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Pyridalyl: A Novel Compound with Excellent Insecticidal Activity, High Selectivity, and Unique Mode of Action

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ABSTRACT

Pyridalyl is a novel synthetic insecticide discovered by Sumitomo Chemical Co. Ltd. and has been developed globally as the trade names, Pleo®, or Sumipleo®. The compound is highly effective against various pests of Lepidoptera, Thysanoptera, Diptera and certain Acari, including resistant strains that are less susceptible or resistant to existing insecticides. The lepidopterous larvae treated with pyridalyl exhibited unique symptoms that were not observed with existing insecticides, suggesting the novel mode of action of pyridalyl. Intoxication study found that pyridalyl has a wide range of effective dosage, and thus the compound can provide an excellent anti-feeding activity against target insect pests. The laboratory and field experiments found that pyridalyl has an excellent selectivity between target insect pests and beneficial arthropods including pollinators, predators and parasitoids. In the cytotoxicological experiments using an insect cell line Sf9, the cytotoxicity of pyridalyl and its analogs on Sf9 cells were highly associated with the insecticidal activity against *S. litura*, indicating that the cytotoxicity of pyridalyl in Sf9 cells reflects its insecticidal action at least in part. The cytotoxicity study also found that pyridalyl displayed obvious cytotoxicity to Sf9 cells whereas it had no effect on a mammalian cell line CHO-K1, thereby high selectivity was observed even at cell-line level. These characteristics of pyridalyl would allow the compound to be one of the most powerful tools for controlling and managing pest insects under IPM- and IRM-based crop protection programs.

Keywords: beneficial arthropods, insecticidal resistance management (IRM), integrated pest management (IPM), Lepidoptera, novel mode of action, selectivity, Thysanoptera

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INTRODUCTION

The problem of insecticide resistance is still one of the biggest issues in applied agrobiology, though several outstanding insecticide classes, such as organophosphates, carbamates, pyrethroids, benzoylureas and neonicotinoids, have been developed so far and achieved a remarkable success for crop protection from a number of insect pests. The discovery of new insecticides with a novel mode of action is one of the most effective tactics for controlling insect pests that are highly resistant to existing products. However, even with the development of a new insecticide having a new mode of action, it is possible that insecticide resistance will be developed after a certain period following its discovery, indicating that an integrated management system including not only chemical insecticide but also other management tools are necessary.

Recently, it is widely considered that the establishment and practical use of integrated pest management (IPM) and insecticide resistant management (IRM) systems can reduce and delay the development of insecticide resistance (e.g. Forrester *et al*. 1993; Kawai 1997; Matthews 1997; Jutsum *et al*. 1998; Bourguet *et al*. 2005; Aggarwal *et al*. 2006). Under these management systems, key natural enemies are conserved for suppressing the populations of insect pests, and hence selective chemical agents that are harmless to the natural enemies were preferentially applied. In addition, there is a growing concern about the toxicity of pesticide chemicals and some pesticides have been restricted for use in certain countries. Regulatory agencies worldwide have placed a premium on crop protection by pesticides having improved toxicological profiles for non-targeted organisms.

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Invited Review

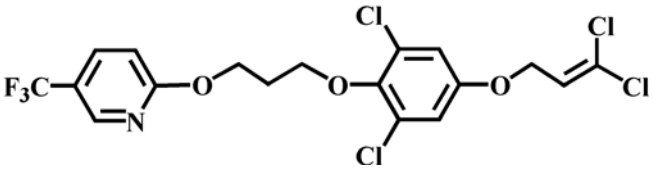

Fig. 1 Chemical structure of pyridalyl.

Therefore, it is desirable to develop new insecticidal agents that are both active against resistant insect pests and safe to humans, environments and beneficial arthropods.

Pyridalyl is a novel synthetic insecticide (**Fig. 1**) discovered at the Agricultural Chemicals Research Laboratory of Sumitomo Chemical Co. Ltd. The compound has been developed globally and is now supplied in several countries under the trade names, Pleo® or Sumipleo®. The compound has a unique chemical structure, dihalopropenyloxy benzene, which is not related to any other existing insecticide classes (Sakamoto *et al*. 2004), showing excellent insecticidal activity against a wide range of insect pests including Lepidoptera (Cook *et al*. 2004, 2005; Isayama *et al*. 2004; Saito *et al.* 2004; Murray *et al*. 2005), Thysanoptera (Isayama *et al*. 2005) and Diptera (Tokumaru *et al*. 2005). In contrast, the compound was found to be safe to non-targeted organisms, especially harmless to beneficial arthropods including pollinators (Isayama *et al*. 2005; Tsuchiya 2005), predators (Tillman and Mulrooney 2000; Hamamura and Shinoda 2004; Isayama *et al*. 2005; Hamamura *et al*. 2006), and parasitoids (Tillman and Mulrooney 2000).

Therefore, pyridalyl is expected to be a powerful tool for controlling and managing insect pests under IPM and IRM programs. Here, in this review article, the biological profiles of pyridalyl as well as its unique mode of action are introduced: i.e. the insecticidal characteristics obtained in laboratory and field experiments; the effects on insect and mammalian cell cultures; and the impacts on non-targeted organisms, especially on beneficial arthropods under laboratory and more practical conditions. In the latter part of this article, the effective use of pyridalyl in IPM and IRM programs is discussed.

INSECTICIDAL CHARACTERISTICS

Insecticidal activity and spectrum

Pyridalyl shows an excellent insecticidal activity against a wide range of lepidopterous insect pests as well as Thysanoptera, Diptera and certain Acari. **Table 1** shows the insecticidal activity of pyridalyl against key lepidopterous insects that are important in a wide variety of agricultural crops including vegetables, fruit trees, ornamentals and field crops. One of the advantages of pyridalyl includes its high levels of effectiveness against numbers of lepidopterous insects across the genus, because different lepidopterous insect species often occur simultaneously in crop fields under practical conditions. Moreover, several lepidopterous insects are known to have developed high resistance to existing insecticides and are becoming more difficult to control, e.g. *Helicoverpa armigera* (Forrester *et al*. 1993; Gunning *et al*. 1996), *Heliothis zea* (Sparks 1981), *Heliothis virescens* (Sparks 1981; Plapp *et al*. 1990; Elzen *et al*. 1992), and *Plutella xylostella* (Hama 1990; Isayama *et al*. 2004).

In addition to the target insect pests of Lepidoptera, pyridalyl is also highly effective against one of the most serious thrips species, *Frankliniella occidentalis* (Thysanoptera) (Isayama *et al*. 2005), and the leafminers of the genus *Liriomyza* (Diptera) (Tokumaru *et al*. 2005). Tokumaru *et al*. (2005) evaluated the insecticidal activity of pyridalyl against three leafminer species, *L. sativae*, *L. trifolii*, and *L. bryoniae*, and found that the compound is quite effective against larval and adult stages of the leafminers by foliar spray and leaf dipping applications, respectively, regardless of the species and the strains used. Moreover, our laboratory experiments revealed that the tomato russet mite, *Aculops lycopersici* (Acari), which occurs specifically on solanaceous crop plants but is recently becoming more important in Japan (Watanabe 1996; Kawai 2003), could be totally controlled by the foliar application of pyridalyl at 100 ppm (**Table 2**), the concentration registered for other insect pests in Japan.

Table 2 Acaricidal activity of pyridalyl against tomato russet mite, *Aculops lycopersici.* Formulated products (10%FL, Sumitomo Chemical for pyridalyl; and 20%FL, Nissan Chemical Industries for pyridaben) were diluted in water, and a sufficient amount of the solution was sprayed onto tomato plants (five-leaf stage), to that 50 mites were released at the lowest leaf seven days before the foliar application. Number of mites occurring on the tomato leaves was scored using microscopes 7 and 14 days after application (DAT).

Chemicals	Conc. (ppm)	Number of mites per leaf [a] 7DAT	14DAT
Pyridalyl	50	0.1 a	4.3 a
	100	0.0 a	0.0 a
Pyridaben	133	0.0 a	0.1 a
UTC	—	45.9 b	311.3 b

[a] Different letters indicate significant differences among treatments on each observation day ($p < 0.05$, Tukey-Kramer HSD test).

Table 1 Insecticidal activity of pyridalyl against various key target insect pests.

Pest species	Stage	Test method	LC_{50} (ppm)	Ref. [a]
Rice leafroller, *Cnaphalocrosis medinalis*	3rd-instar larva	Foliar spray	1.55	i
Tobacco budworm, *Helicoverpa armigera*	3rd-instar larva	Leaf dipping	1.36	i
Cotton bollworm, *Helicoverpa zea*	1st-instar larva	Diet overlay	1.55	ii
	2nd-instar larva	Leaf dipping	3.23	i
Tobacco budworm, *Heliothis virescens*	1st-instar larva	Diet overlay	1.54	ii
	2nd-instar larva	Leaf dipping	4.29	i
Cabbage armyworm, *Mamestra brassicae*	3rd-instar larva	Foliar spray	1.98	i
Common cabbage worm, *Pieris rapae crucivora*	2nd-instar larva	Foliar spray	3.02	i
Diamondback moth, *Plutella xylostella*	3rd-instar larva	Leaf dipping	4.48	i
Beet armyworm, *Spodoptera exigua*	3rd-instar larva	Leaf dipping	0.93	i
Common cutworm, *Spodoptera litura*	2nd-instar larva	Body immersion	0.67	iii
	3rd-instar larva	Body immersion	1.07	iii
	3rd-instar larva	Foliar spray	0.77	i
	4th-instar larva	Body immersion	1.66	iii
	5th-instar larva	Body immersion	2.05	iii
	6th-instar larva	Body immersion	3.30	iii
Western flower thrips, *Frankliniella occidentalis*	2nd-instar larva	Foliar spray	9.49	iii
	Adult	Foliar spray	26.5	iii

[a] Codes indicate the reference literatures: Sakamoto *et al.* (2003) (i); Cook *et al.* (2005) (ii); and Isayama *et al.* (2005) (iii).

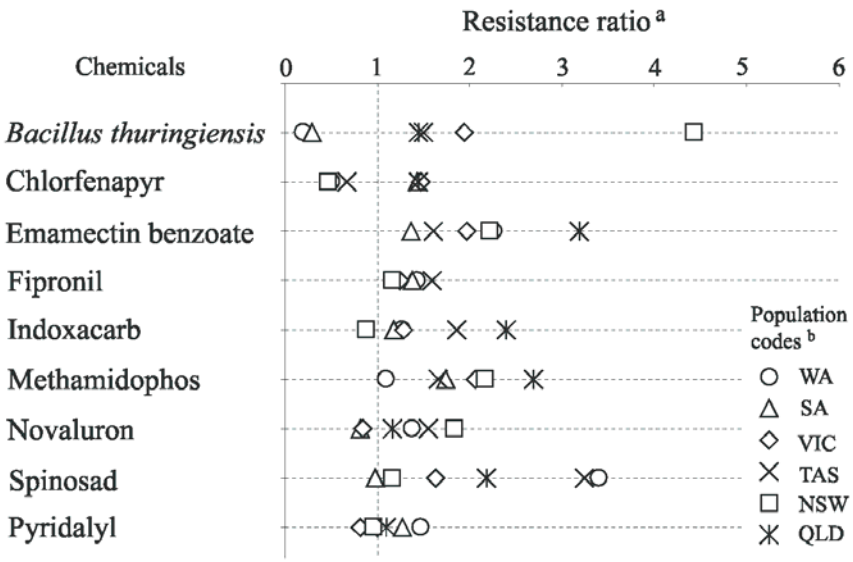

Fig. 2 Level of insecticide resistance of field populations of *Plutella xylostella* derived from wide regions of Australia. [a] Resistance ratios were calculated from relative values of LC_{50} or LC_{95} between field populations and a susceptible laboratory strain, which had been maintained since 1994 in the Institute for Horticultural Development, Knoxfield, Australia. A resistance ratio of 1 means that the field population is equivalent in susceptibility to the laboratory strain. [b] Population codes indicate the state from which the population was collected: Western Australia (WA), South Australia (SA), Victoria (VIC), Tasmania (TAS), New South Wales (NSW), and Queensland (QLD).

To evaluate its practical efficacy, pyridalyl was tested on various strains of pest insects that are resistant or less susceptible to existing insecticides. Sakamoto *et al*. (2004) illustrated that pyridalyl was highly active against a laboratory strain of *Plutella xylostella* which was highly resistant to pyrethroids, organophosphates and benzoylureas. **Fig. 2** shows the resistance level of field populations of *P. xylostella* to several insecticides, which was examined under the national resistance monitoring program during 2000 to 2001 by the Australian Institute for Horticultural Development. The susceptibility to pyridalyl was quite similar among the populations studied, whereas variable levels of susceptibility were found with several other insecticides. In Japan, Isayama *et al*. (2004) studied the insecticide susceptibility of field populations of *P. xylostella* for nearly two decades in Hyogo Prefecture, southwestern Honshu, and illustrated that pyridalyl was highly effective to the multi-resistant populations of *P. xylostella*, against which a number of insecticides including organophosphates, pyrethroids, nereistoxin analogs, and benzoylureas lost their practical efficacy within a few years of being placed on the market. Tokumaru *et al*. (2005) examined the susceptibility of field populations of leafminers to various insecticides consisting of several chemical classes and revealed that pyridalyl was one of the most effective insecticides among the products studied. Previous studies so far found no cross-resistance between pyridalyl and other existing insecticides, suggesting a novel mode of action of pyridalyl.

Intoxication symptoms

Saito *et al*. (2004) observed the symptoms of *Spodoptera litura* larvae intoxicated with lethal or sub-lethal dosages of pyridalyl. The lepidopterous larvae treated with pyridalyl at lethal dosage showed typical symptoms of flaccid paralysis followed by death within several hours after treatment (**Fig. 3**). The intoxicated larvae immediately lost their vigor, mobile activity, and body elasticity with no conspicuous symptoms such as hypercontraction, convulsion and vomiting after the application of pyridayl. In the treatment of sub-lethal dosage, pyridalyl showed another unique symptom on the epidermis at and around the treatment area. In most of the treated larvae, the dorsum area to which pyridalyl topically applied turned darker, resulting in a specific symptom similar to a burn scar after molting (**Fig. 4**). Thereafter, some larvae could not complete ecdysis and thereby died, and the remaining pupa mostly developed into abnormal shapes and failed to emerge as adults (**Fig. 5**). Thus, pyridalyl has a wide range of effective dosages, indicating that the populations of lepidopterous pests can be suppressed by pyridalyl under the conditions where the pests are exposed to lower dosages of the compounds.

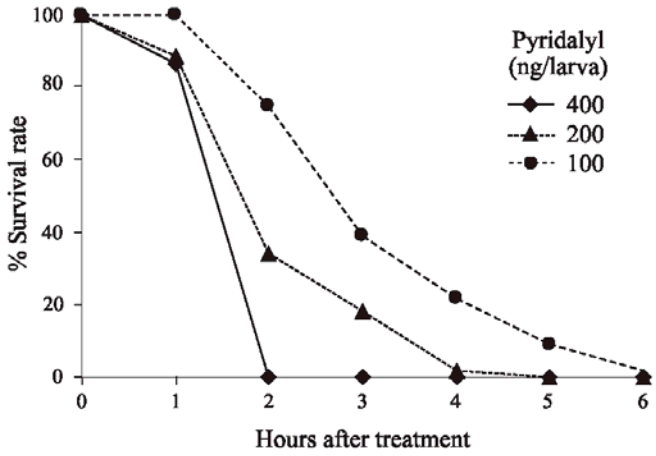

Fig. 3 The acute toxicity of lethal dosage of pyridalyl on fifth-instar larvae of *Spodoptera litura*.

Fig. 4 The symptom of fifth-instar larva of *Spodoptera litura* treated with a sub-lethal dosage of pyridalyl (6.25 ng per larva), as indicated by an arrow.

To investigate whether the unique symptoms of pyridalyl shown above were caused by the interference of cell functions, the ultrastructural changes of organelles in cell tissues of *S. litura* larvae were observed by transmission electron microscopy by Saito *et al*. (2006). When pyridalyl was topically treated on the larval dermis, the compound caused remarkable ultrastructural changes of organelles in the epidermal cells, exhibiting a rapid progression of hydro-

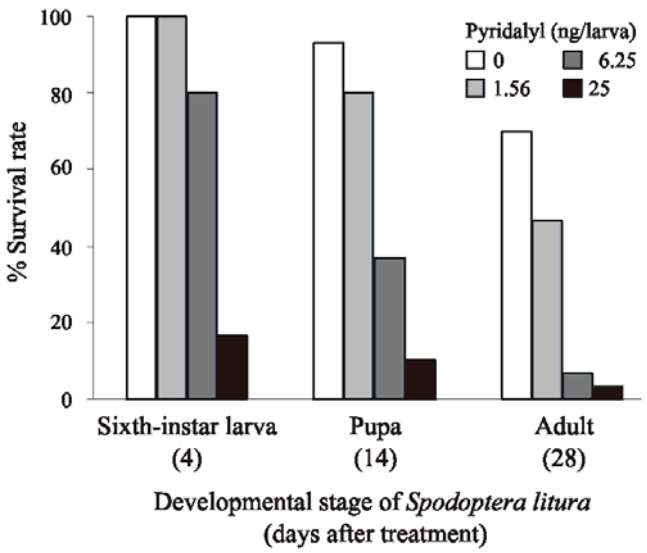

Fig. 5 Effect of sub-lethal dosages of pyridalyl on fifth-instar larvae of *Spodoptera litura*.

pic degeneration and necrosis containing mitochondrial swelling, dilated rough endoplasmic reticulum, dilated Golgi apparatus, shrunken nuclei, and increase of unidentified clear granules (see Fig. 1 in Saito *et al*. 2006). In contrast, no such changes appeared in the dermal cells apart from the treated area and other cell tissues such as the dorsal aorta, muscles, fat tissues, midgut and ganglia. The results indicate that pyridalyl affects only the epidermal cells under the treated dermis. Temporal observation of cell tissues treated with pyridalyl indicated that the time course of the ultrastructural changes in the larval epidermal cells were parallel to the development of intoxication symptoms and the subsequent death of the larvae, suggesting that the ultrastructural effects on epidermal cells could be associated with the insecticidal action of pyridalyl.

Singh and Singh (1984) studied the action of dieldrin, a cyclodiene insecticide, on the ultrastructure of the sixth abdominal ganglion of cockroaches, *Periplaneta americana*, and found that the compound caused mitochondrial swelling with broken cristae in nerve cell bodies and neuropiles, and depletion of synaptic vesicles from presynaptic terminals in the neuropiles. Retnakaran *et al*. (1989) revealed that chlorfluazuron, one of the benzoylphenyl urea chemistries, exhibited characteristic symptoms to the mesothoracic tergite of spruce budworm larvae, *Choristoneura fumiferana*, such as accumulation of large numbers of vesicles with fibrous, protein-like materials inside, loss of microvilli, and plasma membrane plaque. Although only a handful of previous studies are available that investigated the ultrastructural changes caused by insecticides in insect tissues (e.g., Singh and Singh 1984; Hassan and Charnley 1987; Retnakaran *et al.* 1989), pyridalyl exhibited unique and specific symptoms on the lepidopterous larvae and their epidermal cell tissues, that is in high contrast with those observed in the existing insecticides. The findings in intoxication symptoms and ultrastructural analysis also support a novel mode of action of pyridalyl and are informative and helpful for clarifying the mechanisms of insecticidal action of the compound.

Anti-feeding activity

Pyridalyl shows insecticidal activity both by oral and dermal applications, displaying the typical symptoms of decrease of vigor, mobile activity, and body elasticity in the intoxicated larvae. Thereby, suppression of the feeding activity of target insect pests by pyridalyl is very rapid, protecting the crop plants from the insect attack immediately after application. **Fig. 6** shows the degree of feeding damages of cabbage, *Brassica oleracea* var. *capitata*, caused by *S. litura* larvae that were released on the plants (four leaf stage) just after the application of insecticides. Although the mortality did not differ among the treated plots (100%), pyridalyl was more effective in protecting larvae attack than commercial standards (**Fig. 6B**), whereas the cabbage plants treated with commercial standards such as emamectin-benzoate and chlorfenapyr were significantly damaged by the larvae (**Fig. 6C, 6D**). These results show that pyridalyl can provide an excellent anti-feeding activity in addition to the lethal effect, which will lead to the improved commercial value of crops treated with pyridalyl.

Practical efficacy in field experiments

In Japan, field trials by an official institution, the Japan Plant Protection Association, confirmed that pyridalyl is highly effective under practical conditions against various lepidopterous, thysanopterous and dipterous insect pests without any phytotoxicity in a registered dosage (**Table 3**). The field experiment by Isayama *et al*. (2004) showed an excellent efficacy of pyridalyl by foliar application at 100 ppm against *P. xylostella* that is highly resistant to existing insecticides such as pyrethroids, organophosphates and benzoylureas. In Australia, the field experiment conducted by Murray *et al*. (2005) demonstrated that the foliar application of pyridalyl at 50 and 100 g a.i./ha performed quite well for controlling *Helicoverpa* spp. (mainly *H. armigera*) occurring on mungbean, *Vigna radiata*, and fababean, *Vicia faba*. In USA, Cook *et al*. (2004) illustrated that the population of *Spodoptera exigua* was controlled most effectively by the treatment with pyridalyl at the rate 140 g a.i./ha in a field experiment using cotton, *Gossypium hirsutum*. Field trials have been carried out globally over several years to evaluate the practical efficacy of pyridalyl, and now the compound is registered for use in several countries such as

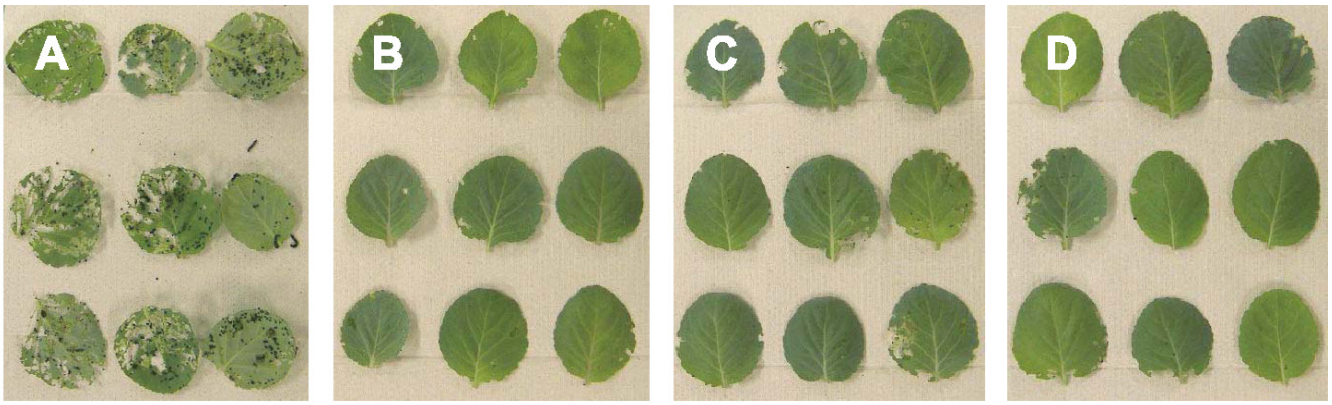

Fig. 6 The degree of feeding damages on cabbage leaves caused by *Spodoptera litura* larvae, observed in untreated control (A), and in the treatment of pyridalyl (B), emamectin-benzoate (C), and chlorfenapyr (D) at their concentrations registered in Japan (B, 100 ppm; C, 10 ppm; D, 50 ppm).

Table 3 List of field trials of pyridalyl conducted by a Japanese official association, Japan Plant Protection Association (JPPA). Efficacy data were obtained from the data book (1998-2006) published by JPPA.

Crop Pest	Conc. (ppm)	Efficacy score [a] A	 B	 C	 D	Evaluated year
Cabbage, *Brassica oleracea* var. *capitata*						
Diamondback moth, *Plutella xylostella*	100	6				1999-2001
Common cabbage worm, *Pieris rapae crucivora*	100	5				2000-2001
Common cutworm, *Spodoptera litura*	100	6	1			1999-2001
Cabbage armyworm, *Mamestra brassicae*	100	4	1	1		1998-2006
Corn earworm, *Helicoverpa armigera*	100	3	2			1998-2001
Cabbage webworm, *Hellula undalis*	100	3	4			2003-2004
Japanese radish, *Raphanus sativus*						
Diamondback moth, *Plutella xylostella*	100	6	3	1		2000-2001
Common cabbage worm, *Pieris rapae crucivora*	100	3	2			2000-2001
Cabbage armyworm, *Mamestra brassicae*	100	5	1			2000-2001
Lettuce, *Lactuca sativa*						
Corn earworm, *Helicoverpa armigera*	100	6	2			1999-2001
Common cutworm, *Spodoptera litura*	100	6	1			2000-2001
Garden pea leafminer, *Chromatomyia horticola*	100	3	3	1		2005-2006
Chinese cabbage, *Brassica rapa* var. *glabra*						
Common cabbage worm, *Pieris rapae crucivora*	100	1	3	1	1	2000-2001
Diamondback moth, *Plutella xylostella*	100	6	2			1999-2005
Cabbage armyworm, *Mamestra brassicae*	100	5	2	1		2000-2001
Broccoli, *Brassica oleracea* var. *italica*						
Diamondback moth, *Plutella xylostella*	100	3				2002-2004
Common cutworm, *Spodoptera litura*	100	3				2004
Tomato, *Solanum lycopersicum*						
Common cutworm, *Spodoptera litura*	100	1	1			2001
Corn earworm, *Helicoverpa armigera*	100	8				1998-2000
Legume leafminer, *Liriomyza trifolii*	100	2				2003-2004
Vegetable leafminer, *Liriomyza sativae*	100	2	1			2003-2004
Bell pepper, *Capsicum annuum* var. *grossum*						
Corn earworm, *Helicoverpa armigera*	100	6	2			1999-2001
Melon thrips, *Thrips palmi*	100		3			2003
Eggplant, *Solanum melongena*						
Corn earworm, *Helicoverpa armigera*	100	4	4			1998-2000
Common cutworm, *Spodoptera litura*	100	5	1			1998-2000
Legume leafminer, *Liriomyza trifolii*	100	1	2			2003
Melon thrips, *Thrips palmi*	100	6		1		1998-2001
Strawberry, *Fragaria ananassa*						
Common cutworm, *Spodoptera litura*	100	5	1			1999-2000
Corn earworm, *Helicoverpa armigera*	100	1	2			2003-2004
Scallion, *Allium fistulosum*						
Beet armyworm, *Spodoptera exigua*	100	4	2			1998-2000
Onion thrips, *Thrips tabaci*	100	2	6	1		2000-2006
Chrysanthemum, *Chrysanthemum morifolium*						
Corn earworm, *Helicoverpa armigera*	100	4	1			2000-2003
Soybean, *Glycine max*						
Common cutworm, *Spodoptera litura*	50	5	1			2002-2005
	100	10				2002-2003
Total		140	54	7	1	

[a] Degree of practical efficacy in each field trial was categorized into the following four scores on the basis of the criteria established by JPPA: highly effective (A); effective (B); moderately effective (C); and less effective (D). Numerical values in the table indicate the number of filed trials.

Korea, Thailand, Malaysia, Turkey and India as well as Japan (see **Appendix**).

IMPACTS ON NON-TARGETED ORGANISMS

The mammalian- and eco-toxicological profiles of pyridalyl are shown in **Table 4**. Although pyridalyl is highly toxic to target insect pests, the compound is thought to be harmless and safe to the non-targeted organisms, displaying high levels of selectivity between insect pests and vertebrates.

Table 5 indicates the effects of pyridalyl on beneficial arthropods including pollinators and natural enemies in laboratory bioassays. Pyridalyl was found to be harmless to both of the indigenous and commercial arthropods that belong to a broad range of taxonomical classification. Hamamura and Shinoda (2004) evaluated the effect of a wide variety of commercial products to an indigenous predatory mite, *Neoseiulus womersleyi*, and two commercial predatory mites, *Phytoseiulus persimilis* and *N. californicus*, by direct spray method, and illustrated that pyridalyl could be considered as one of the safest products for the predatory mites among the 33 compounds studied. Hamamura *et al*. (2006) studied the insecticide susceptibility of the spiderlings of *Pardosa astrigera*, which is considered as an promising natural enemy for diamondback moth, *Plutella xylostella*, in cabbage fields, and found no effect of pyridalyl on the spiderling, arguing that such compounds having insecticidal activity against *Plutella xylostella* but no impact on *Pardosa astrigera* can be useful for IPM-based plant protection. Impacts of pyridalyl on the eclosion of the parasitic wasps were also examined using *Aphidius colemani*, *Encarsia formosa*, and *Neochrysocharis formosa*. When pyridalyl

Table 4 Mammalian toxicity and ecotocxicity data of pyridalyl. Data was obtained from Sakamoto *et al.* (2005).

Study	Species	Results
Mammalian toxicity, acute oral	Rat (SD)	LD_{50} >5000 mg/kg
Mammalian toxicity, acute dermal	Rat (SD)	LD_{50} >5000 mg/kg
Mammalian toxicity, acute inhalation [a]	Rat (SD)	LC_{50} >2010 mg/m^3
Bird toxicity, acute oral	Bobwhite quail	LD_{50} >2250 mg/kg
Fish toxicity, acute	Carp	96 hr LC_{50} >10 mg/L
Daphnia toxicity, acute	*Daphnia magna*	48 hr EC_{50} = 3.8 μg/L
Alga toxicity, acute	*Selenastrum capricornutum*	72 hr EC_{50} >10 mg/L

[a] Four-hour inhalation from nose.

Table 5 Effects of pyridalyl on various beneficial arthropods.

Species name	Ecological role	Conc. (ppm)	Stage	Method [a]	Observation period [b]	Mortality (%)	Ref. [c]
Coleoptera							
Harmonia axyridis	Predator	100	Adult	BI	3	23.3	
Coccinella septempunctata	Predator	100	Adult	BI	4	0	
Hippodamia convergens	Predator	1797	Adult	DS	3	3.3	i
Propylaea japonica	Predator	100	Larva	RC	4	9.1	
Stethorus japonicus	Predator	100	Larva	RC	1	0	
Paederus fuscipes	Predator	100	Adult	RC	2	13.3	
Oligota kashmirica benefica	Predator	100	Adult	RC	1	0	
Anisodactylus punctatipennis	Predator	100	Adult	BI	7	6.7	
Dermaptera							
Anisolabis maritima	Predator	100	Larva	BI	3	12.0	
Neuroptera							
Chrysoperla carnea	Predator	200	Larva	BD	2	10.0	
Hemiptera							
Geocoris punctipes	Predator	1797	Adult	DS	3	10.0	i
Orius strigicollis	Predator	100	Adult	RC	5	16.7	
Orius sauteri	Predator	200	Adult	RC	2	11.8	
Hymenoptera							
Bracon mellitor	Parasitoid	1797	Adult	DS	3	3.3	i
Cardiochiles nigriceps	Parasitoid	1797	Adult	DS	3	0	i
Cotesia marginiventris	Parasitoid	1797	Adult	DS	3	6.7	i
Encarsia formosa	Parasitoid	100	Adult	RC	1	3.1	
Dacnusa sibirica	Parasitoid	100	Adult	RC	1	0	
Neochrysocharis formosa	Parasitoid	100	Adult	RC	2	13.3	
Diglyphus isaea	Parasitoid	100	Adult	RC	1	25.0	
Bombus terrestris	Pollinator	100	Adult	DS	5	10.0	iii
Apis mellifera	Pollinator	100	Adult	DS	6	10.0	
Acari							
Phytoseiulus persimilis	Predator	100	Adult	DS	1	0	ii
Neoseiulus californicus	Predator	100	Adult	DS	1	3.6	ii
Neoseiulus womersleyi	Predator	100	Adult	DS	1	1.7	ii
Neoseiulus cucumeris	Predator	100	Adult	RC	2	1.3	
Araneae							
Pardosa astrigera	Predator	100	Larva	DS	1	0	iv
Ummeliata insecticeps	Predator	100	Adult	BI	3	10.0	
Tetragnatha praedonia	Predator	100	Adult	DS	7	5.0	

[a] Each test method is briefly mentioned as follows: BD (Body Dipping), appropriate amount of test solution was directly applied to insect body using a micro-applicator or fine brush; BI (Body Immersion), whole insect body was immersed into test solution for a definite period of time; DS (Direct Spray), sufficient amount of test solution was directly sprayed to whole insect body using a sprayer or spray tower; RC (Residual Contact), test solution was applied to leaves, cups or vials, then insects were released onto the treated object. Regarding the original data that does not have a reference, the detailed experimental methodology for each species is described below:

Harmonia axyridis, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and ten adults of *H. axyridis* were directly dipped into the solution for 20 sec. Treated insects were put onto a filter paper placed in a plastic cup (9 cm in diameter and 5 cm deep) together with 100 2nd-instar larvae of diamondback moth, *Plutella xylostella*. The cup was covered with a lid and kept at 25ºC. Mortality was assessed three days after the treatment. The test was conducted in three replications;

Coccinella septempunctata, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and ten adults of *C. septempunctata* were directly dipped into the solution for 20 sec. Treated insects were put onto a filter paper placed in a plastic cup (9 cm in diameter and 5 cm deep) together with 50 third-instar larvae of diamondback moth, *Plutella xylostella*. The cup was covered with a lid and kept at 25ºC. Mortality was assessed four days after the treatment. The test was conducted in three replications;

Propylaea japonica, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and 15mL of the solution was sprayed to a eggplant, *Solanum melongena* (five-leaf stage), potted in a plastic cup (90 mL), that was heavily infested with cotton aphid, *Aphis gossypii*, and green peach aphid, *Myzus persicae*. When the treated plants were dried, a treated leaf were cut off and placed on a plastic cup (9 cm in diameter and 5 cm deep), and then one first-instar larva of *P. japonica* was put into the cup. The cup was covered with a lid and kept at 25ºC. Mortality was assessed 4 days after the treatment. The test was conducted in eleven replications;

Stethorus japonicus, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a citrus (*Citrus unshiu*) leaf was dipped into the solution for 20 sec. After dried, the treated leaf was put into a glass vial (3 cm in diameter and 5 cm deep) together with five adults of *S. japonicus*. The vial was covered with a lid and kept at 25ºC. Mortality was assessed 24 hours after the treatment. The test was conducted in three replications;

Paederus fuscipes, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and 2 mL of the solution was applied to a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep) using a pipette. Five adults of *P. fuscipes* were released onto the treated filter paper, and another filter paper folded into four was added to the cup to prevent the interference between the insects. The cup was covered with a lid and kept at 25ºC. Mortality was assessed 48 hours after the treatment. The test was conducted in three replications;

Oligota kashmirica benefica, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a citrus (*Citrus unshiu*) leaf was dipped into the solution for 20 sec. After dried, the treated leaf was put into a glass vial (3 cm in diameter and 5 cm deep) together with five adults of *Oligota kashmirica benefica*. The vial was covered with a lid and kept at 25ºC. Mortality was assessed 24 hours after the treatment. The test was conducted in two replications;

Table 5 (Cont.)

Anisodactylus punctatipennis, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and five adults of *A. punctatipennis* were directly dipped into the solution for 5 sec. Treated insects were put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), and another filter paper folded into four was added to the cup to prevent the interference between the insects. The cup was covered with a lid and kept at 25ºC. Third-instar larvae of diamondback moth, *Plutella xylostella*, were added to the cup accordingly to feed *A. punctatipennis*. Mortality was assessed 7 days after the treatment. The test was conducted in three replications;

Anisolabis maritime, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and eight adults of *Anisodactylus punctatipennis* were directly dipped into the solution for 10 sec. Treated insects were put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), and another filter paper folded into four was added to the cup to prevent the interference between the insects. Cotton swab saturated with water was put into the cup. The cup was covered with a lid and kept at 25ºC. Third-instar larvae of diamondback moth, *Plutella xylostella*, were added to the cup accordingly to feed *A. maritime*. Mortality was assessed 3 days after the treatment. The test was conducted in three replications;

Chrysoperla carnea, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and 100 µL of the solution was applied to second-instar larvae of *C. carnea* using a microapplicator (Burkard). Treated insects were individually put onto a cabbage (*Brassica oleracea* var. *capitata*) leaf placed in a plastic cup (9 cm in diameter and 5 cm deep). The cup was covered with a lid and kept at 25ºC. Eggs of Mediterranean flour moth, *Ephestia kuehniella*, were added to the cup accordingly to feed *C. carnea*. Mortality was assessed 3 days after the treatment. The test was conducted in 20 replications;

Orius strigicollis, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and sufficient amount of the solution was sprayed onto a cucumber, *Cucumis sativus* (one-leaf stage), potted in a 90-mL plastic cup. When the treated plant was dried, a treated leaf was put onto a water-saturated filter paper placed in a plastic cup (6.5 cm in diameter and 3 cm deep), and then 10 adults of *O. strigicollis* were released into the cup. The cup was covered with a lid and kept at 25ºC. Eggs of Mediterranean flour moth, *Ephestia kuehniella*, were added to the cup accordingly to feed *O. strigicollis*. Mortality was assessed 5 days after the treatment. The test was conducted in three replications;

Orius sauteri, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a sufficient amount of the solution was sprayed onto a cabbage, *Brassica oleracea* var. *capitata* (two-leaf stage), potted in a 90-mL plastic cup. When the treated plant was dried, a treated leaf was put into a plastic cup (9 cm in diameter and 8 cm deep), and then 8 adults of *O. sauteri* were released into the cup. The cup was covered with a lid and kept at 25ºC. Eggs of Mediterranean flour moth, *Ephestia kuehniella*, were added to the cup accordingly to feed *O. sauteri*. Mortality was assessed 2 days after the treatment. The test was conducted in four replications;

Encarsia formosa, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a sufficient amount of the solution was sprayed onto a tomato, *Solanum lycopersicum* (three-leaf stage), potted in a 90-mL plastic cup. When the treated plant was dried, a treated leaf was put into a glass vial (3 cm in diameter and 5 cm deep), and then ten adults of *E. formosa* were released into the vial. The vial was covered with a lid and kept at 25ºC. Mortality was assessed 24 hours after the treatment. The test was conducted in three replications;

Dacnusa sibirica, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a cucumber (*Cucumis sativus*) leaf was dipped into the solution for 60 sec. The stem of the treated leaf was covered with a water-saturated cotton, and the leaf was put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), then a cotton swab saturated with honey (5% in water) was added into the cup. Ten adults of *Dacnusa sibirica* were released into the cup, and the cup was covered with a lid and kept at 25ºC. Mortality was assessed 24 hours after the treatment. The test was conducted in three replications;

Neochrysocharis formosa, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a cucumber (*Cucumis sativus*) leaf was dipped into the solution for 60 sec. The stem of the treated leaf was covered with a water-saturated cotton, and the leaf was put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), then a cotton swab saturated with honey (5% in water) was added into the cup. Ten adults of *N. formosa* were released into the cup, and the cup was covered with a lid and kept at 25ºC. Mortality was assessed 48 hours after the treatment. The test was conducted in three replications;

Diglyphus isaea, formulated product of pyridalyl (10%FL, Sumitomo Chemical) was diluted in ion-exchanged water, and a cucumber (*Cucumis sativus*) leaf was dipped into the solution for 60 sec. The stem of the treated leaf was covered with a water-saturated cotton, and the leaf was put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), then a cotton swab saturated with honey (5% in water) was added into the cup. Ten adults of *D. isaea* were released into the cup, and the cup was covered with a lid and kept at 25ºC. Mortality was assessed 24 hours after the treatment. The test was conducted in three replications;

Apis mellifera, five adults of *A. mellifera* were put onto a filter paper placed in a plastic cup (9 cm in diameter and 8 cm deep), and the cup was covered with a wire-wove lid. Formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and 1 mL of the solution was sprayed to the cup using a spray tower (Daiki Rika Kogyo). When the treated cup was dried, the wire-wove lid was removed from the cup, and a pollen grain and a cotton swab saturated with sucrose solution (60% in water) were added into the cup. The cup was covered with a perforated plastic lid and kept at 25ºC. Mortality was assessed 6 days after the treatment. The test was conducted in six replications;

Neoseiulus cucumeris, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and sufficient amount of the solution was sprayed onto a cucumber, *Cucumis sativus* (one-leaf stage), potted in a 90-mL plastic cup. When the treated plant was dried, a treated leaf was cut and put into a plastic cup (9 cm in diameter and 5 cm deep), and 100 adults of *N. cucumeris* were released into the cup. The cup was covered with a lid and kept at 25ºC. Mortality was assessed 2 days after the treatment. The test was conducted in three replications;

Ummeliata insecticeps, formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and adults of *U. insecticeps* were directly dipped into the solution for 20 sec. Treated spiders were individually put onto a filter paper placed in a plastic cup (6.5 cm in diameter and 3cm in depth). The cup was covered with a lid and kept at 25ºC. Mortality was assessed 3 days after the treatment. The test was conducted in 20 replications;

Tetragnatha praedonia, adults of *T. praedonia* were individually put onto a filter paper placed in a plastic cup (6.5 cm in diameter and 3 cm deep). Formulated product of pyridalyl (10% FL, Sumitomo Chemical) was diluted in ion-exchanged water, and 0.2 mL of the solution was sprayed to the cup using a spray tower (Daiki Rika Kogyo). The cup was covered with a lid and kept at 25ºC. Four days after treatment, 0.5 mL of water was added to the cup to feed *T. praedonia.* Mortality was assessed 7 days after treatment. The test was conducted in 20 replications.

[b] Numerical values mean the days after treatment of pyridalyl.

[c] Codes indicate the reference literatures: Tillman and Mulrooney (2000) (i); Hamamura and Shinoda (2004) (ii); Isayama *et al.* (2005) (iii); and Hamamura *et al.* (2006) (iv).

was applied directly to the mummies of their host insects parasitized by the wasps at a registered rate 100 ppm, the rate of adult emergence from the mummies treated with pyridalyl was not different from those in untreated control. Regarding pollinators, the influence of pyridalyl on *Bombus terrestris* was evaluated by Isayama *et al*. (2005) using a direct spray treatment method to their nest, and no harmful impact was observed throughout the experiment (3 weeks). Moreover, the field observations conducted in the official experiment station in Tochigi Prefecture, Japan found no adverse effect of pyridalyl on worker bees of *Apis mellifera* by the direct application of pyridalyl to their beehive (see also Tsuchiya 2005).

Finally, the influence of pyridalyl on natural enemies was examined in more practical situation of pyridalyl use. In a greenhouse crop system with pepper plants, *Capsicum annuum* var. *grossum,* in Japan, we evaluated the impacts of pyridalyl on pirate bugs, *Orius strigicollis*, together with its efficacy against western flower thrips, *Frankliniella occidentalis*. **Fig. 7** shows the temporal change of population density of both insects occurring on the pepper plants after the application of pyridalyl and acrinathrin. In the plots treated with acrinathrin, the number of *O. strigicollis* was extremely low whereas obvious levels of *F. occidentalis* occurred constantly. Meanwhile, considerable numbers of *O. strigicollis* were found on the pepper plants treated with pyridalyl, and as a result, the density of *F. occidentalis* was suppressed at a quite low level during the experimental period by the effective combination of pyridalyl and *O. strigicollis*. Tillman and Mulrooney (2000) recognized an excellent selectivity of pyridalyl to natural enemies by the field experiment of cotton in USA where three natural enemies, *Geocoris punctipes*, *Hippodamia convergens*, and *Coleomegilla maculata*, occurred simultaneously with their host insects. In conclusion, both the laboratory and field experiments described above confirmed that pyridalyl is highly selective between insect pests and beneficial arthropods.

STUDIES ON THE MODE OF ACTION

Cytotoxicity

As demonstrated above, remarkable ultrastructural changes of organelles in the epidermal cells of *S. litura* was observed with pyridalyl treatment and the ultrastructural changes were temporally parallel to the development of intoxication symptoms and the subsequent death of the larvae

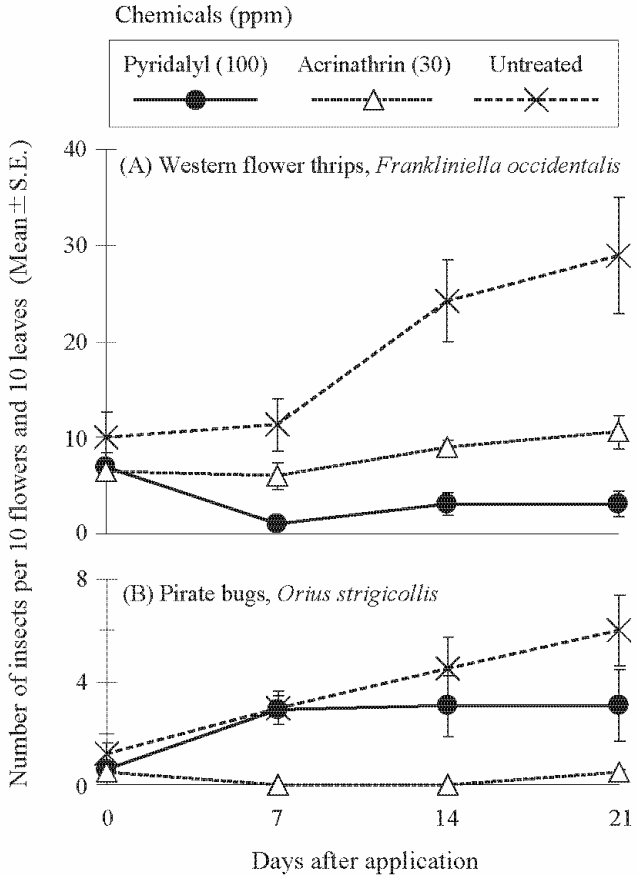

Fig. 7 The number of western flower thrips, *Frankliniella occidentalis*, and pirate bugs, *Orius strigicollis*, occurring on pepper plants treated with pyridalyl and acrinathrin and in untreated control.

caused by the treatment of pyridalyl. Thus, to obtain the clues of the mode of action of pyridalyl, Saito *et al*. (2005) evaluated the cytotoxicity of pyridalyl and its analogs on the cultured Sf9 cell line, which was established from the pupal ovarian tissue of *Spodoptera frugiperda*.

The cell growth of Sf9 was significantly disrupted by the cytotoxic compounds, such as chlorfenapyr (an uncoupler of oxidative phosphorylation in mitochondria), rotenone (an inhibitor of the mitochondrial respiration chain), oligomycin (a F_0F_1-ATPase inhibitor), anisomycin (a disruptor of protein synthesis with blocking peptidyl transferase activity, Liao *et al*. 1997), and 5-fluorouracil (an inhibitor of DNA synthesis by suppressing the activity of thymidylate synthetase, Spears *et al*. 1984; and also a disruptor of ribosomal RNA formation by the incorporation into the RNA, Cory *et al*. 1979). In contrast, the treatments of acephate and methomyl, known inhibitors of acethylcoline esterase, and cypermethrin, which acts on sodium channels in axons of central and peripheral neurons, were found to be non-cytotoxic to Sf9 cells. Pyridalyl significantly inhibited the growth of Sf9 cells and a clear dose-response curve was observed. An analog compound of pyridalyl, which is effective against *S. litura*, similarly suppressed the growth of Sf9 cell, while the other analogs, that show no insecticidal activity against *S. litura*, did not cause any potent inhibitory effects on Sf9 cells, though they have only minor differences in chemical structure compared to pyridalyl. Therefore, the cytotoxicity of pyridalyl and its analogs on Sf9 cells were highly associated with the insecticidal activity against *S. litura*, indicating that the cytotoxicity of pyridalyl in Sf9 cells reflects its insecticidal action at least in part.

Furthermore, Isayama *et al*. (2005) performed similar cytotoxic experiments using CHO-K1 cell culture, which was derived from ovarian tissue of Chinese hamster. The cytotoxicity on CHO-K1 was quite different between rotenone and pyridalyl. Rotenone was found to clearly inhibit the growth of CHO-K1 cells as well as Sf9 cells. In contrast, pyridalyl did not show any inhibitory effects on CHO-K1 cell cultures even by high-dose treatment, though the previous studies found that the compound caused significant cytotoxicity to Sf9 cells. Therefore, the results indicate that pyridalyl exhibits excellent selectivity at the level of cell lines that will contribute to the high level of safety on various non-targeted organisms.

ATP concentrations in cultured cell

ATP levels in cells are influenced by various external factors and hence regarded as a useful tool indicating the conditions of the cell. They can be measured quickly with high sensitivity based on the intensity of the bioluminescence generated from the luciferin-luciferase reaction. Thus, the effects of pyridalyl on the ATP concentration in cultured Sf9 cells were compared to those treated with various cytotoxic substances by Saito (2005).

Fig. 8 shows the temporal change of ATP concentrations in Sf9 cells treated with several substances and insecticides. The ATP levels were apparently reduced within a few hours by the treatment with the substances disrupting mitochondrial respiration, such as acequinocyl, chlorfenapyr, fenpyroxymate and oligomycin. Pyridalyl also significantly decreased the ATP contents in Sf9 cells, but the decline of ATP concentration was not as rapid as those observed in the treatment of the substances acting on mitochondrial respiration mentioned above. In contrast, 5-fluo-

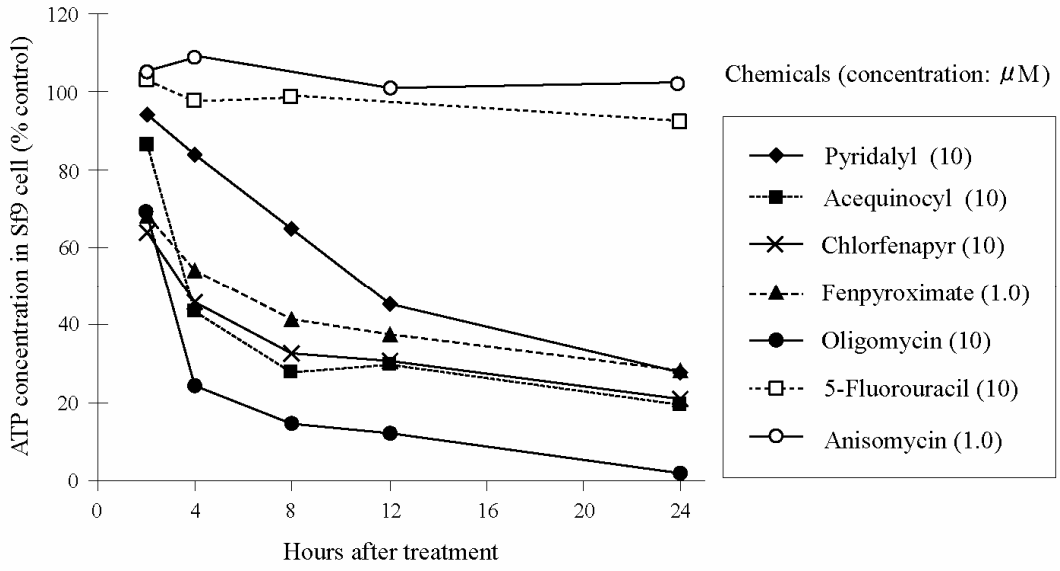

Fig. 8 Temporal changes of ATP concentration in cultured Sf9 cells treated with several chemicals.

rouracil and anisomycin did not show the decline of ATP levels in Sf9 cells, though they significantly suppressed the proliferation of Sf9 cells.

The *in vitro* inhibition assay on mitochondrial respiration conducted by Saito *et al*. (2005) revealed that the oxygen consumption by the mitochondria isolated from the flight muscle of *S. litura* adults was not different between the treatment of pyridalyl and untreated control under any of the conditions studied, indicating that pyridalyl inhibits neither mitochondrial electron transport nor ATP synthesis, and does not act as an uncoupler of mitochondrial respiration. Therefore, we concluded that the cytotoxic effect of pyridalyl on Sf9 cells appeared within several hours after the treatment are involved in neither the direct disruption of mitochondrial energetics nor the mechanism of action of 5-fluorouracil (i.e. an inhibitor of thymidylate synthetase which results in the suppression of DNA synthesis, and also a disruptor of ribosomal RNA formation by incorporation into the RNA) or anisomycin (i.e. a blocker of peptidyl transferase activity which leads the disturbance of protein synthesis).

CONCLUDING REMARKS

The IPM and IRM programs are comprehensive technical systems for pest management that comprise several insect control methods including natural enemies, disruptor of pheromone communication, pest-resistant variety, rotation of non-host plants, cultural control, and chemical agents. The effective combination of these methods will reduce the enormous selection pressure of insecticides acting on insect pests, and will lead to the prevention and/or delay of the development of insecticide resistance. Indigenous natural enemies are conserved and commercial natural enemies are often released additionally onto crop plants to suppress the population density of insect pests under economic injury level. Thus, pesticides with high selectivity could be the key element of IPM- and IRM-based approaches (van Driesche and Bellows 1996).

In Japan, implementation of IPM program is advanced in greenhouse cultivation of fruiting vegetables such as cucumbers, eggplants, peppers, strawberries and tomatoes (Okabayashi 2003; Yamashita and Shimokawaya 2005), in which the cultivation condition can be controlled more easily and natural enemies efficiently settle on crop plants. In these crops, especially for tomatoes, bumblebees of the genus *Bombus* are widely used for pollination, and natural enemies are released and conserved as biological agents for controlling pest insects (Matsuura 1993; Watanabe 1996; Tanaka *et al*. 1998), hence selective pesticides that are harmless both to pollinators and natural enemies are preferred for use. However, under IPM and IRM programs with reduced applications of selective insecticides, crop plants are often damaged by several minor pests such as eriophyid mites of the genus *Aculops*, tarsonemid mites of the genus *Polyphagotarsonemus*, and mealybugs of the genus *Phenacoccus* (Tanaka *et al*. 1998; Okabayashi 2003; Yamashita and Shimokawaya 2005). They rarely occur under the practical control program relied mainly on pesticide control, because pesticides with broad-spectrum activity that are applied for main target pests could simultaneously control the minor pests (Watanabe 1996; Tanaka *et al*. 1998). Thus, it is important to evaluate the impacts of chemical agents on minor insect pests as well as beneficial arthropods under IPM- and IRM-based approach.

As reviewed in this article, previous studies showed that pyridalyl has an excellent selectivity between pest insects and beneficial arthropods both in laboratory and field experiments. The cytotoxicological experiments using cultured cell lines demonstrated that pyridalyl was apparently cytotoxic to insect cell culture whereas it had no effect on mammalian cell line, thereby high selectivity was observed even at cell-line level. The laboratory and field experiments illustrated that pyridalyl is highly active against resistant strains of pest insects as similar to susceptible strains. We also found that the lepidopterous larvae intoxicated with pyridalyl exhibited unique symptoms that were not observed in those treated with existing insecticides. These results suggest that pyridalyl should have a unique and novel mode of action. The characteristics of pyridalyl would allow the compound to be one of the most powerful tools in IPM program. Moreover, another advantage of pyridalyl for IPM agent involves its effectiveness against certain minor pests, because they occur more frequently under the IPM condition with less application of pesticides (Watanabe 1996; Tanaka *et al*. 1998; Okabayashi 2003). To evaluate the practical performance of pyridalyl under IPM program, further feasibility studies will be needed from variable viewpoints such as economical cost-benefit; labor and time needed for pest monitoring and control; potential risk of resistance development; impacts on agro-ecosystem and ambient environment; and practical insecticidal efficacy. These applied approaches will contribute to facilitate the optimum use of pyridalyl in IPM program, improving the quality and efficacy of the management program as a whole.

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REFERENCES

Aggarwal N, Brar DS, Basedow T (2006) Insecticide resistance management of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and its effect on pests and yield of cotton in North India. *Journal of Plant Diseases and Protection* **113**, 120-127

Bourguet D, Desquilbet M, Lemarie S (2005) Regulating insect resistance management: the case of non-Bt corn refuges in the US. *Journal of Environmental Management* **76**, 210-220

Cook DR, Leonard BR, Gore J (2004) Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). *Florida Entomologist* **87**, 433-439

Cook DR, Leonard BR, Gore J, Temple JH (2005) Baseline responses of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), to indoxacarb and pyridalyl. *Journal of Agricultural and Urban Entomology* **22**, 99-109

Cory JG, Breland JC, Carter GL (1979) Effect of 5-fluorouracil on RNA metabolism in *Novikoff hepatoma* cells. *Cancer Research* **39**, 4905-4913

Elzen GW, Leonard BR, Graves JB, Burris E, Micinski S (1992) Resistance to pyrethroid, carbamate, and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. *Journal of Economic Entomology* **85**, 2064-2072

Forrester NW, Cahill M, Bird LJ, Layland JK (1993) Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research: Supplement Series* **1**, R1-132

Gunning RV, Moores GD, Devonshire AL (1996) Insensitive acetylcholinesterase and resistance to thiodicarb in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* **55**, 21-28

Hama H (1990) Current status and countermeasures of insecticide resistance in the diamondback moth. *Proceedings of the Kanto-Tosan Plant Protection Society* **37**, 1-4

Hamamura T, Kohno K, Takeda M (2006) Insecticide susceptibility of *Pardosa astrigera* L. Koch spiderlings. *Japanese Journal of Applied Entomology and Zoology* **50**, 253-255

Hamamura T, Shinoda T (2004) Selection of harmless pesticides to three predaceous mites. *Proceedings of the Kansai Plant Protection Society* **46**, 63-65

Hassan AEM, Charnley AK (1987) The effect of dimilin on the ultrastructure of the integument of *Manduca sexta*. *Journal of Insect Physiology* **33**, 669-676

Isayama S, Ogawa M, Kasamatsu K (2004) Observable changes in diamondback moth's (*Plutella xylostella* L.) susceptibility to insecticides in Iwaoka, Kobe City, Hyogo Prefecture, Japan. *Japanese Journal of Applied Entomology and Zoology* **48**, 337-343

Isayama S, Saito S, Kuroda K, Umeda K, Kasamatsu K (2005) Pyridalyl, a novel insecticide: potency and insecticidal selectivity. *Archives of Insect Biochemistry and Physiology* **58**, 226-233

Jutsum AR, Heaney SP, Perrin BM, Wege PJ (1998) Pesticide resistance: assessment of risk and the development and implementation of effective management strategies. *Pesticide Science* **54**, 435-446

Kawai A (1997) Prospect for integrated pest management in tea cultivation in Japan. *Japan Agricultural Research Quarterly* **31**, 213-217

Kawai A (2003) Recent topics on *Aculops lycopersici*. *Plant Protection* **57**, 99-102
Liao J, Ku N-O, Omary MB (1997) Stress, apoptosis, and mitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. *The Journal of Biological Chemistry* **272**, 17565-17573
Matthews GA (1997) Implementing cotton integrated pest management. *Experimental Agriculture* **33**, 1-14
Matsuura M (1993) Pollination of tomato plants with the introduced bumble bee, *Bombus terrestris*. *Plant Protection* **47**, 173-176
Murray DAH, Lloyd RJ, Hopkinson JE (2005) Efficacy of new insecticides for management of *Helicoverpa* spp. (Lepidoptera: Noctuidae) in Australian grain crops. *Australian Journal of Entomology* **44**, 62-67
Okabayashi T (2003) The development of the practical natural enemy use in the field and its extension. *Plant Protection* **57**, 530-534
Plapp Jr FW, Jackman JA, Campanhola C, Frisbie RE, Graves JB, Lutrell RG, Kitten WF, Wall M (1990) Monitoring and management of pyrethroid resistance in tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. *Journal of Economic Entomology* **83**, 335-341
Retnakaran A, MacDonald A, Nicholson D, Percy-Cunningham J (1989) Ultrastructural and autoradiographic investigation of the interference of chlorfluazuron with cuticle differentiation in the spruce budworm, *Choristoneura fumiferana*. *Pesticide Biochemistry and Physiology* **35**, 172-184
Sakamoto N, Saito S, Hirose T, Suzuki M, Matsuo S, Izumi K, Nagatomi T, Ikegami H, Umeda K, Tsushima K, Matsuo N (2004) The discovery of pyridalyl: a novel insecticidal agent for controlling lepidopterous pests. *Pest Management Science* **60**, 25-34
Sakamoto N, Ueda N, Umeda K, Matsuo S, Haga T, Fujisawa T, Tomigahara Y (2005) Research and development of a novel insecticide 'pyridalyl'. *Research and Development Reports of Sumitomo Chemical* **2005-I**, 33-44
Saito S (2005) Effects of pyridalyl on ATP concentrations in cultured Sf9 cells. *Journal of Pesticide Science* **30**, 403-405
Saito S, Isayama S, Sakamoto N, Umeda K (2004) Insecticidal activity of pyridalyl: acute and sub-acute symptoms in *Spodoptera litura* larvae. *Journal of Pesticide Science* **29**, 372-375
Saito S, Sakamoto N, Umeda K (2005) Effects of pyridalyl, a novel insecticidal agent, on cultured Sf9 cells. *Journal of Pesticide Science* **30**, 17-21
Saito S, Yoshioka T, Umeda K (2006) Ultrastructural effects of pyridalyl, an insecticidal agent, on epidermal cells of *Spodoptera litura* larvae and cultured insect cell Sf9. *Journal of Pesticide Science* **31**, 335-338
Singh GJP, Singh B (1984) Action of dieldrin and *trans*-aldrindiol upon the ultrastructure of the sixth abdominal ganglion of *Periplaneta americana* in relation to their electrophysiological effects. *Pesticide Biochemistry and Physiology* **21**, 102-126
Sparks TC (1981) Development of resistance in *Heliothis zea* and *Heliothis virescens* in North America. *Bulletin of the Entomological Society of America* **27**, 186-192
Spears CP, Gustavsson BG, Mitchell MS, Spicer D, Berne M, Bernstein L, Danenberg PV (1984) Thymidylate synthetase inhibition in malignant tumors and normal liver of patients given intravenous 5-fluorouracil. *Cancer Research* **44**, 4144-4150
Tanaka K, Ueda M, Shibao M (1998) Integrated pest management in tomato greenhouse pollinated by *Bombus terrestris*. *Plant Protection* **52**, 73-76
Tillman PG, Mulrooney JE (2000) Effect of selected insecticide on the natural enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor*, *Cardiochiles nigriceps*, and *Cotesia marginiventris* (Hymenoptera: Braconidae) in cotton. *Journal of Economic Entomology* **93**, 1638-1643
Tokumaru S, Kurita H, Fukui M, Abe Y (2005) Insecticidal susceptibility of *Liriomyza sativae*, *L. trifolii*, and *L. bryoniae* (Diptera: Agromyzidae). *Japanese Journal of Applied Entomology and Zoology* **49**, 1-10
Tsuchiya T (2005) Characteristics of pyridalyl (Pleo®), a novel insecticide. *Journal of Pesticide Science* **30**, 278-281
van Driesche RG, Bellows TS (1996) *Biological Control*, Chapman and Hall, New York, 539 pp
Watanabe M (1996) The extended distribution of the tomato russet mite in Kanto area. *Plant Protection* **50**, 329-330
Yamashita I, Shimokawaya H (2005) Integrated pest management in sweet peppers under protected cultivation. *Plant Protection* **59**, 457-461

JAPANESE ABSTRACT

ピリダリルは住友化学（株）によって発見された新規な殺虫剤であり、プレオ、またはスミプレオの商品名で世界的に販売されてきた。本化合物は、鱗翅目、アザミウマ目、双翅目およびダニ目に属する様々な害虫に高い効力を示し、既存の殺虫剤に感受性が低い、あるいは抵抗性を示す系統に対しても有効である。ピリダリルを処理した鱗翅目害虫の幼虫は、既存の殺虫剤には見られない特異な症状を呈し、ピリダリルが新規な作用機作を有することが示唆された。詳細な症状観察によって、ピリダリルが効果を示す濃度は広範囲であり、また対象害虫に対して極めて高い摂食阻害効果を示すことが明らかになった。一方、ピリダリルは花粉媒介者、捕食者、捕食寄生者などの有用昆虫に対する影響はほとんどなく、対象害虫との間に高い選択性を有する。昆虫培養細胞Sf9を使った細胞毒性試験を行ったところ、ピリダリルとその類縁体の細胞毒性の強さと、ハスモンヨトウに対する殺虫効果との間には高い相関性があることから、Sf9に対する細胞毒性が少なくとも部分的に殺虫効果に関与していると考えられた。Sf9に対する明らかな細胞毒性とは対照的に、ピリダリルは哺乳動物の培養細胞CHO-K1にはほとんど影響を与えず、本化合物の高い選択性は培養細胞レベルでさえ検出された。よって、ピリダリルはその優れた特性によって、総合的害虫管理（IPM）や害虫抵抗性管理（IRM）などのマネジメントシステムに基づいた防除プログラムのもとで、害虫を有効に防除し管理する資材として非常に有用であると考えられた。

Appendix Target crops, target pests, and use rate of pyridalyl registered in several countries.

Country	Crop	Insect	Use rate
Japan	Vegetables	Corn earworm, *Helicoverpa armigera*	100 mg a.i./L
		Common cutworm, *Spodoptera litura*	
		Beet armyworm, *Spodoptera exigua*	
		Diamondback moth, *Plutella xylostella*	
		Common cabbage worm, *Pieris rapae crucivora*	
		Cabbage armyworm, *Mamestra brassicae*	
		Cabbage webworm, *Hellula undalis*	
		Melon thrips, *Thrips palmi*	
		Onion thrips, *Thrips tabaci*	
		Garden pea leafminer, *Chromatomyia horticola*	
		Legume leafminer, *Liriomyza trifolii*	
		Vegetable leafminer, *Liriomyza sativae*	
Korea	Vegetables	Beet armyworm, *Spodoptera exigua*	100 mg a.i./L
		Diamondback moth, *Plutella xylostella*	
Thailand	Vegetables	Diamondback moth, *Plutella xylostella*	100 mg a.i./L
Malaysia	Vegetables	Diamondback moth, *Plutella xylostella*	100 mg a.i./L
Turkey	Cotton	Corn earworm, *Helicoverpa armigera*	100 g a.i./ha
	Tomato	Cotton leafworm, *Spodoptera littoralis*	
India	Cotton	Corn earworm, *Helicoverpa armigera*	100 g a.i./ha
	Vegetables	Common cutworm, *Spodoptera litura*	
		Diamondback moth, *Plutella xylostella*	