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A semi-field testing procedure using the ladybird beetle, *Coccinella septempunctata* L. (Col., Coccinellidae), for assessing the effects of pesticides on non-target leaf-dwelling insects under field exposure conditions

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Abstract: Over a 2-year period, three research facilities examined a semi-field testing procedure to determine its usefulness in predicting potential side-effects of pesticides on non-target leaf-dwelling insects under field exposure conditions. The ladybird beetle *Coccinella septempunctata* was chosen as an indicator species for such insects. The objectives of the research were to examine the amenability of the testing method and to evaluate the reproducibility and reliability of the results. Second instar larvae of the beetles were placed on aphid-infested broad bean plants and then exposed to a spray treatment with one of three insecticide formulations at different application rates. The pesticides used were ME 605 Spritzpulver (WP 40), Metasystox R (EC 250) and Pirimor Granulat (50% a.i. w/w). Larval mortalities and numbers of successful metamorphoses observed in the tests were found to be in good agreement with observations made under agricultural field conditions. The reproductive performance of beetles which developed from the exposed larvae was highly variable between replicate groups although beetles were partly maintained in groups of comparable parent density and sex ratio.

Overall, it is concluded that the proposed test procedure is amenable and that the observed effects on larval survivability and the number of successful metamorphoses can be used to reliably predict harmful effects of pesticides to ladybird beetles in the field. If used as testing endpoint, the reproductive performance should only be qualitatively evaluated, since this sublethal endpoint is apparently subjected to a high natural variability.

1 Introduction

As an indicator species for testing adverse effects of pesticides on leaf-dwelling arthropod species, *Coccinella septempunctata* L. is proposed by European regulatory authorities (e.g. PINSDORF, 1989) and by an international expert group for non-target arthropod registration testing (BARRETT et al., 1994). *C. septempunctata* is a common coccinellid in Europe which is assumed to support the biological control of different aphids due to its polyaphidophagous feeding habit (FISCHER-COLBRIE et al., 1988).

In the past, a great number of pesticides have been examined for their potential adverse effects on *C. septempunctata* in laboratory tests (HASSAN et al., 1987; 1988; 1991). In these tests a worst case exposure was simulated by confining early instar larvae on glass plates, which were coated with a freshly dried spray deposit (PINSDORF, 1989). Although direct spray treatments of the test organisms may have greater impacts than dried spray deposits, the exposure conditions in these tests are assumed to be so harsh that the observed effects are higher than the highest potential impact occurring under field exposure conditions where several factors mitigate the exposure of organisms to those contaminants (BARRETT et al., 1994). This assumption is largely supported by comparatively testing pesticides for their impacts on beneficial arthropods in standardized laboratory and individually designed field tests (e.g. HASSAN et al., 1987; VOGT, 1994).

Laboratory tests are economically valuable tools to reliably identify pesticides which are harmless for non-target arthropods. On the other hand, adverse effects observed in these tests cannot directly be extrapolated to the field situation due to the intentionally intensified exposure conditions in the laboratory tests. Pesticides which cause adverse effects in the laboratory have to be further investigated to examine whether the identified hazard potential represents a true risk to non-target arthropods under realistic field exposure conditions. Field tests, however, require large resources and are often difficult to interpret due to indirect effects such as food depletion. Small scale semi-field tests appear more cost effective and better to standardize in regard to prey-predator relationship and exposure conditions. Therefore, semi-field tests are assumed to be an appropriate intermediate testing step between the artificial worst case exposure assay in the laboratory and a large scale field study (BARRETT et al., 1994).

This paper describes a standardized method for testing effects of pesticides to *C. septempunctata* under field exposure conditions which is similar to what has been proposed by BIGLER and WALDBURGER (1988) for *Chry-*

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soperla carnea Steph (Neurop., Chrysopidae). In addition, results are presented from a preliminary validation testing programme where three research facilities closely followed the proposed test protocol.

2 Material and methods

2.1 Test organisms

Testing was performed on 3–4-day-old larvae of the ladybird beetle *C. septempunctata*. The larvae were raised from egg clutches which were either purchased from commercial suppliers or harvested from a laboratory stock culture. Egg clutches were stored at 20 ± 2°C, 40–70% R.H., and a 16 h photoperiod >1000 lux in plastic Petri dishes until larval hatch. For mass rearing methods of *C. septempunctata*, the following literature can be reviewed: PINSDORF, 1989; SAMSOE-PETERSEN et al., 1989; HASSAN et al., 1993).

2.2 Test plants

The broad bean, *Vicia faba* var. *major*, was recognized as an appropriate plant for testing. It was easily cultivated and allowed an effective assessment on the number of alive ladybird larvae during the test. In addition, *V. faba* var. *major* was used for the mass-rearing of *Acyrthosiphon pisum*, *Aphis fabae* and *Megoura viciae* (Hom., Aphididae) which were used as prey organisms during the test. A change of the host plant during the test was therefore not required which was assumed to be supportive for aphid growth.

2.3 Test cages

Test cages consisted of a seed tray (approximately 55 × 35 and 6 cm high) planted with broad beans. Seed trays were placed into a drainage tray (approximately 60 × 40 and 6 cm high) which was provided with an outlet and could be filled with water if required. In this way plants could be supplied during the test with water without affecting spray deposits on the leaves.

After spray treatments, frames (made from stainless steel or plastic) with a size of about 50 × 30 cm = 0.15 m² and between 14 and 25 cm high were placed on the seed trays such that the frame walls enclosed the central area planted with bean plants. Care was taken to leave sufficient space between plants and frame walls. The upper borders of the steel frames were twisted to the inner side to prevent beetle larvae from escape. Where plastic frames were used, the inner sides of the walls were coated with Fluon. Then, test cages were placed into gauze tents (about 2 m × 2 m and 2 m high) or covered with rectangular enclosures made of nylon gauze (about 47 × 28 and 50 cm high) to exclude raptors like birds and small mammals as well as parasitoids. Test cages were subjected to outdoor weather conditions until larvae had entered the pupal stage.

2.4 Test compounds and application rates

For the preliminary validation testing, three insecticidal products were chosen: ME 605 Spritzpulver (40% w/w Methylparathion), Metasystox R (25% w/v Oxydemeton-methyl) and Pirimor Granulat (50% w/w Pirimicarb). At their typical use rates, two of the three test products (ME 605 Spritzpulver, Metasystox R) are classified as harmful to foliage-dwelling insects (e.g. THEILING and CROFT, 1988; GRANDE et al., 1989; ZOEBELEIN, 1988) while Pirimor Granulat is supposed to be harmless or only moderately harmful (FRANZ et al., 1980; GRÄPEL, 1982; HASSAN et al., 1983; BOLLER et al., 1989). In the ring tests, Pirimor Granulat was tested at 0.3 kg/ha. This application rate had no significant harmful effects on *C. septempunctata* under laboratory exposure conditions (GRÄPEL, 1982). ME 605 Spritzpulver and Metasystox R were tested at rates of 0.3 and 1.0 kg/ha and 0.3, 1.2 and 2.0 l/ha, respectively. All products were dissolved in tap water as under agricultural practice and applied to the test cages in a water volume of 300 l/ha.

2.5 Preparation of test animals, plants and prey aphids

Approximately 10 days prior to application, between 40 and 70 broad bean seeds (*V. faba* var. *major*) were sown in the centre of a seed tray with a distance of 10 cm to the tray margin. After emergence of the cotyledons (about 7 days later) seedlings were artificially infested with pea aphids (*A. pisum*, *A. fabae* and *M. viciae*) as a food source for the test animals during testing.

In the late afternoon of the day prior to spray treatments, 2–3-day-old ladybird larvae were removed from the egg store dishes. Larvae were then maintained without food until treatment to attract them to the aphid-infested plant tips where exposure to the spray fluid during treatment was assumed to be highest. (Due to their cannibalistic behaviour, it is recommended to maintain larvae solitarily (e.g. in micro titer plates at 14° ± 2°C) during the starvation period.)

2.6 Plant and prey population management

To keep broad beans in the desired well-hydrated state, the drainage trays were periodically (depending upon natural precipitation) filled with tap water. The tray had a hole which was sealed by a rubber stopper during water supply.

Cages were regularly re-infested with fresh pea aphids (each working day up to about 10 days after application) to counter the effects of food depletion (all test compounds showed an aphicidal activity). This was considered necessary since otherwise food depletion could pretend impacts of even harmless compounds. Extra aphids were supplied by either placing infested bean leaves/stalks (not treated) on the top of the bean plants (research facility 1 and 3) or by brushing off aphid-infested plants above the test cages (research facility 2). The latter appears less appropriate since many of the brushed-off aphids dropped on the ground where they could be killed by ground spray deposits before being able to climb the plants.

2.7 Bioassay procedure

One hour prior to application, 15–20 randomly assigned ladybird beetle larvae were carefully placed on the aphid-infested broad beans within the seed trays. The plastic/steel frames were placed on the seed tray beforehand to prevent larvae from escape. Shortly prior to application, plastic/steel frames were removed to avoid contamination of frame walls with spray deposits. Immediately after treatment, the plastic/steel frames were returned to the seed trays and the whole device was placed into the drainage trays. Then, test cages were either equipped with nylon gauze enclosures (research facility 1) or placed into gauze tents (research facilities 2 and 3). In their final position, test cages were arranged in a block design.

2.8 Application of the test compounds

Control and active treatments were carried out either in the field (research facility 1) or in the laboratory (research facilities 2 and 3). The distance between the spray nozzles and the plant tips was between 40 and 50 cm. Research facility 1 used a hand-operated spray boom (Agrotop spray boom with three Lurmark 03F110 nozzles) for spray treatments. Application rate was controlled by adjusting walking speed. The applied

spray fluid volume corresponded to 300 l/ha. Spray fluid was delivered at a constant pressure of 2.0 bar.

Research facility 2 and 3 performed treatments by using a laboratory spray-cabinet which delivered the spray via two spray nozzles. The test compounds were delivered in 300 l water per hectare at a constant pressure of about 2.0 bar. After application, the treated trays were immediately subjected to outdoor weather conditions.

2.9 Assessment of mortality and behaviour

The location and condition of the ladybird beetle larvae was first recorded ≈2 h after application. They were classified as being:

live: alive and apparently unaffected
moribund/dead: on the ground, either immobile or twitching slightly.

Further checks were made on each other day. After appearance of the first pupa, checks were made daily and, in research facilities 1 and 3, all pupae found were transferred to the laboratory (leaves carrying pupae were clipped). Pupae were stored in plastic Petri dishes (9 cm diameter) at 21–26°C, 30–80% air humidity, and a 16 h photoperiod >1000 lux until beetles emerged. In research facility 2, recovered pupae remained in the outdoor cages until emergence of the beetles. After the last visible pupa/beetle was recovered the broad bean plants were harvested and carefully checked for remaining larvae/pupae. Recovered larvae were transferred to the laboratory and fed with pea aphids in petri dishes until they had pupated.

2.10 Fecundity assessments

After emergence, beetles were transferred to breeding cages (between 0.1–0.2 m^2 and 20–30 cm high) which consisted of glass or macrolon with side and rear walls of nylon netting. Cages were equipped with removable front walls or cover lids to allow access to the inner side. Cage floors were lined with filter paper. One or two aphid-infested potted broad bean plants were placed into each breeding cage to provide beetles with fresh aphids. Beetles were (bi)weekly transferred to clean cages. Broad bean plants were exchanged when the aphid population was depleted. Cages were maintained at 21–26°C, 30–80% air humidity, and a 16 h photoperiod >1000 lux. Approximately 7 days after emergence of the last beetle, the sex of each beetle was determined by microscope. In research facilities 2 and 3 all beetles subjected to the same treatment were pooled and placed in a single breeding cage. In research facility 1 beetles of each treatment were pooled and subsequently allocated to subgroups of up to 20 individuals with comparable population density and sex ratio. Subgroups were placed in separate breeding cages. Fan-folded filter papers or black paper sheets, rolled up to form a cylinder, were provided as an oviposition site. The filter papers/paper sheets were placed between leaves of the bean plants and on the bottom of the cages. Filter papers/paper sheets which contained deposited eggs were removed daily except weekends and replaced. The sampled eggs were counted and stored in Petri dishes (1 egg-clutch per dish) until larval hatch.

2.11 Climatic conditions during the study

Weather conditions during the outdoor part of the study were continuously monitored by adjacent weather stations. During the indoor phase, temperature and air humidity were monitored by thermohygrometers.

2.12 Evaluation of the results

Mortality was defined as the number of larvae which failed to complete the metamorphosis or which remained unrecovered, relative to the initial number of larvae. Larvae/pupae found to have been cannibalized or which were inadvertently killed during the evaluation procedure were discarded from calculation. Mortality observed in groups of the active treatments was corrected for control mortality using the equation of SCHNEIDER-ORELLI (1947):

$$M\,[\%] = [(t - c)/(100 - c)] \times 100,$$

where M [%] = relative mortality, c = % mortality in controls and t = % mortality in treatment groups.

As an index for the reproductive performance of the adult beetles the average number of fertilized eggs (F) per female was calculated using the formula:

$$F = n \times h/100,$$

where F = number of fertilized eggs per female, n = number of eggs laid per female and h = hatch rate in [%].

The reproductive performance (R) of beetles exposed during their larval development to an active treatment was corrected by the equation of ABBOTT (1925):

$$R\,[\%] = [(F(c) - F(t))/F(c)] \times 100,$$

where R [%] = relative reproductive performance, F(c) = number of fertilized eggs/female in controls and F(t) = number of fertilized eggs/female in treatment groups.

3 Results

3.1 Recovery rates of pupae in the water-treated control groups

Under the environmental conditions prevailing during the tests, larvae required between 9 and 26 days to enter the pupal stage. The number of pupae recovered in the control cages by the three research facilities averaged between 62 and 85% relative to the initially entered larvae (table 1). No statistically significant correlation (t = 0.89–2.26; FG = 8; P > 0.05–0.2) could be found between recovery rate, as an indicator of larval survival, and abiotic conditions prevailing during testing (fig. 1).

Table 1. *Recovery rates of* C. septempunctata *larvae/pupae in the water-treated control cages. In the test, 15–20 3–4-day-old larvae were placed in each test cage (n = 4) on aphid-infested broad bean plants and exposed to a spray treatment with tap water. Larvae were recovered after they had pupated. Figures summarize results from ring tests performed by three research facilities over 2 years*

Research facility	No. of tests performed	Percentage of recovered larvae/pupae (% of entered larvae)*
Facility 1	5	70 ± 18 (44–94)
Facility 2	2	62 (59–65)
Facility 3	3	85 ± 12 (68–94)

* Means ± SD. Minimum and maximum values are given in parenthesis.

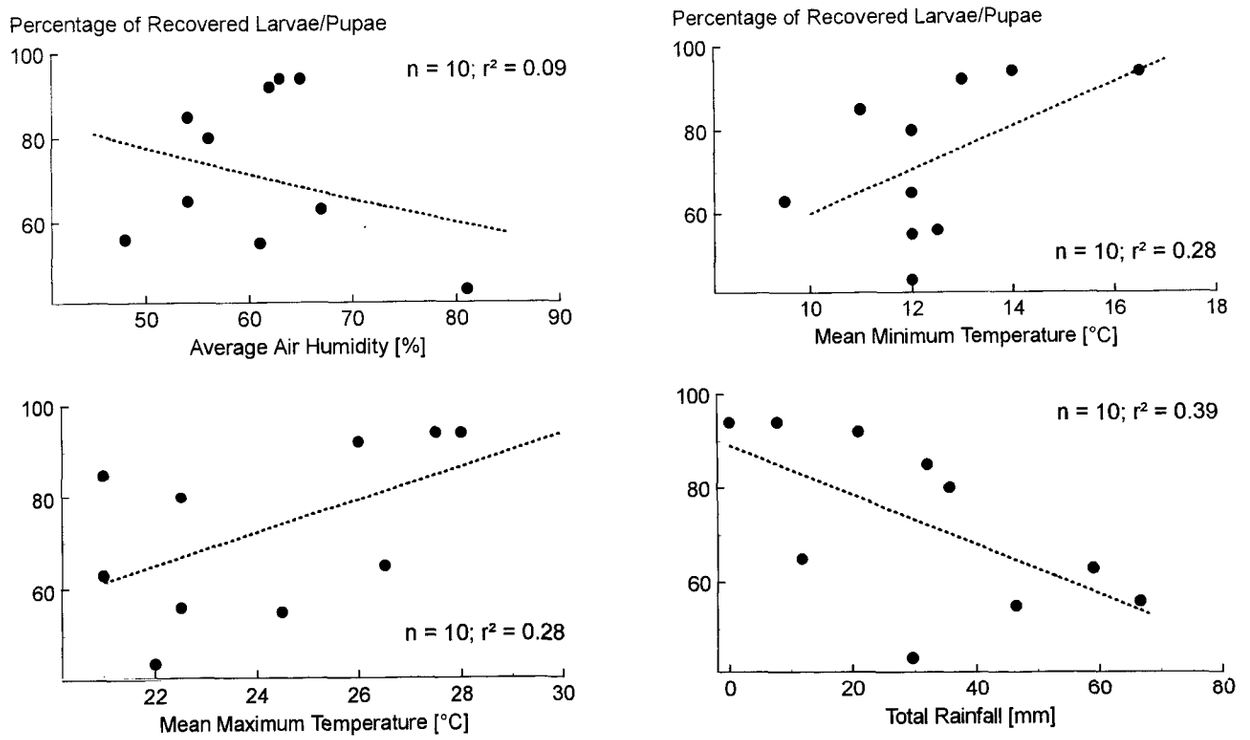

Fig. 1. Influence of abiotic conditions prevailing during testing on the recovery rate of larvae/pupae of C. septempunctata *in the water-treated control cages. In the test, 15–20 early instar larvae were placed on aphid-infested broad bean plants in outdoor test cages (*n = *4) and treated with tap water. Larvae were recovered after they had pupated*

3.2 Treatment-related effects on larval mortality

The effects of the three tested insecticides (ME 605 Spritzpulver, Metasystox R and Pirimor Granulat) on the survivability of *C. septempunctata* larvae are shown in table 2. In the groups treated with 1 kg/ha ME 605 Spritzpulver no larva/pupa was recovered alive at termination of the outdoor part of the study in any of the three testing facilities. Recovery was also strongly reduced at the lower rate of 0.3 kg/ha.

Metasystox R also consistently caused a nearly 100% larval mortality at application rates of 1.2 and 2 l/ha. At the lower application rate of 0.3 l/ha, up to 40% of the larvae could successfully enter the pupal stage (table 2)

A treatment with Pirimor Granulat increased the larval mortality up to 44% relative to the untreated controls (table 2). In one case, where the recovery rate in the control remained below 50%, a slightly higher number of survivors were found in the treated plots compared to the control plots (relative mortality: −2.3%).

3.3 Treatment-related effects on the number of metamorphosed beetles

Between 88 and 92% of the larval/pupal stages of *C. septempunctata* recovered from control groups could successfully complete their metamorphosis (table 3). The rate of metamorphosis was apparently not

Table 2. *Survivability of* C. septempunctata *larvae subjected to one of six insecticidal spray treatments under field exposure conditions. Fifteen to 20 early instar larvae were placed on aphid-infested broad bean plants in outdoor test cages (*n = *4) and exposed to the spray treatment. Larvae were recovered after they had pupated. Mortality rates (= % not recovered larvae) observed in treatment groups were corrected for the respective control mortality according to the formula of* Schneider and Orelli, *1947 (see paragraph 2). Figures summarize results from ring tests performed by three research facilities over 2 years*

Treatment	No. of tests performed	Corrected preimaginal mortality (% not recovered larvae)*
ME 605® Spritzpulver (0.3 kg/ha)	3	97 ± 2 (95–100)
ME 605® Spritzpulver (1.0 kg/ha)	4	100
Metasystox® R (0.3 l/ha)	3	80 ± 17 (59–100)
Metasystox® R (1.2 l/ha)	3	100
Metasystox® R (2.0 l/ha)	2	96 (91–100)
Pirimor® Granulat (0.3 kg/ha)	4	18 ± 18 (−2–44)

* Means ± SD. Minimum and maximum values are given in parenthesis.

Table 3. *Emergence rate of ladybird beetles (*C. septempunctata*) from pupae recovered in the test cages. In the test, 15–20 early instar larvae were placed on aphid-infested broad bean plants in outdoor cages (*n = 4*) and exposed to one of six insecticidal spray treatments. Larvae were recovered after they had pupated. Emergence of beetles from pupal stages was followed under controlled laboratory conditions except in research facility 2 where pupae remained in the test cages until beetles had hatched. Figures summarize results from ring tests performed by three research facilities over 2 years*

Research facility	Percentage of emerged beetles (% from recovered pupae)* Water treated controls	Groups exposed to 0.3 kg/ha Pirimor
Facility 1	88 ± 5 (82–93; n = 5)	87 (86–89; n = 2)
Facility 2	90 (89–90; n = 2)	89
Facility 3	92 ± 4 (87–96; n = 3)	81

* Means ± SD. Minimum and maximum values are given in parenthesis.

adversely affected when the pupal stages remained in the outdoor cages until emergence of the beetles (see data of research facility 2 in table 3).

From the survivors of the groups subjected to a spray treatment with 0.3 kg/ha ME 605 Spritzpulver and 0.3 l/ha Metasystox R none could successfully complete the metamorphosis.

In the groups, treated with 0.3 kg/ha Pirimor Granulat, the percentage of beetles which emerged from recovered pupae ranged between 81 and 89% (table 3). Thus, a spray treatment of *C. septempunctata* with 0.3 kg/ha Pirimor Granulat in an early larval instar had apparently no impact on metamorphosis.

3.4 Treatment-related effects on the reproductive performance of the emerged beetles

The number of eggs laid per female beetle per day varied widely both, between the three research facilities and between the different test runs within the same facility (table 4). High variations were even recorded between subgroups of the same test run where beetles were maintained under almost identical test conditions.

Including all control data sets, the number of eggs laid per female per day ranged from 3.1–15.4. Egg laying performance of ladybird females differed not statistically significant between the two test years as indicated by an analysis of data from research facility 1 ($n = 13$; $P = 0.89$; Mann–Whitney U test).

For a better understanding of the causal factors for the observed variability in the reproductive performance, the general pattern of egg deposition and the potential effects of population density and sex ratio on the reproductive performance were examined in more detail. Figure 2 shows a pattern of egg deposition as typically observed in the performed test runs. In 6 out of 8 cages analysed, egg deposition was clearly synchronized between the females with conspicuous peaks in egg deposition activity (Wald-Wolfowitz iteration test: Büning and Trenkler, 1978).

In one cage a high number of individuals escaped

Table 4. *Reproductive performance of ladybird beetles (*C. septempunctata*) emerged from larvae which had been subjected to a spray treatment with either tap water (300 l/ha) or 0.3 kg/ha Pirimor® Granulat under field exposure conditions. Beetles from replicate cages of the same treatment were pooled. Within each test run, research facility 1 randomly allocated beetles subjected to the same treatment group into subgroups with comparable parent density and sex ratio. The reproductive performance of the adult stages was examined under standardized laboratory conditions over 33–49 days. Figures summarize results from ring tests performed by three research facilities over 2 years*

Research facility	Number of (sub-) groups examined	Initial no. of beetles per cage*	Initial sex ratio* (males : fem.)	No. of eggs per female per day*	Fertilization rate* (% larval hatch)
Water treated controls					
Facility 1	13	19 ± 3 (12–33)	0.9 ± 0.3 (0.5–1.3)	10.0 ± 3.1 (6.5–15.4)	51 ± 27 (8–84)
Facility 2	1	42	1.0	8.6	45
Facility 3	2	43 (33–53)	1.0 (0.9–1.2)	3.6 (3.1–4.2)	82 (80–84)
Groups exposed to 0.3 kg/ha Pirimor					
Facility 1	3	15 ± 1 (14–16)	1.4 (0.8–1.8)	14.9 ± 2.6 (11.4–17.5)	64 ± 8 (57–74)
Facility 2	1	31	0.9	21.2	57
Facility 3	1	25	0.9	7.4	88

* Means ± SD. Minimum and maximum values are given in parenthesis.

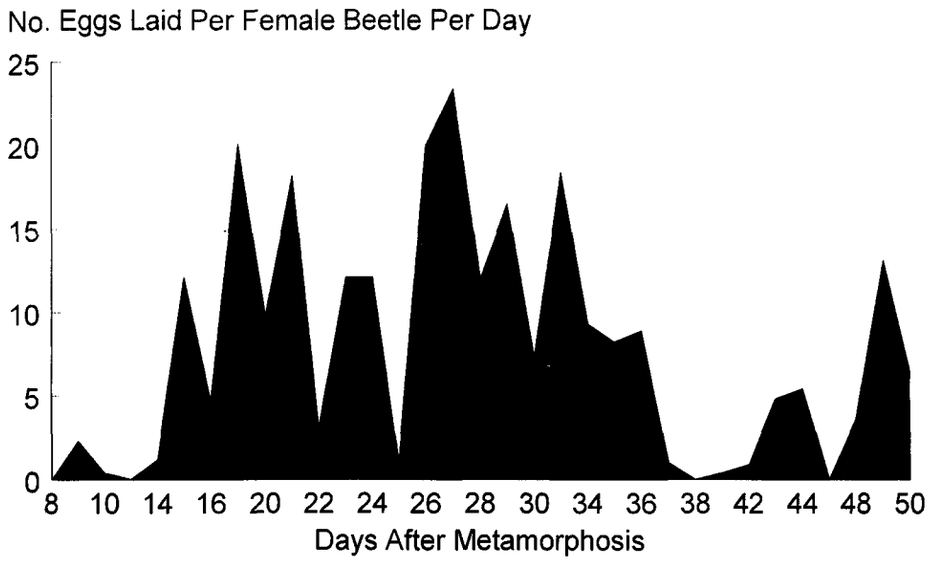

Fig. 2. Typical pattern of egg deposition of ladybird beetles as observed under the standardized laboratory conditions over a 42 day period. Beetles emerged from larvae which had been exposed to a spray treatment with tap water (300 l/ha) under outdoor conditions. Adults were sampled, pooled, and allocated to subgroups of about 20 individuals with equalized sex ratios. Each subgroup was placed into a breeding cage which was equipped with aphid-infested broad bean plants as food source. Fan-folded filter papers served as oviposition sites. Eggs were removed and counted daily except weekends

inadvertently from the breeding cage during an evaluation event. The remaining individuals were merged with those of an other subgroup of the same treatment. Shortly after merging, the reproductive activity became completely suppressed (fig. 3). After about 2 weeks, egg deposition recommenced with a higher reproductive output as observed in the initial phase.

No significant ($t = 1.82$; $FG = 14$; $P > 0.05$) correlation was found between the sex ratio as established in the breeding cages and the number of eggs laid per female. In contrast, parent density seemed to adversely affect this evaluation criterion. If the number of eggs laid per female was plotted against the number of individuals per breeding cage, a significant (all facilities: $t = 3.18$; $FG = 14$; $P < 0.01$; facility 1: $t = 2.22$; $FG = 11$; $P < 0.05$) reduction in the number of eggs laid per female with increasing parent density was indicated (fig. 4).

No difference was observed between the reproductive performance of the control and the Pirimor Granulat group (table 5). If the average number of eggs laid per female per day in the pertinent control groups was used as a basis for comparison and assumed to be 100%, the relative egg laying performance of females from the Pirimor Granulat group averaged 179%.

Concerning the fertilization rates of eggs there was no difference between the water-treated control groups and the group exposed to Pirimor Granulat. However,

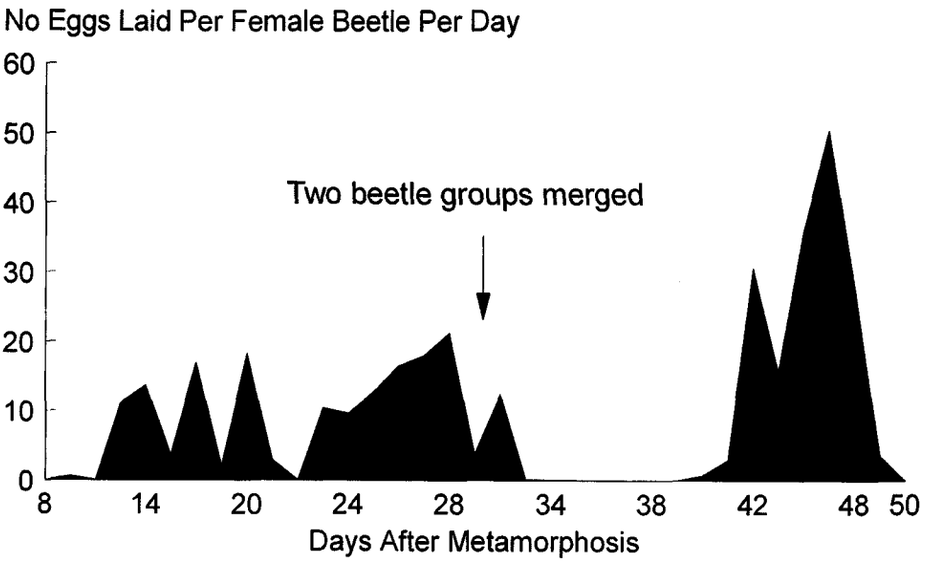

Fig. 3. Changes of the egg deposition pattern of ladybird beetles after merging with conspecifics subjected to identical preimaginal treatments. For details of the testing procedure, see legend to fig. 2

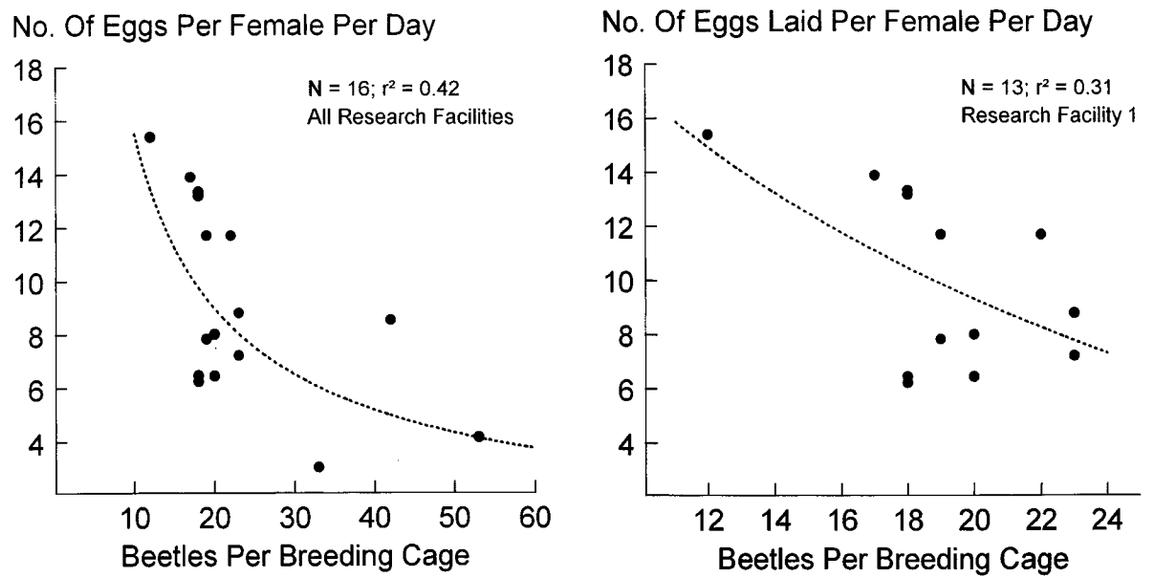

Fig. 4. Effects of parent density as established in the breeding cages on the number of eggs laid per female beetle per day. For details of the testing procedure, see legend to fig. 2

even between subgroups of the water-treated controls fertilization rates fluctuated widely ranging from 8% to 84% (table 4). If the fertilization rate was plotted against the sex ratio established within the breeding cages a significant ($t = 8.57$, $FG = 14$; $P < 0.0001$) positive correlation was observed for the data from research facility 1 (fig. 5). However, this correlation has to be considered with caution since all data pairs with sex ratios greater than 1 originate from test year 1993 and data pairs with sex ratios smaller than 1 from test year 1994. Thus, the observed correlation may result from differences in population fitness rather than from a true effect of the sex ratio.

4 Discussion

In laboratory toxicity tests on beneficial arthropods, exposure of the test organisms to the test compound is intentionally intensified to detect any potential impact of the test compound on the test species (HASSAN, 1984). In this way, safe compounds can be reliably and economically identified. However, adverse effects recorded at this test level can not directly be extrapolated to field conditions where several factors will substantially mitigate the exposure (e.g. translocation of deposited residues into plant tissues, precipitation events, forced evaporation, UV degradation). For evaluation whether the hazard potential observed in laboratory tests presents a true risk to non-target arthropods compounds have to be tested under exposure conditions more realistic to the field. Field studies are very labour-intensive and, due to indirect effects, often difficult to interpret. Thus, the development of more standardized test procedures, which include major exposure-mitigating factors, is considered as an appropriate approach for a revised evaluation of compounds assessed to be harmful under laboratory worst case exposure conditions (BARRETT et al., 1994).

In support of this approach a preliminary ring test was performed by three research facilities to examine the amenability and reliability of a semi-field test protocol for assessing the effects of pesticides to leaf-dwelling insects under field exposure conditions. The chosen test design is similar to that proposed by BIGLER and WALDBURGER (1988) for testing effects of pesticides on *Chrysoperla carnea* Steph. under semi-field test conditions.

No correlation was found between larval survivability and single abiotic factors such as air humidity, minimum-maximum temperatures, and precipitation. However, in combination abiotic conditions may well have an impact on larval survivability. Both, lower temperatures and higher precipitation rates adversely affected the recovery rate and both conditions frequently appear in combination under natural weather conditions. Thus, for ring tests performed under outdoor conditions some variability in test performance between contributing research facilities has to be anticipated due to differences in the weather conditions prevailing during testing. If such weather-related influences on test performance are considered, data on larval survivability and rate of beetle emergence recorded for the control groups were largely comparable between the three research facilities. This allows the conclusion that the test protocol was

Table 5. *Egg-laying performance of* C. septempunctata *beetles emerged from larvae which had been subjected to a spray treatment with Pirimor® Granulat (0.3 kg/ha). Figures give the reproductive performance of beetles from the Pirimor treatment group relative to the pertinent water-treated control group. The number of eggs laid in the pertinent control groups was set to 100%*

Research facility	No. of tests performed	Treatment-related effect (% control)
Facility 1	2	108–156
Facility 2	1	247
Facility 3	1	206

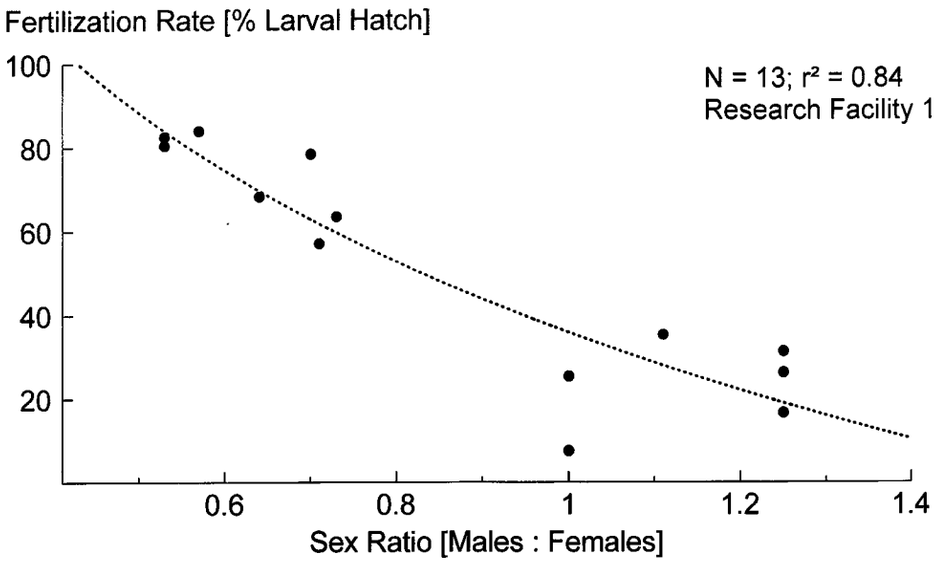

Fig. 5. Effects of sex ratio as established in the breeding cages on fertilization rates of eggs laid by female ladybird beetles under standardized laboratory conditions on fan-folded filter papers. For details of the testing procedure, see legend to fig. 2

amenable and sufficiently robust to elaborate usable results. This conclusion is further supported by the largely comparable effects recorded in the three research facilities for the three test compounds. Based on survivability of the preimaginal stages in the treatment groups the examined test compounds would have been labelled in a largely consistent manner: ME 605 Spritzpulver and Metasystox R would have been labelled as harmful for non-target leaf-dwelling insects as represented by ladybird beetles whereas Pirimor Granulat would have been labelled as harmless or only slightly harmful to those insects. This classification of the test compounds is in good agreement with findings from studies performed under realistic agricultural exposure conditions (e.g. Methylparathion: GRANDE et al., 1989; Oxydemeton-methyl: ZOEBELEIN, 1988; Pirimicarb: TREVIZOLI and GRAVENA, 1979).

The inter-laboratory variability in the preimaginal mortality rate of *C. septempunctata* observed for the treatment with 0.3 kg/ha Pirimor Granulat is more likely to be attributed to indirect effects, e.g. reduction of prey quality and/or quantity, rather than related to direct toxic effects of this compound. This assumption is based on the finding that, at the tested application rate, Pirimor Granulat had no impact on *C. septempunctata* larvae even under the more stringent laboratory exposure conditions (GRÄPEL, 1982). Although the semi-field test protocol requested to provide daily fresh aphids to counter such indirect effects the number of freshly supplied aphids and/or the weather-related persistence of the spray deposits may have differed between the three research facilities. Thus, ladybird larvae may have suffered temporarily from food deprivation and this could have increased the rate of cannibalism. This potential interference between adverse effects of the test compound on the test organism and on its prey emphasizes the necessity for carefully recording not only the response of the test organism to the spray deposit but also that of the supplied prey species. If adverse effects on the prey aphids are observed, fresh aphids should be supplied in excess to counter indirect effects related to food deprivation. On the first sight, differentiation between indirect and direct effects appears irrelevant for species which are specialized to prey upon aphids: either die from intoxication or from starvation. However, the proposed test organism represents an indicator species for other leaf-dwelling non-target insects which may be less specialized and will be able to survive by using an other food source.

Besides aphicides, herbicides may raise problems when tested in the proposed fashion. As already recommended by BIGLER and WALDBURGER (1988), the plant species has to be changed in cases where a herbicide is anticipated to damage broad bean plants. In those cases, BIGLER and WALDBURGER (1988) recommend barley plants (*Hordeum distichon*) with *Metopolophium dirhodum* Wlk. (Hom., Aphididae) as prey aphid. The suitability of barley plants will be tested in the near future.

Fecundity of female beetles and fertilization rates of deposited eggs were highly variable both within and between different test runs. This observation is in agreement with findings from an earlier analysis of laboratory data (SCHMUCK et al., 1996) where the reproductive performance of the adults are examined in a comparable way (PINSDORF, 1989). The authors concluded that population density and sex ratio established within the breeding cages could have an influence on the reproductive performance of beneficial insects. In the present study, population density was found to potentially influence the reproductive output of female *C. septempunctata*. However, even between groups comprised of beetles from the same stock culture, which were maintained under largely identical test conditions and held a comparable parent density conspicuous differences in the reproductive performance were observed. Thus, differences in the reproductive performance between the control and the treatments groups have to be considered very carefully since this evaluation criterion is appar-

ently subjected to a high test- and/or species-inherent variability. At this time, only a qualitative rather than a quantitative assessment of this sublethal endpoint can be recommended. A qualitative evaluation could be based on historical data which define limits within the reproductive performance can typically be anticipated for a healthy population of ladybird beetles under the described test conditions.

Regarding the technical aspects of the current test proposal, some further points should be raised to amend standardization and simplify the method. Firstly, spray deposits are of course very vulnerable to precipitation events. Therefore, it may be recommendable for routine testing to protect test cages from precipitation during the first days after application to ensure exposure of test organisms to spray deposits. Secondly, egg laying activities start typically between 1 and 2 weeks after emerging of the adult stages and continue over a period of $\geqslant 5$ weeks. According to our data base peaks of egg deposition are mostly observed during weeks 3 and 5 after the last beetle had hatched. Since recording of the reproductive performance is the most labour-intensive part of the study it appears cost-effective to limit recording of this sublethal endpoint to this period. A minimum number of eggs laid within this 3 week period can be defined for the control group (see below) to ensure that the reproductive performance in the treatment groups can reliably be evaluated. If the minimum number of eggs in control groups is not achieved within the 3 weeks, recording may be extended accordingly.

The quality of the test run performed according to the described study design can partly be evaluated by validity criteria which have to be met by control groups. From our current results, we would propose the following minimum criteria which have to be achieved in the control groups: $\geqslant 60\%$ recovery rate for larval/pupal stages, $\geqslant 80\%$ emergence rate of adult stages from recovered larvae/pupae and a minimum number of five eggs per viable female per day of weeks 3 to 5 after the last beetle had emerged. Whether these preliminary validity criteria are sufficiently robust has to be proven by the now scheduled validation ring testing programme of an Joint Testing Initiative of IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants), BART (Beneficial Arthropod Registration Testing Group), COMET (Commercial Ecotoxicological Testing Group) and EPPO (European and Mediterranean Plant protection Organization).

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