Development of the Novel Insecticide Spinetoram (DIANA®)

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Spinetoram is a new chemical in the spinosyn class of insecticides. It is a semi-synthetic spinosyn discovered in modification studies of fermenting substances of *Saccharopolyspora spinosa* by Dow AgroSciences LLC.

Spinetoram has good insecticidal properties such as broad insecticidal spectrum, rapid action and short pre-harvest interval. Sumitomo Chemical Co., Ltd. started its development in Japan from 2005. Its formulated products, DIANA® SC, DIANA® WDG and STOUT® DANTOTSU® DIANA® box granule, have been registered since March 2011.

Its insecticidal properties, the best application timing for the control of cabbage insect pests and the safety assessment of spinetoram are introduced in this paper.

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Introduction

Various types of agricultural insecticides have been developed to date. However, there are many cases of emergence of resistant insect pests against various insecticides due to long-term use or continuous application of insecticides for crop protection. In order to suppress such resistance development, it is generally recommended to implement rotation programs which include some insecticides with different modes of action. Therefore, plant protection associations and agricultural research institutes in many districts have proposed "rotation application," referring to the mode of action classification by the Insecticide Resistance Action Committee (IRAC). Nevertheless, there are not enough efficacious insecticides to implement rotation programs for all crops, and consequently agricultural chemical companies are competing with each other in the development of novel insecticides.

Spinetoram (DIANA®) has preferable properties such as a broad insecticidal spectrum and excellent insecticide activity against insect pests which are resistant to various existing insecticides. It can therefore be expected that spinetoram will make a great contribution to the stable production and quality improvement of agricultural products. Its development process, insecticidal properties, efficacy against insect pests and safety assessment are introduced in this paper.

* Currently belongs to: Crop Protection Division, Domestic

Development Process

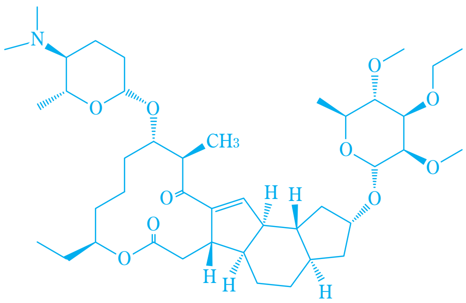
Spinetoram is a derivative of biological active substances (spinosyns) produced by the soil actinomycete *Saccharopolyspora spinosa*, and is a semi-synthetic spinosyn discovered during modification studies of natural substances by Dow AgroSciences LLC.[1)]

Regarding spinetoram development, foreign countries have preceded Japan. Spinetoram's pesticide registration was approved in New Zealand in 2007 as the world's first registration,[2)] and was followed by the U.S.A., Canada, Mexico, South Korea, Malaysia, Pakistan[3)] and Australia,[4)] and so on to date.

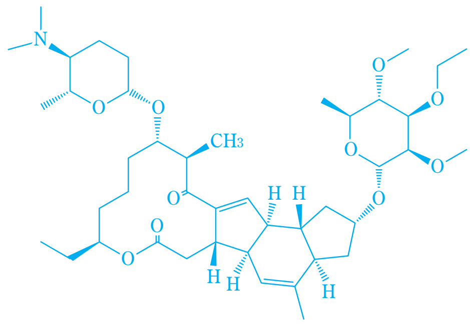
In Japan, Sumitomo Chemical Co., Ltd. concluded a joint development agreement with Dow Agrosciences in 2005. Since then Sumitomo Chemical has studied the insecticidal properties of spinetoram against Japanese crops and insect pests, and appropriate application based on them. In 2006 Sumitomo Chemical started to evaluate the efficacy on vegetables, tea plants, orchard trees and paddy rice through the Japan Plant Protection Association under the development codes "S-1947SC," "S-1947WDG" and "S-8640 box granules". On March 29, 2011, these formulated products were registered as the following agricultural insecticides: spinetoram water-dispensable powder insecticide (brand names: DIANA®SC and DIANA®WDG); and clothianidin, spinetoram and isotianil granules (brand names: STOUT®, DANTOTSU® and DIANA® box granules).

Active Ingredient

The active ingredient spinetoram is composed of two chemical compounds spinetoram-J and-L, both of which have a macrocyclic lactone structure (**Fig. 1**).



spinetoram-J



spinetoram-L

Common name	spinetoram	
	<spinetoram-J>	<spinetoram-L>
IUPAC name	(1*S*,2*R*,5*R*,7*R*,9*R*,10*S*,14*R*,15*S*,19*S*)-7-(6-deoxy-3-*O*-ethyl- = 2,4-di-*O*-methyl-α-L-mannopyranosyloxy)-15-[(2*R*,5*S*,6*R*)- = 5-(dimethylamino)tetrahydro-6-methylpyran-2-yloxy]-19-ethyl- = 14-methyl-20-oxatetracyclo[10.10.0.0^{2,10}.0^{5,9}]docosa-11-ene- = 13,21-dione	(1*S*,2*S*,5*R*,7*S*,9*S*,10*S*,14*R*,15*S*,19*S*)-7-(6-deoxy-3-*O*-ethyl- = 2,4-di-*O*-methyl-α-L-mannopyranosyloxy)-15-[(2*R*,5*S*,6*R*)- = 5-(dimethylamino)tetrahydro-6-methylpyran-2-yloxy]-19-ethyl- = 4,14-dimethyl-20-oxatetracyclo[10.10.0.0^{2,10}.0^{5,9}]docosa- = 3,11-diene-13,21-dione
Molecular formula	$C_{42}H_{69}NO_{10}$	$C_{43}H_{69}NO_{10}$
Molecular weight	748.02	760.03
Appearance	White powder	White-Yellow solid
Log Pow	4.09±0.16(pH7)	4.49±0.09 (pH7)
Solubility in water	10 mg/L (20°C)	31.9 mg/L (20°C)
Vapor pressure	5.3×10^{-5}Pa (20°C)	2.1×10^{-5}Pa (20°C)

Fig. 1 Chemical and physical properties of spinetoram

Table 1 Insecticidal activity of spinetoram on major pests

Order	Pests			Crops	Methods	Observation	LC50 (ppm)
	Name	Scientific Name	Growth Stage				
Lepidoptera	Diamondback moth	*Plutella xylostella*	3rd instar larva	Cabbage	dipping (leaf)	4DAT*	0.01ppm
	Common cutworm	*Spodoptera litura*	3rd instar larva	Cabbage	dipping (leaf)	4DAT	1.17ppm
	Common white	*Pieris rapae crucivora*	mid instar larva	Cabbage	dipping (leaf)	4DAT	0.02ppm
	Cotton bollworm	*Helicoverpa armigera*	3rd instar larva	Cabbage	dipping (leaf)	4DAT	0.08ppm
	Cabbage looper	*Trichoplusia ni*	3rd instar larva	Cabbage	dipping (leaf)	4DAT	0.01ppm
	Smaller tea tortrix	*Adoxophyes honmai*	mid instar larva	Tea	dipping (leaf)	10DAT	0.94ppm
	Oriental tea tortrix	*Homona magnanima*	3rd instar larva	Tea	dipping (leaf)	4DAT	0.87ppm
	Summer fruit tortrix	*Adoxophyes orana fasciata*	3rd instar larva	Apple	forliar spray	4DAT	0.11ppm
	Rice leafroller	*Cnaphalocrocis medinalis*	late instar larva	Rice	dipping (leaf)	4DAT	0.06ppm
Thysanoptera	Melon thrips	*Thrips palmi*	adult	Cucumber	dipping (leaf)	3DAT	0.019ppm
	Yellow tea thrips	*Scirtothrips dorsalis*	adult	Tea	dipping (leaf)	3DAT	0.038ppm

Order	Pests			Crops	Methods	Observation	Mortality
	Name	Scientific Name	Growth Stage				
Diptera	Tomato leafminer	*Liriomyza sativae*	early instar larva	Cucumber	dipping (leaf)	3DAT	23ppm:100%
	Pea leafminer	*Liriomyza huidobrensis*	early instar larva	Cucumber	dipping (leaf)	3DAT	23ppm:100%
Hemiptera	Sweeto potato whitefly (biotype Q)	*Bemisia tabaci*	first instar nymph	Cabbage	dipping (leaf & insect)	4DAT	47ppm: 98%

* Days after treatment

Insecticidal Properties

1. Insecticidal Spectrum

Spinetoram has excellent insecticidal activity against thrips, leafminer flies and whiteflies in addition to lepidopteran insects such as the diamondback moth *Plutella xylostella*, leafroller and so on (**Table 1**). Therefore, because spinetoram has a broad insecticidal spectrum toward major insect pests, it can be assumed that even amid the infestation of multiple pest species (e.g., lepidopteran insects and thrips), spinetoram can show good control against them at the same time.

2. Mode of Action

It is considered that spinetoram affects nicotinic acetylecholine receptors and γ-aminobutyric acid (GABA) receptors existing on postsynaptic membranes in insect nervous systems, thereby causing abnormal neural transmission (**Fig. 2**). Spinosyns, including spinetoram, are classified in Group 5 of the MoA classification by the IRAC (**Fig. 3**).

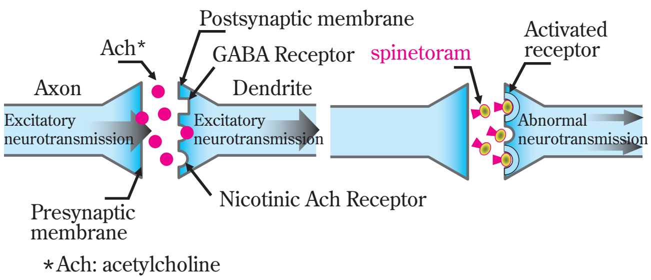


Fig. 2 Mode of action of spinetoram

From the perspective of resistance management, it is recommended to avoid the continuous use of spinetoram and spinosad (the latter of which has been classified in the same MoA group as spinetoram) and instead conduct a rotation application with insecticides that are classified in other groups.

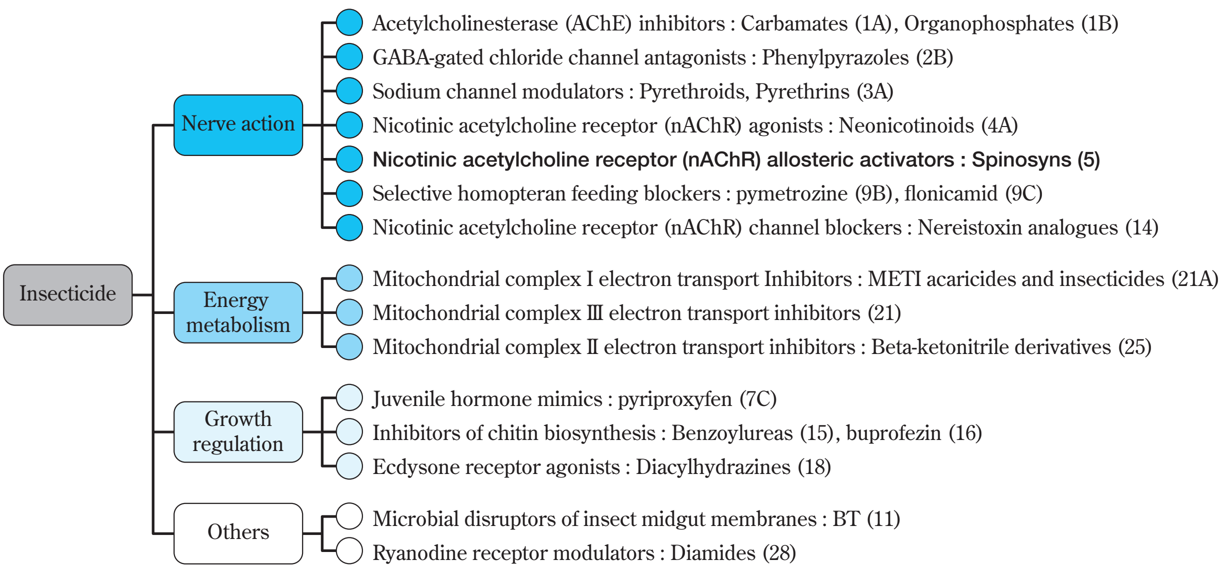
3. Effects against Insect Pests Resistant to Existing Insecticides

Because the insecticidal resistance problem is a very important one to solve, newly developed compounds are desired not to show cross resistance with existing insecticides. Therefore, we have evaluated spinetoram's insecticidal activity on *Plutella xylostella,* which has resistance toward organophosphates, chitin biosynthesis inhibitors and synthetic pyrethroids; and smaller tea tortrix *Adoxophyes honmai,* which shows resistance toward ecdysone receptor agonists and diamides.

In the results it has been confirmed that spinetoram shows superior insecticidal activity against these insect pests (**Fig. 4**). It has therefore been concluded that spinetoram is also effective against insect pests which are resistant to the above existing insecticides in crop protection.

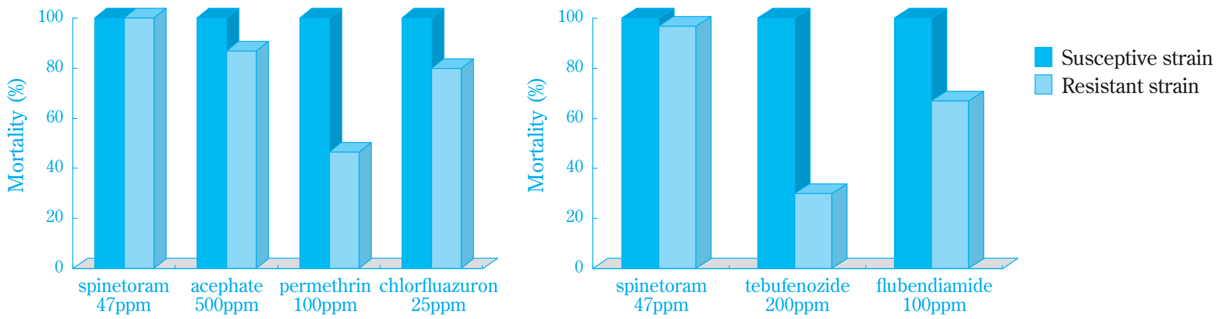
4. Speed of Insecticidal Action and Suppression of Feeding Damage

The study on the speed of insecticidal action by spinetoram was carried out using *Plutella xylostella* as the subject group. The larvae released onto spinetoram-treated cabbage leaves stopped moving in two hours and



Source : IRAC MoA Classification ver.7.1 [6)]

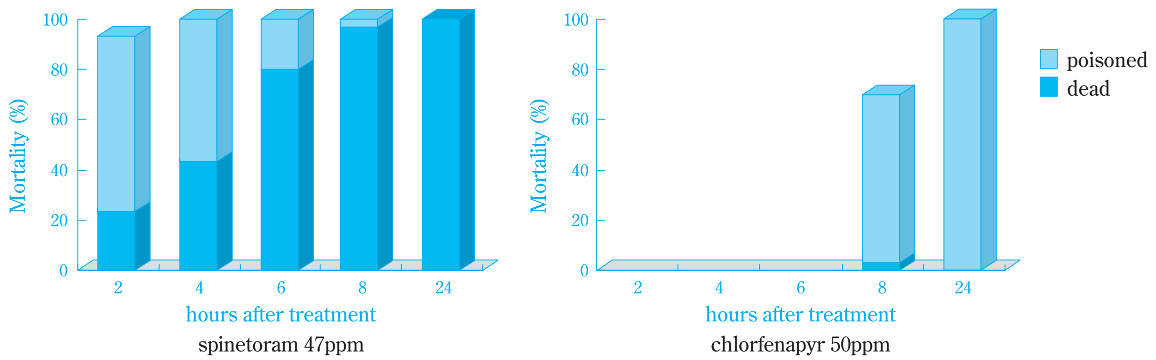
Fig. 3 Major MoA of insecticides



Insect : Diamondback moth (*Plutella xylostella*)
Plant : Cabbage
Method : feeding leaves dipped into diluted solution of insecticide

Insect : Smaller tea tortrix (*Adoxophyes honmai*)
Plant : Tea
Method : feeding leaves dipped into diluted solution of insecticide

Fig. 4 Insecticidal activity on lepidopteran insects resistant to various insecticides



Insect : Diamondback moth (*Plutella xylostella*)
Plant : Cabbage
Method : feeding leaves dipped into diluted solution of insecticide

Fig. 5 Rapid action of spinetoram on *Plutella xylostella*

Insect : Diamondback moth (*Plutella xylostella*)
Plant : Cabbage
Method : feeding leaves dipped into diluted solution of insecticide
Observation: 24 hours after treatment

Fig. 6 Suppression of feeding damage of *Plutella xylostella*

died immediately after that (**Fig. 5**). Furthermore, they stopped feeding immediately after the release on treated leaves (**Fig. 6**). The quick action of spinetoram described above is considered to be a desirable characteristic for a crop protection agent.

5. Insecticidal Activity in Each Growth Stage

Spinetoram demonstrates superior insecticidal activity against a wide range of growth stages from eggs, larvae which are from first instar to late instar, and adults of lepidopteran insect pests such as *Pluttella xylostella* and

Table 2 Insecticidal activity of spinetoram on variou growth stage

Pests	Mortality (%)				
	Egg[1)]	Larva[2)]			Adult[3)]
		1st instar	3rd instar	last instar[4)]	
Diamondback moth[a)] (*Plutella xylostella*)	88.2	100	100	100	100
Summer fruit tortrix[b)] (*Adoxophes orana fasciata*)	93.1	100	100	100	100

a) applied spinetoram 47ppm
b) applied spinetoram 25ppm

1) dipped eggs into diluted water of spinetoram for 10 sec.
2) feeding leaves dipped into diluted solution of spinetoram for 60 sec.
3) foliar spray or dry film method
4) Diamondback moth : 4th instar, Summer fruit tortrix : 5th instar

Adoxophes orana fasciata, as shown in **Table 2**. Moreover, it shows superior insecticidal activity against young larvae and adults of dipteran insect pests such as *Liriomyza sativae*.

In many cases insect pests at various growth stages occur simultaneously in the field. It is suggested that spinetoram could show excellent control efficacy in crop protection in such cases because of its superior insecticidal activity against various growth stages of insect pests.

6. Intake Pathway for Insect Pests

Generally, there are two routes for intake pathway of the active ingredient (AI) of an insecticide: One of these is where the AI directly attaches to the insect's body surface and permeates into the body through the cuticular layer; another is where the AI is taken into the insect's body when the insect feeds on treated crops. The first route is referred to as "contact toxicity" and the second one is referred to as "dietary toxicity". A study on the intake pathway of spinetoram was conducted using the common cutworm *Spodoptera litura*. It has been confirmed that spinetoram demonstrates insecticidal activity through both the contact toxicity and the dietary toxicity pathways (**Table 3**). According to this property, it can be assumed that spinetoram can control not only insects which are directly sprayed but also those which feed on treated leaves.

Table 3 Dietary and contact toxicity of spinetoram

Chemicals	LC_{50} (ppm)	
	Dietary toxicity[1)]	Contact toxicity[2)]
spinetoram	1.92	3.65

Pest : Common cutworm (*Spodoptera litura*)
Crop : Cabbage
Method : 1) feeding leaves dipped into diluted solution of spinetoram
2) Insect body dipping

7. Movement in Plant

Depending on the shape of the plant, insecticides can only be readily applied on the upper sides of leaves but can not easily be applied on the undersides of leaves. Therefore, application can be uneven in some cases. However, if the AI has "translaminar activity", which is the ability of the AI to penetrate the leaf cuticle and move into the leaf tissue, it can be expected to show good control efficacy on insect pests even though the insecticide is not applied evenly.

We studied the insecticidal activity of spinetoram against *Spodoptera litura* larvae which were released on the underside of the leaf which was treated with spinetoram on the upper side of the leaf. In this study, spinetoram showed superior insecticidal activity (**Fig. 7**), thus it was confirmed that spinetoram had translaminar activity (**Fig. 7**).

Moreover, if the AI possesses "systemic activity", which is the ability of the AI to penetrate plants and move to various parts of the plant, the plant itself will obtain insecticidal activity. Therefore spinetoram can provide laborsaving control by soil application. In paddy rice, the lepidopteran insect pests are successfully controlled by application of granules over the soil in the nursery boxes.

8. Pre-harvesting Interval (PHI)

Spinetoram has been registered in the Pesticide Registration for use on vegetables, tea trees, orchard trees, paddy rice, flowers and ornamental plants. Its PHI is one day for all target categories under which spinetoram was registered, except for paddy rice and tea trees. In the case of vegetables that are able to be harvested on a daily basis, such as cucumbers, egg plants, tomatoes

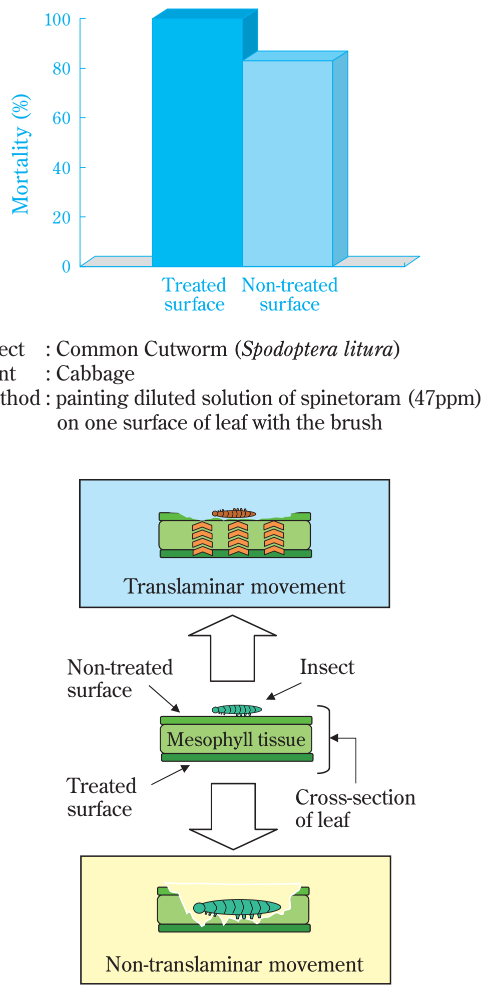


Fig. 7 Translaminar activity of spinetoram on cabbage

and strawberries, these crops often require pest control during the harvest period. Because spinetoram has a short half-life on plant bodies, characteristically it can be used without concern for PHI. Furthermore, we intend to register spinetoram with a PHI of one day for other farm crops (which will be described later).

9. Phytotoxicity

Basically, no phytotoxicity has been observed in the official trials to date, and thus spinetoram's high safety toward various types of crops has been confirmed.

Recommended Application Timing and Efficacy

Based on knowledge regarding the previously described insecticidal properties, we investigated the optimal application timing of spinetoram to achieve the best effects in major crop areas through all stages of spinetoram commercialization from registration application to market launch.

The following describes the result of the study on cabbage which is a major leaf vegetable crop as an example.

1. Relationship of Crop Growth Stage and Insect Infestation Timing

In Japan, major cabbage production districts include the Aichi, Gunma and Chiba prefectures.[7] These cabbages can be generally classified into two types based on the cultivation season. One type is cultivated in highlands and is harvested in summer, and the other type is cultivated in lowlands and is harvested from autumn to winter/spring. We will introduce the relationship between plant growth stage and insect infestation timing, and crop protection by spinetoram, taking cabbages harvested from autumn to winter in lowlands as an example.

On this type of cabbage production, the cultivation model as shown **Fig. 8** is the typical cultivation model for autumn/winter harvest cabbages and various types of insect pests will infest the cabbages according to their growth stages. More specifically, during the period between transplanting through to the growing period, the plants are infested by *Hellulla undalis*, which causes damage to the shoots. However, as growth progresses the plants are infested by *Spodoptera litura* and *Pieris rapae*, which cause damage to leaves. Furthermore, in recent years there has been an increase in the number of reports regarding damage caused by *Thrips tabaci*, which is known as a major pest for welsh onions and onions.

2. Application Timing of Spinetoram to Achieve the Best Effect

The most desirable insecticidal properties of spinetoram vary depending on the crop growth stages.

In the case shown in **Fig. 8**, *Hellulla undalis*, which is considered a major pest during the period from transplanting to the early growing stage, will burrow into the shoots and feed the shoots. Because the shoot has a complex shape, thus making it difficult to apply insecticide to the shoots, it is ideal to spray an insecticide having translarminar activity during this timing. Moreover, because the plant body size is small in this growth stage, if feeding damage is significant it will later affect the growth of the plant. Therefore quick action for suppression of feeding damage is a preferable property.

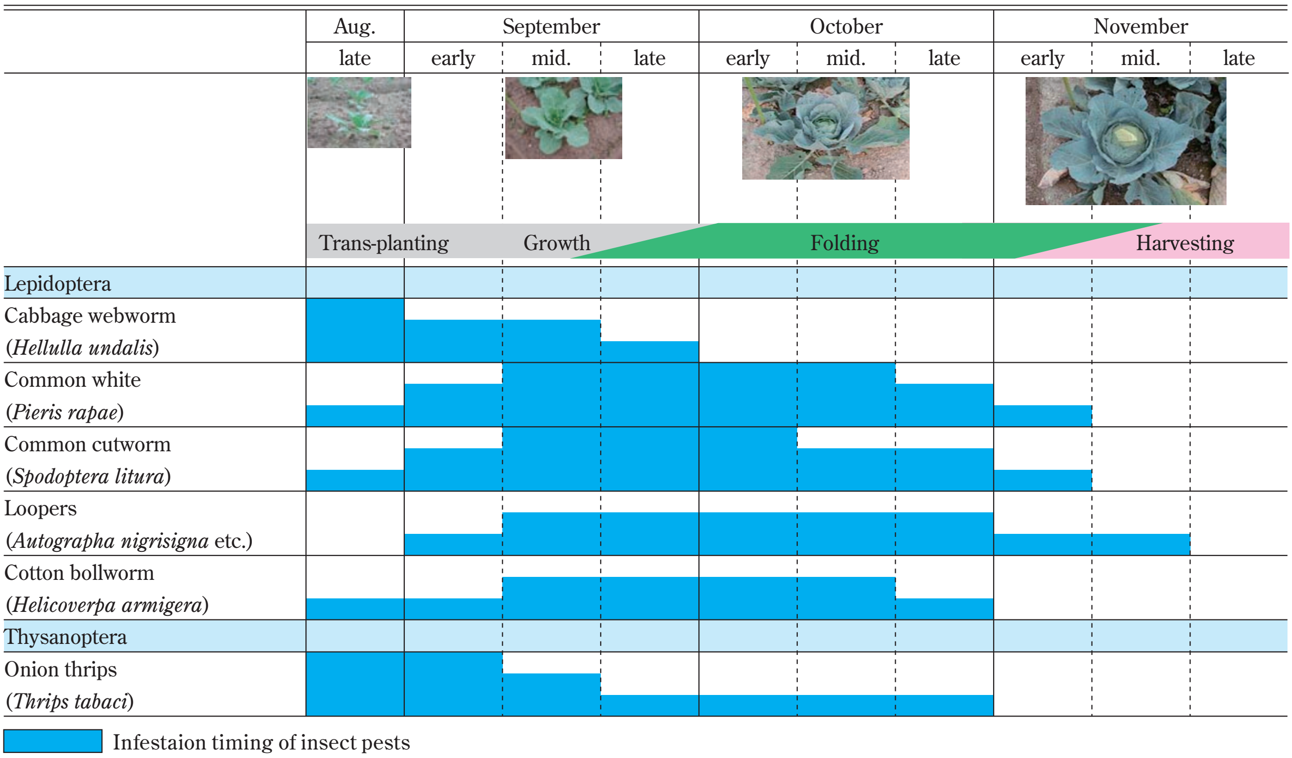


Fig. 8 Infestation timing of insect pests on cabbage in autumn

Similarly, during the period from the beginning until the first half of the heading stage of cabbages, *Pieris rapae* and *Spodoptera litura*, which is are major insect pests during this timing, are relatively large in size and feed a lot of leaves. Therefore the rapid action to suppress feeding damage is a preferable insecticidal property at this timing.

During the period from the second half of the heading stage until harvest time, the insecticide application will not be uniform since intra-row spacing becomes tighter with the enlargement of the head, thus causing thick, grown leaves to overlay each other. It is therefore preferable that insecticides have translaminar activity. And it is also important that the PHI of the insecticide is minimal through the perspective of residue in crops because this time is close to the harvest time.

Spinetoram has all of the preferable insecticidal properties for each crop growth stage as mentioned above. Therefore it is assumed that spinetoram can be used at any growth stage from transplanting until harvesting. However, it is desirable to apply spinetoram during a single cropping period when its properties can be most effectively utilized, because the same insecticide can be used only once or twice in a single cropping period based on the perspective of avoiding continuous applications of one insecticide to prevent resistance development. It is assumed that spinetoram would possess its greatest effect during the period in which lepidopteran insects and thrips infest on cabbage concurrently. We therefore evaluated the efficacy of spinetoram on *Hellulla undalis* and *Thrips tabaci* during a period from transplanting through to the early growth stage.

In these studies, it has been confirmed that spinetoram showed an excellent efficacy against *Hellulla undalis*, being equal to or superior to standard agents such as emamectin benzoate, flubendiamide, and chlorantraniliprole (**Fig. 9**). It has also been confirmed to show an excellent efficacy against *Trhips tabaci*, being equal to or superior to standard agents such as phenthoate, acetamiprid, and tolfenpyrad (**Fig. 10**).

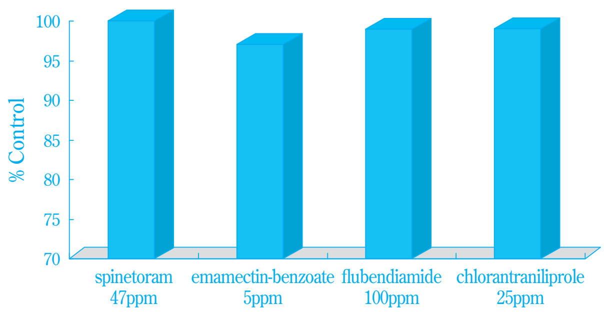


Fig. 9 Efficacy against *Hellulla undalis* on cabbage by foliar spray

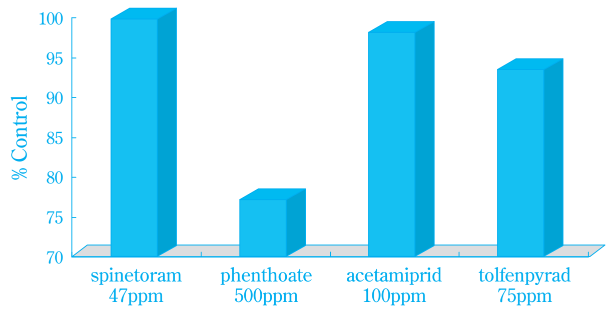


Fig. 10 Efficacy against *Thrips tabaci* on cabbage by foliar spray

Based on the above study results, it was surmised that the application timing to achieve the greatest effects of spinetoram was from the stage of transplanting through to the growing stage for the model shown in **Fig. 8**.

Thus the most effective spinetoram application timing can be determined for each crop type by means of various studies. However, the application timing must be adjusted according to the local district in order to maximize the effect, because the growth stages of crop and insect pest infestation are closely related to the local topography and climate, whereby situations vary according to the districts.

Toxicity/Metabolism/Residue

1. Mammalian Toxicity

(1) Acute Toxicity, Irritation and Skin Sensitization

In the acute oral/dermal/inhalation toxicity studies conducted on the technical grade (TG), 25% water-dispersible granules (WDG), 11.7% water-dispersible granules (SC) and 0.5% granules (GR) of spinetoram, no mortality or severe toxic symptoms were observed even at high dose levels, thus showing weak acute toxicity in all formulations. Minimal or mild eye irritation was observed in all formulations. No skin irritation was observed in spinetoram TG, and minimal skin irritation was observed in spinetoram 25%WDG, 11.7%SC and 0.5%GR. Although spinetoram TG demonstrated weak skin sensitization in the LLNA (Local Lymph Node Assay), the results of the LLNA or Buehler tests were negative for 25%WDG, 11.7%SC and 0.5%GR (**Table 4**).

(2) Subacute, Chronic Toxicity and Carcinogenicity

In the results of subacute and chronic toxicity and carcinogenicity studies using rats, mice and dogs (**Table 5**), when spinetoram TG was repeatedly administered, slight vacuolation and the aggregates of

Table 4 Acute toxicity summary of spinetoram

	spinetoram	spinetoram 25%WDG	spinetoram 11.7%SC	spinetoram 0.5%GR
Test type				
Acute oral (LD_{50})	> 5000 mg/kg (rat)	> 5000 mg/kg (rat)	> 5000 mg/kg (rat)	> 2000 mg/kg (rat)
Acute dermal (LD_{50})	> 5000 mg/kg (rat)	> 5000 mg/kg (rat)	> 5000 mg/kg (rat)	> 2000 mg/kg (rat)
Inhalation (LC_{50})	5500 mg/m^3 of air (rat) (4-hour, nose only exposure)	–	–	–
Eye irritation	Minimally irritant (rabbit)	Mildly irritant (rabbit)	Mildly irritant (rabbit)	Minimally irritant (rabbit)
Skin irritation	Non-irritant (rabbit)	Minimally irritant (rabbit)	Minimally irritant (rabbit)	Minimally irritant (rabbit)
Skin sensitization	Weak sensitizer (mouse)	Non-sensitizer (mouse)	Non-sensitizer (mouse)	Non-sensitizer (guinea pig)

Table 5 Subacute and chronic toxicity summary of spinetoram

Species	Administration route and duration	Dose (ppm)	NOAEL (mg/kg/day)
Rat	Oral (in diet), 13 weeks	120, 500, 1000, 2000, 4000	Male: 32.4 (500ppm) Female: 9.5 (120ppm)
Rat	Oral (in diet), 24 months	50, 250, 500, 750	Male: 10.8 (250ppm) Female: 13.2 (250ppm) No carcinogenicity
Dog	Oral (in diet), 13 weeks	150, 300, 900	Male: 5.73 (150ppm) Female: 4.97 (150ppm)
Dog	Oral (in diet), 12 months	50, 100, 200	Male: 2.96 (100ppm) Female: 2.49 (100ppm)
Mouse	Oral (in diet), 18 months	25, 80, 150, 300	Male: 18.8 (150ppm) Female: 23.9 (150ppm) No carcinogenicity

Table 6 Developmental and reproductive toxicity summary of spinetoram

Study	Species	Administration route and duration	Dose (mg/kg/day)	NOAEL (mg/kg/day)	
Developmental toxicity	Rat	Oral (gavage) Days 6-20 of gestation	30, 100, 300	Maternal	Systemic NOAEL: 100 Developmental NOAEL: 300
				Fetal	300
	Rabbit	Oral (gavage) Days 7-27 of gestation	2.5, 10, 60	Maternal	Systemic NOAEL: 10 Developmental NOAEL: 60
				Fetal	60
Two-generation reproductive toxicity	Rat	Oral (in diet)	3, 10, 75	Parental	Systemic NOAEL: 10 Reproductive NOAEL: 10
				Offsprings	Systemic NOAEL: 10

Table 7 Neurotoxicity summary of spinetoram

Species	Administration route and duration	Dose	NOAEL (mg/kg/day)
Rat	Acute oral (gavage)	200, 630, 2000 (mg/kg/day)	>2000 mg/kg/day
Rat	Oral (in diet), 12 months	50, 250, 500, 750 (ppm)	Male: 36.7 (750ppm) Female: 44.3 (750ppm)

macrophages-histiocytes, which are considered to be phospholipidosis of a low toxicological significance, were observed in multiple tissues such as lymph nodes. It is generally considered that there is a low possibility that the vacuolation caused by phospholipidosis would induce cell dysfunction. It was therefore concluded that these aggregates did not indicate a loss of cell or tissue function. Other than those findings, skeletal muscle degeneration, retinal degeneration, anemia and arteritis were observed. Skeletal muscle degeneration was a localized slight change observed at high dose, and it did not affect motor function. Moreover, retinal degeneration was a change observed at high dose, but neither did it show any effect that would suggest dysfunction. Anemia was accompanied by regenerative changes, but it did not cause deterioration of general conditions. Arteritis seen in dogs was not a severe change that could cause deterioration in their general condition, and the change was spontaneously observed in experimental animals. Furthermore, carcinogenicity was seen neither in rats nor mice.

(3) Reproductive and Developmental Toxicity

In rat and rabbit teratogenicity studies, no teratogenicity was observed in fetuses. In rat two-generation reproductive toxicity study, abnormal parturition, decreased viability rate, increased post implantation loss and decreased body weight were observed in offspring. These findings were noted at the dose levels at which maternal toxicity was observed. The no-observed-adverse-effect level (NOAEL) was 10 mg/kg/day for both general toxicity and reproductive toxicity (**Table 6**).

(4) Neurotoxicity

From the results of acute neurotoxicity study and one-year chronic neurotoxicity study, no specific neurotoxic effect was observed (**Table 7**).

(5) Genotoxicity

The results of the following studies turned out to be negative (**Table 8**): a reverse mutation assay using *Salmonella typhimurium* and *Escherichia coli*; a gene mutation assay using Chinese hamster ovary cells; an *in vitro* chromosomal aberration assay using rat lymphocytes; and a micronucleus assay using mice.

Table 8 Mutagenicity summary of spinetoram

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA100, TA1535 and TA1537 −/+S9 mix: 1.00 – 5000 µg/plate *E. coli* WP2uvrA −/+S9 mix: 33.3 – 5000 µg/plate	Negative
Gene mutation	Chinese hamster ovary cells −/+S9 mix: 10 – 320 µg/mL	Negative
In vitro chromosomal aberration	Rat lymphocytes −/+S9 mix: 10 – 80 µg/mL	Negative
Micronucleus	CD-1 mice 500, 1000, 2000 mg/kg	Negative

2. Metabolism in Animals and Plants

(1) Metabolism in Animals

^{14}C-labeled spinetoram-J and -L were rapidly absorbed after oral administration to rats and distributed into tissues. They were rapidly metabolized, and most of ^{14}C was excreted into feces, thus showing no residual/accumulative properties. The major metabolic pathway of spinetoram-J and -L is a glutathione conjugation of the parent compound, and glutathione conjugation of the metabolites—which was caused by *N*-demethylation, *O*-deethylation, deglycosylation and hydroxylation of the parent compound—was also observed. The oral absorption of spinetoram-J and -L in rats was 71% and 75%, respectively.

(2) Metabolism in Plants

Plant metabolism studies using the ^{14}C-labeled spinetoram-J and -L demonstrated that their metabolic pathways were almost the same in four different types of crops (lettuce, radish, apple and paddy rice), *via* *N*-demethylation, *N*-formylation and cleavage or opening of the macrolide ring system.

3. Environmental Fate and Residue

(1) Degradation in Water

^{14}C-labeled spinetoram-J and -L were stable in buffer solutions at pH 5 and 7, while they gradually degraded *via* *N*-demethylation at pH 9. The degradation half-life of spinetoram-J could not be accurately calculated due to its slow degradation, but that of spinetoram-L (25°C) was estimated to be 154 days at pH 9. Moreover, the degradation of the ^{14}C-labeled spinetoram in the buffer solution (pH 7) and natural water (pH 8.5) was significantly accelerated by illumination, leading to the formation of numerous polar degradation products *via* *N*-demethylation, elimination of the forosamine moiety and cleavage of the macrolide ring system. The estimated photolytic half-lives under natural sunlight at Tokyo in spring were 2.2 and 0.94 days for spinetoram-J in the buffer solution and natural water, respectively, and those of spinetoram-L were 0.99 and 0.50 days.

(2) Metabolism in Soil

^{14}C-labeled spinetoram-J and -L were aerobically degraded in flooded soil with half-lives (25°C) of 193 and 456 days, respectively. They were metabolized *via* *N*-demethylation, and eventually decomposed and were firmly bounded by the soil residues and mineralized to CO_2. In aerobic soil as well, the ^{14}C-labeled spinetoram was metabolized through a similar pathway, and the half-lives (25°C) ranged from 8 to 29 days (spinetoram-J) and 3 to 17 days (spinetoram-L). Furthermore, the degradation of the ^{14}C-labeled spinetoram on the soil surface was accelerated by illumination, and the photodegradation half-lives were estimated to be 116 days (spinetoram-J) and 18 days (spinetoram-L) under natural sunlight at north latitude 40 degrees in summer.

(3) Field Dissipation

Aquatic field dissipation studies were conducted in two paddy fields with soil texture (origin) of light clay (volcanic ash soil) and sandy clay loam (residual soil). The fields were treated once with spinetoram 0.5%GR at the rate of 5 kg/10a. The dissipation half-lives were estimated to be 1 to 95 days with the maximum residue concentrations of 0.441 to 1.35 mg/kg.

Terrestrial field dissipation studies were conducted by applying a 1000-fold diluted solution of spinetoram 11.7%SC twice at a rate of 300 L/10a onto two upland fields with soil texture (origin) of light clay (volcanic ash soil) and sand (aeolian soil). The dissipation half-lives were estimated to be 9 to 14 days with the maximum residue concentrations of 0.394 to 0.576 mg/kg.

(4) Mobility in Soil

The mean soil adsorption coefficients (K_{Foc} (ads)) normalized with the soil organic carbon content were calculated for spinetoram, using the Freundlich adsorption isotherm, to be 2290 mL/g (spinetoram-J) and 2690 mL/g (spinetoram-L), which suggested their low mobility in soils.

(5) Paddy Field Lysimeter

A paddy field lysimeter study was conducted by transplanting rice seedlings with a single treatment of spinetoram 0.5%GR at the rate of 50 g/box (1 kg/10a) in nursery boxes. The residue concentrations in the surface water and leachate were less than the limit of quantification (0.0010 mg/L) throughout the study.

(6) Residue in Crops

Residue trials for tea plants were conducted with a single application of 2500-fold diluted solution of spinetoram 11.7%SC at the rate of 300 L/10a, and the maximum mean residue was 1.26 ppm.

Residue trials were conducted for tomatoes, cherry tomatoes, eggplants, cabbages, lettuces, leaf lettuces, boston lettuces, welsh onions, and strawberries. The same diluted formulation as that in tea residue trials was applied twice with a seven-day interval at the rate of 200 - 300 L/10a to the six crops. The maximum mean residues ranged from 0.05 to 4.30 ppm.

Residue trials for apples, Japanese pears and peaches were conducted with two applications of 5000-fold diluted formulation of spinetoram 25%WDG with a seven-day interval at the rate of 300 - 500 L/10a. The maximum mean residues ranged from less than the limit of quantification (0.02 ppm) to 0.14 ppm.

Residue trials for rice were conducted with a single treatment of spinetoram 0.5%GR to nursery boxes at the rate of 50 g/box (1 kg/10a). The residue in rice grain and straw were less than the limit of quantification (0.02 ppm).

(7) Residues in Rotational Crops

One filed rotational crop study was conducted by cultivating Japanese radishes and wheat as rotational crops in fields where rice plants were cultivated as a primary crop. Spinetoram 0.5%GR was applied to rice seedlings in nursery boxes at the rate of 50 g/box (1 kg/10a) and then, the seedlings were transplanted into the fields. The residues in both rotational crops were less than the limit of quantification (0.02 ppm).

Another field rotational crop study was conducted by cultivating turnips and cucumbers as rotational crops in fields after the cultivation of tomatoes treated twice with 2500-fold diluted formulation of spinetoram 11.7%SC at the rate of 300 L/10a with a seven-day interval. The residues in both rotational crops were less than the limit of quantification (0.02 ppm).

4. Effects on Non-Target Organisms

Table 9 summarizes the results of ecotoxicological studies on aquatic organisms, honeybees, silkworms, natural enemy insects and birds.

(1) Effects on Aquatic Organisms

The acute toxicity values (LC_{50}/EC_{50}) of spinetoram TG in carp, *Daphnia magna* and freshwater green algae were 3.9, >3.17 and 1.060 mg/L, respectively. Furthermore, the corresponding toxicity values of spinetoram 25%WDG in these aquatic organisms were 24, >24 and 19 mg/L, respectively. The toxicity values of spinetoram 11.7%SC in these aquatic organisms were 100, >54 and 530 mg/L, respectively. The toxicity values of spinetoram 0.5%GR in these aquatic organisms were >1000, >1000 and >1000 mg/L, respectively.

Table 9 Eco-toxicological summary of spinetoram on non-target organisms

Test substance	Test species		Test type	Results
spinetoram	Aquatic organisms	Carp	Acute (96 hr)	LC_{50} = 3.9 mg/L
		Daphnia magna	Acute (48 hr)	EC_{50} > 3.17 mg/L
		Green alga*1	Acute (72 hr)	E_rC_{50} = 1.060 mg/L
	Honeybee	*Apis mellifera*	Acute contact (48 hr)	LD_{50} = 24.8 ng/bee
	Bird	Bobwhite quail	Acute oral	LD_{50} > 2250 mg/kg
spinetoram 25%WDG	Aquatic organisms	Carp	Acute (96 hr)	LC_{50} = 24 mg/L
		Daphnia magna	Acute (48 hr)	EC_{50} > 24 mg/L
		Green alga*1	Acute (72 hr)	E_rC_{50} = 19 mg/L
	Honeybee	*Apis mellifera*	Residual toxicity test*2	≤ 3 days
	Silkworm	*Bombyx mori*	Residual toxicity test*3	≤ 31 days
spinetoram 11.7%SC	Aquatic organisms	Carp	Acute (96 hr)	LC_{50} = 100 mg/L
		Daphnia magna	Acute (48 hr)	EC_{50} > 54 mg/L
		Green alga*1	Acute (72 hr)	E_rC_{50} = 530 mg/L
	Honeybee	*Apis mellifera*	Residual toxicity test*4	≤ 7 days
	Natural enemy insects	*Paederus fuscipes* (adult)	Acute contact (48 hr)	mortality 0% (at 50 mg a.i./L)
		Harmonia axyridis (larvae)	Acute contact (48 hr)	mortality 3.3% (at 50 mg a.i./L)
		Chrysoperla carnea (larvae)	Acute contact (96 hr)	mortality 10% (at 50 mg a.i./L)
spinetoram 0.5%GR	Aquatic organisms	Carp	Acute (96 hr)	LC_{50} > 1000 mg/L
		Daphnia magna	Acute (48 hr)	EC_{50} > 1000 mg/L
		Green alga*1	Acute (72 hr)	E_rC_{50} > 1000 mg/L

*1: *Pseudokirchneriella subcapitata*
*2: Potted *Catharanthus roseus* plant sprayed with 50 mg a.i./L solution and aged under outdoor condition
*3: Mulberry plant sprayed with 50 mg a.i./L solution
*4: Potted strawberry plant sprayed with 50 mg a.i./L solution and aged in greenhouse

Those values were significantly higher than the predicted environmental concentrations, which suggested no significant effects of spinetoram on aquatic organisms in practical use.

(2) Effects on Honeybees, Silkworms and Natural Enemy Insects

The acute contact toxicity value (LD_{50}) of spinetoram TG on honeybees (*Apis mellifera*) was 24.8 ng/bee, originating from its insecticidal activity. However, the durations of residual toxicity were short, ranging from 3 to 7 days according to residual toxicity tests, supposing the possible exposure in realistic field conditions, which were conducted using plants sprayed with the spinetoram formulations by the foliage treatment method. The duration of residual toxicity of the spinetoram formulation on silkworms was 31 days or less in the residual toxicity tests. The mortality of natural enemy insects, *Paederus fuscipes* (adults), *Harmonia axyridis* (larvae) and *Chrysoperla carnea* (larvae), ranged from 0 to 10% in the acute toxicity tests. Based on these results, no significant effects of spinetoram on honeybees, silkworms and natural enemy insects are considered during practical use.

(3) Effects on Birds

The acute oral toxicity of spinetoram TG to bobwhite quail was weak at an LD_{50} value of >2250 mg/kg.

Table 10 Domestic registration of spinetoram 11.7% SC

Target Crops	Target Pests	Dilution rate	Spray volume (L/10a)	PHI*1	Maximum number of applications*2	Application method
Tomato Grape tomato	Common cutworm	2500 ~ 5000	100 ~ 300	1 day	2	Foliar spray
	Cotton bollworm					
	Leafminer flies					
	Whiteflies	2500				
	Thrips					
Egg plant	Whiteflies					
	Common cutworm	2500~5000				
	Cotton bollworm					
	Thrips					
	Leafminer flies					
Cabbage	Diamondback moth					
	Common white					
	Cabbage webworm					
	Common cutworm					
	Cabbage armyworm					
	Loopers					
	Cotton bollworm					
	Thrips					
Welsh onion	Thrips					
	Beet armyworm					
	Stone leek leafminer					
Lettuce Leaf lettuce	Leafminer flies					
	Cotton bollworm					
	Common cutworm					
Strawberry	Thrips					
	Common cutworm					
Tea	Yellow tea thrips		200 ~ 400	7 day	1	
	Smaller tea tortrix					
	Oriental tea tortrix					
	Tea leafroller					
	Mugwort looper					
	Camellia spiny whitefly					
Chrysanthemum	Thrips		100 ~ 300	—	2	
	Cotton bollworm					
	Leafminer flies					
Ornamentals (not included chrysanthemum)	Cotton bollworm					
	Leafminer flies					

Registration situation as of February 22nd, 2012
*1 Pre-harvesting interval *2 per one cropping period

Table 11 Domestic registration of spinetoram 25% WDG

Target Crops	Target Pests	Dilution rate	Spray volume (L/10a)	PHI*1	Maximum number of applications*2	Application method
Apple	Apple leafminer Fruits moth Tortrix Mugwort looper Woolly worm	5000~10000	200 ~ 700	1 day	2	Foliar spray
Peach	Fruits moth Peach leafminer					
Pear	Tortrix Fruits moth					
	Pear psyllid	5000				

Registration situation as of March 7th, 2012
*1 Pre-harvesting interval *2 per one year

Table 12 Domestic registration of spinetoram 0.5% - clothianidin 0.8% - isotianil 2.0% GR

Target Crops	Target pests & diseases	Application weight	Application timing	Maximum number of applications*	Application method
rice (nursery box)	Blast Bacterial leaf blight Bacterial grain rot Brown spot Rice leaf beetle Planthoppers Green rice leafhopper Rice skipper Green rice caterpillar Rice stem borer Rice leafroller Rice water weevil	50g/box	Three days before transplanting ~ Transplanting	1	Drop granule uniformly from above in a nursery box

Registration situation as of March 7th, 2012
* per one cropping preiod

Accordingly, no significant effects of spinetoram on birds are considered during practical use.

Based on the above test results, one can surmise that spinetoram has low acute toxicity toward mammals. Thus it does not have any adverse effects on the next generation such as carcinogenicity and teratogenicity, nor does it have an adverse effect on fertility, even if it is taken for a long period of time.

Furthermore, based on the environmental fate and the effects on non-target organisms, the usage of spinetoram is unlikely to pose an unacceptable risk to the environment.

Registration Details

Tables 10, 11 and **12** show the details of spinetoram registration (as of March 7, 2012).

We are planning to expand the range of application of spinetoram to green peppers, Japanese radishes, Chinese cabbages, broccolis, Japanese mustard spinaches, cauliflowers, melons, cucumbers, onions, asparaguses, grapes, citrus fruits, yellow peaches, Japanese plums, nectarines, and blueberries. We also plan to expand the scope of target insect pests.

Conclusion

Because spinetoram is effective against a broad range of insect pests, including thrips, leafminer flies and whiteflies in addition to lepidopteran insects, it can effectively control multiple numbers of important insect pests that coexist in crop production sites. Furthermore, because the PHI of spinetoram is minimal and it quickly prevents damages to crops, it is easy for farmers to use this insecticide. Thus we expect that spinetoram will make a great contribution to the stable production and quality improvement of agricultural products.

For the future dissemination of spinetoram, we will continue to propose its effective applications suitable for insect pest control situations of each district based on its insecticidal properties.

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