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Development of a novel fungicide, pyraziflumid

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Pyraziflumid is a novel succinate dehydrogenase inhibitor (SDHI) fungicide discovered and developed by Nihon Nohyaku Co., Ltd. It exhibits excellent fungicidal activities against a broad range of plant diseases and has a favorable safety profile for the Integrated Pest Management (IPM) program. This compound was found by researching the unique chemical derivatives, 3-(trifluoromethyl)pyrazine-2-carboxamides, and has good biological properties, such as preventive, residual and curative activity, and rain-fastness. Pyraziflumid was registered and launched in Japan in 2018. It was registered in South Korea in 2018 and is now under development in other countries. This paper describes the discovery, synthesis, biological activity, safety profile and mode of action of pyraziflumid.

Keywords: pyraziflumid, NNF-0721, carboxamide, fungicide, SDHI, IPM.

Introduction

In the global market of agriculture, controlling many kinds of plant diseases is important to maintain and increase crop yields. For protection from fungal diseases, many kinds of fungicides have been developed and have contributed to practical agricultural production. However, there is still much demand for new agrochemical products that have excellent biological activity as well as being safe for mammals and the environment, with the flexibility to fit various crop-cultivation methods.

Pyraziflumid is a novel fungicide discovered and developed by Nihon Nohyaku Co., Ltd. It is an SDHI with a unique chemical structure containing the 3-(trifluoromethyl)pyrazine-2-carboxamide group (Fig. 1) and classified as code 7 by the Fungicide Resistance Action Committee (FRAC). Pyraziflumid shows excellent biological activity against ascomycete and basidiomycete fungi and exhibits good controlling activity against many diseases in the field. Its efficacy was evaluated in official trials of the Japan Plant Protection Association (JPPA) from 2012, code number NNF-0721, and obtained registration in March 2018. Three commercial products were launched, PARADE®20FL for vegetables, PARADE®15FL for fruits crop, and DECIDE®FL for turf in Japan. Furthermore, pyraziflumid was registered in South Korea in 2018 and is under development in other countries.

1. History of Invention

1.1. Discovery of the lead compound

In 1990, BC723 was discovered by Mitsubishi Kasei Corporation. Its mode of action was succinate dehydrogenase inhibition, and it showed fungicidal activity not only against basidiomycetes but also against ascomycetes.[1–3] It was the first SDHI fungicide with practical activity against ascomycete diseases such as gray mold and powdery mildew.

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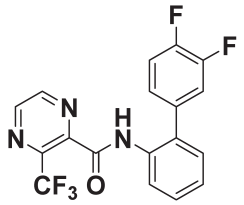

Fig. 1. Chemical structure of pyraziflumid.

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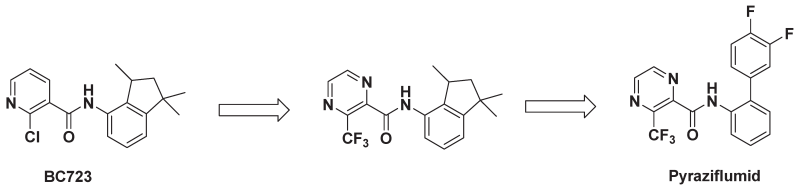
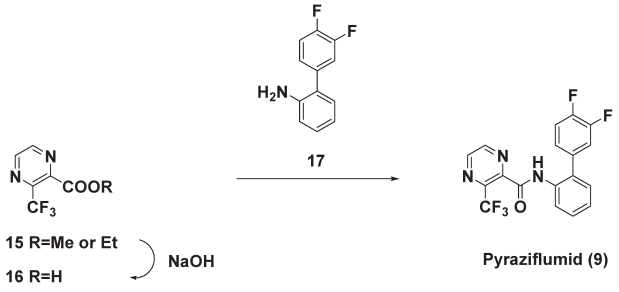


Fig. 2. Discovery of pyraziflumid.

cal Corporation was transferred to Nihon Nohyaku Co., Ltd., in 2002, we began the research to find the next generation of SDHIs that have excellent abilities to control plant diseases and good properties as environmentally friendly agrochemicals. Based on our previous studies on BC723 derivatives,[3] we supposed that 3-(trifluoromethyl)pyrazine-2-carboxamides would have good potential as candidates for a lead compound (Fig. 2). In the course of our new acaricide discovery research, we synthesized 3-(trifluoromethyl)pyrazine-2-carboxylic acid as a novel compound and employed it in synthesizing pyrazine derivatives. We found that 3-(trifluoromethyl)pyrazine-2-carboxamide (**3**) tended to be more active than pyridine carboxamides not only against gray mold and brown rust, but also against powdery mildew as shown in Table 1. This activity enhancement seemed to be caused by the substituent effect of the trifluoromethyl group peculiar to 3-substituted pyrazine-2-carboxamides.[4]

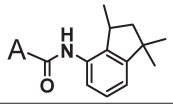
1.2. Structure–activity relationship

It has been reported that some *N*-(biphenyl-2-yl)carboxamides may exhibit biological activities against plant diseases similar to those of *N*-(1,1,3-trimethylindan-4-yl)carboxamides.[5] Therefore, we extended the search range for fungicidal activity to include *N*-(various substituted biphenyl-2-yl)-3-(trifluoromethyl)-pyrazine carboxamides. The fungicidal activities are shown in Table 2. Their structure–activity relationships suggested that the control activity against gray mold might be specifically governed by the substituent effect at the 4′-position (**4**, **5**, **6**), whereas the activity against brown rust could be influenced by the 3′-position substituents (**7**, **8**). The regiospecific substituent effects at the 4′- or 3′-position seemed to be independent and to contribute additively to the activity against gray mold and brown rust (**9**, **10**, **11**, **12**). With respect to the control activity against powdery mildew, a regiospecific effect of the substituents was not observed, but disubstituted derivatives including a fluorine atom at any position exhibited high activity (**9**, **11**, **12**, **13**, **14**). These results on structure–activity relationships through the evaluation of control activity against three diseases demonstrate that *N*-(3′,4′-difluorobiphenyl-2-yl)-3-(trifluoromethyl)pyrazine-2-carboxamide, pyraziflumid (**9**), is the most well-balanced compound as a fungicide from the viewpoint of unique regiospecific substituent effects.[4]



Scheme 1. Synthesis of pyraziflumid (**9**).

Table 1. *N*-(1,2,3-Trimethylindan-4-yl)pyridine- or pyrazine-carboxamides and their biological activities



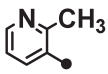
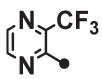
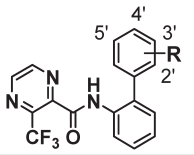
Compound No.	A	mp(°C)	Fungicidal activity EC_{80} (mg a.i./L)		
			Gray mold (Cucumber)	Brown rust (Wheat)	Powdery mildew (Barley)
1		123.5–124.5	20–100	1–10	200
2 (BC723)		133–134	10–40	1–10	200
3		169.1–170.1	10–40	<1	1–10

Table 2. *N*-(various substituted biphenyl-2-yl)-3-(trifluoromethyl)pyrazine-2-carboxamides and their biological activities



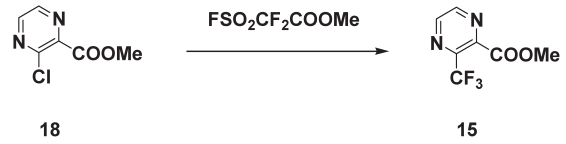
Compound No.	R	mp(°C)	Fungicidal activity EC_{80} (mg a.i./L)		
			Gray mold (Cucumber)	Brown rust (Wheat)	Powdery mildew (Barley)
4	4′-F	133–134	2–10	20–100	2–10
5	4′-Cl	145–146	2–10	2–10	5–20
6	4′-Br	156–157	2–10	20–100	0.5–2
7	3′-F	69–70	10–40	2–10	2–10
8	3′-Cl	110.5–111.5	5–20	0.5–2	2–10
9 (Pyraziflumid)	3′,4′-F_2	119–120	2–10	0.5–2	0.5–2
10	3′,4′-Cl_2	132–132.6	2–10	0.5–2	2–10
11	3′-F,4′-Cl	128–129	2–10	2–10	0.5–2
12	3′-F,4′-CF_3	142.5–143.5	5–20	0.5–2	0.5–2
13	3′,5′-F_2	119–120	5–20	2–10	0.5–2
14	2′,4′-F_2	117–118	2–10	5–20	0.5–2

2. Synthesis

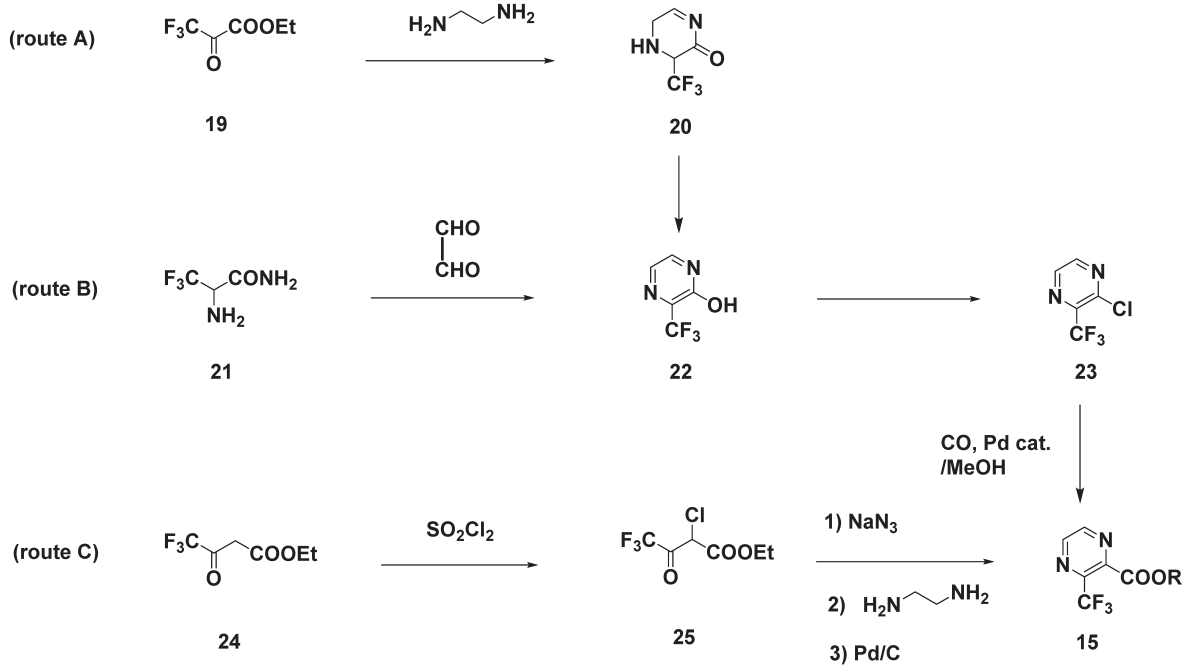
Pyraziflumid can be prepared by the reaction of 3-(trifluoromethyl)- 2-pyrazine carboxylic acid ester (**15**) with 3′,4′-biphenylamine (**17**) or condensation between 3-(trifluoromethyl)-2-pyrazine carboxylic acid (**16**) and 3′,4′-biphenylamine (**17**) (Scheme 1).

The key intermediate ester (**15**) was initially synthesized by the reaction of 3-chloro-2-pyrazine carboxylic acid ester (**18**) with a trifluoromethylation reagent (Scheme 2).

As shown below, we have developed new economically advantageous synthetic routes that avoid the use of expensive trifluoromethylation reagents as follows: (A) the route *via* condensation between ethylenediamine and diketone (**19**), (B) the route *via* condensation between glyoxal with the amino acid amide (**21**), and (C) the route *via* condensation between a keto ester (**24**) and ethylenediamine.[6–9](Scheme 3)



Scheme 2. Preparation of trifluoromethylpyrazine ester (**15**).



Scheme 3. New preparation methods of trifluoromethylpyrazine ester (**15**).

3. Physical and Chemical Properties

Common name: Pyraziflumid
Experimental code: NNF-0721
CAS registry no.: 942515-63-1
Chemical name: *N*-(3′,4′-difluorobiphenyl-2-yl)-3-(trifluoromethyl)pyrazine-2-carboxamide
Molecular formula: $C_{18}H_{10}F_5N_3O$
Molecular weight: 379.28
Appearance: pale yellow crystal
Melting point: 119°C
Solubility in water: 2.32 mg/L (20°C)
Partition coefficient: $\log P_{o/w} = 3.51$ (25°C)

Table 3. Inhibition of mycelial growth by pyraziflumid against phytopathogenic fungi

Pathogen		EC_{50} value (mg a.i./L)
Ascomycota	*Ascochyta fabae*	0.11
	Botrytis cinerea	0.004[a)]
	Cladosporium cucumerinum	0.057
	Colletotrichum gloeosporioides	>100
	Colletotrichum orbiculare	>100
	Corynespora cassiicola	0.045 [b)]
	Diaporthe citri	>100
	Didymella bryoniae	0.040
	Diplocarpon mali	0.040
	Elsinoe fawcetti	0.014
	Elsinoe ampelina	0.016
	Fusarium oxysporum f.sp.*lycopersici*	>100
	Monilinia fructicola	0.11
	Monilinia mali	0.005
	Mycosphaerella pomi	0.85
	Passalora fulva	<0.16 [c)] (MIC)
	Penicillium digitatum	>100
	Pestalotiopsis menezesiana	>100
	Pseudocercospora vitis	0.087
	Pseudocercosporella capsellae	0.61
	Sclerotium cepivorum	0.041
	Sclerotinia sclerotiorum	0.023
	Valsa ceratosperma	4.7
	Venturia inaequalis	0.045
	Venturia nashicola	0.80
	Verticillium dahlia	11
	Zygophiala jamaicensis	0.041
Deutromycota	*Alternaria brassicicola*	0.057
	Alternaria kikuchiana	0.058
	Alternaria porri	0.040
	Alternaria solani	0.15
	Mycosphaerella arachidis	0.12
	Mycovellosiella nattrassii	0.1 [c)] (MIC)
	Phoma lingam	<0.03
	Phomopsis fukushii	>100
Basidiomycota	*Rhizoctonia solani*	0.20
	Sclerotium rolfsii	0.34
Oomycota	*Phytopthora infestans*	>100 [d)]

Agar plate assay using PDA medium except for footnotes below. [a)] YBA agar paper disk method.[10] [b)] YBA medium. [c)] YB medium. [d)] Rye medium.

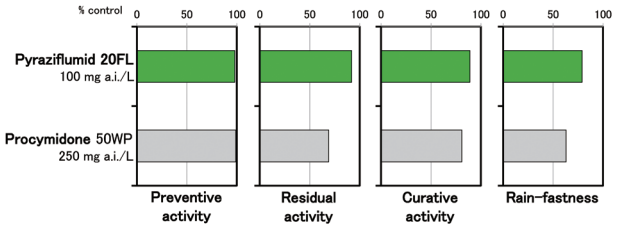


Fig. 3. Biological properties of pyraziflumid against Sclerotinia rot of cabbage (detached leaf test). Preventive activity : mycelium agar plug of *Sclerotinia sclerotiorum* was inoculated 1 day after the application of fungicides. Residual activity : mycelium agar plug of *S. sclerotiorum* was inoculated 14 days after the application of fungicides. Curative activity : *S. sclerotiorum* was inoculated 1 day before the application of fungicides. Rain-fastness : artificial rainfall (50 mm/2 hr) was treated after the application of fungicides and *S. sclerotiorum* was inoculated after drying.

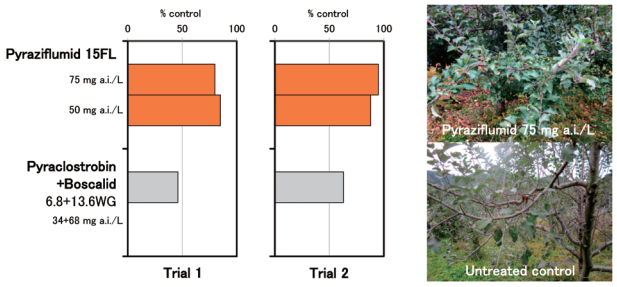


Fig. 5. Field trials of PARADE®15FL against apple marrsonina blotch. Trial 1: Location; Aomori pref., Japan 2010, % infected and fallen leaves of control; 61.5%, Treatment date; June 10, 25, July 9, Assessment date; November 4. Trial 2: Location; Aomori pref., Japan 2013, % infected and fallen leaves of control; 100.0%, Treatment date; June 18, July 2, 17, August 1, Assessment date; October 24. Both trials were treated 200 L/10a water volume as registered in Japan.

4. Biological Activity

4.1. Antifungal spectrum

The antifungal activity of pyraziflumid is summarized in Table 3. Pyraziflumid showed high antifungal activity based on the inhibition of mycelium growth and spore germination in the agar plate assay using PDA (potato dextrose agar), YBA (yeast extract, Bacto peptone and sodium acetate) and YB (yeast extract and Bacto peptone) media. Its EC_{50} values were lower than 0.1 mg a.i./L against ascomycetes fungi such as *Botrytis cinerea*, *Cladosporium cucumerinum*, *Corynespora cassiicola*, *Didymella bryoniae*, *Diplocarpon mali*, *Elsinoë fawcetti*, *Elsinoë ampelina*, *Monilinia mali*, *Pseudocercospora vitis*, *Sclerotium cepivorum*, *Sclerotinia sclerotiorum*, *Venturia inaequalis* and *Zygophiala jamaicensis*. The antifungal activity of the compound was also confirmed against deutromycetes such as *Alternaria* spp. and *Phoma lingam* and basidiomycetes such as *Rhizoctonia solani* and *Sclerotium rolfsii*. However, pyraziflumid did not show any antifungal activity at 100 mg a.i./L against *Colletotrichum* spp., *Diaporthe citri*, *Fusarium oxysporum*, *Penicillium digitatum*, *Pestalotiopsis menezesiana*, *Phomopsis fukushii*, or *Phytophthora infestans*.

Besides the effect on mycelium growth and spore germination, pyraziflumid exhibits inhibition activity against the apothecium formation of *Sclerotinia sclerotiorum*.[11]

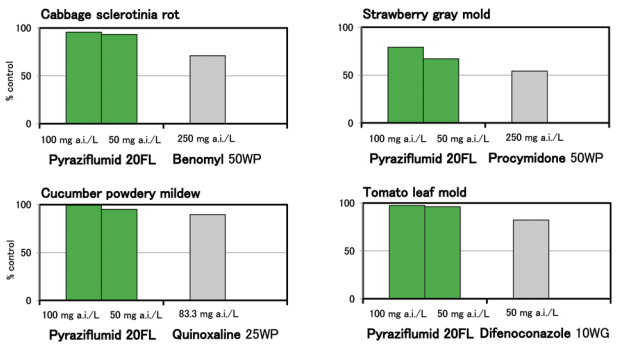


Fig. 4. Field trials of PARADE®20FL against vegetable crop diseases. Cabbage sclerotinia rot: Location; Gunma pref., Japan 2014, Disease index of control; 24.7, JPPA* official trial. Strawberry gray mold: Location; Osaka pref., Japan 2013, Disease index of control; 55.9, Nihon Nohyaku research center. Cucumber powdery mildew: Location; Ibaragi. Pref., Japan 2013, Disease index of control; 61.2, JPPA official trial. Tomato leaf mold: Location; Ibaragi pref., Japan 2014, Disease index of control; 60.9, JPPA official trial All trials were treated 100–300 L/10 a water volume as registered in Japan. *JPPA: Japan Crop Protection Association

4.2. Biological properties

In order to evaluate the biological properties of pyraziflumid, detached-leaf tests against sclerotinia rot of cabbage were conducted using a mycelium agar plug of *Sclerotinia sclerotiorum* and its excellent and stable efficacy such as preventive, residual and curative efficacy, and rain-fastness was confirmed (Fig. 3).[12]

4.3. Field trials

The results of field trials conducted by JPPA and Nihon Nohyaku Co., Ltd., are shown in Figs. 4 and 5. Pyraziflumid showed good efficacy against sclerotinia rot of cabbage, gray mold of strawberry, powdery mildew of cucumber and leaf mold of tomato (Fig. 4). Pyraziflumid exhibited a particularly high efficacy against sclerotinia rot of leafy vegetable, fruiting vegetable, bean and onion crops (data not shown).

In order to confirm its long-lasting effect on apple diseases, field trials were conducted in Aomori Prefecture in 2010 and 2013. In the results, pyraziflumid showed excellent residual activity against Marssonina blotch (Fig. 5). In addition, practical efficacy against other diseases of apple and other fruit crops, such as scab, brown rot and ring rot, was confirmed.[12,13]

Table 4. SDH inhibitory activity of pyraziflumid derived from phytopathogenic fungi, crop plants and mammal

Classification		Scientific name	IC_{50} (nM)
Phytopathogenic fungi	Ascomycetes	*Sclerotinia sclerotiorum*	1.8 (median of 24 strains)
		Sclerotinia minor	0.7
		Botrytis cinerea	4.1
		Blumeria graminis	5.8
		Venturia inaequalis	18.9
		Alternaria brassicae	2.1
		Magnaporthe oryzae	2,500
	Basidiomycetes	*Puccinia recondita*	14.4
		Rhizoctonia solani	5.9
	Oomycetes	*Pythium aphanidermatum*	>8,000
Crop plants	Brassicaceae	*Brassica oleracea* (Cabbage)	>32,000
	Solanaceae	*Solanum lycopersicum* (Tomato)	>32,000
	Cucurbitaceae	*Cucumis sativus* (Cucumber)	>32,000
Mammal	Mouse	*Mus musculus*	>32,000

5. Safety Properties

Pyraziflumid and its formulated products showed low acute mammalian toxicity and no critical eye or skin irritation. Though the formulation product of pyraziflumid caused mild skin sensitization, it was classified as an out of category in the Globally Harmonized System (GHS). In prolonged exposure studies such as those for carcinogenicity, teratogenicity, reproductive toxicity, and genotoxicity, it was confirmed that pyraziflumid did not adversely affect human health.

Acute fish and aquatic invertebrates toxicity of pyraziflumid was low, and it was less bioaccumulative. Consequently, pyraziflumid is considered to be environmentally safe as long as it is used according to its usage standards.

6. Mode of Action

6.1. *Inhibitory activity of succinate dehydrogenase derived from Sclerotinia sclerotiorum*

We predicted that pyraziflumide's mode of action would be the inhibition of succinate dehydrogenase (SDH) in mitochondria complex II, based on information obtained from a previous study on BC723 and the similarity of chemical structure of both compounds. The dichloroindophenol (DCIP) method[14] was carried out in order to confirm and measure the inhibitory activity of pyraziflumid. Sclerotia of *Sclerotinia sclerotiorum* were collected from fields in Japan and used as samples for inhibitory activity evaluation.

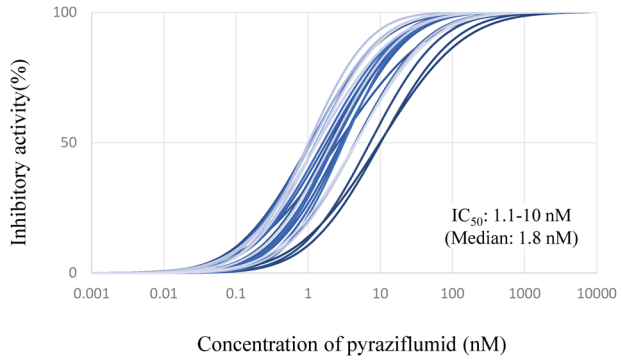


Fig. 6. Inhibition curve of SDH derived from *Sclerotinia sclerotiorum* by pyraziflumid.

In the evaluation of 24 strains of *S. sclerotiorum*, pyraziflumid showed inhibitory activity against SDH, depending on the concentration of the compound, and its IC_{50} values were 1.1–10 nM (the median value was 1.8 nM) (Fig. 6).[15]

6.2. *Inhibitory activity of succinate dehydrogenase derived from other phytopathogenic fungi, crop plants and animals*

Pyraziflumid also exhibited inhibitory activity against various phytopathogenic fungi (Table 4). The IC_{50} values of pyraziflumid against ascomycetes, *Sclerotinia minor*, *Botrytis cinerea*, *Blumeria graminis, Venturia inaequalis* and *Alternaria brassicae* were 0.7–18.9 nM. Against the basidiomycetes, *Puccinia recondita* and *Rhizoctonia solani*, they were 14.4 and 5.9 nM, respectively. In contrast, no inhibitory activity of pyraziflumid was observed against the SDH derived from the oomycete *Pythium aphanidermatum*. Against nontarget SDHs derived from crop plants, such as cabbage, tomato and cucumber, and mice, pyraziflumid did not show any inhibitory activity. These results indicate that pyraziflumid has high target-based selectivity between phytopathogenic fungi and nontarget species. This selectivity might be responsible for pyraziflumid's low risk of phytotoxicity and low acute toxicity to mammals.

Concluding Remarks

The novel SDHI fungicide pyraziflumid was discovered by consolidating the research based on our past knowledge of BC723-

related studies and recent findings obtained during the discovery of pyflubumide as an acaricide.

As of May 2020, pyraziflumid has been registered in Japan and South Korea, and it has been commercialized in Japan since 2018. Regarding PARADE®, the trade name of pyraziflumid as a commercial fungicide in Japan, we are now working to expand the registration to many kinds of crops and diseases. Moreover, excellent systemic activity of PARADE® enabled us to obtain registration for drench application to the nursery tray of leafy vegetables and to prevent infection with sclerotinia rot after transplanting. The promotion of this labor-saving technique would increase the efficiency of practical agricultural production.

Pyraziflumid is now in preparation for registration in many other countries and is expected to contribute to stable agricultural production throughout the world.

Acknowledgements

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