# Toxicity of Selective Insecticides to Neochrysocharis formosa (Westwood) (Hymenoptera: Eulophidae), a Parasitoid of the American Serpentine Leafminer Liriomyxa trifolii (Burgess) (Diptera: Agrizomydae)

Tran, Dang Hoa
Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences,Kyushu University

Takagi, Masami

Takasu, Keiji
Laboratory of Bioresources and Management, Division of Bioresources and Management, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

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# Toxicity of Selective Insecticides to *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a Parasitoid of the American Serpentine Leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agrizomydae)

## Dang Hoa TRAN[1], Masami TAKAGI* and Keiji TAKASU[2]

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of
Applied Genetics and Pest Management, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
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The susceptibilities of *Neochrysocharis formosa*, a larval parasitoid of the American ser-
pentine leafminer *Liriomyza trifolii*, to three insecticides (imidacloprid, pymetrozine and
lufenuron) were investigated in the laboratory. Individual parasitoids were placed in the grass
vials whose internal surface was coated with the insecticides. For 24 h exposure, the $LC_{50}$ values
were 0.033 $\mu$g/0.5 ml for imidacloprid, 75.57 $\mu$g/0.5 ml for pymetrozine and 0.417 $\mu\ell$/0.5 ml for
lufenuron. For imidacloprid and lufenuron, these values were 775.5 and 14.9 times lower than
the recommended concentrations, respectively. Even in the concentrations lower than the $LC_{50}$,
parasitoid survival rapidly decreased with time, and the longevity of parasitoid females was also
reduced. These results suggested that all of imidacloprid, pymetrozine and lufenuron were
harmful to *N. formosa*.

## INTRODUCTION

Insect pest management relies on both natural enemies and insecticides as is a typical
of many agroecosystems. Pesticides have long overshadowed importance of natural
enemies in pest management programs. The frequent use of insecticides to manage these
pests may destroy natural enemies and encourage pest resurgence or a secondary pest
outbreak. Pesticides can exert two different types of effects on natural enemies. Lethal
effects are expressed as acute or chronic mortality arising from contact with a pesticide.
Sublethal effects, in contact, are often chronic and are expressed as some change in the
insect's life history attributes, such as its fecundity, longevity, developmental time, egg
viability, consumption rates, behavior, and so forth (Ruberson *et al.*, 1998). Lethal effects
are manifested as short–term mortality and often the greatest impact on natural enemies
(Johnson and Tabashnik, 1999).

Natural enemies and pesticides can be effectively integrated with adequate knowl-
edge of the pesticides to be used and its effects on natural enemy populations (Jepson,
1989; Croft, 1990, Greathead, 1995). Understanding the impact of pesticides usually

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[1] Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics
and Pest Management, Graduate School of Bioresource and Bioenvironmental Science, Kyushu
University
[2] Laboratory of Bioresources and Management, Division of Bioresources and Management, Department
of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University
* Corresponding author (E–mail: mtakagi@agr.kyushu–u.ac.jp)

requires a variety of bioassays to determine the selectivity of pesticides against the natural enemies, and their role in the ecology of pest management programs (Croft, 1990, Hassan, 1989). Pioneering work has been carried out in Europe to develop standardized tests for measuring the toxicity of pesticides to beneficial arthropods using a sequential procedure progressing from exposure in the laboratory to field trials (Hassan, 1989).

*Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), native to North and South America, is a serious pest of numerous ornamental and vegetable crops worldwide (Parrella, 1987; Spencer, 1989, 1990). Over 40 species of parasitoids have been recovered from *Liriomyza* spp. leafminers in the world (Waterhouse and Norris, 1987), including 27 species in Japan (Konishi, 1998). *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) is an endoparasitoid that attacks larvae of leafminers and eggs of sawflies (Yoshimoto, 1978; Hasson, 1990, 1995). In Kyushu, *N. formosa* is predominant among parasitoids attacking *L. trifolii*, and has been recognized as an effective biological agent of leafminers in tomato, bean and eggplant (Saito *et al.*, 1996; Arakaki *et al.*, 1998; Ohno *et al.*, 1999 and Maryana, 2000). Therefore, inundative release of *N. formosa* show promise for suppression of *L. trifolii* in those crops where insecticide use has been reduced.

In this study, a series of tests were conducted on females of *N. formosa* to determine their sensitivities to different insecticides (imidacloprid, pymetrozine and lufenuron). Imidacloprid and pymetrozine are commonly used for controlling sucking pests such as *Aphis gossypi, Brevioryne brassicaei, Bemisia argentifolii, Thrips tabaci* and *Caliothrips brasiliensis* (Bethke and Redak, 1997; Marquini *et al.*, 2002; Sechser *et al.*, 2002; Ester *et al.*, 2003). Lufenuron is currently available for lepidopteran pests including *Spodoptera littoralis, Phthorimaea operculella* and *Lacanobia oleracae* (Javaid *et al.*, 1999; Edomwande *et al.*, 2000; Whiting *et al.*, 2000; Butter *et al.*, 2003). These pests and the leafminers often co-exist in fields of various vegetables and ornamental crops, including tomato, melon, cucumbers, eggplants, green pepper, peach, chrysanthemum, apple and strawberry (Sibanda *et al.*, 2000; van Lenteren, 2000; Marquini *et al.*, 2002). The purpose was to determine which insecticide was least toxic to the parasitoid, and therefore best suited for use in an IPM program.

## MATERIALS AND METHODS

**Insect pest**

*L. trifolii* was reared on the kidney bean, *Phaseolus vulgaris* L., in the same manner as described by Giang and Ueno (2002) and Tran *et al.* (2004). A single seed of this plant was sown in a plastic pot (7.5 cm in diameter) kept at 25°C and 60–70% humidity under constant light. One week after germination, a tray (32 cm×44 cm×6 cm) containing 24 potted plants was placed on a shelf (200 cm×60 cm×50 cm) covered with a fine nylon mesh. Leafminer adults were released inside the mesh and allowed to oviposit on the plants for 24 h. Thereafter, the potted plants were maintained under the same conditions until all leafminer larvae feeding on the plants reach the last instar. The leaves containing final–instar larvae were cut off and kept in a polyethylene terephthalate (PET) bottle (1.5 l in volume) to gain adult leafminers.

**Parasitoid**

The asexual strain of the parasitoid *N. formosa* used for the present study originated from a culture that was reared from *L. trifolli* mines collected in August 1997 from Kagoshima Prefecture by the Fukuoka Agricultural Research Center, Fukuoka, Japan. This parasitoid was maintained with the final– instars of *L. trifolii* at 25 °C with 60–70% humidity and 16L: 8D. Each leaf of kidney bean plants (15–20 cm in height) was infected with 30–50 second and third instar larvae of *L. trifolii*. For parasitization, 6 host–infested plants and a piece of tissue paper (2 cm×2 cm) saturated with a honey solution were placed in a plastic cage (35 cm×20 cm×25 cm) covered with a fine nylon mesh. About 100–300 parasitoids were introduced into the cage. After exposure for 24 h, these plants were relocated to a plastic container (60 cm×50 cm×40 cm) until pupation of the parasitoids (approximately 6 days after parasitism). The kidney bean leaves with parasitoid pupae were removed from the plant stems and placed into a PET bottle (1 l in volume). Emergence of parasitoids was checked daily. Female wasps were provided honey immediately after emergence.

**Insecticides**

We tested the insecticides listed in Table 1. They were selected on the basis of their current and potential use for the management of key insect pests on vegetable crops.

**Table 1.** Insecticides tested for toxicity to *N. formosa*

| Common name | Trade name | Formu- lation[a] | Recommended concentrations ($\mu$g[b] or $\mu l$[c]/ 0.5 ml) | Main targets |
|---|---|---|---|---|
| Imidacloprid | Admire | WP | 25[b] | Aphids, leafhoper, flea beetle, whitefly. |
| Pymetrozine | Chess | WP | 62.5[b] | Aphids, whitefly |
| Lufenuron | Match | EC | 6.25[c] | Lepidoptera, thrips, rust mites. |

[a] EC, emulsifiable; WP, wettable power

**Bioassay**

Toxicity measurements were made by exposing parasitoids to insecticide coats on the inner surfaces on 20 ml grass vials (28 mm×60 mm). The coats were prepared by pipetting 0.5 ml insecticide solution with acetone into the vials, and manually rotating the vials on their sides until the solvent evaporated. The vials coated only acetone were used for control. Wasp females were individually placed in each vial along with a 1 cm square of cotton soaked in 30% honey in water. Exposed females were kept at 25 °C, 60–70% humidity and 16L: 8D light period.

*Dose–response bioassay*

Preliminary tests were used to obtain the approximate LC$_{50}$ for each insecticide. A 50 ml stock solution was prepared for each insecticide with concentration reflecting recommended field rates by producers (Table 1). The solution was made by diluting insecticide with Acetone 300, 99.5+% (GC). A series of concentrations for each insecticide was

made by adding acetone to a 1 ml stock solution. The ranges of doses tested for each insecticide were: imidacloprid, 0.0125 to 2.5 $\mu$g/vial; pymetrozine, 6.25 to 87.5 $\mu$g/vial and lufenuron, 0.301 to 6.25 $\mu\ell$/vial. Mortality determinations were made 24 h after initial exposure. Twenty parasitoid females were tested at each insecticide concentration.

*Time–response bioassay*

Serial time– dose–response bioassays was used to determine response of the females to different doses of each insecticide in the glass vials. The ranges of doses tested for each insecticide were made by diluting insecticide with acetone until being dose equivalent to 1, 2, 5 and 10 times lower than $LC_{50}$–dose obtained from the dose–response bioassays. Survival was determined at 24 h, 48 h, 72 h, 96 h and 120 h after initial exposure. Alive females were maintained and monitored daily until all wasps had died to determine their longevity. The piece of soaked cotton was replaced daily to provide fresh food to the female. Fifty females were tested at each insecticide concentration

## Data analysis

Dose– response data were analyzed by probit analysis program PriProbit ver. 1.63 (Sakuma, M, 1998). Serial time– dose data were analyzed using the survival analysis, Kaplan–Meier test, JMP4J (SAS Institute Inc. 2000). The longevity of females was analyzed by a one–way ANOVA, and means were separated by Fisher's PLSD test, StatView ver. 5.0 (SAS Institute Inc. 1998).

## RESULTS

### Dose–response

Results of the probit analysis of dose response data ($LC_{50}$, slopes and intercepts of the dosage–mortality lines) for *N. formosa* females are given in Table 2. The data show a wide range in response to different insecticides. The ranges from most toxic (imidacloprid) to least toxic (pymetrozine) are 2,290–fold at the $LC_{50}$ level. Imidaclopid and lufenuron were more specifically toxic to the females. $LC_{50}$ values of lufenuron and imidaclopid were much lower than the recommended concentrations 14.9 and 757.5 times, respectively. For pymetrozine, $LC_{50}$ value was a little higher than the recommended concentration.

**Table 2.** Probit parameters of dose respones of *N. formosa*

| Common name | Intercept[a] | Slope[a] | $LC_{50}$[b] ($\mu$g or $\mu\ell$[c]/ vial) | $\chi^2$[c] | Ratio: recommended concentration/$LC_{50}$ |
|---|---|---|---|---|---|
| Imidacloprid | 2.73 (0.35) | 1.84 (0.28) | 0.033 (0.021–0.045) | 9.13 ( 6,0.167) | 757.5 |
| Pymetrozine | −4.86 (0.94) | 2.59 (0.53) | 75.59 (62.01–101.74) | 11.93 (6, 0.130) | 0.8 |
| Lufenuron | 0.85 (0.12) | 2.26 (0.44) | 0.417 (0.244–0.57) | 5.95 (5, 0.311) | 14.9 |

[a] ($\pm$standard error); [b] (95% fiducial limits); [c] (df, p)

## Time–response

The cumulative survival in the present of insecticides depended on the duration of exposure (Fig. 1). When the females were exposed to treated insecticides, the cumulative survival decreased with time, and the survival was different between insecticide concentrations (imidaclopid: $X^2=146.1$, $df=4$, $P<0.0001$; pymetrozine: $X^2=159.5$, $df=4$, $P<0.0001$; lufenuron: $X^2=157.7$, $df=4$, $P<0.0001$).
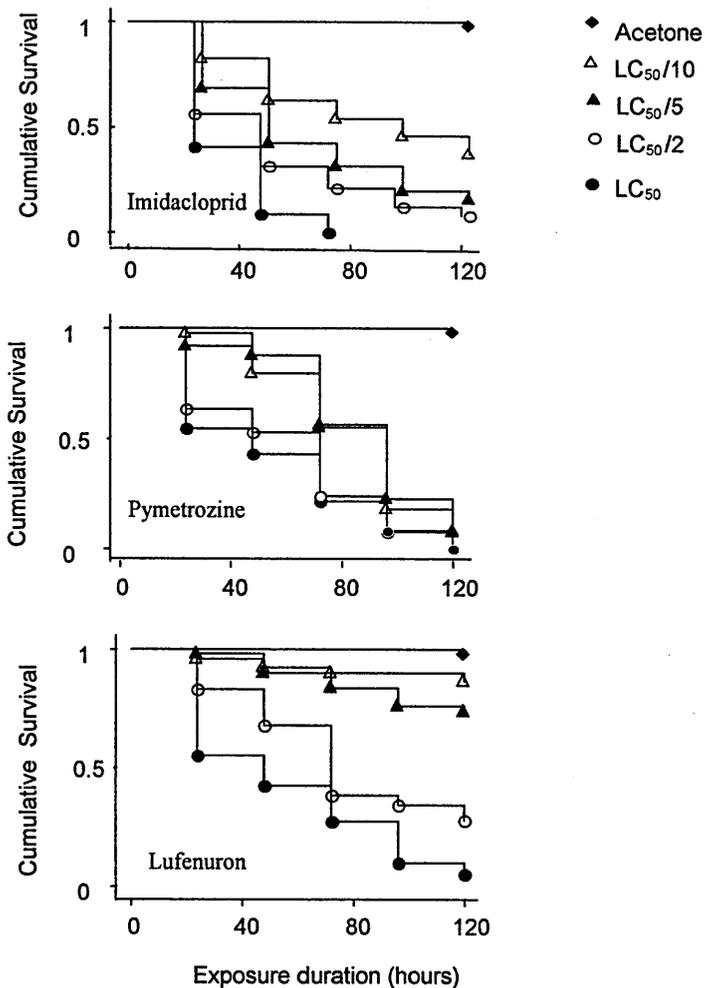


**Fig. 1.** Cumulative survival of female *N. formosa* that survived treatments with different concentrations of imidaclopid ($X^2=146.1$, $df=4$, $P<0.0001$), pymetrozine ($X^2=159.5$, $df=4$, $P<0.0001$), lufenuron ($X^2=157.7$, $df=4$, $P<0.0001$). Survival Analysis: Kaplan– Meier test, JMP4J, SAS Institute 2000.

Exposure to all concentrations of imidaclopid and pymetrozine resulted in decreasing the cumulative survival. Meanwhile, exposure to lufenuron resulted in little effect on survival until a threshold was reached ($LC_{50}/5$), whereby exposure to $LC_{50}$ and $LC_{50}/2$ resulted in a high level of mortality within the first 5 days.

**Effect of different doses of insecticides on longevity**

When the females were exposed to treated insecticides, the mean longevity decreased with increasing concentration. The females' longevity in all insecticide treatments was shorter than control treatment (Table 3).

There were highly significant differences (imidaclopid, $F=51.2$, $df=4$, 240, $P<0.0001$; pymetrozine, $F=115.1$, $df=4$, 216, $P<0.0001$; lufenuron, $F=115.1$, $df=4$, 216, $P<0.0001$) in the mean longevity of females exposed to the different concentrations of all insecticides.

The mean longevity was no significantly different among the treatments of $LC_{50}$, $LC_{50}/2$ and $LC_{50}/5$ of imidaclopid ($P>0.05$) and pymetrozine ($P>0.05$), and among the treatments of $LC_{50}/5$, $LC_{50}/10$ of lufenuron and acetone ($P>0.05$).

## DISCUSSION

Beneficial arthropods can be exposed to pesticides by direct contact, by indirect contact with residues on plant surfaces, or by the ingestion of pesticide–contaminated prey or host (Jepson, 1989). In most studies, pesticide effects have been evaluated by exposure of the natural enemy to a range of pesticide concentrations (Desneux *et al.*, 2004; Youn *et al.*, 2003; Sanon *et al.*, 2002; Akol *et al.*, 2002, Yokoyama, 1984).

The females of *N. formosa* were very susceptible to imidaclopid, and its $LC_{50}$ value was very low ($0.033\,\mu g/0.5\,ml$). The $LC_{50}$ values allowed to rank the insecticides in order of increasing toxicity: pymetrozine, lufenuron, imidacloprid. Imidacloprid was about 2290 times more toxic than pymetrozine. Moreover, the $LC_{50}$ values were lower than recommended field rates for imidacloprid and lufenuron. When testing commercial products, the ratio between the field recommended rate and $LD_{50}$ (or $LC_{50}$) gives an indication of the risk (Youn *et al.*, 2003; Desneux *et al.*, 2004). Desneux *et al.* (2004) used this quotient for evaluating the risk of pesticides to *Aphidius ervi*, a generalist parasitoid of aphids.

**Table 3.** Effect of different doses of insecticides on longevity of *N. formosa* females. (day, mean ±SD)

| Dose | Common name | | |
|------|-------------|---|---|
| | Imidacloprid | Pymetrozine | Lufenuron |
| $LC_{50}$ | 1.54±0.65a | 2.56±1.3a | 2.37±1.46a |
| $LC_{50}/2$ | 2.44±1.99ab | 2.78±1.15a | 4.84±4.82b |
| $LC_{50}/5$ | 3.31±3.33b | 3.68±1.25b | 10.41±5.81c |
| $LC_{50}/10$ | 5.37±4.93c | 3.7 ±1.44b | 11.4 ±5.18c |
| Acetone | 10.74±3.94d | 10.74±3.94c | 10.74±3.94c |

Means with the same letters within a column are not significantly different by Fisher's PLSD after one–way ANOVA, $P<0.05$.

The ratios may also allow to compare the risk to *N. formosa* among three treated insecticides. The ratio was equal to 0.8 for pymetrozine, 14.9 for lufenuron, and 775.5 for imidacloprid, presenting the highest risk. Imidacloprid was the most harmful insecticide to the parasitoid because of high toxicity and risk.

Previous studies indicated the decline of parasitoid populations caused by imidacloprid, lufenuron and pymetrozine application at field recommended rates (Ozawa, *et al.*, 1998; Côsoli *et al.*, 2001; Rebek and Sadof, 2003; Rogers and Potter, 2003). This study was evidence for a range of possible trends in susceptibility to those insecticides at low concentrations in the laboratory. Statistical comparison of both cumulative survival and mean longevity suggested the presence of sublethal effects of the tested insecticides on *N. formosa*. Exposure to imidacloprid, lufenuron and pymetrozine led to a concentration –dependent decline in survival (Fig. 1). Increasing insecticide concentrations caused the high level of parasitoid mortality. A comparison of the survivorship curves suggested that the cumulative survival rapidly decreased after exposure to imidacloprid for 24 h, pymetrozine and lufenuron (at $LC_{50}$ and $LC_{50}/2$) for 48 h. Meanwhile, the survivorship curves of females exposed to lufenuron at $LC_{50}/5$ and $LC_{50}/10$ shared patterns of stability and decline similar to those of the acetone control. The data indicated that pymetrozine and lufenuron at low concentrations ($LC_{50}/5$ and $LC_{50}/10$) did not have acute effects on *N. formosa* females, though those chronic effects caused the females' mortality increased after 48 h exposure.

Those insecticides also appeared to elicit a concentration–dependent decline in the mean longevity of *N. formosa* females (Table 3). Longevity of the females was greatly reduced by both imidacloprid and pymetrozine, and did not exceed 5.4 days in imidacloprid treatments, 3.7 days in pymetrozine treatments, compared to 10.7 days in control treatment. This agrees with the finding by Stapel *et al.* (2000) that longevity in the parasitoid *Microplitis croceipes* adults exposed to extrafloral nectar of cotton treated with imidacloprid was reduced. The high activity of imidacloprid is brought about by its binding to nicotinergic acetylcholine receptors in the insect nervous system (Marquini *et al.*, 2002). The precise mode of action of pymetrozine is unknown, but treated insects quickly stop feeding and die of starvation (Harrewijn and Kayser, 1997). Thus, imidacloprid and pymetrizine had chronic toxic to *N. formosa* resulted in the reduction of the parasitoid's longevity.

At low concentrations ($LC_{50}/5$ and $LC_{50}/10$), lufenuron did not affect longevity in *N. formosa* females. Since lufenuron works as a chitin– synthesis inhibitor by interfering with the synthesis of chitin, it causes parasitoid mortality before the parasitoid became a mature stage (Côsoli *et al.*, 2001). The interference is perhaps not expected in this study since chitin synthesis is absent or occurs at a very low level in adults (Wilson and Cryan, 1997). It demonstrated that lufenuron at low concentrations was less toxic to the females.

The results of the present study suggest that a ranking system based on actual and chronic effects of treated insecticide toxicity on *N. formosa* was in the following relative order: imidacloprid＞pymetrozine＞lufenuron. In addition to chronic effects of these insecticides, a previous study showed that imidacloprid and lufenuron reduced host searching efficiency of *N. formosa* females (Tran *et al.*, 2004). As a result, the treated insecticides are potential constraints on the effectiveness of *N. formosa* as a biological

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control agent of leafminers. However, both studies were conducted in the laboratory conditions, and thus tested insecticides are expected to be less harmful when apply in the field conditions.


## ACKNOWLEDGMENTS

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