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GENERAL CHARACTERISTICS OF FENPYRAZAMINE, A NOVEL FUNGICIDAL COMPOUND FOR CONTROLLING GRAY MOLD

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RESUMÉ

Fenpyrazamine est une nouvelle substance active fongicide découverte par Sumitomo Chemical Co., Ltd. Elle fait preuve d'un bon profil toxicologique et d'une très faible toxicité sur de nombreux auxiliaires. Elle possède une activité fongicide sur de nombreux micromycètes *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Monilia fructicola* et *Pseudocercosporella herpotrichoides*, grâce à l'inhibition de leur croissance mycélienne. La résistance croisée observée de la fenpyrazamine et du fenhexamid suggère que les deux substances partagent le même mode d'action. La caractérisation de l'efficacité de la fenpyrazamine sur *B. cinerea* a été réalisée sur concombre et sur vigne. Elle a permis de montrer que, outre une bonne efficacité préventive, elle avait une bonne activité translaminaire, et inhibait le développement des lésions. Ainsi la fenpyrazamine fait preuve d'une bonne efficacité dans des conditions très variées.

Mots-clés : fenpyrazamine, *Botrytis cinerea*, fongicide, caractéristiques, mode d'action

SUMMARY

Fenpyrazamine is a novel fungicidal compound discovered and developed by Sumitomo Chemical Co., Ltd. Antifungal activity was tested and resulted in strong inhibition on mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Monilia fructicola* and *Pseudocercosporella herpotrichoides*. Fenpyrazamine showed cross-resistance with fenhexamid, and it suggests fenpyrazamine has the same mode of action as fenhexamid. Efficacy characteristics of fenpyrazamine were evaluated against cucumber or grapevines gray mold, and fenpyrazamine showed not only high preventive efficacy but also translaminar activity, inhibition of lesion development activity. It suggests fenpyrazamine provides high efficacy under various conditions. Furthermore, fenpyrazamine showed good toxicological

profiles and low adverse effect on various beneficials. It is concluded that fenpyrazamine will provide a new option for controlling gray mold with good toxicological and efficacy characteristics

Key words: fenpyrazamine, *Botrytis cinerea*, fungicide, caractéristics, mode of action.

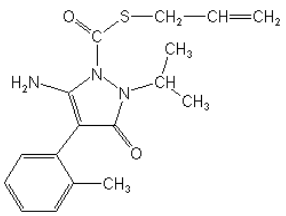
INTRODUCTION

Gray mold, caused by *Botrytis cinerea*, is not only a destructive disease in various crops, but also depredates the quality of the harvest. For controlling the disease, many fungicides are developed and provide excellent control, and the farmers implement programs of treatment with an alternation of mode of action to limit the selection of resistant strains and also limit the depreciation of harvest quality. Furthermore, safety for environment and non-target organisms is required for today's agrochemicals.

Fenpyrazamine is a novel fungicidal compound discovered and developed by Sumitomo Chemical Co., Ltd. and shows strong activity against *B. cinerea*. In this article, physicochemical properties, toxicological profiles, mode of action and efficacy characteristics of fenpyrazamine are described.

Physical and chemical properties of fenpyrazamine (Table I)

Table I: Physical and chemical Properties of fenpyrazamine

Code name	S-2188
Chemical name （IUPAC）	S-allyl 5-amino-2-isopropyl-4-(2-methylphenyl)-3-oxo-2,3-dihydropyrazole-1-carbothioate
Chemical structure	
Molecular formula	C17H21N3O2S
Molecular weight	331.43 g/mol
Physical state	Solid （at 25 °C）
Melting point	116.4 °C
Vapor pressure	2.89×10-8 Pa （at 25 °C）
Octanol-water	3.52 （at 25 °C）

partition coefficient	
Water solubility	20.4 mg/L （at 20 °C）
Adsorption coefficient	KFoc(ads) 112 - 731 ml/g

Toxicological profiles of fenpyrazamine 50 WG (Table II and III)

Table II: Toxicological profile

Acute oral LD50	Rat	>2000 mg/kg
Acute dermal LD50	Rat	>2000 mg/kg
Acute inhalation LD50	Rat	>4.84 mg/L
Eye irritation	Rabbit	No irritant
Skin irritation	Rabbit	No irritant
Mutagenicity		No mutagenic potential (Ames test)
Teratogenicity	Rat	Not a teratogen
Carcinogenicity	Rat	Not a carcinogenic

Table III: Eco-toxicological profile

Acute single oral LD50	Bobwhite quail (C. virginianus)	> 2000 mg a.i./kg
Short-term (8-day) dietary LC50	Bobwhite quail (C. virginianus)	> 5,000 mg a.i./kg diet
Short-term (10-day) dietary LC50	Mallard Duck (A. platyrhynchos)	> 5,000 mg a.i./kg diet
Acute toxicity (14 day) LC50	Earthworm (Eisenia foetida)	> 800 mg a.i./kg soil
Acute oral LD50	Honey bee (Apis ellifera)	> 100 µg a.i./a bee

Toxicity to beneficials of fenpyrazamine (Table IV and V)

To confirm the absence of impact on beneficials, a number of studies were carried on several crops. On protected vegetables, it is a specifically important point, as beneficial are regularly introduced in the greenhouse to control natural enemies like white flies, thrips, mites,…The potential impact of fenpyrazamine 50WG on beneficials has been evaluated through two different types of studies :

- residual effect of fenpyrazamine 50WG on the parasitic wasps *Encarsia formosa* and *Eretmocerus eremicus*
- direct contact effect on *Chrysoperla carnea*, *Macrolophus caliginosus*, *Nesidiocoris tenuis, Orius laevigatus*, *Encarsia formosa* and *Eretmocerus eremicus*

Fenpyrazamine 50WG was tested at 0.24% concentration, which is the maximum concentration that could be used in the field (1.2kg in 500 liters). At such a rate, fenpyrazamine 50WG was classified in OILB category 1.

Table IV: Impact on beneficials

Tested organisms	**fenpyrazamine 50WG 0.24%**	**Bifenthrine SC 0.1%**
Chrysoperla carnea	1	4
Encarsia formosa	1	4
Eretmocerus eremicus	1	4
Macrolophus caliginosus	1	4
Nesidiocoris tenuis	1	4
Orius laevigatus	1	4

Category 1: <30 % inhibition

Category 2: 30-79 % inhibition

Category 3: 80-99% inhibition

Category 4: >99 % inhibition

On grape, the impact of one application of fenpyrazamine 50WG at 600 g a.i. /ha on *Kampimodromus aberrans* was tested. The toxic reference is deltamethrine 1.5EW used at 18 g a.i. /ha. Assessments on the number of mobile forms (on 25 leaves) are done before the application of the products, and then 1-2-3 weeks after the application and up to 2 months after application. The residual population is the ratio (x100) of number of mobile forms on treated / untreated. The products are then classified as Non Toxic (NT), Moderately Toxic (MT) or Toxic (T) at different times after the application, according to the residual population (>60%, 30-59% or <30%)

Fenpyrazamine 50WG was classified as NT all over the trial. The population of predatory mites has increased on the plots treated with fenpyrazamine 50WG in the same proportion as on the control plots. Fenpyrazamine 50WG can be classified as Neutral on *Kampimodromus aberrans* (Table V).

Table V: Impact of one application of fenpyrazamine 50WG at 600 g a.i. /ha on the population of *Kampimodromus aberrans*

Treatment	**Assessment timing**	**No. of mobile forms**	**% reduction**	**Residual population**	**Classification**
Control	0DAA	41.8			
fenpyrazamine 50WG		41.8			
delthamethrin 1.5EW		41.8			
Control	7DAA	26.8			
fenpyrazamine 50WG		35.6	-32.8	132.8	NT
delthamethrin 1.5EW		9.4	64.9	35.1	MT
Control	14DAA	40.2			
fenpyrazamine 50WG		62.4	-55.2	155.2	NT
delthamethrin 1.5EW		9.4	76.6	23.4	T
Control	21DAA	65.6			
fenpyrazamine 50WG		73.6	-12.2	112.2	NT
delthamethrin 1.5EW		6.6	89.9	10.1	T
Control	45DAA	133.6			
fenpyrazamine 50WG		108.6	18.7	81.3	NT
delthamethrin 1.5EW		10.8	91.9	8.1	T
Control	60DAA	150.4			
fenpyrazamine 50WG		211.8	-40.8	140.8	NT
delthamethrin 1.5EW		19.8	86.8	13.2	T

Antifungal activity of fenpyrazamine

Inhibition on mycelial growth of plant pathogenic fungi was tested. Fenpyrazamine has strong antifungal activity against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Pseudocercosporella herpotrichoides* and *Monilia fructicola* .On the other hand, efficacies of fenpyrazamine against other genus fungi were not so high (Table VI). Fenpyrazamine also has a strong antifungal activity against a number of *Botrytis*, *Scletorinia* and *Monilia* spp. (Table VII).

Table VI: Antifungal activity of fenpyrazamine against various plant pathogens

Tested pathogen	EC50 (ppm)	Tested pathogen	EC50 (ppm)
Botrytis cinerea	0.03	*Sclerotinia sclerotiorum*	0.11
Monilia fructicola	0.02	*Pseudocercosporella herpotrichoides*	0.002
Phytophthora capsici	> 50	*Pythium aphanidermatum*	> 50
Fusarium oxysporum f. sp. *lycopersici*	> 50	*Colletotrichum lagenarium*	> 50
Phoma betae	2.4	*Corticium rolfsii*	31.1
Mycosphaerella melonis	5	*Ustilago nuda*	> 50
Alternaria alternata apple pathotype	11.4	*Rhizoctonia solani*	> 50

Table VII: Antifungal activity of fenpyrazamine against various *Botrytis*, *Sclerotinia* and *Monilia* spp.

Tested pathogen	EC50 (ppm)	EC90 (ppm)
Botrytis allii	0.03	0.67
Botrytis byssoidea	0.01	0.05
Botrytis elliptica	0.09	1.75
Botrytis paeoniae	0.02	0.05
Botrytis tulipae	0.03	0.67
Sclerotinia minor	0.05	0.25
Sclerotinia trifoliorum	0.01	0.04
Monilia kusanoi	0.04	0.19
Monilia mume	0.01	0.06

***In vivo* preliminary tests**

Preventive efficacy of fenpyrazamine was evaluated with pot test; on the following diseases; cucumber (gray mold and powdery mildew), wheat (eyespot, leaf blotch, brown rust and powdery mildew), rice (blast), tomato (late blight). Seedlings of each crop were sprayed with fenpyrazamine at 200 ppm and inoculated with a pathogen. Assessment was conducted several days after inoculation. Fenpyrazamine showed strong preventive efficacy against gray mold on cucumber and eyespot on wheat. Preventive efficacy of fenpyrazamine against the other diseases was not so high.

Table VIII: Preliminary efficacy tests with fenpyrazamine on seedlings.

	Cucumber			Wheat				Rice		Tomato
Dosage (ppm)	Botrytris	Powdery mildew	Eyespot	Powdery mildew	Fusarium	Septoria	Brown Rust	Blast	Shelth blight	Late blight
200	5	0	5	1	0	0	0	0	0	0
50	5	0	5	0	0	0	0	0	0	0
12.5	4	0	4	0	0	0	0	0	0	0

Index for assessment:

5 = 100% disease

4 = 99-90% disease

3 = 89-70% disease

2 = 69-50% disease

1 = 49-25% disease

0 = 24-0% disease

Mode of action

No germination of conidia of *Botrytis cinerea* was affected by fenpyrazamine. However, germ tube elongation was strongly inhibited even at low concentration (Table IX). And fenpyrazamine treated germ tubes showed morphological change such as swelling, which is similar to that treated with ergo-sterol biosynthesis inhibitors. Unsaponifiable lipids of *B. cinerea* incubated in presence of fenpyrazamine were analyzed by gas chromatography. The chromatographic properties were different from those from tebuconazole-treated *B. cinerea* which indicate a mode of action different from tebuconazole one's. Cross-resistance test was conducted, and fenpyrazamine showed no cross-resistance with benzimidazole, dicarboximide, carboxamide, and strobirulin fungicides, although showed cross-resistance with a hydroxyanilides fungicide, fenhexamid. Accordingly, fenpyrazamine is supposed to have the same target enzyme with fenhexamid. Further tests for elucidating this hypothesis are now on progress.

Table IX: Inhibition on germ tube elongation and spore germination of *Botrytis cinerea*

	fenpyrazamine (ppm)		
	0.1	0.2	0.5
Germ tube elongation(1)	±	+	+
Spore germination(2)	-	-	-

-: 0-50% Inhibition, ±: 51-75 %inhibition, +: 76-100% inhibition

(1)Inhibition was based on germ tube length of treated and untreated conidia after incubation on PDA for 18 hours at 18 °C.

(2)Inhibition was based on germination rate of treated and untreated conidia after incubation on PDA for 18 hours at 18 °C.

Efficacy characteristics in pot trials against *Botrytis cinerea*

Preventive efficacy, translaminar activity and inhibition of lesion development activity were assessed against *Botrytis cinerea* on cucumber leaves. Moreover, rainfastness was assessed on field grapevines. Fenpyrazamine showed not only high preventive efficacy but also translaminar, inhibition of lesion development activity and rainfastness. It is expected fenpyrazamine shows high efficacy under various conditions.

Preventive efficacy on cucumber plants

Cucumber plants were grown in pots. At about 4 leaf-stage, plants were sprayed with different doses of fungicides (from 0.8 to 50g ai/ha) at the equivalent volume of 1000 l/ha. Treated leaves were inoculated 24 hours after the fungicide spray, with a piece of PDA (0.9% agar) gel containing *Botrytis* spores. Plants were allowed to grow for 5 days at 15°C under high humidity. The radius of the lesion was then measured and the efficacy calculated.

Fenpyrazamine showed an excellent preventive efficacy on gray mold, equal or better than already existing products (Table X).

Table X: Preventive efficacy on cucumber gray mold

Treatment	Dosage (g a.i./ha)	Lesion radius (mm)			% control
		Replication 1	Replication 2	Average	
Fenpyrazamine 50WG	50	0	0	0.0	100
	12.5	0	0	0.0	100
	3.1	0	2	1.0	91.3
	0.8	6.3	6.3	6.3	45.0
fenhexamid 50WG	50	0	0	0.0	100
	12.5	0	0	0.0	100
	3.1	0	0	0.0	100
	0.8	10	10.5	10.3	10.5
boscalid 50WG	50	1.3	1.5	1.4	87.8
	12.5	2.3	2.6	2.5	78.6
	3.1	2.6	3	2.8	75.5
	0.8	7.6	6.5	7.1	38.4
Untreated		11.3	11.6	11.5	

Translaminar activity

The axial surface of cucumber leaf was treated with fenpyrazamine 50WG. One day after treatment, the abaxial surface of the leaf was inoculated by PDA medium containing spores of *Botrytis cinerea*. The same protocol was carried out with boscalid and fenhexamid. Plants were allowed to grow for 7 days at 15°C under high humidity, and the radius of lesion was measured.

We observed 95% disease control with a treatment of fenpyrazamine at 600g a.i. /ha versus 70% for boscalid and 69% for fenhexamid at their registered rate (Table XI).

Table XI: Translaminar activity on cucumber gray mold

Treatment	Dosage (g a.i./ha)	Lesion radius (mm)			% control
		Replication 1	Replication 2	Average	
fenpyrazamine 50WG	600	1	1	1	95.1
	300	1.3	3	2.2	89.5
	100	4.9	4.8	4.9	76.4
fenhexamid 50WG	750	6.3	6.5	6.4	68.9
	375	7	9.3	8.2	60.3
	125	10.3	10.3	10.3	49.9
boscalid 50WG	600	6	6.3	6.2	70.1
	300	7.8	7.3	7.6	63.3
	100	8.3	9.5	8.9	56.7
Untreated	-	19.6	21.5	20.6	

Inhibition of lesion development activity

Inoculation of *Botrytis* spores was conducted at first. After growing for 33 hr at 23 °C, fungicides were treated with spray volume 1000L/ha. Plants were allowed to grow for 5 days at 15°C under high humidity. The radius of the lesion was then measured.

As a result, fenpyrazamine 50WG showed excellent Inhibition of lesion development activity (Table XII).

Table XII: Inhibition of lesion development activity on cucumber gray mold

Treatment	Dosage (g a.i./ha)	Lesion radius (mm)			% control
		Replication 1	Replication 2	Average	
fenpyrazamine 50WG	600	0.75	1	0.88	95.3
	300	0.8	0.9	0.85	95.4
fenhexamid 50WG	750	12.8	11.3	12.1	35.3
Untreated		19.6	17.5	18.6	

On the top of that, fenpyrazamine demonstrated a good rainfastness on vineyards (Armengaud *et al.*, 2012b) and on vegetables (Armengaud *et al.*, 2012a)

Field trial against *Botrytis cinerea* **on grapevines**

Table XIII: Field trial efficacy – 2 treatments – 2008 – 3 trials – CEB method n° 37

Trial		**Trial 1**		**Trial 2**		**Trial 3**			
Application date (first appl.)		22.06.08	22.06.08	25.06.08	25.06.08	18.06.08	18.06.08		
Application date (last appl.)		02.09.08	02.09.08	18.09.08	18.09.08	09.09.08	09.09.08		
Crop GS at last application		GS 69	GS 69	GS 67	GS 67	GS 67	GS 67	Mean of 3 trials	
Crop GS at last application		GS 85	GS 85	GS 85	GS 85	GS 85	GS 85		
Assessment type		Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Target assessed		Bunches	Bunches	Bunches	Bunches	Bunches	Bunches		
Assessment date		22.09.08	22.09.08	10.11.08	10.11.08	31.10.08	31.10.08		
Spray volume (l/ha)		209	209	300	300	125	125		
Treatment	**g a.i./ha**								
Untreated	**-**	[64.8%]	[23.4%]	[81.0%]	[19.95%]	[69.5%]	[13.3%]	[71.8%]	[18.88%]
S-2188 50WG	**600**	64.5%	78.0%	84.6%	80.7%	71.5%	83.2%	73.5%	80.6%
Ref. 1	**1000**	37.0%	54.9%	93.8%	94.0%	62.6%	60.1%	64.5%	69.7%
Ref. 2	**1350**	53.7%	65.8%	87.7%	78.2%	66.9%	70.0%	69.4%	71.3%

S-2188 50 WG demonstrates an efficacy at the level of the references used in such 3 trials in programs with two applications the first one at flowering crop stage and the second one at ripening. S-2188 50 WG is an interesting new tool to be used in program to control gray mold in vineyard (Armengaud *et al.*, 2012b)

CONCLUSION

Fenpyrazamine is a novel fungicidal compound discovered and developed by Sumitomo Chemical Co., Ltd. Antifungal activity was tested and resulted in strong inhibition on mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Monilia fructicola* and *Pseudocercosporella herpotrichoides*. Fenpyrazamine showed cross-resistance with fenhexamid, and it suggests fenpyrazamine has the same mode of action as fenhexamid. Efficacy characteristics of fenpyrazamine were evaluated against cucumber or grapevines gray mold, and fenpyrazamine showed not only high preventive efficacy but also translaminar activity, and inhibition of lesion development activity. Fenpyrazamine provides high efficacy under various conditions on *Botrytis cinerea* in vineyards and vegetables crops. Furthermore, fenpyrazamine showed good toxicological profiles and low adverse effect on various beneficials. S2188 50 WG will be a new interesting tool to preserve the quality of the bunches and the vegetables when it will be used in a program.

In a first step fenpyrazamine will be develop on vine and vegetables to control gray mold. In a second step, we can examine further developments on other crops like orchards.

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