Toxicity of Pesticides to *Chrysocharis pentheus* (Walker) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), Larval Parasitoids of *Liriomyza* species (Diptera: Agromyzidae)

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Abstract

In this study, we evaluated the toxicity of 27 insecticides and 16 fungicides to two adult parasitoids of the *Liriomyza* species, *Chrysocharis pentheus* (Walker) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), in the laboratory. Among the insecticides tested, insect growth regulators, *Bacillus thuringiensis*, sodium oleate, pymetrozine, quinoxaline compound, pyridalyl, azadirachtin, and cyflumetofen were non-toxic. Adults exposed to indoxacarb were afflicted until 48 h after the treatment. Neonicotinoid and organophosphate insecticides were very toxic to the two parasitoid species. Among the unclassified insecticides tested, emamectin benzoate, spinosad, tolfenpyrad, and pyridaben were toxic. Almost all tested fungicides were either not toxic or less toxic than the insecticides. The residual toxicity of nitenpyram and pymetrozine granules was less than that of acephate, clothianidin, and dinotefuran. The toxicity of granular insecticides appeared earlier in warm weather and their residual toxicity did not last as long as compared to cool weather. Among the diluted insecticides, the residual toxicity of spinosad, emamectin benzoate and pyridaben was shorter than that of tolfenpyrad, acetamiprid, and dinotefuran. On the basis of our results and those previous studies, we discussed pesticides that were feasible for tomato IPM programs using biological control of the *Liriomyza* species in greenhouses.

Key words: *Chrysocharis pentheus*; *Neochrysocharis formosa*; parasitoid; pesticide; toxicity; *Liriomyza*

INTRODUCTION

The leaf miners *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) have invaded Japan from foreign countries after the 1990s (Sasakawa, 1993; Iwasaki et al., 2000). These pests severely damage many vegetable crops and commercial flowers in Japan and other countries. European parasitoids of *Liriomyza* species are sold as biopesticides worldwide and are recognized as effective biocontrol agents in Japan (Tatara et al., 1993; Ozawa et al., 1999). However, many indigenous parasitoid species are known as natural enemies of *Liriomyza* species. Some of these parasitoids are effective natural enemies for *Liriomyza* species on tomato in the field and greenhouse, *Solanum lycopersicum* L. and gerbera plants (Saito et al., 1996; Ohno et al., 1999). In Japan, many parasitoids of the *Liriomyza* species also attack the garden pea leafminer, *Chromatomyia horticola* (Goureau) (Takada and Kamijo, 1979). A biological control method using indigenous parasitoids was developed for tomatoes cultivated in greenhouses; the parasitoids were introduced into the greenhouse with parasitized garden pea leafminers in the leaves and stems of beans (Doi et al., 2005). However, chemical control has been a major strategy for pests even in the

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integrated pest management (IPM) program for tomatoes. To utilize natural enemies, it is important to evaluate the effects of pesticides on parasitoids. The dominant parasitoids of *Liriomyza* species on tomato plants in greenhouses in Shizuoka prefecture are *Chrysocharis pentheus* （Walker） (Hymenoptera: Eulophidae), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) (Doi et al., 2005). The effects of pesticides on *D. isaea*, *H. varicornis,* and *N. formosa* have previously been reported (Ozawa et al., 1998; Yamamura and Takemoto, 2001; Katayama and Ozawa, 2003a,b; Matsumura, 2003b; Bjorksten and Robinson, 2005; Tran et al., 2005; Shimomoto, 2006). However, some new pesticides to control tomato pest have registered each year in Japan. The objective of this study was to determine the toxicity of pesticides commonly used for tomatoes to the adult of two important parasitoids, *C. pentheus* and *N. Formosa,* under laboratory conditions. Furthermore, we evaluated the toxic duration after the pesticide treatment under greenhouse conditions to identify feasible pesticides for tomato IPM programs using the parasitoids of the leafminer *Liriomyza* species as the biological agent.

MATERIAL AND METHODS

Wasp collection. *L. trifolii* was collected from gerbera (*Gerbera jamesonii* Hook (Asteraceae)) grown in Hamamatsu, Shizuoka prefecture in 1991, and *C. pentheus* and *N. formosa* were collected from *C. horticola* infested leaves of garden pea, *Pisum sativum* L. at the Shizuoka Prefectural Research Institute of Agriculture and Forestry in 2005. They were continuously reared on the kidney bean, *Phaseolus vulgaris* L. (Fabaceae) as a host under constant laboratory conditions. *L. trifolii* adults were released on kidney beans plants with two leaves by spraying with a 20% honey solution, planting them in a plastic pot, and maintaining them in an acrylic cage (40 × 40 × 40cm, with 100-mesh netting on the both sides) at 25℃ and a photoperiod of 16L8D. Adult parasitoids were released 5 d after the flies were released and kept under the same conditions. The adult parasitoids were collected within one week after emergence.

Pesticides. Tables 1 and 2 list the pesticides selected for the bioassay experiments. We tested 25 insecticides and 16 fungicides registered as tomato cultures in Japan. We also tested two pesticides, azadirachtin and cyflumetofen, which are currently undergoing registration. Insecticides included five insect growth regulators (IGR), two preparations of *Bacillus thuringiensis* (BT), six neonicotinoid insecticides, two organophosphates, one antibiotic, and 11 other types of insecticides. Fungicides used for the experiment included three sterol biosynthesis inhibitors, two benzoimidazoles, two inorganic compounds, one dicarboximide, anilinopyrimidine, methoxyacrylate, three mixed formulations of fungicides, and three other types of fungicides. Table 3 lists the insecticides examined in the greenhouse experiments. We tested five granule formulation insecticides for their residual toxicity on tomatoes planted in a greenhouse, and six insecticides were tested for their residual toxicity after spraying in the greenhouse. We mainly chose the spraying insecticides that have high control effects for tomato plants but have high toxicity to the parasitoids, for selecting of feasible insecticides in an accidental occurrence of the pest under the IPM program.

Pesticide bioassays. Adult mortality in relation to pesticides was tested with *C. pentheus* and *N. formosa* using the method described by Ozawa et al. (1998). Each pesticide (0.1ml) was diluted to the appropriate concentration with acetone (99.5%) before being poured into a glass tube (9cc); the glass tubes were immediately rotated for the solution adhering uniformly on inside of them. Because the wettable powder and suspension concentrate pesticides were difficult to dissolve, they were diluted with water (at 10% the amount of acetone) before adding acetone. Seven to ten adult parasitoids were released into the dried glass tubes. The bottom end of each glass tube was covered with a slip of Tetron gauze. A drop of 50% honey solution, as food for the adult parasitoids, was smeared on the Tetron gauze. The glass tubes were maintained at 25℃ and a photoperiod of 16L8D. Parasitoids mortality was recorded at 24 h after the treatment. If there was afflicting

Table 1. Insecticides used in the bioassay experiment

Type	Common name	Trade name (Formulation[1])	ingredient (%)	rate	Main targets pest for tomato
Insect Growth Regulator	Cyromazin	Trigarde (L)	3.8	1,000	Legume leafminer
	Buprofezin	Applaud (WP)	25	1,000	Whitefly
	Chlorfluazuron	Atabron (EC)	5	2,000	Tobacco bud worm Common cut worm
	Lufenuron	Match (EC)	5	3,000	Tobacco bud worm Common cut worm Tomato erineum mite
	Flufenoxuron	Cascade (EC)	10	2,000	Tobacco bud worm Common cut worm
Neonicotinoid	Dinotefuran	Starkle (SG)	20	3,000	Whitefly
	Acetamiprid	Mospiran (SP)	20	2,000	Aphid Whitefly
	Nitenpyram	Bestgard (SG)	10	1,000	Thrips Aphid Whitefly
	Clothianidin	Dantotsu (SG)	16	2,000	Aphid Whitefly Legume leafminer
	Imidacloprid	Admire (WDG)	10	2,000	Aphid Whitefly
	Thiamethoxiam	Actara (WDG)	10	2,000	Whitefly
Bacillus thuringiensis	BT subsp. Kurstaki	Delphin (WDG)	10	1,000	Tobacco bud worm Common cut worm
	BT subsp. Kurstaki	Guardjet (WP)	7	1,000	Tobacco bud worm
Antibiotics	Milbemectin	Koromaito (EC)	1	1,500	Whitefly Legume leafminer Tomato erineum mite
Organophosphates	Diazinon	Diazinon (EC)	40	1,000	Aphid Spider mite
	MEP Fenitrothion	Smichion (EC)	50	2,000	Aphid
Other	Emamectin Benzoate	Affirm (EC)	1	2,000	Tobacco bud worm Tomato erineum mite
	Spinosad	Spinoace (WDG)	25	5,000	Thrips Tobacco bud worm Legume leafminer
	Sodium oleate	Oleate (L)	20	100	Aphid
	Pymetrozine	Chess (WP)	25	3,000	Aphid Whitefly
	Tolfenpyrad	Hachi-Hachi (EC)	15	1,000	Whitefly Legume leafminer Western flower thrips
	Pyridaben	Sunmite (FL)	20	1,000	Whitefly Tomato erineum mite
	Indoxacarb	Tornade (FL)	10	2,000	Tobacco bud worm Common cut worm
	Quinoxaline compound	Morestan (WP)	25	1,500	Greenhouse whitefly Tomato erineum mite
	Pyridalyl	Pleo (FL)	10	1,000	Tobacco bud worm Common cut worm Legume leafminer
	Azadirachtin	- (EC)	4.5	1,500	-
	Cyflumetofen	Danisaraba (FL)	20	1,000	-

[1] EC: emulsifiable concentrate, FL: flowable, L: liquid formulation, SG: water soluble granule, SP: water soluble powder, WDG: water dispersible granule , WP: wettable powder

Table 2. Fungicides used in the bioassay experiment

Type	Common name	Trade name (Formulation[1])	ingredient (%)	rate	Main targets pest for tomato
Sterol biosynthesis inhibitor	Fenarimol	Rubigen (WP)	12	6,000	Leaf mold
	Triflumizole	Trifumin (WP)	30	3,000	Leaf mold Cercospora leaf mold
	Tetraconazole	Salvatore (SP)	11.6	2,000	Leaf mold
Benzoimidazole	Thiophanate-Methyl	Topsin M (SC)	40	1,000	Leaf mold Gray mold Stem rot
	Benomyl	Benlate (WP)	50	3,000	Leaf mold Gray mold Stem rot
Dicarboximide	Iprodione	Robral500 (SC)	40	1,000	Gray mold
Anilinopyrimidine	Mepanipyrim	Furupica (FL)	40	2,000	Gray mold
Methoxyacrylate	Azoxystrobin	Amistar (FL)	20	2,000	Leaf mold Gray mold
Inorganic compound	Potassium bicarbonate	Caligreen (L)	80	800	Gray mold Powdery mildew
	Sulfur	Iou (FL)	52	400	Powdery mildew
Others	TPN	Daconil1000 (FL)	40	1,000	Leaf mold Early blight Late blight
	Cyazofamid	Ranman (FL)	9.4	1,000	Late blight
	Fludioxonil	Savior20 (FL)	20	1,000	Gray mold
Mix compound	Kasugamycin and capper mixtur	kasumin-bordeaux (WP)	75.6+5.7	1,000	Leaf mold
	Mancozeb and metalaxyl	Ridomil MZ (WP)	55+10	750	Late blight
	Fenhexamid and fludioxonil	Justmeet (WDG)	20+50	2,000	Gray mold Early blight Late blight

[1] FL: flowable, L: liquid formulation, SC: suspension concentrate, SP: water soluble powder, WDG: water dispersible granule, WP: wettable powder

individual, the mortality of the parasitoids was recorded again at 48 h after the treatment. Each bioassay was replicated three times. The controls were treated with acetone (99.5%). Recorded mortalities were corrected by Abbott's equation (Abbott, 1925).

Residual toxicity of granular insecticides on parasitoids in the greenhouse. Residual toxicity tests were carried out at the Shizuoka Prefectural Research Institute of Agriculture and Forestry in 2005 and 2006. The insecticides were inoculated by pricking-in-hole treatment at the appropriate doses for tomato saplings of the variety ‘House Momotaro’. No insecticide was applied to the control. Three tomato trees were used for each insecticide treatment and the control, were planted in an unheated greenhouse in biscuit pots (30 cm in diameter and 25 cm in height); after the treatment, the top leaf of each tomato tree was marked with string twisted around the petiole. The residual toxicity of the insecticides was tested according to the method described by Ozawa et al. (1998). We collected three leaflets per tomato tree under the marked leaf, then sprayed 10% honey solution on the leaflet surface using a hand sprayer and then placed each leaflet in a plastic petri dish (9 cm in diameter and 1 cm in height). After the honey solution dried, seven to ten adult parasitoids were released in the petri dishes, which were maintained at 25℃ and a photoperiod of 16L8D. Parasitoid mortality was recorded at 48 h after the release and was corrected by Abbott's equation (Abbott, 1925). Each residual insecticide test was replicated three times. The seeding dates of the tomatoes were October 4, 2005, and April 5, 2006. The insecticide treatments were conducted on November 4, 2005 (four insecticides) and May 1, 2006(three insecticides). Parasitoid mortality was examined every 7 d for 49 d in 2005, and for 56 d in 2006 after the treatment.

Table 3. Insecticides used in residual test

Common name	Trade name (Formulation[1)])	Ingredient	Treated date	Rate	Main targets pest for tomato
Granual formulation test					
Nitenpyram	Bestgard (G)	1%	Nov. 4, 2005 & May 1, 2006	2 g per plant	Legume leafminer Whitefly Aphid
Acephate	Orutoran (G)	5%	Nov. 4, 2005	2 g	Whitefly Aphid
Pymetrozine	Chess (G)	3%	Nov. 4, 2005	1 g	Whitefly Aphid
Clothianidin	Dantotsu (G)	0.5%	Nov. 4, 2005 & May 1, 2006	2 g	Legume leafminer Whitefly Aphid
Dinotefuran	Starkle (G)	1%	May 1, 2006	2 g	Legume leafminer Whitefly Aphid
Splaying test					
Spinosad	Spinoace (WDG)	25%	Nov. 7, 2005 & May 15, 2006	5,000 fold	Thrips Tobacco bud worm Legume leafminer
Tolfenpyrad	Hachi-Hachi (EC)	15%	Nov. 7, 2005	1,000	Whitefly Legume leafminer Western flower thrips
Acetamiprid	Mospiran (SP)	20%	Nov. 7, 2005	2,000	Aphid Whitefly
Emamectin Benzoate	Affirm (EC)	1%	May 15, 2006	2,000	Tobacco bud worm Tomato erineum mite
Pyridaben	Sunmite (FL)	20%	May 15, 2006	1,000	Whitefly Tomato erineum mite
Dinotefuran	Starkle (SG)	20%	May 15, 2006	3,000	Whitefly

[1)] EC: emulsifiable concentrate, FL: flowable, G: granule, SG: water soluble granule, SP: water soluble powder, WDG: water dis persible granule

Residual toxicity after spraying insecticide on the parasitoids in the greenhouse. Insecticides of appropriate concentrations were sprayed on tomato trees after planting and parasitoid mortalities were examined after treatment. No insecticide was applied to the control. Three tomato trees were used for each insecticide treatment and the control, were planted on November 4, 2005 and May 1, 2006 for the tests and the insecticides were applied on November 7, 2005 (three insecticides) and May 8, 2006 (four insecticides). Parasitoid mortality was recorded at 48 h after the release and was examined at every 7 d for 35 d after treatment. Some insecticides exhibited continuously low mortality in twice or more after spraying and were therefore omitted from subsequent bioassays. Other experimental procedures were the same as described for the previous experiment.

RESULTS

Pesticide bioassays. The corrected mortalities of *C. pentheus* and *N. formosa* exposed to the different insecticides are shown in Table 4. The average mortalities in the control for *C. pentheus* and *N. formosa* were 3.2% and 2.6%, respectively. The insecticides had a similar effect on mortality in both parasitoids. Mortalities from exposure to IGR and BT insecticides were not significantly different from the control ($p > 0.05$, Fisher's exact test with False Discovery Rate (FDR) correction (Benjamini and Hochberg, 1995)).

Organophosphates produced 100% mortality and neonicotinoids produced almost 100% in both parasitoids. Mortality from exposure to milbemectin was significantly higher than in the control ($p < 0.05$) but lower than that from exposure to neonicotinoids and organophosphates. Among the other types of the insecticides, mortalities caused by sodium oleate, pymetrozine, quinoxaline compound, pyridalyl, azadirachtin, and cyflumetofen were equivalent to the control ($p > 0.05$), whereas the mortalities caused by emamectin benzoate, spinosad, tolfenpyrad, and pyridaben were almost 100% which were significantly higher than the control ($p < 0.05$). Although the mortality of parasitoids exposed to indoxacarb was not significantly different from the control ($p > 0.05$), almost all of the parasitoids were afflicted at 24 h and even at 48 h after treatment.

Table 4. Corrected mortality of adults of *C. pentheus* and *N. formosa* at 24h after exposed in insecticides

Type	Common name	% corrected mortality	
		C. pentheus	*N. formosa*
Insect Growth Regulator	Cyromazin	0	9.7
	Buprofezin	3.0	0
	Chlorfluazuron	5.9	4.8
	Lufenuron	0	0
	Flufenoxuron	0	1.8
Neonicotinoid	Dinotefuran	100*	100*
	Acetamiprid	100*	100*
	Nitenpyram	100*	100*
	Clothianidin	95.4*	95.4*
	Imidacloprid	100*	100*
	Thiamethoxiam	84.7*	100*
Bacillus thuringiensis	BT subsp. Kurstaki(Delphin)	13.2	0
	BT subsp. Kurstaki(Gurdjet)	0	0
Antibiotics	Milbemectin	56.5*	63.9*
Organophosphates	Diazinon	100*	100*
	Fenitrothion	100*	100*
Others	Emamectin Benzoate	100*	100*
	Spinosad	100*	100*
	Sodium oleate	0	17.9
	Pymetrozine	0	0
	Tolfenpyrad	100*	100*
	Pyridaben	100*	95.2*
	Indoxacarb	3.0[1)] 27.0[2)]	0[1)] 0[2)]
	Quinoxaline compound	9.5	9.5
	Pyridalyl	0	4.2
	Azadirachtin	0	0
	Cyflumetofen	0	0

[1)] All survive individuals were afflicted.
[2)] Mortality at 48h after treatment. All survive individuals were afflicted.
*Significant different from control at $p < 0.05$ by Fisher's exact test with False Discovery Rate (FDR) correction.

Mortalities due to exposure to fungicides are shown in Table 5. The fungicides had a lesser effect on the mortalities of the two parasitoid species than the insecticides. There were differences in mortalities of some fungicides between *C. pentheus* and *N. formosa*. *N. formosa* was more susceptible to sulfur than *C. pentheus,* while *C. pentheus* showed higher mortality from exposure to benomyl than *N. formosa*. The mortalities of *C. pentheus* produced by benomyl and potassium bicarbonate and the mortalities of *N. formosa* by potassium bicarbonate and sulfur, were significantly higher than in the control ($p < 0.05$).

Table 5. Corrected mortality of adults of *C. pentheus* and *N. formosa* at 24h after exposed in fungicides

Type	Chemical name	% corrected mortality	
		C. pentheus	*N. formosa*
Sterol biosynthesis inhibitor	Fenarimol	0	21.7
	Triflumizole	7.9	11.4
	Tetraconazole	3.7	14.0
Benzoimidazole	Thiophanate-methyl	0	4.2
	Benomyl	40.8*	12.5
Dicarboximide	Iprodione	0	0
Anilinopyrimidine	Mepanipyrim	0	0
Methoxyacrylate	Azoxystrobin	0	0
Inorganic compound	Potassium bicarbonate	41.9*	68.2*
	Sulfur	16.9	46.3*
Others	TPN	0	0
	Cyazofamid	0	3.3
	Fludioxonil	0	3.3
Mix compound	Kasugamycin and capper mixtur	0	3.9
	Mancozeb and metalaxyl	0	0
	Fenhexamid and fludioxonil	0	0

*Significantly different from control at $p < 0.05$ by Fisher's exact test with False Discovery Rate (FDR) correction

Residual toxicity of granular insecticides on parasitoids in the greenhouse. The percentage mortalities in the two parasitoid species after treatment with granular insecticides during planting in 2005 and 2006 are shown in Fig. 1. The average mortalities in the control were 1.4% in 2005 and 3.1% in 2006 for *C. pentheus*, and 0% in 2005 and 1.9% in 2006 for *N. formosa*. In 2005, the mortalities of *C. pentheus* due to exposure to the four tested insecticides at 7 d after treatment were not significantly different from the control ($p > 0.05$, Fisher's exact test with FDR correction). However, the mortalities of *N. formosa* caused by acephate and clothianidin at 7 d after treatment were significantly higher than the control ($p < 0.05$). *C. pentheus* exposed to nitenpyram exhibited low mortality after treatment and the mortalities were not significantly different from the control at any point during the experiment ($p > 0.05$). *N. formosa* also exhibited almost low mortality after treatment with nitenpyram, except for 14 d after treatment. For most insecticides, mortalities in the two parasitoids were the highest at 14 d after treatment with the exception of *C. pentheus* exposed to clothianidin and *N. formosa* exposed to acephate, which were the highest at 21 d and 7 d after treatment, respectively. Mortalities that occurred after pymetrozine treatment were not significantly different from the control ($p > 0.05$). Mortalities of both parasitoids caused by clothianidin and acephate remained high until 42 d after treatment, and were not significantly different from the control at 49 d after treatment ($p > 0.05$). In

2006, we examined three insecticides. The mortalities of both parasitoids caused by the three insecticides were the highest at 7 d after treatment. *C. pentheus* exposed to nitenpyram exhibited low mortality at 14 d after treatment and no significant differences from the control ($p > 0.05$). On the other hand, *N. formosa* exposed to nitenpyram exhibited significantly higher mortality at 7 d and 14 d after treatment ($p < 0.05$), but we did not observe any significant differences from the control 21 d after treatment ($p > 0.05$). Because the mortalities of the two parasitoids were also low at 28 d after treatment, subsequent bioassays were not conducted. The mortalities produced by clothianidin and dinotefuran, which are the same type of insecticide as nitenpyram, remained high for 40 d after treatments.

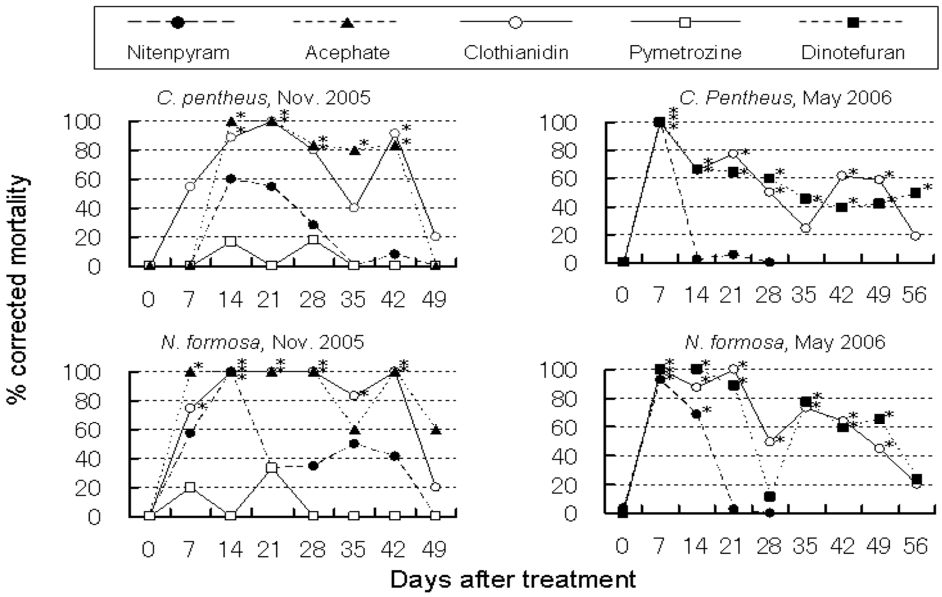

Fig. 1. Residual toxicity of granule insecticides to adult *C. pentheus* and *N. formosa* on tomato leaves. The granular insecticides were applied using the pricking-in-hole treatment at the appropriate doses for planting tomato saplings (variety 'House Momotaro') in a greenhouse, in November 2005 and in May 2006. "0 days after treatment" means that the bioassay was conducted within a day after treatment. * indicates that the mortality was significantly different from control at $p < 0.05$ by Fisher's exact test with False Discovery Rate (FDR) correction.

Residual toxicity after spraying insecticide on the parasitoids in the greenhouse. The changes in the mortalities of the two parasitoid species after spraying with insecticides are shown in Fig. 2. The average mortalities in the control were 4.2% in 2005 and 1.9% in 2006 for *C. pentheus* and 0% in 2005 and 2.8% in 2006 for *N. formosa*. Among the three insecticides in 2005, the residual toxicities of acetamiprid and spinosad differed between *C. pentheus* and *N. formosa*. *C. pentheus* exhibited high mortality after acetamiprid spraying ($p < 0.05$, Fisher's exact test with FDR correction); however, the mortality of *N. formosa* became equivalent to the control at 14 d after spraying ($p > 0.05$). The mortalities of both parasitoids immediately after spraying with spinosad were significantly higher than the control ($p < 0.05$); however, after 7 d, only the mortality of *C. pentheus* was higher than that of the control ($p < 0.05$), and after 14 d, the mortalities of both parasitoids were not difference from that of the control ($p > 0.05$). Mortality after spraying with tolfenpyrad was remained high throughout the experiment. We examined four insecticides in 2006. Tested insecticides except dinotefuran exhibited continuously low mortality at 21 d and 28 d after spraying and were therefore omitted from subsequent bioassays. Whereas the mortalities of *C. pentheus* were significantly different from the control until 7 d after the pyridaben spraying ($p < 0.05$), the mortality of *N. formosa* after spraying was not significantly different from the control at any point during the experiment ($p > 0.05$). The mortalities of both parasitoids immediately after the spraying with emamectin benzoate were higher than that of the control ($p < 0.05$). After this point, the mortality decreased rapidly and approached that of the control ($p > 0.05$). The effect of spinosad spraying in 2006 indicated a tendency similar to that in 2005. The mortalities immediately after spraying with spinosad and at 7 d were higher for both parasitoids as compared to that of the control ($p < 0.05$), but this difference was not maintained at 14 d after the spraying ($p > 0.05$). The mortalities of both parasitoids caused by spraying with dinotefuran were higher than that of the control throughout experiment ($p < 0.05$).

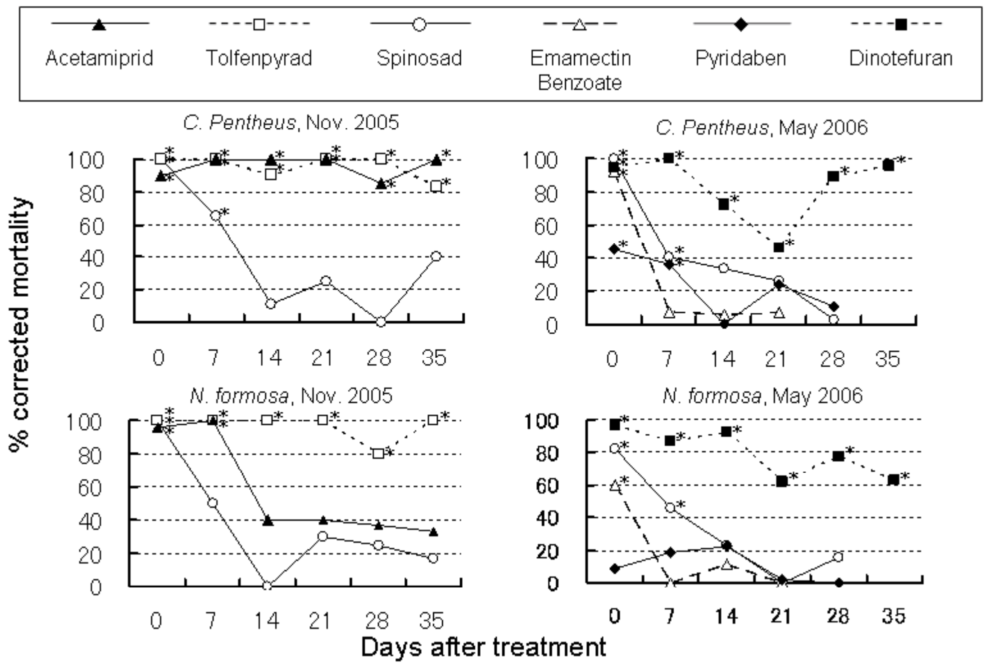

Fig. 2. Residual toxicity of insecticides to adult *C. pentheus* and *N. formosa* on tomato leaves. The insecticides were sprayed on tomato saplings (variety 'House Momotaro') in a greenhouse, in November 2005 and in May 2006. "0 days after treatment" means that the bioassay was conducted within a day after spraying. * indicates that mortality was significantly different from control at $p < 0.05$ by Fisher's exact test with False Discover Rate (FDR) correction.

DISCUSSION

It is important to select pesticides that are harmonious with biological controls in IPM programs. In the current study, the bioassay experiment identified a number of pesticides that are non-toxic to two major parasitoids adult of the *Liriomyza* species, *C. pentheus* and *N. formosa*. Both parasitoids responded in a manner similar to that of the insecticides. Among the insecticides tested, IGR and BT were low in toxicity. Among other insecticides, sodium oleate, pymetrozine, quinoxaline compound, azadirachtin and cyflumetofen also showed low toxicity to the parasitoids. On the other hand, neonicotinoid and organophosphate insecticides showed high toxicity to the adult parasitoids, as did a number of other insecticides, including emamectin benzoate, spinosad, tolfenpyrad and pyridaben. This result is fairly consistent with previous studies on *D. isaea* (Ozawa et al., 1998), *N. formosa* (Yamamura and Takemoto, 2001; Shimomoto, 2006), and *H. varicornis* (Katayama and Ozawa, 2003a). Indoxacarb appears to be toxic to parasitoids because all individuals were afflicted and the majority was moribund within 48h after treatment. However, Shimomoto (2006) reported that indoxacarb was not toxic to adults of *N. formosa*. The discrepancy between Shimomoto's results and our study is unclear. Mafi and Ohbayashi (2006) demonstrated that imidacloprid, thiamethoxam, acetamiprid, and clothianidin, which were belonged to the class of neonicotinoids insecticides, were less effective on *C. pentheus*. It is also not clear why their results do not coincide with ours; however, the difference may be due to the fact that they used parasitoids collected from parasitized citrus leafminers (*Phyllocnistis citrea*). Flufenoxuron, an IGR found to be non-toxic in our study, was found to affect the progeny of *H. varicornis* adults (Katayama and Ozawa 2003b) but non that of *D. isaea* adults (Ozawa et al., 1998). Because these two species and those used in our study belong to the same family (Eulophidae), it is necessary to check the effects in the progeny of *C. pentheus* and *N. formosa* for effective use in tomato IPM programs. Furthermore, Tran et al. (2004) reported that lufenuron affected the oviposition behavior of *N. formosa*. IGR should be evaluated not only through bioassays but also in terms of the insects' behavior and survival of progeny. However, their total effects on the parasitoids showed lower than that of neonicotinoid or organophosphate, and at present there are few harmless pesticides to the parasitoids, therefore, IGR should be selected for tomato IPM programs.

C. pentheus and *N. formosa* responded differently to fungicides. Although benomyl was slightly toxic to *N. formosa*, sulfur was slightly toxic *C. pentheus*, and potassium bicarbonate was slightly toxic to both parasitoids, their mortalities were lower than those of pesticides. These results are consistent with previous work on *D. isaea* (Ozawa et al. 1998). Because most of the examined fungicides are no or less toxic, tomato farmers can safely use many kinds of fungicides under the IPM programs.

Some pests might occur accidentally in tomato IPM programs. Among these pests, whiteflies, leafminers, lepidoptera larvae, and tomato erineum mites can cause severe damage and must be controlled. However, few pesticides are effective against pests yet harmless to the natural enemies. If the duration of residual toxicity of a pesticide to the parasitoid is short, farmers can re-release the parasitoids in short intervals. Therefore, it is important to identify pesticides with a short period of residual toxicity to the parasitoids among the toxic pesticides in laboratory tests. Among the examined insecticides, the shortest residual effects on mortality were found with emamectin benzoate, pyridaben and spinosad. Most of the insecticides examined in our study showed similar toxicity tendencies to those in previous studies with *N. formosa* (Yamamura and Takemoto, 2001; Shimomoto, 2006), *D. isaea* (Ozawa, 1998), and *H. varicornis* (Katayama and Ozawa, 2003). However, Shimomoto (2006) reported that spinosad has long-term residual effects on *N. formosa* exceeding one month. We found that the mortality caused by spinosad was less than 20% at 14 d after the treatment. The reason for the difference between Shimomoto's results and ours is unclear; further research needs to be conducted to clarify this discrepancy.

The application of granular insecticides when planting tomato saplings is a useful method to control the early occurrence of insect pests. Differences between *C. pentheus* and *N. formosa* in residual toxicity were observed in some insecticides. However, the residual toxicity was not consistently high for each species. Among the tested insecticides, pymetrozine showed low residual toxicity to the parasitoids throughout the experiment and mortalities caused by nitenpyram decreased at 21 d after treatment. In contrast, other tested insecticides showed toxicity over 40 d after the planting. Other studies have shown similar results for the toxic effect of acephate and clothianidin on *N. formosa* (Shimomoto, 2006), acephate on *D. isaea* (Ozawa, 1998), and acephate, nitenpyram and clothianidin on *H. varicornis* (Katayama and Ozawa, 2003). However, the toxic periods differed among the studies. For instance, in greenhouse study using eggplant in July, Shimomoto (2006) reported that the mortality of adult *N. formosa* treated with acephate fell bellow 50% at 19 d after treatment. Because eggplant usually takes up much water than tomato (Kamoto et al.,1974) and warm weather condition accelerated it, it is consider that the residual toxicity in his study is shorter than that of our result. Currently, most serious disease that threatens tomato cultivation in Japan is the tomato yellow leaf curl disease, which is associated with the whitefly vector *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Honda,2006). It is difficult to use the natural enemy of whitefly because quite low control thresholds are required for preventing virus transmission. Under these circumstances, the *Liriomyza* species is the main target pest for biological control in tomato IPM. For preventing virus transmission, granular insecticide application with planting is an important control method for the whitefly (Honda, 2006). Among tested granular insecticides, nitenpyram is useful in IPM programs because it provides a high control effect on the *Liriomyza* species and the whitefly (Tokumaru,2005; Ohya and Uekusa,2009).

In this study, the residual toxicity of granular insecticides showed variation with seasons. The highest mortalities for most tested insecticides were observed at 14 d after treatment in November 2005, but at 7 d in May 2006. Generally, the effect of granular insecticides is associated with the quantity of water taken up by the plant; therefore, the toxic effect might appear early if the rate of water uptake is increased. Furthermore, water uptake increases with the radiant energy from the sun and an increase in saturation deficit. Saturation deficit will increase with a rise in air temperature, even at the same relative humidity. We did not measure average air temperature and radiant energy in the greenhouse for these experiments; therefore, we based our discussion on those constantly measured data at the Shizuoka Prefectural Research Institute of Agriculture and Forestry. The average air temperatures and radiant energy 7 d, 14 d and 30 d after the treatment were higher in May than in November (Table 6). Therefore, it is possible that the residual effect of the granular insecticides on the parasitoids decreases with high air temperature and the much radiant energy of sunlight after application.

Table 6. Average air temperature and cumulative radiant energy of sunlight after insecticide treatment at Shizuoka Prefectural Research Institute of Agriculture and Forestry.

	Nov. 2005	May 2006
Average air temperature (℃)		
7 days	17.5	20.5
14 days	16.1	20.7
30 days	14.3	21.4
Cumulative radiant energy(MJ/m^2)		
7 days	75.4	128.6
14 days	138.1	213.5
30 days	302.5	471.7

On the basis of our results and those previous studies, we recommend some pesticides for tomato IPM programs using biological control of the *Liriomyza* species in greenhouses. A granular formulation of nitenpyram applies with the planting. The feasible pesticides spray for major pest including less residual toxic or less effect pesticide as follows; almost all fungicides for tomato deceases, pymetrozine, quinoxaline compound, sodium oleate and nitenpyram for the whitefly; IGR, BT and emamectin benzoate for the lepidoptera pest; emamectin benzoate, pyridaben and lufenuron for the tomato erineum mite; IGR, spinosad and pyridalyl for the *Liriomyza* species. Azadirachtin and cyflumetofen were also less toxic to the parasitoids and could be potentially feasible pesticides.

New pesticides will register with tomato pests in succession and the response to pesticides differs between parasitoid species; moreover, the dominant species of parasitoid of the *Liriomyza* species differs according to location (Saito et al., 1996; Ohno et al., 1999; Matsumura et al., 2003). Hence, further studies like theses are warranted to improve tomato IPM program in Japan.

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