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Teflubenzuron effects on the red mason bee: a preliminary test set up in microcosm

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Abstract

The effects of a commercial insecticide formulation of teflubenzuron (Nomolt®) have been tested inside a plexiglas structured microcosm.

This artificial ecosystem has been articulated in two cells: one of them was used for analysis and the other one as a control environment. Each cell had light and aeration control; temperature and humidity control was not provided.

The test species have been chosen in order to represent different trophic levels: to create a terrestrial food chain, detritus consumers have been introduced into the microcosm, in association with the primary producers *Trifolium repens* L. var. *sylvestris* and *Phacelia tanacetifolia* Benth. and the primary consumer *Osmia rufa* L. An aquatic chain was also created inside each cell.

On this artificial ecosystem, teflubenzuron has been tested for chronic toxicity by spraying, on the flowering vegetation inside the analysis cell, an aqueous solution containing 0.15 ml of Nomolt® per litre.

Before spraying, 11 *O. rufa* males just emerged and 15 *O. rufa* female cocoons have been introduced in the biological community of each cell.

Results have been statistically analyzed. Teflubenzuron produced a rapid lethal effect on *O. rufa* emergence from most of the cocoons and it influenced adversely different behavioural aspects of the adults. This kind of damage increased during the testing period, and, after 7 days all the red mason bees contained in the analysis cell were died.

In the control cell the survivorship of the bees was 13 day; their conditions were good until the end of their lives, but this result is considerably shorter than the life period recorded for other *Osmia* bees in past experiments.

Probably this is due to temperature and humidity control missing; after this experience it is rather evident that this kind of regulation is essential for microcosms.

Another problem to be recorded for future experiences is that, in presence of a water pond, these solitary bees tend to throw themselves in it and they often die, even if small wooden floats are placed on the water surface.

Teflubenzuron seems to affect emergence, behaviour and survival of *O. rufa* bees in a microcosm environment.

Key words: teflubenzuron, *Omia rufa* L., microcosm, non-target effects, behaviour.

Introduction

Recent rapid increases in the sophistication of ecological experiments have paved the way for attempts to study the population dynamics in ecosystem processes of a community under specified environmental conditions (Lawton *et al*., 1993).

Microcosms as biological models have played a central role in the development of contemporary ecological thought (Huffaker, 1958; Tilman, 1977). They have shown, in many circumstances, their validity in order to test the effects of pollutants on biological communities, made up of different species simultaneously present inside their structure. The study of these simplified ecosystems is providing provocative insights into ecological principles as well as issues of environmental management and global stability (Beyers and Odum, 1993).

In fact it is possible to check, in these confined systems, the effects due to the application of different xenobiotic chemicals in relation with time, inducing non-penalizing life conditions for the introduced species and giving precise informations about individual responses to treatments. Moreover microcosms are easily replicated and offer precise control over environmental factors as well as the ability to manipulate and control spatial heterogeneity; their validity and their proven power in addressing many current and future problems in ecology makes them an important and useful tool (Drake *et al*., 1996).

In order to develop ecological indicators and strategies for environmental monitoring and assessment of contaminants, a first attempt to use a microcosm to test the effects of toluene and carbon monoxide on edaphic, aquatic and aerial organisms, has been done by Fiat Auto and the University of Turin (Badino *et al*., 2000; Ferrazzi *et al*., 2002). This plexiglas structured laboratory microcosm was articulated in two cells (experimental and control), each having a capacity of 1.5 m^3, and contained a terrestrial ecosystem and an aquatic one.

The two cells are now in the Environmental Quality Laboratory of Di.Va.P.R.A., where, despite technical problems connected with temperature control, they are used to test the effects of insecticides on Megachilidae, solitary bees known to be very important as pollinators (Kevan, 1999; Pinzauti, 2000), and other species.

In this experiment it was decided to use this microcosm for a preliminary test, to assess the effects of the IGR insecticide commercialised as Nomolt® (teflubenzuron), on different Arthropoda species, on other invertebrates and on unicellular algae.

The danger of pesticides, especially insecticides, to pollinators is well documented and understood (Johansen and Mayer, 1990; Bortolotti *et al*., 2001); most studies on pesticides toxicity and hazards to pollinators have dealt with honey bees (Porrini *et al*., 2002), but these are poor bioindicators for effects on other pollinators, even bees (Kevan and Plowright, 1995).

In fact, even if previous experiences showed that teflubenzuron does not affect survival of adults of the horn faced bee (*Osmia cornifrons* Radoszkowski) (Kawashima, 1989), of *Bombus terrestris* (L.) (Patetta *et al*., 2002) and of the honey bee (*Apis mellifera* L.) (Gromisz and Gromisz, 1996), the use of IGR insecticides to contain above all juvenile stages of endopterigota insects is not recommended in presence of pollinators because of negative effects induced on brood of social apoidea insects as honey bees and bumble bees (Wael *et al*., 1995; Gromisz and Gromisz, 1996).

Nevertheless we still do not know much about its possible impact on solitary apoidea, whose environmental role is so much important that they are reared in order to improve pollination in orchards and greenhouses.

Materials and methods

In the plexiglas structured microcosm set up in cooperation with Fiat Auto and articulated in two identical cells, only aeration and lightness were controlled during this experiment. Temperature and humidity control was not provided to check the feasibility of avoiding this regulation in microcosms experiment.

Many test species have been chosen in relation to the effects of the pollutant on the different trophic levels: to create a biological community, inside each cell detritus consumers have been introduced (the crustacean isopod *Porcellio scaber* Latr. and the earthworm *Eisenia foetida* Sav.) in association with the primary producers *Trifolium repens* L. var. *sylvestris* and *Phacelia tanacetifolia* Benth. and the primary consumer *Osmia rufa* L.

An aquatic chain was also created by introducing the unicellular alga *Selenastrum capricornutum* Printz and the crustacean cladoceran *Daphnia magna* Straus.

Each cell allowed the isolation from the outside environment, the input of the desired dosage of chemical and continuous monitoring of the different organisms.

Among the different test species, we take into consideration *O. rufa*; the red mason bees used in this experiment were obtained with intensive rearing at the Department C.D.S.L., Sez. Entomologia Agraria, University of Pisa and kindly provided by dr Mauro Pinzauti.

Inside each cell a small cane bundle, suitable for *Osmia* nest-building, was prepared.

Honey with pollen was introduced inside each cell in order to supply food for the red mason bees.

About Megachilidae, 11 *O. rufa* males have been introduced in each cell after emergence, when females were going to come out from cocoons; in order to arrange at least the same number of females, 15 feminine cocoons have also been included.

Considering the contemporary presence of the red mason bees and of an aquatic environment, cork pieces have been placed on the water surface, in order to help fallen bees to get out of the water tank.On this artificial ecosystem teflubenzuron was tested for chronic toxicity. After introducing the Apoidea insects, an aqueous solution containing 0.15 ml/l of Nomolt® was sprayed inside the experimental cell on the flowering vegetation. It was introduced only one time at the beginning of the test. The concentration tested corresponds to the dose indicated in the Nomolt® instruction sheet against *Leptinotarsa decemlineata* (Say) on potatoes and eggplants.

Surveys were carried out every two days and results have been statistically analysed using the Fisher exact test, the choice of which has been done considering the small samples analyzed.

The proportion of observations in the different categories which define the contingency table was compared in order to check if it was significantly different ($P \leq 0,05$), with regard to what is expected from random occurrence.

Results

The whole experiment lasted 13 days. Data obtained during the test, with regards to *O. rufa*, are presented in table 1.

The IGR insecticide influenced different biological parameters of *O. rufa*, producing a rapid lethal effect on emergence from most of the cocoons, on survival and behavioural aspects of the adults. This kind of damage increased during the testing period, and, after 7 days, all the red mason bees contained in the analysis cell were died.

Table 1. Data obtained during the test with regards to *O. rufa* (T=treated cell; C=control cell).

Date	5/4/2002		7/4/2002		9/4/2002		11/4/2002		13/4/2002		15/4/2002		17/4/2002	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Vital Cocoons	15	15	13	5	0	0	0	0	0	0	0	0	0	0
Alive females	0	0	8	1	5	0	3	0	0	0	0	0	0	0
Alive males	11	11	9	8	8	3	7	0	6	0	2	0	0	0
Dead females	0	0	5	4	3	1	2	0	3	0	0	0	0	0
Dead males	0	0	2	3	1	5	1	3	1	0	4	0	2	0
Dead organisms	0	0	7	7	4	6	3	3	4	0	4	0	2	0

A significant difference between the control environment and the experimental one was recorded in relation with emergence; in fact in the treated cell only 5 female bees emerged from the 15 cocoons; while in the control one 13 bees emerged.

A difference in the number of females emerged in the two cells was noticed.

During the first two surveys the difference between the mortality of females in the two cells was significant, but then it was nullified by a high level of female mortality in both the cells.Males were generally more vital and active than females and they lived longer. During the third and the fourth surveys there was a significant difference between the number of dead males in the two cells. Behavioural differences have been recorded: in the control cell the flight was normal, but nest visiting was not noticed. In the test cell, red mason bees showed increasing damage and had difficult movements, involuntary mandible trembling, ligula evaginated, hairless body, repeated attempts to clean legs, antennae and the whole body.

In the control cell the survivorship of the bees was 13 days; their conditions were good until the end of their lives, but this result is considerably shorter than the life period recorded for other *Osmia* bees in past experiments (Badino *et al*., 2000; Ferrazzi *et al*., 2002). The high level of humidity inside the two cells, connected with temperature control missing, has probably influenced activity and survival of the red mason bees. In both the cells, mortality of males and females was partially due to the presence of water: in fact, despite the presence of the cork pieces, bees often fell into the tank and were not able to climb onto the pieces or, when on them, they were not able to fly. Probably humidity did not allow fallen bees to dry their wings.

Discussion and conclusions

The insect growth regulator teflubenzuron, known to have a broad spectrum of activity, low environmental impact and low toxicity against the more common beneficial arthropods, including honeybees (Shell Agricoltura, 1991), has influenced different biological parameters of *O. rufa*: emergence, survival, behaviour.

Previous works did not underline damage on Apoidea adults, while effects on pre-imaginal phases have already been observed (Kawashima, 1989; Wael *et al*., 1995; Gromisz and Gromisz, 1996). At present the importance of behavioural parameters as a response to pollutants in *Osmia* has never been considered.

It is well-known that teflubenzuron interferes in the synthesis of chitin, and this is particularly evident in juvenile stages; nevertheless in adult *O. rufa* some pertinent effects maybe due to chitin synthesis inhibition, as hair removal, were observed; it is also possible to consider a chymothrypsin inhibition (Corbett *et al*., 1984), maybe due to food contamination inside the cells.

In this preliminary test, *O. rufa* mortality could have been influenced by environmental parameters. After this experience it is rather evident that arranging the microcosm cells from the temperature and humidity point of view will be indispensable for future microcosms experiments involving the contemporary presence of terrestrial and aquatic ecosystems.

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