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Title:

Insecticide susceptibility and carboxylesterase activity in leafminers (Diptera: Agromyzidae) and their associated hymenopteran parasitoids

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ABSTRACT

Laboratory cultures of three dipteran leafminers, *Liriomyza trifolii*, *L. sativae* and *Chromatomyia horticola*, and their four most common hymenopteran parasitoids in Japan, *Diglyphus isaea*, *Chrysocharis pentheus*, *Neochrysocharis formosa* and *Hemiptarsenus varicornis*, were evaluated for their susceptibility to three conventional insecticides following topical application. All the leafminers were far less sensitive to malathion and moderately less sensitive to permethrin compared with the parasitoids. The marked disparity in insecticide susceptibility convincingly explains the increased abundance of leafminers in the field after applications of these insecticides. *Liriomyza trifolii* and *C. horticola* were also less sensitive to emamectin-benzoate than the parasitoids, although *L. sativae* was as sensitive as the parasitoids. We also evaluated the susceptibility of a more minor parasitoid species attacking leafminers in Japan, *Halticoptera circulus*, to malathion and it was less sensitive than the four more common parasitoid species and may, therefore, be suitable for augmentative biological control of leafminers in IPM programmes that include insecticides. In addition, carboxylesterase activity of each insect species was determined and correlations with their insecticide susceptibility discussed.

Keywords:

Liriomyza spp.

Chromatomyia horticola

Hymenopteran parasitoid

Halticoptera circulus

Insecticide susceptibility

Carboxylesterase

1. Introduction

The leafminers *Liriomyza trifolii* (Burgess), *Liriomyza sativae* Blanchard and *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) are widespread and important pests of vegetables and ornamental crops (Spencer, 1990). In Japan, the invasive species, *L. trifolii*, was first observed in 1990 and causes serious damage on a wide variety of crops including tomato, celery, chrysanthemum and gerbera (Saito, 1993a). In 1999 a second invasive leafminer, *L. sativae*, was first recorded in Japan and has subsequently also become damaging on crops such as cucumber and tomato (Iwasaki et al., 2000). *Chromatomyia horticola* is an indigenous leafminer but has become an increasingly serious pest, particularly on garden pea and lettuce, since the 1990s (Saito, 2004). While initially leafminers were generally considered only as secondary pests, their pest status has increased and been compounded by the development of insecticide resistance (Parrella and Keil, 1984; Saito et al., 1992, 2008a; Saito, 2004; Tokumaru et al., 2005) and by declining populations of hymenopteran parasitoids following applications of broad-spectrum insecticides in the field (e.g. Hernández et al., 2011; Kaspí and Parella, 2005; Johnson et al., 1980; Oatman and Kennedy, 1976; Saito, 2004; Saito et al., 1996, 2008a; Salvo and Valladares, 2007; Trumble and Toscano, 1983; Weintraub, 2001). It has been hypothesised that coincidental marked increases in leafminer populations and associated declines in parasitoid numbers following broad-spectrum insecticide may be due to differences in their relative susceptibility to the applied insecticides. However, there are few studies that have directly quantified the susceptibility of both leafminers and parasitoids at the same time (Priyono et al., 2004) and these studies are an essential foundation to understanding and predicting the relative dynamics of their populations in the field.

The objective of this study was to directly quantify, in the laboratory, the differences in susceptibility between these three leafminer species and their four most abundant parasitoids in Japan, *Diglyphus isaea* (Walker), *Chrysocharis pentheus* (Walker), *Neochrysocharis formosa* (Westwood) and *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) (Amano et al., 2008; Saito et al., 1996, 2008a,b) to topical applications of three model insecticides representing organophosphates, pyrethroids and macrolides respectively. Another parasitoid, *Halticoptera circulus* (Walker) (Hymenoptera: Pteromalidae), which is a minor species in leafminer populations in Japan (Amano et al., 2008; Saito et al., 1996, 2008a,b), was included in this study because, interestingly, populations of this species do not always decline following

insecticide applications (Johnson et al., 1980; Saito et al., 2008a; Trumble and Toscano, 1983). Furthermore, carboxylesterase activity, a well-known mechanism for insecticide detoxification in insects (Oakeshott et al., 2005), was also examined in these leafminer and parasitoid species and the potential relationship between carboxylesterase activity and susceptibility to insecticides is discussed.

2. Material and methods

2.1. Insect cultures

The leafminers, *L. trifolii*, *L. sativae* and *C. horticola*, were collected in Shizuoka, Japan, in 1991, 2004, and 2005, respectively, and subsequently maintained on potted kidney bean plants. Five hymenopteran parasitoid species were used in this study: *D. isaea*, *C. pentheus*, *N. formosa*, *H. varicornis* and *H. circulus*. With the exception of *H. circulus*, which was collected from the leafminer *Liriomyza chinensis* (Kato) in Nara, Japan, in 2009, all the parasitoids were collected in Shizuoka; *D. isaea* and *C. pentheus* from *C. horticola* in 2007, *N. formosa* from *L. sativae* in 2004 and *H. varicornis* from *L. trifolii* in 1995. The parasitoid cultures were maintained on *L. trifolii* larvae, except *H. circulus*, which was maintained on *C. horticola* larvae. Only 2–3-day-old adult leafminers and parasitoids were used in experiments. Body weights of living individual adults of each species were estimated from the mean weight of 100 leafminers or parasitoids (following anaesthetisation by exposure to CO₂) weighed together using a precision analytical balance.

2.2. Insecticide bioassays

Three synthetic insecticides (Wako Pure Chemical, Japan) were evaluated in the bioassays: malathion (99% a.i., organophosphate), permethrin (94% a.i., pyrethroid) and emamectin-benzoate (81% a.i., macrolide). Each insecticide was dissolved in acetone (99.5%) and five different doses were prepared by a series of ten-fold dilutions: 0.001, 0.01, 0.1, 1 and 10 mg/mL for malathion and 0.0001, 0.001, 0.01, 0.1 and 1 mg/mL for permethrin and emamectin-benzoate. These insecticide solutions were applied directly, using a topical applicator (Auto Microapplicator, Burkard Manufacturing, UK), to the dorsal surface of the thorax of individual adult leafminers or their parasitoids that had been anaesthetised by brief exposure to CO₂. The volume of each insecticide solution applied was 0.086 µl for a single leafminer and 0.017 µl for a single

parasitoid; these volumes had been adjusted according to species weight to account for their contrasting body sizes. Approximately 30 adults were treated for each replicate of each dose of insecticide. For each dose, exposed adults were placed together in Petri dishes and incubated at $23 \pm 1^\circ\text{C}$ for 3 h after which time the numbers of living and dead individuals were counted and they were all sexed. Each dose had three replicates and the data for each replicate were used individually (i.e. not pooled) to estimate the LD_{50} value. Following exclusion of doses achieving 100% mortality or mortality close to the control, there remained three to five doses per insecticide, each represented as an amount (ng) of insecticide per body weight (mg) of each insect. The control treatment consisted of applying acetone only, which resulted in $< 5\%$ mortality for all insect species. The LD_{50} values were estimated using Probit analysis (SPSS, 2009) of the dose-mortality data, after correction for control mortality using Abbott's formula. *Halticoptera circulus* was only evaluated for its susceptibility to malathion, because thereafter the culture unfortunately collapsed. For each species a tolerance ratio (TR) to each insecticide was also calculated ($= \text{LD}_{50}$ value of each insect divided by the LD_{50} value of the most susceptible insect). The statistical significance of differences in insecticide susceptibility amongst the insect species was estimated by comparing 95% FL of the LD_{50} value; non-overlapping 95% FLs were considered significantly different to each other.

2.3. Carboxylesterase activity

The experiments were conducted as follows, according to the methods of Saito (1993b) in which the carboxylesterase activity of individual aphids was determined. A single leafminer or parasitoid adult was crushed on a concave ground-glass plate using a glass rod in 300 μl of 1/15 mol/L phosphate buffer (pH 7.2). Then, 50 μl of this homogenate was added to a further 2.9 ml of phosphate buffer, before 50 μl of 0.03 mol/L α -naphthyl acetate dissolved in ethanol was added as a substrate. After incubation for 10 min at 30°C , α -naphthol, a product of the hydrolysed substrate, was stained by the addition of 1 ml of 1% Fast Blue RR:5% sodium lauryl sulfate (2:5 by volume) and the absorbance measured at 600 nm using a spectrophotometer (U-1900, Hitachi High-Technologies Co., Japan). A standard curve was prepared using α -naphthol instead of the homogenate, and the carboxylesterase activity in the samples was given as the amount of α -naphthol.

The amount of protein in each sample was also determined by the Bradford's method using Coomassie Brilliant Blue G-250 (CBB) (Wako Pure Chemical) (Tojo, 1980) as follows. Two-hundred microliters of tissue homogenate were mixed with 1.3 ml of distilled water and 1.5 ml of 0.02% CBB in 17% phosphoric acid. The absorbance of the CBB, which selectively binds protein, was then measured at 595 nm using a spectrophotometer. The amount of product was determined using a standard curve prepared using 98% bovine serum albumin (Wako Pure Chemical) dissolved in 1/15 mol/L phosphate buffer (pH 7.2). Carboxylesterase activity was then estimated as the amount of α -naphthol (nmol) per quantity of protein (μ g). Data for each insect were statistically analysed using activities of all individuals by ANOVA followed by a Tukey HSD-test at $P = 0.05$ (SPSS, 2009).

3. Results and Discussion

3.1. Susceptibility to insecticides

For malathion, the leafminer species had significantly higher LD₅₀ values than the four common parasitoid species (*D. isaea*, *C. pentheus*, *N. formosa* and *H. varicornis*) (non-overlapping 95% FLs) and the resulting large tolerance ratios (TRs) ranged from 670 to 12800 compared with 1 to 6.7 for the parasitoids (Table 1). This marked disparity in susceptibility to malathion may convincingly explain the increased abundance of *L. trifolii* (Saito et al., 1996; Trumble and Toscano, 1983), *L. sativae* (Johnson et al., 1980; Oatman and Kennedy, 1976) and *C. horticola* (Saito et al., 2008a) compared with their common parasitoids, in the field following application of organophosphates.

The other parasitoid, *H. circulus*, had significantly higher LD₅₀ values for malathion than the other parasitoids tested in this study (non-overlapping 95% FLs) and relatively large TRs of 333 for females and 302 for males. Compared to the leafminers, the parasitoid, *H. circulus*, had a significantly lower LD₅₀ value than *L. trifolii* (non-overlapping 95% FLs) but a similar LD₅₀ value (overlapping 95% FLs) to *L. sativae* and *C. horticola*. This may account for field reports suggesting that *H. circulus* populations do not always decline following application of organophosphates (Johnson et al., 1980; Saito et al., 2008a; Trumble and Toscano, 1983)

For permethrin, the LD₅₀ values for leafminers were not significantly different to the LD₅₀ values of their common parasitoids (overlapping 95% FL) although, overall, leafminer TRs were generally larger

(7.9 to 41.7) than the TRs of their common parasitoids (1.0 to 18.1) (Table 2). This difference may still be sufficient to favour leafminer survival over parasitoid survival following application of this insecticide in the field (Saito et al., 1996, 2008a; Schuster and Price, 1985) but would require further study to confirm.

For emamectin-benzoate, the LD₅₀ value for *L. sativae* was low and not significantly different to the common parasitoids (overlapping 95% FLs). As a result the TRs were also small: 8.7 for females and 3.7 for males of *L. sativae* and between 1.0 and 19.3 for the parasitoids. In contrast the LD₅₀ values for *L. trifolii* (excluding males) and *C. horticola* were significantly higher than for the common parasitoids (non-overlapping 95% FLs) and resulted in relatively large TRs (279 to 479) (Table 3). Even though we found large differences in susceptibility to emamectin-benzoate between two of the leafminer species and their common parasitoids, in Japan, increased leafminer populations following applications of emamectin-benzoate have not been reported. While the LD₅₀ values for emamectin-benzoate against the more tolerant leafminer species were high, they were still much lower than the equivalent LD₅₀ values for malathion, which may account for the field observations. In fact, effective control of leafminers by emamectin-benzoate (at the recommended field rate) has been confirmed in both laboratory tests (Saito, 2004; Tokumaru et al., 2005) and a field trial (Saito, 1997) and the insecticide is used commercially for leafminer control in Japan.

Amongst the parasitoid species, *H. circulus* appeared to have the lowest susceptibility to malathion compared with the other parasitoids tested in this study supporting field reports that found no decline in populations of *H. circulus* after application of the organophosphates, malathion (Saito et al., 2008a) or metamidophos (Trumble and Toscano, 1983). In addition, *H. circulus* populations appeared not to decline after permethrin applications in the field (Saito et al., 2008a). Together these findings suggest that this parasitoid is, at least partially tolerant to malathion, and potentially, also permethrin, although we did not evaluate its susceptibility to permethrin in this study and so cannot confirm this. While *H. circulus* was still significantly more sensitive to malathion than *L. trifolii*, as described above, its relative tolerance might make it more suited for augmentative biological control of leafminers within IPM programmes using organophosphates and pyrethroids, than the other parasitoids. However, further studies on the detailed biology of this parasitoid would be needed before this could be confirmed and they could be used practically. For the other parasitoids that were more sensitive to insecticides, conservation biological

control approaches including the use of more selective insecticides would be more appropriate. Selective insecticides include flufenoxuron and cyromazine which are effective against leafminers but less toxic against parasitoids than other insecticides (Bjorksten and Robinson, 2005; Saito et al., 1992, 1996). Both augmentation and conservative strategies using parasitoids could contribute to economically viable control of insecticide-tolerant leafminers, especially if used together.

3.2. Carboxylesterase activity

Mean carboxylesterase activity in leafminers ranged from 20.7 (female *C. horticola*) to 60.3 nmol/μg (female *L. trifolii*), with the highest activity of 248.2 nmol/μg observed in one female *L. trifolii* (Table 4). In contrast, the mean carboxylesterase activity in the four common parasitoid species (*D. isaea*, *C. pentheus*, *N. formosa* and *H. varicornis*) was < 10 nmol/μg with no individuals exhibiting activities > 40 nmol/μg. The other parasitoid *H. circulus* exhibited mean carboxylesterase activity of 30.8 and 31.6 nmol/μg for females and males, respectively. When the mean carboxylesterase activity was compared among all the insect species tested in this study, *Liriomyza* spp. leafminers exhibited significantly higher carboxylesterase activity than all the parasitoids except *H. circulus* ($F = 25.29$; $df = 14, 404$; $P < 0.05$). However, carboxylesterase activity in the leafminer *C. horticola* was not significantly different to the parasitoids ($P > 0.05$), excluding male *D. isaea* ($P < 0.05$). It is possible, therefore, that carboxylesterase activity in *Liriomyza* spp. and *H. circulus* may be related to their tolerance to malathion.

In *Liriomyza* spp., insensitive target acetylcholinesterase and oxidative metabolism are thought to confer tolerance to organophosphates and pyrethroids, respectively (Broadbent and Pree, 1989; Damayanthi and Karunaratne, 2005; Keil and Parrella, 1990). In contrast, carboxylesterase and oxidative detoxification are reported to be important for organophosphate and/or pyrethroid tolerance in hymenopteran parasitoids including the leafminer parasitoid *Diglyphus begini* (Ashmead) (Baker, 1994; Baker et al., 1998; Perez-Mendoza et al., 2000; Rathman et al., 1992). Our study suggests that elevated carboxylesterase activity may be important for conferring malathion tolerance in *Liriomyza* spp. leafminers and the parasitoid *H. circulus* but that other mechanisms may be more important for malathion tolerance in the leafminer *C. horticola*. *In vitro* studies on the enzyme are necessary in order to fully characterize enzyme-conferring tolerance in leafminers and *H. circulus*.

224

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229 **References**

- 230 Amano, K., Suzuki, A., Hiromori, H., Saito, T., 2008. Relative abundance of parasitoids reared during field
231 exposure of sentinel larvae of the leafminers *Liriomyza trifolii* (Burgess), *L. sativae* Blanchard, and
232 *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae). Appl. Entomol. Zool. 43, 625–630.
- 233 Baker, J.E., 1994. Resistance mechanism in the Bamberg strain of *Anisopteromalus calandrae*
234 (Hymenoptera: Pteromalidae). J. Entomol. Sci. 29, 580-584.
- 235 Baker, J.E., Fabrick, J.A., Zhu, K.Y., 1998. Characterization of esterase in malathion-resistant and
236 susceptible strains of the pteromalid parasitoid *Anisopteromalus calandrae*. Insect Biochem. Mol. Biol.
237 28, 1039-1050.
- 238 Bjorksten, T.A., Robinson, M., 2005. Juvenile and sublethal effects of selected pesticides on the leafminer
239 parasitoids *Hemiptarsenus varicornis* and *Diglyphus isaea* (Hymenoptera: Eulophidae) from Australia.
240 J. Econ. Entomol. 98, 1831-1838.
- 241 Broadbent, A.B., Pree, D.J., 1989. Resistance to pyrazophos in the serpentine leafminer *Liriomyza trifolii*
242 (Burgess) (Diptera: Agromyzidae) in Ontario greenhouses. Can. Entomol. 121, 47-53.
- 243 Damayanthi, B.T., Karunaratne, S.H.P.P., 2005. Biochemical characterization of insecticide resistance in
244 insect pests of vegetables and predatory ladybird beetles. J. Natn. Sci. Foundation Sri. Lanka 33,
245 115-122.
- 246 Hernández, R., Harris, M., Liu, T-X., 2011. Impact of insecticides on parasitoids of the leafminer,
247 *Liriomyza trifolii*, in pepper in south Texas. J. Insect Sci. 11, 1-14.
- 248 Iwasaki, A., Kasuga, K., Iwaizumi, R., Sasakawa, M., 2000. A newly recorded pest, *Liriomyza sativae*
249 Blanchard in Japan. Plant Prot. 54, 12-17 (in Japanese).
- 250 Johnson, M.W., Oatman, E.R., Wyman, J.A., 1980. Effects of insecticides on populations of the vegetable
251 leafminer and associated parasites on summer pole tomatoes. J. Econ. Entomol. 73, 61-66.

252 Kaspi, R., Parrella, M.P. 2005. Abamectin compatibility with the leafminer parasitoid *Diglyphus isaea*. Biol.
 253 Con. 35, 172-179.

254 Keil, C.B., Parrella, M.P., 1990. Characterization of insecticide resistance in two colonies of *Liriomyza*
 255 *trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. 83, 18-26.

256 Oakeshott, J.G., Claudianos, C., Campbell, P.M., Newcomb, R.D., Russell, R.J., 2005. Biochemical
 257 genetics and genomics of insect esterase, in: Lawrence, I.G., Kostas, I., Sarjeet, S.G. (Eds.),
 258 Comprehensive Molecular Insect Science, vol. 5. Elsevier, Oxford, pp. 309–381.

259 Oatman, E.R., Kennedy, G.G., 1976. Methomyl induced outbreak of *Liriomyza sativae* on tomato. J. Econ.
 260 Entomol. 69, 667-668.

261 Parrella, M.P., Keil, C.B., 1984. Insect pest management: the lesson of *Liriomyza*. Bull. Entomol. Soc. Am.
 262 30, 22-25.

263 Perez-Mendoza, J., Fabrick, J.A., Zhu, K.Y., Baker, J.E., 2000. Alterations in esterase are associated with
 264 malathion resistance in *Habrobracon hebetor* (Hymenoptera: Braconidae). J. Econ. Entomol. 93,
 265 31-37.

266 Priyono, D., Rauf, A., Robinson, M., Bjorksten, T., Hoffmann, A.A., 2004. Toxicity of chemicals commonly
 267 used in Indonesian vegetable crops to *Liriomyza huidobrensis* populations and the Indonesian
 268 parasitoids *Hemiptarsenus varicornis*, *Opius* sp., and *Gronotoma micromorpha*, as well as the
 269 Australian parasitoids *Hemiptarsenus varicornis* and *Diglyphus isaea*. J. Econ. Entomol. 97,
 270 1191-1197.

271 Rathman, R.J., Johnson, M.W., Rosenheim, J.A., Tabashnik, B.E., Purcell, M., 1992. Sexual differences in
 272 insecticide susceptibility and synergism with piperonyl butoxide in the leafminer parasitoid *Diglyphus*
 273 *begini* (Hymenoptera: Eulophidae). J. Econ. Entomol. 85, 15-20.

274 Saito. T., 1993a. Occurrence of the leafminer, *Liriomyza trifolii* (Burgess), and its control in Japan.
 275 Agrochem. Japan 62, 1-3.

276 Saito T., 1993b. Insecticide resistance of the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae),
 277 VI. Qualitative variation of aliesterase activity. Appl. Entomol. Zool. 28, 263–265.

278 Saito T., 1997. Control of American serpentine leafminer, *Liriomyza trifolii* (Burgess), by emamectin
 279 benzoate. Ann. Rept. Kanto-Tosan Pl. Prot. Soc. 44, 239-240 (in Japanese).

280 Saito, T., 2004. Insecticide susceptibility of the leafminer, *Chromatomyia horticola* (Goureau) (Diptera:
281 Agromyzidae). Appl. Entomol. Zool. 39, 203-208.

282 Saito, T., Oishi, T., Ikeda, F., Sawaki, T., 1992. Effect of insecticides on the serpentine leafminer, *Liriomyza*
283 *trifolii* (Burgess) (Diptera: Agromyzidae). Jpn. J. Appl. Entomol. Zool. 36, 183-191 (in Japanese with
284 English summary).

285 Saito, T., Ikeda, F., Ozawa, A., 1996. Effect of pesticides on parasitoid complex of serpentine leafminer
286 *Liriomyza trifolii* (Burgess) in Shizuoka Prefecture. Jpn. J. Appl. Entomol. Zool. 40, 127-133 (in
287 Japanese with English summary).

288 Saito, T., Doi, M., Katayama, H., Kaneko, S., Tagami, Y., Sugiyama, K., 2008a. Seasonal abundance of
289 hymenopteran parasitoids of the leafminer *Chromatomyia horticola* (Diptera: Agromyzidae) and the
290 impact of insecticide applications on parasitoids in garden pea field. Appl. Entomol. Zool. 43, 617-624.

291 Saito, T., Doi, M., Tagami, Y., Sugiyama, K., 2008b. Hymenopterous parasitoids of the exotic leafminers,
292 *Liriomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Shizuoka
293 Prefecture, Japan. Jpn. J. Appl. Entomol. Zool. 52, 225-229 (in Japanese with English abstract).

294 Salvo, A., Valladares, G.R., 2007. Leafminer parasitoids and pest management. Cien. Inv. Agr. 34, 125-142.

295 Schuster, D.J., Price, J.F., 1985. Impact of insecticides on lepidopterous larval control and leafminer
296 parasite emergence on tomato. Proc. Fla. State Hort. Soc. 98, 248-251.

297 Spencer, K.A., 1990. Host Specialization in the World Agromyzidae (Diptera). Kluwer Academic
298 Publishers, London.

299 SPSS, 2009. PASW Statistics 18. SPSS Inc., Chicago.

300 Tojo, S., 1980. Fractional analysis of nucleic acids, protein and ureic acids. in: Yoshitake, N. (Ed.),
301 Experiment on Insects, II. Japan Scientific Societies Press, Tokyo, pp. 23-28 (in Japanese).

302 Tokumaru, S., Kurita, H., Fukui, M., Abe, Y., 2005. Insecticide susceptibility of *Liriomyza sativae*, *L.*
303 *trifolii* and *L. bryoniae* (Diptera: Agromyzidae). Jpn. J. Appl. Entomol. Zool. 49, 1-10 (in Japanese
304 with English summary).

305 Trumble, J.T., Toscano, N.C., 1983. Impact of methamidophos and methomyl on populations of *Liriomyza*
306 species (Diptera: Agromyzidae) and associated parasites in celery. Can. Entomol. 115, 1415-1420.

307 Weintraub, P.G., 2001. Effects of cyromazine and abamectin on the pea leafminer *Liriomyza huidobrensis*

308 (Diptera: Agromyzidae) and its parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) in potatoes.
309 Crop Prot. 20, 207-213.

LD₅₀ values for malathion and carboxylesterase activities

Leafminer

Liriomyza trifolii
Liriomyza sativae
Chromatomyia horticola

Parasitoid

Diglyphus isaea
Chrysocharis pentheus
Neochrysocharis formosa
Hemiptarsenus varicornis

Halticoptera circulus

Table 1

Susceptibility of leafminers and their associated parasitoids to malathion.

Insects	Sex	n	LD ₅₀ (95% FL) (ng/mg)	Slope $\pm$ SE	χ^2	TR
Leafminers						
<i>Liriomyza trifolii</i>	Female	226	255 (147–418) a	1.19 $\pm$ 0.19	6.02	12800
	Male	249	229 (112–422) a	0.83 $\pm$ 0.14	6.08	11500
<i>Liriomyza sativae</i>	Female	272	107 (13–1140) ab	0.37 $\pm$ 0.11	1.07	5350
	Male	221	24.3 (4.6–96.2) b	0.42 $\pm$ 0.08	0.52	1220
<i>Chromatomyia horticola</i>	Female	203	29.7 (5.6–165.3) ab	0.40 $\pm$ 0.08	0.62	1490
	Male	216	13.4 (2.3–62.3) b	0.39 $\pm$ 0.07	1.54	670
Parasitoids						
<i>Diglyphus isaea</i>	Female	221	0.133 (0.063–0.272) c	0.81 $\pm$ 0.12	3.86	6.7
	Male	275	0.026 (0.009–0.050) d	1.19 $\pm$ 0.27	0.46	1.3
<i>Chrysocharis pentheus</i>	Female	219	0.068 (0.037–0.116) cd	1.15 $\pm$ 0.18	5.72	3.4
	Male	260	0.020 (0.011–0.039) d	1.65 $\pm$ 0.34	1.56	1.0
<i>Neochrysocharis formosa</i>	Female	200	0.049 (0.005–0.167) cd	0.51 $\pm$ 0.13	1.14	2.5
<i>Hemiptarsenus varicornis</i>	Female	199	0.067 (0.010–0.173) cd	0.72 $\pm$ 0.18	0.59	3.4
	Male	271	0.049 (0.001–0.176) cd	0.83 $\pm$ 0.27	0.83	2.4
<i>Halticoptera circulus</i>	Female	284	6.65 (2.42–19.69) b	0.95 $\pm$ 0.25	0.59	333
	Male	236	6.03 (1.92–18.27) b	1.18 $\pm$ 0.33	1.29	302

LD₅₀ values followed by the same letter are not significantly different based on overlapping 95% FL. Only adult females of *N. formosa* were used as this species has thelytokous parthenogenesis. TR = LD₅₀ value of each insect divided by the LD₅₀ value of the most susceptible insect (male *C. pentheus*).

Table 2

Susceptibility of leafminers and their associated parasitoids to permethrin.

Insects	Sex	n	LD ₅₀ (95% FL) (ng/mg)	Slope ± SE	χ^2	TR
Leafminer						
<i>Liriomyza trifolii</i>	Female	225	0.776 (0.344–1.745) ab	0.74 ± 0.12	2.09	22.2
	Male	290	0.278 (0.080–0.883) ab	0.62 ± 0.17	1.23	7.9
<i>Liriomyza sativae</i>	Female	275	1.214 (0.342–3.039) ab	0.79 ± 0.20	2.69	34.6
	Male	294	1.464 (0.694–2.694) a	1.12 ± 0.21	0.61	41.7
<i>Chromatomyia horticola</i>	Female	287	0.803 (0.104–2.747) ab	0.54 ± 0.17	0.33	22.9
	Male	282	0.375 (0.020–1.708) ab	0.43 ± 0.19	0.84	10.7
Parasitoid						
<i>Diglyphus isaea</i>	Female	274	0.635 (0.275–1.652) ab	0.85 ± 0.20	1.17	18.1
	Male	286	0.231 (0.080–0.466) b	1.03 ± 0.22	1.87	6.6
<i>Chrysocharis pentheus</i>	Female	199	0.111 (0.053–0.236) b	0.91 ± 0.18	3.40	3.2
	Male	256	0.035 (0.007–0.086) b	1.02 ± 0.26	0.81	1.0
<i>Neochrysocharis formosa</i>	Female	219	0.071 (0.026–0.158) b	0.81 ± 0.12	0.61	2.0
<i>Hemiptarsenus varicornis</i>	Female	231	0.082 (0.043–0.151) b	1.03 ± 0.15	0.91	2.3
	Male	288	0.087 (0.042–0.187) b	0.99 ± 0.19	3.35	2.5

LD₅₀ values followed by the same letter are not significantly different based on overlapping 95% FL. Only adult females of *N. formosa* were used as this species has thelytokous parthenogenesis. TR = LD₅₀ value of each insect divided by the LD₅₀ value of the most susceptible insect (male *C. pentheus*).

Table 3

Susceptibility of leafminers and their associated parasitoids to emamectin-benzoate.

Insects	Sex	n	LD ₅₀ (95% FL) (ng/mg)	Slope $\pm$ SE	χ^2	TR
Leafminer						
<i>Liriomyza trifolii</i>	Female	285	9.11 (4.16–18.14) a	1.06 $\pm$ 0.21	3.40	479
	Male	295	2.13 (0.32–5.08) ab	0.94 $\pm$ 0.24	0.68	112
<i>Liriomyza sativae</i>	Female	225	0.166 (0.054–0.390) bc	0.66 $\pm$ 0.13	0.92	8.7
	Male	292	0.071 (0.009–0.211) c	0.58 $\pm$ 0.16	5.32	3.7
<i>Chromatomyia horticola</i>	Female	233	6.39 (2.25–14.17) a	0.93 $\pm$ 0.22	4.23	336
	Male	287	5.31 (1.86–11.19) a	0.94 $\pm$ 0.19	0.42	279
Parasitoid						
<i>Diglyphus isaea</i>	Female	290	0.367 (0.098–1.791) bc	0.51 $\pm$ 0.12	6.11	19.3
	Male	278	0.198 (0.034–0.503) bc	0.76 $\pm$ 0.21	0.23	10.4
<i>Chrysocharis pentheus</i>	Female	219	0.071 (0.026–0.158) c	0.81 $\pm$ 0.12	0.61	3.7
	Male	261	0.019 (0.004–0.103) c	0.51 $\pm$ 0.14	0.49	1.0
<i>Neochrysocharis formosa</i>	Female	220	0.080 (0.021–0.260) c	0.49 $\pm$ 0.10	1.67	4.2
<i>Hemiptarsenus varicornis</i>	Female	241	0.200 (0.061–0.551) bc	0.53 $\pm$ 0.08	1.88	10.5
	Male	254	0.272 (0.008–1.241) bc	0.55 $\pm$ 0.18	0.71	14.3

LD₅₀ values followed by the same letter are not significantly different based on overlapping 95% FL. Only adult females of *N. formosa* were used as this species has thelytokous parthenogenesis. TR = LD₅₀ value of each insect divided by the LD₅₀ value of the most susceptible insect (male *C. pentheus*).

Table 4

Carboxylesterase activity (nmol/μg) in leafminers and their associated parasitoids.

Insects	Sex	n	Mean ± SE	Range
Leafminer				
<i>Liriomyza trifolii</i>	Female	30	60.3 ± 10.2 a	8.7–248.2
	Male	30	58.0 ± 6.0 a	14.3–153.4
<i>Liriomyza sativae</i>	Female	30	44.5 ± 5.4 ab	13.5–145.0
	Male	30	37.1 ± 3.6 bc	2.6–90.9
<i>Chromatomyia horticola</i>	Female	30	20.7 ± 1.3 cd	8.3–41.1
	Male	30	24.4 ± 1.9 cd	9.9–58.9
Parasitoid				
<i>Diglyphus isaea</i>	Female	30	7.1 ± 1.2 de	0.6–24.6
	Male	30	4.6 ± 0.4 e	1.6–11.6
<i>Chrysocharis pentheus</i>	Female	30	9.9 ± 1.4 de	1.6–33.6
	Male	30	7.4 ± 1.0 de	1.4–26.1
<i>Neochrysocharis formosa</i>	Female	30	6.2 ± 0.5 de	0.6–11.7
<i>Hemiptarsenus varicornis</i>	Female	30	9.7 ± 1.4 de	1.1–30.4
	Male	30	5.2 ± 0.6 de	0.4–13.7
<i>Halticoptera circulus</i>	Female	17	30.8 ± 3.7 bc	12.6–70.0
	Male	12	31.6 ± 4.2 bc	10.5–64.2

Mean carboxylesterase activities followed by the same letter are not significantly different ($P > 0.05$; Tukey HSD-test).

Only adult females of *N. formosa* were used as this species has thelytokous parthenogenesis.