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Comparative sensitivity of four ladybird species to five pesticides

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Abstract: Since 2003, four ladybird species have been found in open fields in Belgium: the three native species *Coccinella septempunctata* (L.), *Adalia bipunctata* (L.), *Propylea quatuordecim-punctata* (L.) and the invasive species *Harmonia axyridis* (Pallas). As this last species could be a problem in the future for native species, experiments were carried out to assess its sensitivity to pesticides compared to native species, to determine whether the use of pesticides in agricultural ecosystems could give an advantage to *H. axyridis*. The results also provide information on the sensitivity of the four species and whether it is possible to extrapolate data obtained for one species to another. The LR_{50} of 5 pesticides (three insecticides: imidacloprid, zeta-cypermethrin, triazamate and two fungicides: spiroxamine and metalaxyl-M + fluazinam) was assessed on glass plates. Products were tested on basis of a range-finder test (5 doses in a dilution range 5-10x + control, 10 larvae per test unit) and a definitive test (5 doses in an adapted range to ideally cover 0-100 % mortality + control, 20 larvae per unit). The larvae used for the tests were 2-3 day old and were confined for 7 days on glass plates. Mortalities were recorded daily and final assessment was made after 7 days of exposure.

The LR_{50} results (ml of formulated product/ha ± sd) after 7 days of exposure can be summarised as follows:

	Impulse	Epok	Aztec	Fury	Confidor
C. 7-punctata	1155.5 ± 79.0	681.2 ± 158.8	54.1 ± 8.7	0.4766 ± 0.064	300.5 ± 56.7
P. 14-punctata	810.0 ± 54.8	211.6 ± 51.2	75.7 ± 8.5	0.0279 ± 0.0032	1.26 ± 0.27
A. bipunctata	1123.4 ± 74.5	36.1 ± 9.9	21.8 ± 2.9	0.0118 ± 0.0014	0.54 ± 0.11
H. axyridis	1584.4 ± 111.6	68.3 ± 17.7	143.4 ± 18.4	0.0437 ± 0.0058	0.49 ± 0.11

Comparison of LR_{50} values show no clear relationship between species tested and sensitivity to the different pesticides. *A. bipunctata* was most of the time the most sensitive species, but there were exceptions, for example in the case of Impulse (*P. 14-punctata*) and Confidor (*H. axyridis*). However, if the results obtained with *C. septempunctata* are omitted, the sensitivity of the three other species is more or less comparable, with LR_{50} ratio from the least sensitive to the most sensitive in a range of 1–6fold. *C. septempunctata* was most of the time the least sensitive species. For Confidor, the LR_{50} of *C. 7-punctata* was up to 600 times higher than that of *H. axyridis*, the most sensitive species. These results suggest a possible resistance mechanism for this species.

Keywords: ladybirds, *Coccinella septempunctata*, *Adalia bipunctata*, *Propylea quatuordecim-punctata*, *Harmonia axyridis,* LR_{50}, triazamate, imidacloprid, fluazinam + metalaxyl-M, zeta-cypermethrin, spiroxamine, pesticides, plant protection products.

Introduction

Ladybirds are important aphid predators in agro-ecosystems. Their actions limit the development of several aphids known to be serious pest problems in many crops and reduce the need for insecticide treatments (Hodek, 1973; Rautapää, 1976; Chambers *et al.*, 1983; Latteur & Oger, 1987; Poehling & Borgemeister, 1989). In open fields, *Coccinella* *septempunctata*, *Propylea quatuordecimpunctata* and *Adalia bipunctata* were the the most common species in several crops (Dedrijver *et al.*, 1985; Honek, 1995; Jansen, 2000).

As for other beneficial insects encountered in agriculture, ladybirds are exposed to pesticides. An intensive work has been undertaken to assess possible side-effects of these compounds on ladybirds, generating a sum of data. However, the data are most of the time limited to one or a few species, because of standardization of test methods and species. This is particularly the case with studies realized in the context of product registration at the European level, where *C. septempunctata* is the recommended ladybird species (Anonymous, 1994, 2000). Even in an IPM context, data are only generated on a limited number of species. By example, in the different Joint Pesticide Testing Programs organized by the IOBC, a sum of results were obtained on *C. septempunctata* (Hassan *et al.*, 1987) or *Semiadalia undecim-punctata* (Col.; Coccinellidae) (Hassan *et al.*, 1988, 1991, 1994; Sterk *et al.* 1999) or *H. axyridis* (Hassan *et al.*, 1988). The problem with this standardization is that there is actually very little information on comparative sensitivity of the different ladybird species and the possibility to extrapolate the results obtained on one specific species to another one is limited.

In 2003, the multicolored Asian ladybeetle *H. axyridis* was found for the first time in Belgium in potato crops (Jansen *et al.*, 2004). The presence of this species was confirmed in 2004 and 2005 (Jansen, unpublished). Several studies have shown that the Asian ladybeetle was very aggressive and able to dominate *C. septempunctata* and *A. bipunctata*, both by competition for exploitation of food resources and by direct predation (Yasuda *et al.*, 2001; Kajita *et al.*, 2000; Hautier, 2003; Hokkanen *et al.*, 2003). The Asian ladybeetle is well known in the USA to be a problematic invasive species, with problems on indigenous lady-bird community (Lamana & Miller, 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Brown, 2003; Alyokhin & Sewell, 2004). Depending on the competitive advantage of *H. axyridis* compared to native species and the presence of all these ladybirds in open fields where pesticides are regularly sprayed, the possible differences in sensitivity of these species to various pesticides can play a role in the establishment and development of the Asian multicolored ladybird.

In order to determine whether pesticide side-effect data can be extrapolated from one ladybird species to another and to determine if the use of pesticides in the field, where the multicolored Asian ladybeetle is found, could give a competitive advantage to this species, a specific research program was initiated to compare the sensitivity of different pesticides to four ladybird beetle species, including the invasive species *H. axyridis* and the native species *C. 7-punctata*, *P. 14-punctata* and *A. bipunctata*.

Material and methods

Plant protection products

Table 1 lists the five pesticides selected for testing. These products are all known to be toxic to ladybirds, at least on glass plates, and are used in crops where ladybirds may be important aphid predators. They all belong to a different chemical class.

Mass rearing of the ladybirds and toxicity tests

The four ladybird species were reared in the laboratory in a same way. Adult ladybirds were kept in plastic cages (40 cm x 30 cm x 20 cm) with 20-40 adults per cage. The bottom of the cages was protected by a piece of filter paper. Pea aphids (*Acyrtosiphon pisum*) on cut french bean plants, honeybee pollen and cut blackwheat (*Fagopyrum esculentum*) flowers were offered ad libitum as food. A piece of paper was put into the cages for egg laying. Three times per week beetles were transferred into new cages and their food renewed, except the blackwheat flowers which were only changed when needed. Patches of eggs were harvested and kept on petri dishes for hatching. Young larvae were kept on petri dishes and fed with pea aphids till the pupal stage when they were then used for the mass rearing or used for the tests. All the rearings were done in climatic chambers at 20°C ± 2°C and 60-90%RH, with sodium lamp lightning on a basis of 16h light/8h dark.

Table 1: List of plant protection products used in this study

Commercial name	active(s) ingredient(s)	a.s./l or kg	Formulation	Chemical class
Aztek *	triazamate	140 g	EW	carbamate
Confidor	imidacloprid	200 g	SL	neonicotinoid
Epok	fluazinam + metalaxyl-M	400 g + 200 g	EC	dinitroaniline + acylalanine
Fury 10EW	zeta-cypermethrin	100 g	EW	pyrethroid
Impulse	spiroxamine	500 g	EC	strobilurin

* spelt Aztec in summary

Mass rearings of ladybirds were initiated with adults sampled in field hedges and fallow lands in agricultural ecosystems. *A. bipunctata* rearing started in 1995, *C. 7-punctata* in 1998 and *H. axyridis* and *P. 14-punctata* in 2003. New adults (10-20) were introduced each year to renew the rearing. About 50-100 adults of each species were kept in a same time to produce a sufficient number of larvae for the tests.

For the toxicity test, products were applied with a Burgerjon spray tower (Burgerjon, 1956) onto small glass discs (∅ 5cm) at a rate of 200 l ± 10% of spray mixture/ha. Spray deposit was assessed by gravimetry. One to two hours after application, when pesticide residues were dry, the glass plates were put in small plastic dishes and covered with a plastic ring coated with Fluon GP1 to prevent the beetle from escaping. One 2-3 day old ladybird larvae was then released into each unit. The larvae were kept for 7 days in these units. Pea aphids were added daily and larval mortality was checked every day.

The products were tested first in a range-finder test with 5 doses in a 5-10x dilution rate and a control, with 10 larvae per rate. According to the results obtained in the range-finder, a definitive test was conducted with a narrower dilution range, generally 2x-5x dilution rate according to product, and 20 larva per rate. Mortality obtained for these 5 doses was corrected for control mortality (Abbott, 1926) and used to calculate LR_{50} values using Probit analysis (Minitab 13.20) and fitting a log 10 normal distribution model.

Results and discussions

The LR_{50} values of the five tested products for the 4 ladybird species are illustrated by Figures 1-5. LR_{50} values are expressed in ml formulated product/ha and given with 95% confidence interval. Numbers followed by the same letter are not significantly different (P<0.05). Control mortality reached a mean of 4.5% for all final tests (species x product), with a maximum of 15.0% in one test out of 20.

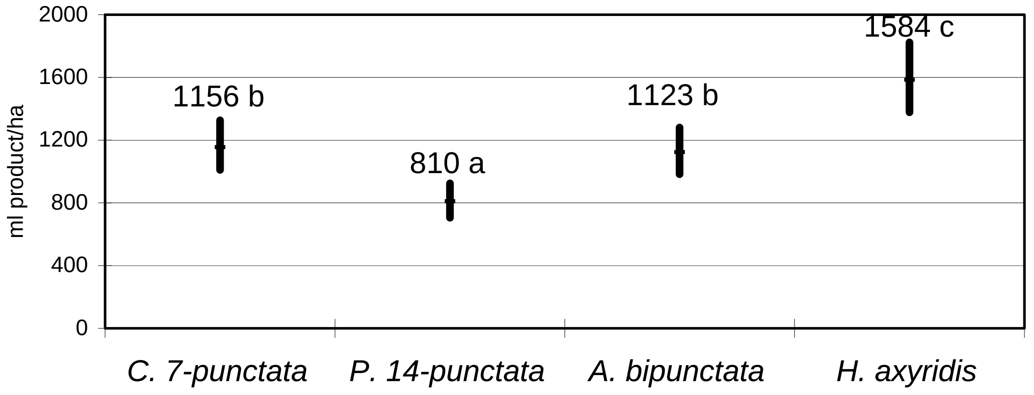

Figure 1: LR50 of Impulse (spiroxamine) for 4 ladybird species

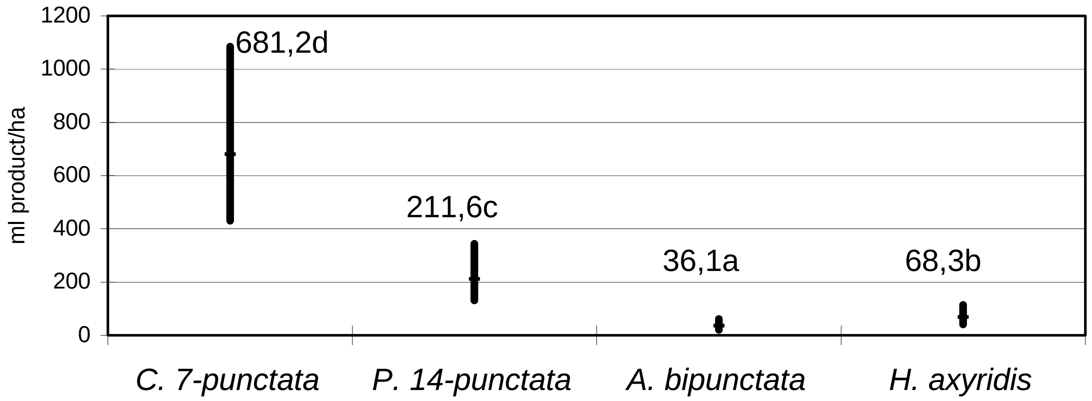

Figure 2: LR50 of Epok (fluazinam + metalaxyl-M) for 4 ladybird species

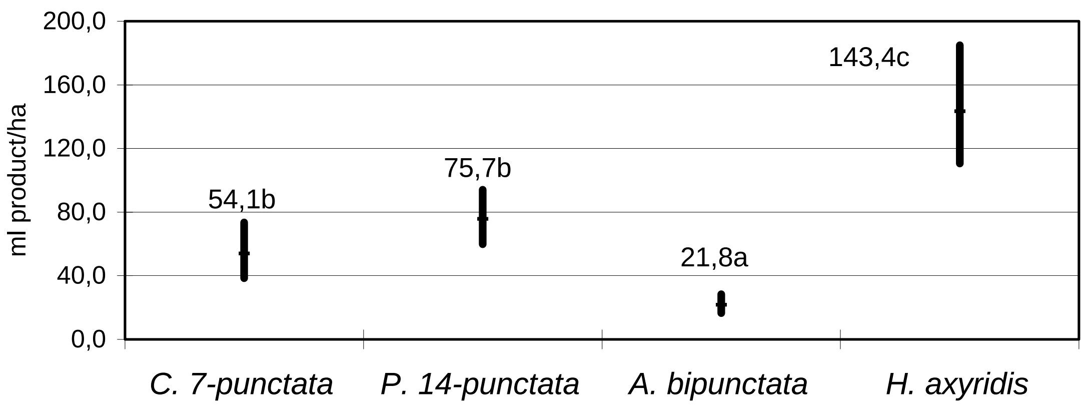

Figure 3: LR50 of Aztec (triazamate) for 4 ladybird species

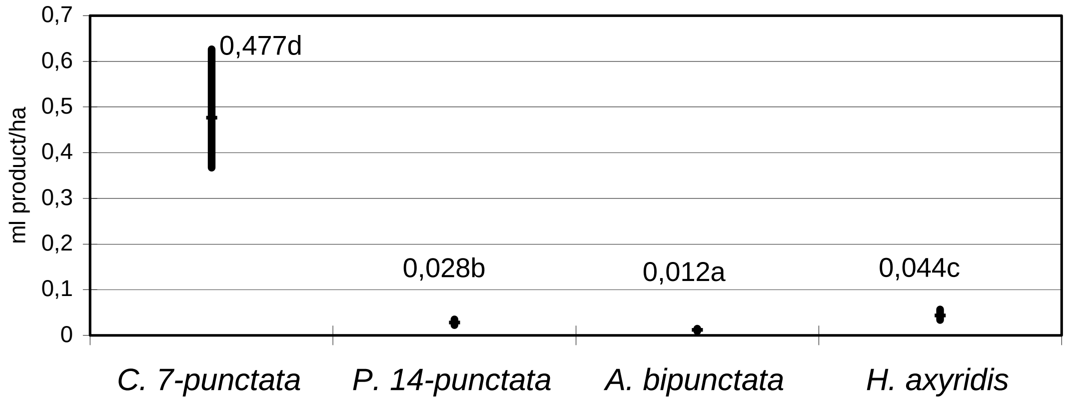

Figure 4: LR50 of Fury 10EW (zeta-cypermethrin) for 4 ladybird species

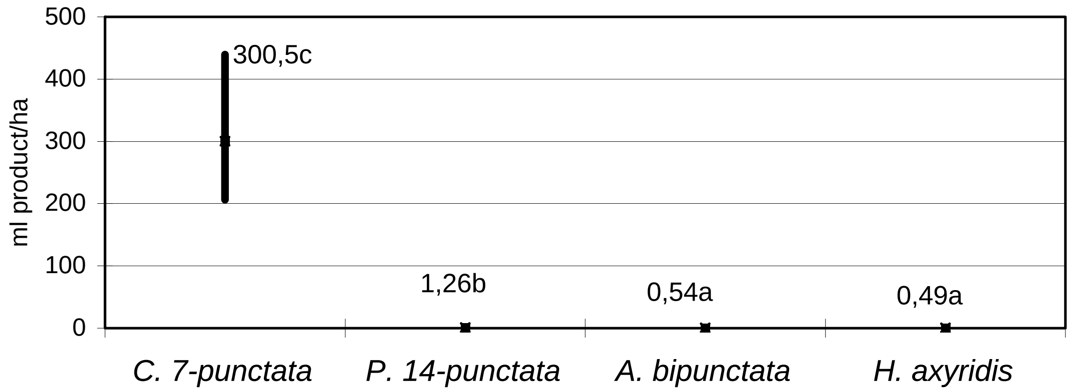

Figure 5: LR50 of Confidor (imidacloprid) for 4 ladybird species

The response of different ladybird species to each product was in some cases very similar, as for Impulse, but in most cases there were great differences between species for the same product, for example for Fury 10EW and Confidor. A comparison of the LR_{50} values (most sensitive species LR_{50}=1), given in Table 2, showed that the LR_{50} range reached a factor of 40 and 600 for these two compounds, respectively, while the range was limited to 2 for Impulse. If *C. 7-punctata* results are omitted, the range was more limited, between 1-2 (Impulse, Confidor) to 1-6 (Fury, Aztec, Epok).

Table 2: Comparative ratio of LR_{50} values of five products for 4 ladybird species. Most sensitive species, LR_{50}=1. Glass plate test with larvae.

	Impulse	Epok	Aztec	Fury	Confidor
C. 7-punctata	1.43	19.17	2.49	40.39	611.79
P. 14-punctata	1	5.87	3.48	2.37	2.57
A. bipunctata	1.39	1	1	1	1.09
H. axyridis	1.96	1.89	6.59	3.70	1

A sensitivity classification of the ladybird species based on LR_{50} (Table 3) showed that generally *A. bipunctata* was the most sensitive species and *C. 7-punctata* the least. *P. 14-punctata* and *H. axyridis* sensitivity was intermediate compared to the two other species. However, this standing only gave an indication and clearly showed that it is impossible to generalize.

Table 3: Sensitivity classification of 4 ladybird species to 5 products. 1=most sentitive to 4= least sensitive. Glass plate test with larvae.

	Impulse	Epok	Aztec	Fury	Confidor	Mean
A. bipunctata	2	1	1	1	2	1,4
P. 14-punctata	1	3	3	2	3	2,4
H. axyridis	4	2	4	3	1	2,8
C. 7-punctata	3	4	2	4	4	3,4

Discussion

Comparison of the LR_{50} results shows that the sensitivity of *H. axyridis* to pesticides seems to be similar to that of *A. bipunctata* and *P. 14-punctata* on glass plates. Comparative LR_{50} values were in a range of 1-6 and even for some products, *H. axyridis* was more sensitive than *P. 14-punctata* and *A. bipunctata.* In comparison with *C. 7-punctata*, *H. axyridis* was clearly more sensitive, but several questions must be answered concerning results obtained with the seven-spot ladybird. These results are different than those obtained in the context of the 4th Joint Pesticide Testing Program, where *H. axyridis* appeared to be less sensitive to several pesticides than *S. 11-notata*, a European native species (Hassan *et al.*, 1988). However, although the products and the doses tested were similar, the methods differed, making it difficult to make a direct comparison of the results. These results suggest that the use of pesticides in the field is unlikely to confer a competitive advantage on *H. axyridis.* However, under field conditions the phenology of insects directly influences possible exposure to pesticide. Thus, treatment positioning and presence of *H. axyridis* in the field, (that arrive a little later than other species), can modify this trend. Importance of searching activity of the larvae and aphid ingestion can also modify pesticide uptake, both by contact and by ingestion of contaminated food. Higher activity and voracity of *H. axyridis* compared to other species would be expected to increase pesticide toxicity.

The results of this study suggest that extrapolation of results obtained for one species to another one can be complicated. As a general trend *A. bipunctata* appears to be the most sensitive species and products that are harmless for this species could be expected to be harmless for the other species. *C. 7-punctata* was the least sensitive species and products that were harmful for this ladybird will probably be harmful for the other species. The LR_{50} values were variable in range according to the product tested, with sometimes a very good correlation between species, by example for Impulse, with LR_{50} ratio 1-2 or to a lesser extend for Aztec, with LR_{50} ratio 1-6. For other products, correlation was not good, with LR_{50} ratio up to 600, making extrapolation between species hazardous. However, most of high LR_{50} ratios came from *C. 7-punctata* results. If these results are omitted, correlation of results and extrapolation seems to be better, with LR_{50} ratios of 1-2 to 1-6 at maximum. If an indicator species for standard tests must be selected, *A. bipunctata* and *H. axyridis* are the best candidates: *A. bipunctata* because of its sensitivity and *H. axyridis* because of its handling and ease of rearing and the possible extrapolation of results to the other species. *P. 14-punctata*, which was not the least sensitive species and is not so easy to produce in laboratory, is not a candidate for selection as an indicator species.

Results obtained for *C. 7-punctata* suggest that this species is not an useful indicator species. With LR_{50} ratio of up to 600 on glass plates, there is a high risk to rate harmless for ladybirds a product that is harmless for *C. 7-punctata* alone. The results obtained with Fury and Confidor, with high differences between the seven spot ladybird and the other ones, while these differences do not appear so clearly with other products, are the indication of a possible resistance mechanism developed by the strain used for the test. The *C. 7-punctata* rearing of the laboratory was started and renewed with adults sampled in field margins, hedges and plants in an agroecosystem and the ladybird population was probably exposed to a range of pesticides over many years, including imidacloprid and pyrethroid insecticides. But it was strange that only the *C. 7-punctata* strain could develop resistance while the other species, that were sampled more or less in the same places, could not. A set of experiments with *C. 7-punctata* strains from other origins are needed to discover whether the results of this study apply to the species or just to the particular strain tested. According to these results the usefulness of *C. 7-punctata* as an indicator species for side effects should be reconsidered.

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