although it may not be practical to undertake replicated field tests in more than one field, the problem of reinvasion from control areas needs to be considered in the interpretation of test results.

The findings above provide evidence that invertebrates are dispersive in farmland, but that the rate of dispersal may be affected by the structure of the habitat. Metapopulation models of the type proposed by Jepson and Sherratt (1991) may provide a basis for investigating the effects of different patterns of spraying over farming systems in the future. This is important since it might be argued from the findings above that a widely used pesticide of intermediate toxicity could be more harmful than a very toxic chemical that is only used to a limited extent. Jepson and Sherratt (1991) propose the risk of local extinction as a suitable criterion of hazard.

2.4 The risk of pest resurgence and the effects of scale

It can be argued that the effects of scale need to be taken into account in interpreting the consequences of adverse side-effects upon pest species; especially with respect to the likelyhood of resurgence occuring. There is evidence for example, for reduced predatory capacity within intensive pesticide treatment regimes in cereals (Burn, 1992). This comes from predator exclusion studies, which demonstrated a reduced capacity to limit aphid populations and from reductions in the rate and level of removal of artificial prey from baited cards that were placed within each treatment. Analogous data comes from more conventional, within-field experimentation (Duffield and Baker, 1990; Duffield, 1991) where apparent aphid resurgence has been detected in the centres of large, treated areas where predator reinvasion has been delayed. The conclusion from both of these studies is that reduced predator population densities may increase the probability of damaging pest outbreaks over time especially on larger scales of treatment. The risk of pest resurgence could be considered to be one of the criteria by which pesticide hazard should be judged. The effects of scale of treatment on resurgence also therefore need to be considered in the interpretation of test results.

3. CONCLUSIONS

A more questioning approach is required in the design and interpretation of field-based tests. At present they may quantify short term hazard however, the realism of within-field studies requires further investigation with respect to patterns of reinvasions by epigeal species. The role of field boundaries in the recovery process needs to be taken into account and the effects of patterns of intended use needs to be considered and discussed in guidelines for the interpretation of data. In the future, the risk of local extinction for key species could be a criterion of hazard, as could the likelyhood of pest resurgence. Modelling may assist the development of both these approaches. At present, these tests are best seen solely as a

comparative excercise to discriminate test compounds from toxic standards. A further tier of data aquisition, such as long-term monitoring during an initial period of registration approval, supplemented by models which incorporate patterns of use, might then provide a realistic prediction of hazard.

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