Research and Development of a Novel Fungicide 'Mandestrobin'

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Mandestrobin is a novel fungicide having a methoxyacetamid structure which has been commercially developed by Sumitomo Chemical Co., Ltd. Mandestrobin has good fungicidal properties, such as a broad antifungal activity spectrum, preventive efficacy, curative efficacy, translaminar ability, long lasting activity, good rainfastness, and low risk of phytotoxicity against major crops. Mandestrobin also shows safer profiles for human health and the environment. In Japan, a formulated product, SCLEA® flowable, has been registered since 2015 and was launched in 2016. Mandestrobin is also under commercial development in EU countries, USA, Canada, Brazil, Australia and Korea, with trademarks such as INTUITY®.

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Introduction

Strobilurins are known as quinone outside inhibitors (QoI in the following), which are one type of cellular respiration inhibitor for pathogenic microorganisms in plants, and there are 20 compounds having the mode of action (MoA) described just in the FRAC MoA poster.[1] Analyzing worldwide sales of fungicides by MoA, QoI fungicides are the largest at approximately 23%[2] of all fungicides and are the most important agricultural fungicide group. Naturally, they are an important group in Japan, and even at present, in the 20th year since the initial domestic launch of strobilurin (kresoxim-methyl, BASF SE) in 1997, they keep a market share of approximately 10%[3] Under these circumstances, mandestrobin (common name acquired in April 2013) is the first strobilurin compound developed by Sumitomo Chemical (**Fig. 1**). This compound was registered as an agricultural chemical in September 2015 in Japan, and sales began in January 2016 as "SCLEA® flowable" (40% mandestrobin flowable). Overseas, it was registered as an agricultural technical product in South Korea in October 2015 and is currently awaiting approval of registration as a fungicide formulation. After receiving approval as an agricultural chemical active ingredient from the EU in December 2015, it has moved to the stage of being evaluated by the regulatory authorities aiming at registration in the various EU countries. It was approved for registration as both a technical product and a fungicide formulation in Canada in May 2016. Applications for registration as an agricultural chemical have been completed in the US, Brazil, Australia and other countries, and registration is being awaited, but evaluations and development are progressing for other countries aiming at expansion into other regions. Several trade names are planned according to region and use, with "INTUITY®" being typical.

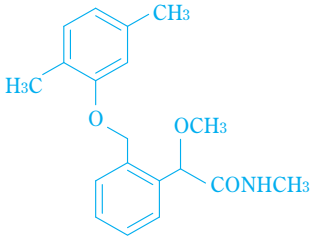


Fig. 1 Chemical structure of mandestrobin

As strong points of mandestrobin, there are its effectiveness on a wide variety of crop diseases centered on sclerotinia rot, fruit tree scab, etc., which are very damaging, and furthermore, the applicability for a wide range of crop cultivation scenes because of a low risk of phytotoxicity for crops. In this article we will report on the efficacy the manufacturing method, physical chemi-

cal properties, formulations and safety for mammals and the environment of mandestrobin.

Development Process

Mandestrobin was discovered by Shionogi & Co., Ltd. and is a novel methoxyacetamide based compound characterized by a mandelic acid structure. Evaluations on introducing this compound into Sumitomo Chemical began in 2004 through the mediation of Sumitomo Corporation. Initially, the racemic form (mandestrobin) and the optical isomer were evaluated in parallel. In the end, mandestrobin was selected because of comprehensively better efficacy against sclerotinia rot of rapeseed (white stem rot) in Europe, and Sumitomo Chemical agreed to the transfer of related patents (owned by Sumitomo Corporation at the time).

Manufacturing Methods

Mandestrobin has a backbone derived from mandelic acid. There are many synthesis routes to obtain mandestrobin from inexpensive starting materials as is shown in **Fig. 2**.

For example, in the processes to manufacture the mandelic acid derivative, there can be two-carbon-increasing reactions starting from aryl halide and one-carbon-increasing reactions of carboxylic acid or an aldehyde, etc. After vigorous investigations, a manufacturing method for mandestrobin with high yields was chosen and established.

Biological Effects

1. Antifungal spectrum

Mandestrobin has a broad spectrum and exhibits antifungal activity for many filamentous fungi that are important crop pathogens. High antifungal activity is exhibited for the *Sclerotinia* genus (*Sclerotina sclerotiorum*, etc.) and *Monilinia* genus (*Monilinia fructicola*, etc.) of the *Sclerotiniaceae*, the *Venturia* genus (*Venturia*

Table 1 Antifungal activity of mandestrobin

Class	Fungal species	EC_{50} (ppm)
Ascomycetes	*Sclerotinia sclerotiorum*	0.022
	Monilinia fructicola	0.034
	Monilinia laxa	0.016
	Monilinia fructigena	0.075
	Venturia inaequalis	0.0082
	Venturia nashicola	0.065
	Diplocarpon mali	0.062
	Diaporthe citri	0.18
Fungi Imperfecti	*Phomopsis sp.*	0.019
	Phomopsis vexanes	0.014
	Phomopsis fukusii	0.043
	Alternaria alternata	0.55
	Botrytis cinerea	0.024
	Colletotrichum gossypii	0.084
	Colletotrichum phaseolorum	0.13
	Colletotrichum simmondsii	0.061
	Corynespora cassiicola	0.073
	Cercospora kikuchii	0.42
	Septoria glycines	0.93
Basidiomycetes	*Rhizoctonia solani*	0.45
Oomycetes	*Pythium graminicola*	> 5.0

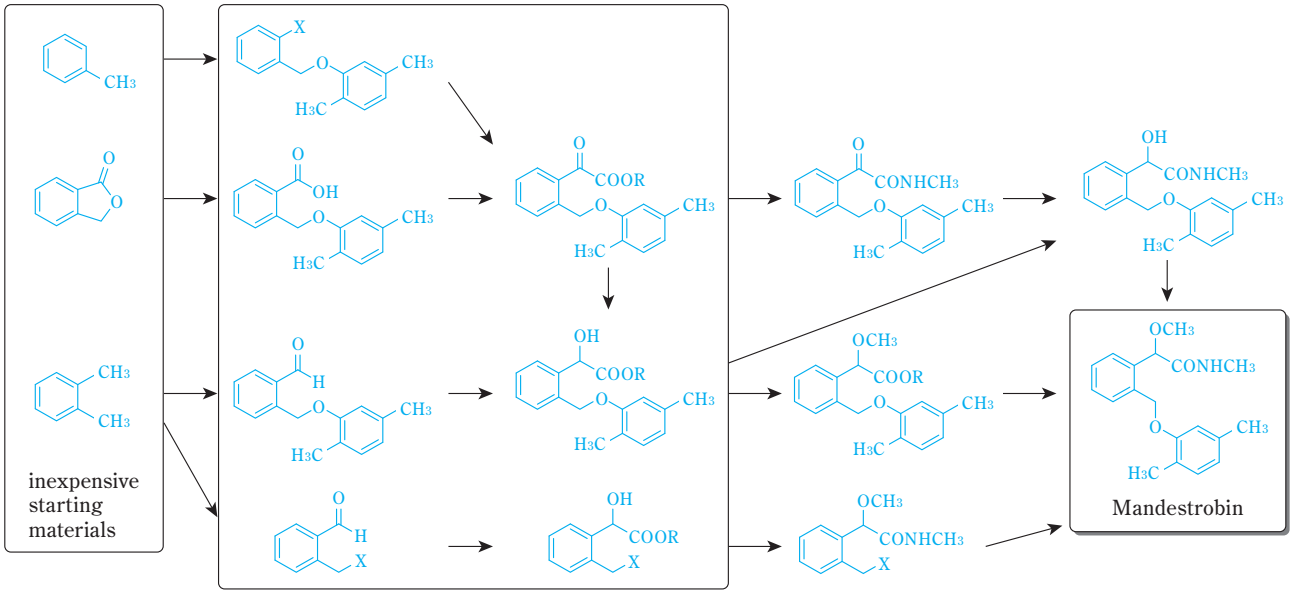


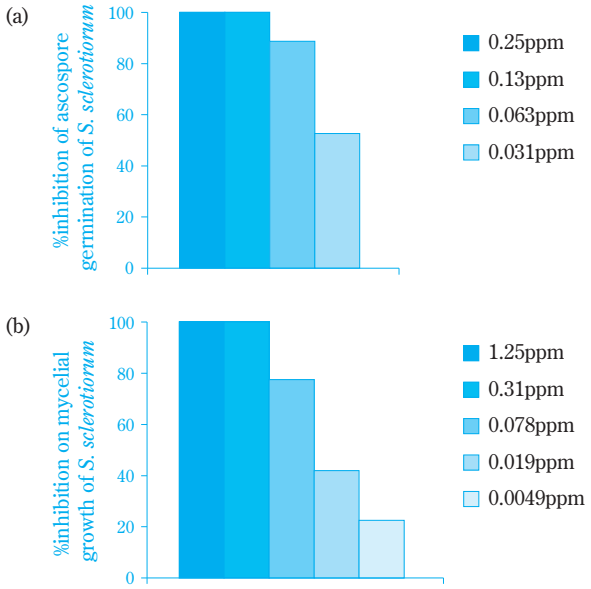
Fig. 2 Synthesis route of mandestrobin

nashicola, etc.) and other *Venturiaceae*, and fungi of the *Phomopsis* genus, which are deuteromycetes, etc. (**Table 1**).

2. Characteristics of action

(1) Inhibition of spore germination and inhibition of mycelial growth in *Sclerotinia sclerotiorum*

Water agar containing 0.031 – 0.25 ppm mandestrobin was inoculated with ascospores of *Sclerotinia sclerotiorum*, and after culturing for 24 hours in a dark location at 18°C, the rate of ascospore germination was observed using a microscope. As a result, mandestrobin exhibited a high level of germination inhibiting activity, the minimum inhibiting concentration (MIC) being at 0.13 ppm (**Fig. 3** (a)). In addition, a PDA medium (100 ppm of methyl salicylhydroxamic acid (SHAM)) containing 0.0049 – 1.25 ppm mandestrobin was inoculated with mycelial *Sclerotinia sclerotiorum* agar pieces, cultured for 48 hours at 18°C and mycelial growth measured. As a result, mandestrobin exhibited a high level of mycelial growth inhibiting effect, and the MIC for mycelial growth was 0.31 ppm (**Fig. 3** (b)).



(a) Medium: water agar, Incubation temperature: 18°C, Incubation time: 24hours
(b) Medium: PDA containing 100ppm of SHAM, Incubation temperature: 18°C, Incubation time: 48hours

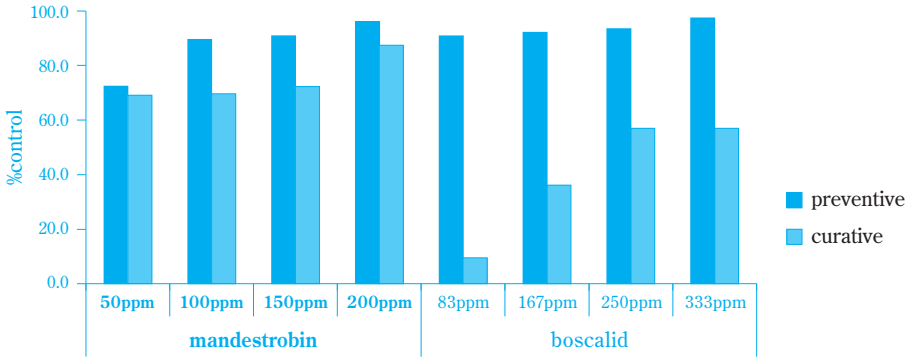
Fig. 3 Inhibitory activity of mandestrobin against (a) ascospore germination and (b) mycelial growth of *S. sclerotiorum*

(2) Preventative effect

In indoor potted tests, mandestrobin exhibited a high level of effectiveness of approximately 90% control of soybean sclerotinia rot (caused by *Sclerotinia sclerotiorum*) even at half the concentration registered for vegetables (200 ppm) in Japan (**Fig. 4**). It also had the high effect of 97% control on pear scab (caused by *Venturia nashicola*) at the lower limit concentration (133 ppm) for the disease in Japan (**Fig. 5**).

(3) Curative effect

In curative effect tests (tests of treatment with fungicide after infection by pathogenic fungus), mandestrobin exhibited a high level of effect of 90% control or greater on soybean sclerotinia rot at the concentration (200 ppm) registered in Japan for the disease, and even at a low concentration of 1/4 of the registered concentration, the efficacy was around 70% control (**Fig. 4**). In addition, it exhibited a high level of effect with 80% control or greater for pear scab at the registered lower limit con-



Reference compound: boscalid as commercial standard
Preventive: *S. sclerotiorum* was inoculated 1 day after fungicide application.
Curative: *S. sclerotiorum* was inoculated 2 days before fungicide application.
Assessment: radius of each lesion was measured (3 replications per treatment).

Fig. 4 Preventive and curative efficacy of mandestrobin against sclerotinia rot on soybean

centration (133 ppm) (**Fig. 5**). As a result, mandestrobin exhibits the ability to control onset at the initial stages of infection by pathogenic fungi. With this curative activity, mandestrobin would be expected to show good efficacy in controlling scletorinia rot on vegetables even with application after 1st symptoms.

(4) Translaminar activity

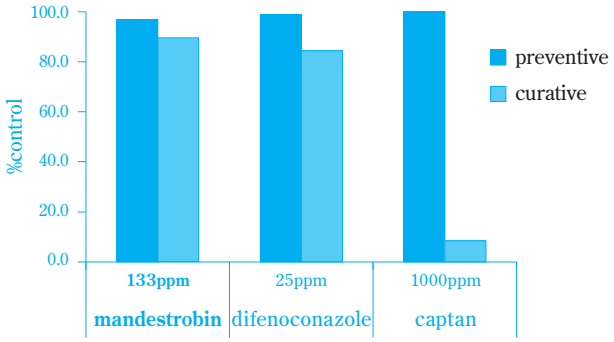
In translaminar activity tests (tests in which leaf surfaces were treated with the fungicide and the abaxial side of surfaces of the leaves were inoculated with the pathogen), mandestrobin had an effect of 70% control or greater on soybeans even at half the concentration (200 ppm) registered in Japan for *Sclerotinia sclerotiorum* (Sclerotinia rot) (**Fig. 6**). As a result, mandestrobin was absorbed into the plant body quickly after treatment and showed movement even to the untreated surfaces. With the trans-laminar ability, mandestrobin would be expected to show a good efficacy in controlling gray mold in cases of a little uneven application in the field.

(5) Long lasting efficacy

In greenhouse 21 day long lasting efficacy tests on soybeans, mandestrobin had a high level of effect of 100% control even at 2/3 of the concentration (200 ppm) registered in Japan for *Sclerotinia sclerotiorum* (Sclerotinia rot) (**Fig. 7**).

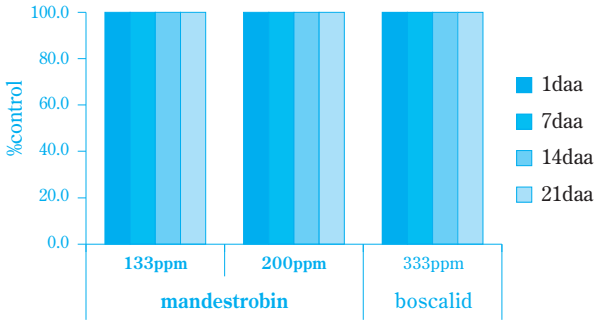
(6) Rain fastness

In rain resistance tests, mandestrobin exhibited a high level of effect of 80 or more % control on soybeans for Sclerotinia rot on soybeans at the concentration (200 ppm) registered in Japan even under conditions where



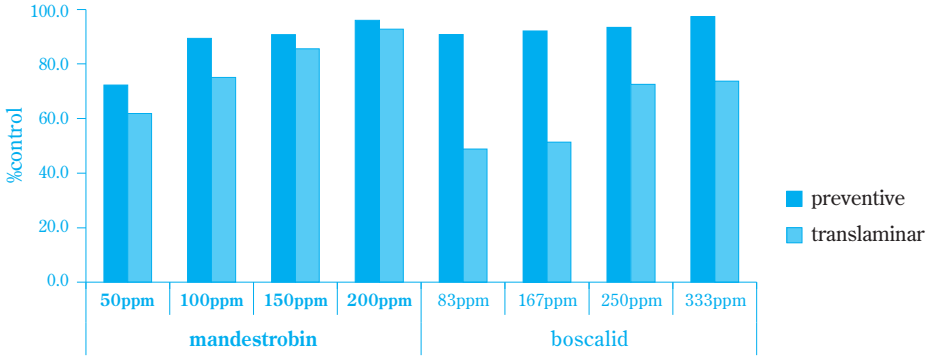
Reference compounds: difenoconazole as commercial standard effective both in preventive and curative condition. captan is the one effective in preventive condition.
Preventive: *V. nashicola* was inoculated 2 days after fungicide application.
Curative: *V. nashicola* was inoculated 6 days before fungicide application.

Fig. 5 Preventive and curative efficacy of mandestrobin against scab on Japanese pear



Reference compound: boscalid as commercial standard
S. sclerotiorum was inoculated 1,7,14 and 21 day(s) after application (daa).

Fig. 7 Residual activity of mandestrobin against sclerotinia rot on soybean



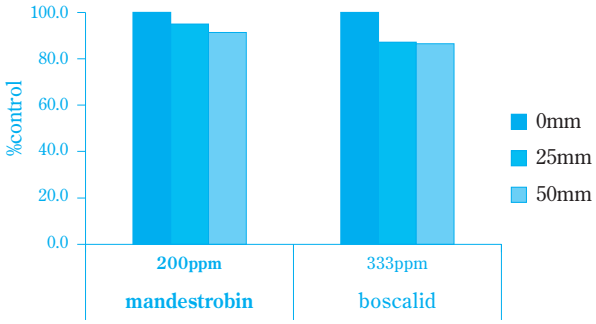
Reference compound: boscalid as commercial standard
Application: foliar application against adaxial side of leaves was conducted.
Inoculation: *S. sclerotiorum* was inoculated on adaxial side (preventive) or abaxial side (translaminar) of leaves 1 day after the application.

Fig. 6 Translaminar activity of mandestrobin against sclerotinia rot on soybean

artificial rain treatment was carried out at a total of 50 mm (25 mm/hour) one day after spraying (**Fig. 8**). In addition, under conditions where artificial rain was implemented for a total of 25 mm (25 mm/hour) two days after spraying, a high level effect of 70% or greater at the registered lower limit concentration (133 ppm) and 90% or greater at the registered upper limit (200 ppm) was exhibited for pear scab (**Fig. 9**).

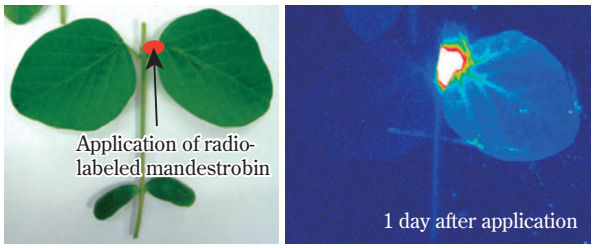
(7) Systemic activity

^{14}C labeled mandestrobin was smeared on the bases of primary soybean leaves and cucumber leaves and the dynamics of the mandestrobin in the plant bodies were examined with an autoradiograph. Movement to the tips of the leaves was confirmed one day after treatment, and it was apparent that mandestrobin had certain systemic activity from the bases to the tips of leaves (**Fig. 10**, **11**). When plants are growing thickly in the field, unevenness of spraying easily occurs leaf by leaf. Or when the grower loses the preferable application timing, curative activity is needed for good disease control. Also in such cases, it can be inferred that the systemic activity of



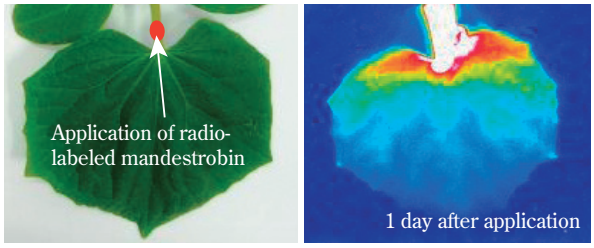
Reference compound: boscalid as commercial standard
Rainfall: artificial rainfall was conducted at 1 day after fungicide application. The amount of the rainfall adjusted to 25mm/hour to 50mm/2hours.
Inoculation: *S. sclerotiorum* was inoculated after the droplet on the leaf dried.

Fig. 8 Rainfastness of mandestrobin against sclerotinia rot on soybean



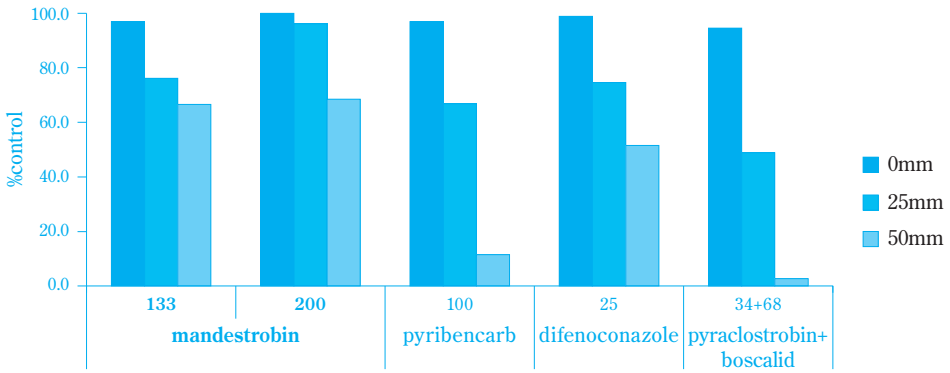
Application: radio-labeled mandestrobin suspension was applied on the petiole.
Assessment: 1 day after application, radioactivity was visualized by imaging analyzer.

Fig. 10 Autoradiograph of soybean treated with radio-labeled mandestrobin



Application: radio-labeled mandestrobin suspension was applied on the petiole.
Assessment: 1 day after application, radioactivity was visualized by imaging analyzer.

Fig. 11 Autoradiograph of cucumber treated with radio-labeled mandestrobin



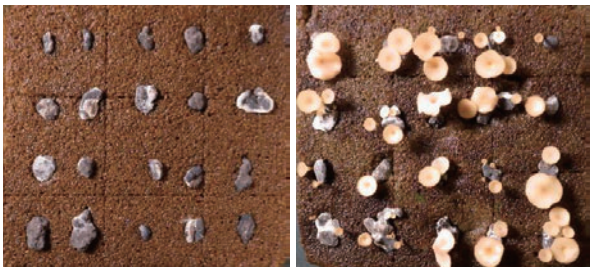
Reference compounds: pyribencarb, difenoconazole and pyraclostrobin+boscalid as commercial standards
Rainfall: artificial rainfall was conducted at 2 days after application. The amount of the rainfall adjusted to 25mm/hour to 50mm/2hours.
Inoculation: *V. nashicola* was inoculated 1 day after artificial rainfall.

Fig. 9 Rainfastness of mandestrobin against scab on Japanese pear

mandestrobin as described above contributes to superior effects under these conditions.

(8) Inhibitory activity in apothecial development of *Sclerotinia sclerotiorum*

Under suitable conditions, apothecia of *Sclerotinia sclerotiorum* are formed from sclerotia, and ascospores, which are the primary source of infection, are formed abundantly on the apothecia. Sclerotia were immersed for 1 minute in a liquid containing mandestrobin diluted to 12.5–200 ppm, and apothecial development was evaluated. As a result, apothecial development was completely inhibited by mandestrobin at 12.5 ppm (**Fig. 12**). From the inhibitory effect on apothecia formation by mandestrobin it can be inferred that the amount of the initial source of infection will be reduced.



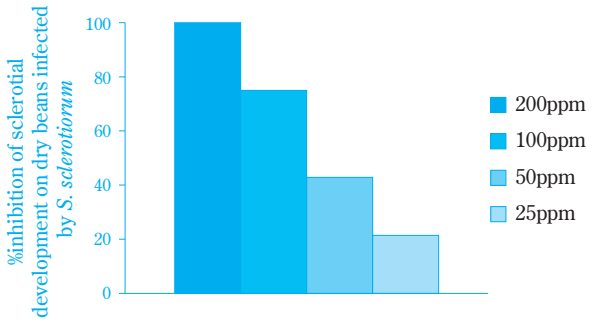
mandestrobin 12.5ppm　　Untreated

Treatment: sclerotia of *S. sclerotiorum* were immersed into mandestrobin suspension for 1 minute.
Incubation: 1month at 15°C.
Assessment: the apothecial development on sclerotia (20 sclerotia/treatment) was evaluated. (UTC: 100%).

Fig. 12 Rate of apothecial development on sclerotia treated and untreated with mandestrobin

(9) Inhibition of sclerotia production

Sclerotia are formed on diseased plants by *Sclerotinia sclerotiorum*, and then the sclerotia overwinter and become the source of infection in the following year. Dry beans were inoculated with *Sclerotinia sclerotiorum* ascospores, and five days after inoculation when symptoms had been sufficiently developed, the plants were treated with mandestrobin by spraying and the inhibitory effect on sclerotia production was evaluated. As a result, mandestrobin exhibited a concentration-dependent inhibitory effect on sclerotia production and the MIC was the registered concentration of 200 ppm (**Fig. 13**). It can be inferred that the inhibitory effect of mandestrobin on sclerotium formation will reduce the overwintering source of infection and be



Inoculation: ascospore suspension was inoculated to dry beans.
Application: 5 days after inoculation, mandestrobin suspension was applied to dry beans.
Assessment: 16 days after incubation, the number of sclerotia on infected dry beans was counted. (UTC average: 9.3 sclerotia/plant)

Fig. 13 Inhibitory activity of mandestrobin against sclerotia production on dry beans infected by *S. sclerotiorum*

able to contribute to a reduction in pressure from the onset of the disease.

(10) Effects on *Sclerotinia sclerotiorum* life cycle

Mandestrobin has an inhibitory effect on almost all stages of the life cycle of *Sclerotinia sclerotiorum* such as spore germination, mycelial growth, sclerotia production and apothecial development, and effects on *Sclerotinia sclerotiorum* can be expected with various timing in practical situations (**Fig. 14**).

(11) Mechanism of action

It can be inferred that mandestrobin has a mitochondria complex-III inhibitory action on plant pathogens as its main MoA, from the structure and spectrum. To demonstrate this, the mitochondria electron transfer system inhibitory activity of mandestrobin was evaluated. We used a crude mitochondria fraction for *Sclerotinia sclerotiorum* and evaluated (i) electron transfer system inhibitory activity on complex-I to complex-III using NADH as substrate and (ii) electron transfer system inhibitory activity on complex-II to complex-III using cytochrome c reduction activity. In these tests, mandestrobin exhibited a high level of inhibitory activity (EC_{50} of (i) 99 nM and (ii) 50 nM) in all cases (**Fig. 15** (a), **Fig. 15** (b)). From the results above, it is clear that the MoA of mandestrobin is complex-III inhibition.

Moreover, QoI and quinone inside inhibitors (QiI) are known to be among the complex-III inhibitors, but because mandestrobin has partial structures in common with QoI fungicides, it can be inferred that it is a QoI agent.

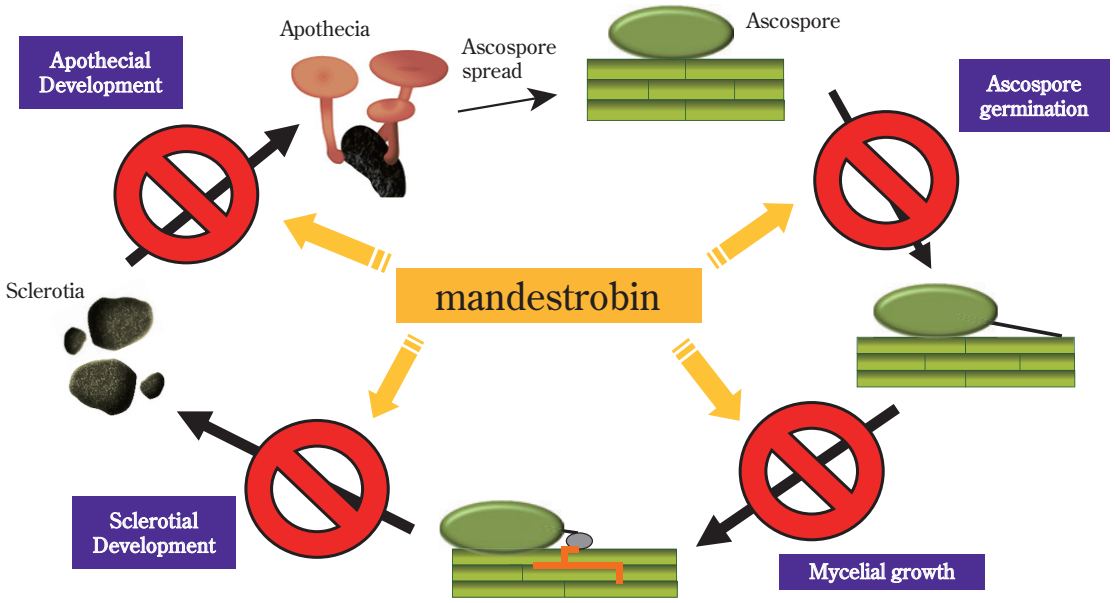
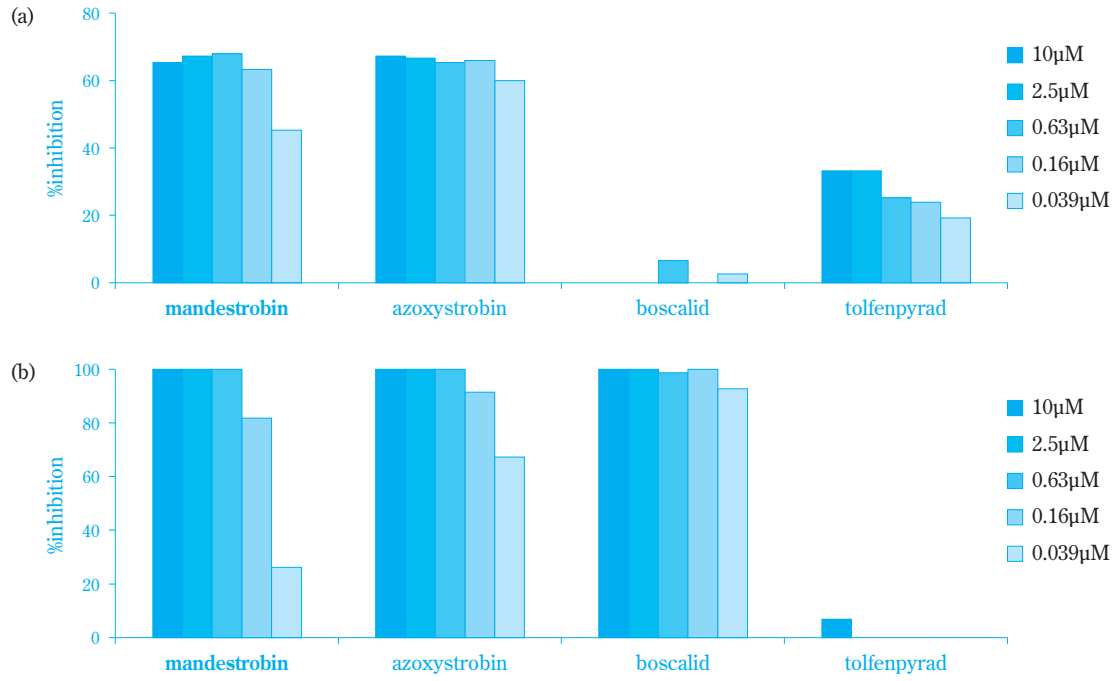


Fig. 14 Inhibitory sites of mandestrobin in the life cycle of *S. sclerotiorum*



Reference compounds : azoxystrobin as Complex-Ⅲ inhibitor, boscalid as Complex-Ⅱ inhibitor and tolfenpyrad as Complex-I inhibitor.
Test condition : (a) NADH was added into crude mitochondrial fraction of *S. sclerotiorum* with tested fungicides, and incubated for 35minutes in 25°C.
(b) cytochrome C was added into crude mitochondrial fraction of *S. sclerotiorum* with tested fungicides, and incubated for 35minutes in 25°C.

Fig. 15 Inhibitory activity of mandestrobin against electron transport chain on mitochondria extracted from *S. sclerotiorum*

(12) Safety for crops

When existing strobilurin fungicides are applied in excess amounts, yellowing of leaves, abnormal growth and other phytotoxic effects are known to be caused in cases of low plant vigor and cases of treatment at growth stages with high sensitivity to chemicals. On the other hand, no phytotoxic damage that creates practical problems has been found for mandestrobin in double-amount phytotoxicity tests (**Table 2**). In particular, mandestrobin could be applied even in the flowering stage of pears where the risk of phytotoxicity is high (**Table 3**).

In addition, when the mandestrobin electron transfer inhibiting action was evaluated using a crude mitochon-

Table 2 Phytotoxicity of mandestrobin on crops

Crop	Phytotoxicity
Apple	—
Japanese pear	—
Vine	—
Tea	—
Cherry	—
Peach	—
Persimmon	—
Japanese apricot	—
Soybean	—
Drybean	—
Azuki bean	—
Lettuce	—
Cabbage	—
Tomato	—
Eggplant	—
Cucumber	—
Rice	—
Water melone	—
Melone	—

Foliar application of mandestrobin 400ppm to the plant in field.
— : No phytotoxicity

Table 3 Phytotoxicity of mandestrobin on Japanese pear

Product	Conc.	Growth stage of Japanese pear		
		Before flowering	Petal fall	10 days after end of flowering
mandestrobin	200ppm	—	—	—

Location: Ibaraki prefecture, Japan
Application: Foliar application, 1 time at each growth stage.
Variety: Kosui
— : No phytotoxicity

Table 4 Inhibitory activity of mandestrobin and other QoIs against electron transport chain from Complex-Ⅱ to Complex-Ⅲ of mitochondria extracted from tomato and *S. sclerotiorum*

Compound	IC_{50} (nM)		L/S*
	Lycopersicon esculentum (Tomato)	*S. sclerotiorum*	
mandestrobin	12000	50	240
azoxystrobin	1400	30	47
kresoxim-methyl	440	10	44

*L: IC_{50} against mitochondria extracted from *Lycopersicon esculentum*
S: IC_{50} against mitochondria extracted *S. sclerotiorum*

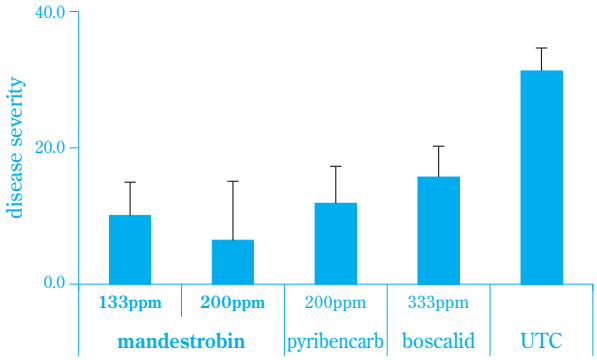
dria fraction from tomatoes, the inhibitory activity on tomato mitochondria (EC_{50} 12,000 nM) was much lower than that (EC_{50} 50 nM) for *Sclerotinia sclerotiorum* mitochondria (**Table 4**). In other words, the phytotoxic damage of mandestrobin to plant mitochondria is low, and this high selectivity can be thought of as one cause of the low phytotoxicity risk with mandestrobin.

3. Practical evaluation

(1) Sclerotinia rot in dry beans

Dry beans are infected by *Sclerotinia sclerotiorum* in their flowers and pods from the flowering stage, and the yield can be devastatingly decreased. There is always risk of infection during the flowering stage, and in Hokkaido, which is the largest dry bean producing area in Japan, control must be implemented 2 to 3 times starting in the early flowering stage. This test treatment was carried out only once at the end of flowering, so the tests were carried out under conditions presuming a requirement for preventative effect, curative effect and residual effect.

As a result, mandestrobin exhibited a high level of preventative effect with intermediate onset conditions of 85% occurrence and a severity of 31 when untreated in field tests on sclerotium rot in dry beans, and it can be considered as being highly practical for preventing this disease (**Fig. 16**).



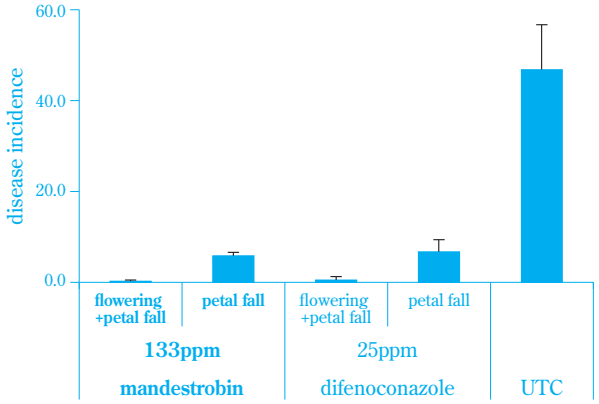
Location: Hyogo prefecture, Japan
Application: single foliar application was conducted at the end of flowering.
Reference compounds: pyribencarb and boscalid as commercial standard
Assessment: assessment was conducted on 19 days after fungicide application.
disease criteria of each plant was assessed according to following disease index. (26 lants/replication, 3 to 4 replications/treatment).
disease index
0; no symptom. 1; 25% of plant was infected,
2; 50% of plant was infected, 3; 75% of plant was infected.
4; whole parts of plant was infected.
disease severity index was calculated according to formula (I).
formula (I)=100*Σ(k * n)/(4 * N)
k; disease index, n; number of plant of each disease index,
N; total number of plant of each replication
Error bar: standard deviation

Fig. 16 Efficacy against sclerotinia rot on dry bean in field conditions

(2) Pear scab

Prevention of pear scab in Japan is most important during the early growth stages and before and after flowering in particular.[4), 5)] On the other hand, it is said that the latency period from infection to outbreak is comparatively long[6)] for pear scab, and not only prevention in the flowering stage, but also curative effects during the latency period have been found to be important. Therefore, we thought that this would be a field where the performance of mandestrobin, which has a superior curative effect for this disease, would clearly be reflected. Thus, in this test, treatment was carried out three days before confirmation of 1st symptoms, and the curative performance of this agent was evaluated.

As a result, mandestrobin exhibited a high level of effectiveness on pear scab (**Fig. 17**) even in heavy disease pressure conditions with an incidence of greater than 47% in an untreated plot. From the above, this agent can be thought to be highly practical.



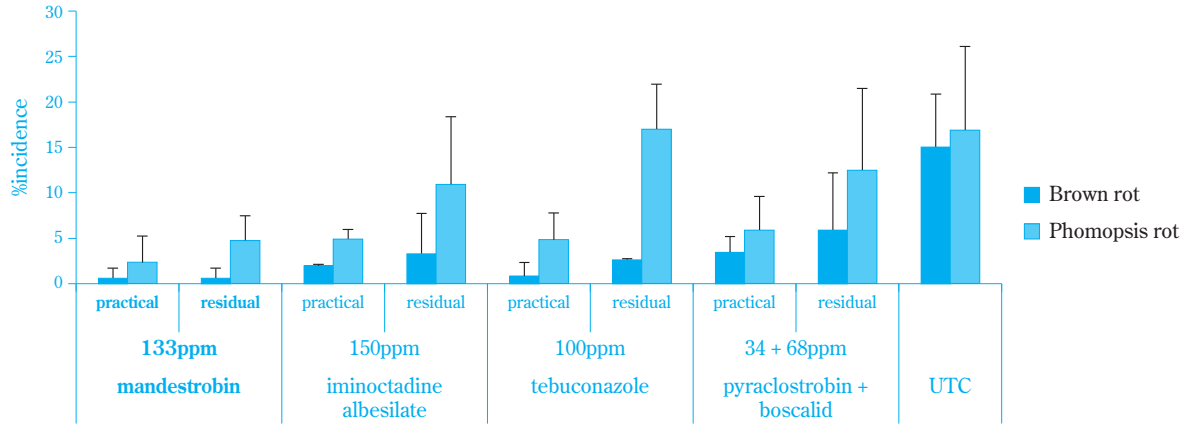
Location: Hyogo prefecture, Japan
Application: foliar application. 1 time in flowering, or 2 times at flowering and petal fall period.
Reference compound: difenoconazole as commercial standard
Assessment: incidence on flower was assessed 13 days after final fungicide application (124-166 flowers/replication, 3replications/treatment).
Error bar: standard deviation

Fig. 17 Efficacy against scab on Japanese pear in field conditions

(3) Brown rot in peaches, phomopsis rot in peaches

Brown rot and phomopsis rot in peaches occur not only during cultivation, but also during shipping and transport as well as after consumers have purchased the fruit, and they can be thought of as some of the most important diseases in peaches. With the trend of reducing labor for farmers in the field of fruit cultivation in recent years, there has been an emphasis on the idea of reductions in agricultural chemicals to reduce the number of applications, and efficacy characteristics are becoming important for exhibiting efficacy when sprayed in a 2 times longer interval than conventional application (double interval). Thus, efficacy evaluations were carried out with mandestrobin on brown rot and phomopsis rot in peaches.

As a result, mandestrobin had a high level of preventative effect for both diseases under both conventional



Location: Hyogo prefecture, Japan
Application: foliar applications was started when fruits became about half final size.
–practical; number of application was 6 time (6/11, 6/19, 6/27, 7/4, 7/11, 7/23)
–residual; number of application was 3 time (6/11, 6/27, 7/11)
Reference compounds: iminoctadine albesilate, tebuconazole and pyraclostrobin+boscalid as commercial standard
Assessment: disease incidence of harvested fruits was assessed after 7 days strage at 25°C (assesses 35-68fruits/replications, 3replications/treatment).
Error bar: standard deviation

Fig. 18 Efficacy against brown rot and phomopsis rot on peach in field conditions

and doubled interval conditions, even though there was a low rate of disease incidence at a little more than 15% when untreated for each disease (**Fig. 18**).

In addition to mandestrobin being highly practical for brown rot and phomopsis rot in peaches from the above, it can be thought of as having a residual effect capable of handling double interval treatment.

Formulation

For the Japanese market, SCLEA® flowable (40% mandestrobin, **Fig. 19**) for fruit and vegetable use was registered in September 2015 and launched in January 2016. This product which is Sumitomo Chemical's first strobilurin fungicide has an effect on various fungi and is especially effective against sclerotinia rot, and it is a high-safety product with little irritation and odor. In addition, this product has superior pourability because of a low viscosity as with most of our conventional flowable formulations.

Fig. 19 SCLEA® flowable (250mL)

Pesticide formulations used for fruits and vegetables are mostly diluted with water before spraying. The dispersibility and the inhibition of foam generation of SCLEA® flowable when diluted with water are superior compared with conventional flowable formulations. Therefore, this product is easy to handle from the viewpoint of the preparation of spray solution and the confirmation of liquid level in a sprayer tank. In addition, since the amount of foam is small, tank cleaning after operations is made easy and can be said to be an advantage of this product. The results (**Table 5**) of a screening study to find an optimal combination system of adjuvants (dispersant and antifoaming agent) and results (**Table 6**) of a comparison study on persistent foam with competitors' products are shown.

On the other hand, there is an issue that must be overcome that water-insoluble co-formulants in the formulation may adhere to the surface of crops and remain as residue. A nearly stain-free formulation after spraying is required in order not to reduce the commercial value. To evaluate the degree of the stain on the crop surface, a model test was conducted by using a paraffin film which resembles a wax layer on the crop surface, and a formulation recipe was established (**Table 7**).

As mentioned above, this product is a superior flowable formulation because it is easy to handle with little foaming during dilution with water and residue on the surface of crops after spraying is small. **Table 8** shows typical physico-chemical properties of SCLEA® flowable. The physico-chemical properties and storage stability of this product are extremely excellent.

Table 5 Screening study to find an optimum combination system of dispersant and antifoamer

Formulations*1	SCLEA® flowable (with combination system A)	Mandestrobin flowable with combination system B	Mandestrobin flowable with combination system C	Mandestrobin flowable with combination system D
Persistent foam*2 (mL)	0	13	13	22

*1 Each mandestrobin formulation is used in different combination systems of dispersant and antifoamer.
*2 250 mL cylinder, 53.6 ppm water, 500 times, 20°C, 1 min.

Table 6 Comparison study on persistent foam with competitors' products

Formulations	SCLEA® flowable	Commercial standard-1	Commercial standard-2
Persistent foam* (mL)	0	20	22

* 250 mL cylinder, 53.6 ppm water, 500 times, 20°C, 1 min.

Table 7 Comparative study of pollution level on surface of paraffin film

Formulations*1	SCLEA® flowable (with combination system A)	Mandestrobin flowable with combination system E	Mandestrobin flowable with combination system F	Mandestrobin flowable with combination system G
Pollution level*2	– –	–	+	+

*1 Each mandestrobin formulation is used in different combination systems of dispersant and antifoamer.
*2 – – : nearly pollution-free, – : essentially non polluted, + : polluted

Table 8 Physical and chemical properties of SCLEA® flowable

Items	Typical value (Methods)
Appearance	Whitish viscous liquid (Visual observation)
Density	1.08 g/mL (20°C)
pH	7.4 (Electric pH meter, neat)
Viscosity	1515 mPa · s (Brookfield viscometer, Spindle No.2, 6 rpm, 25°C)
Suspensibility	99 % (250 mL cylinder, 53.6 ppm water, 500 times, 20°C, 15 min.)
Persistent foam	0 mL (250 mL cylinder, 53.6 ppm water, 500 times, 20°C, 1 min.)
Stability	Physical and chemical properties after storage at ambient temperature for 4 years were very stable

The formulation development for overseas markets is now ongoing to aim at quickly launching mandestrobin formulations which meet the needs in various fields in global markets.

Toxicity, Metabolism and Persistence

1. Toxicity in mammals

(1) Acute toxicity, irritation and skin sensitization

In the acute oral, dermal and inhalation toxicity tests of mandestrobin TG and mandestrobin 40% suspension concentrate (40% SC), there were no mortality or severe toxicity symptoms even at high doses, and acute toxicity was low for all routes of administration. Mandestrobin TG was classified as a mild eye irritant and mandestrobin 40% SC was extremely slight so as to be classified as a practically non-eye irritant. No skin irritation was observed for both mandestrobin TG and mandestrobin 40% SC. Skin sensitization was negative for both in the maximization test for mandestrobin TG and in the Buehler test for mandestrobin 40% SC (**Table 9**).

(2) Subacute toxicity, chronic toxicity and carcinogenicity

In the subacute and chronic toxicity as well as carcinogenicity tests in rats, mice and dogs, the suppression of body weight gain and food consumption were caused by repeated doses of mandestrobin TG. In the liver, both increased organ weight and hepatocellular hypertrophy were found along with the changes in blood biochemical parameters suggesting effects on liver function, and these were thought to be of toxicological significance (**Table 10**). No carcinogenicity was found in rats or mice.

(3) Reproductive and developmental toxicity

In the developmental toxicity tests using rats and rabbits, no teratogenicity was found in fetuses. In the two-generation reproductive toxicity test using rats, no effect was found on reproductive performance and nursing behavior. For the offspring, a slight delay in sexual maturation was found, but it could be considered to be secondary effect of the growth retardation related to maternal toxicity (**Table 11**).

Table 9 Acute toxicity summary of mandestrobin

Test type	mandestrobin	mandestrobin 40%SC
Rat acute oral (LD_{50})	> 2000 mg/kg	> 2000 mg/kg
Rat acute dermal (LD_{50})	> 2000 mg/kg	> 2000 mg/kg
Rat inhalation (LC_{50})	> 4964 mg/m^3 of air (4-hour, nose only exposure)	—
Eye irritation (Rabbit)	Mild irritant	Practically non-irritant
Skin irritation (Rabbit)	Non-irritant	Non-irritant
Skin sensitization (Guinea pig)	Non-sensitizer	Non-sensitizer

Table 10 Subacute and chronic toxicity summary of mandestrobin

Species	Administration route and duration	Dose	NOAEL (mg/kg/day)
Rat	Dermal, 28 days	100, 300, 1000 mg/kg/day	Male: 1000 Female: 1000
Rat	Oral (in diet), 13 weeks	800, 4000, 10000, 20000 ppm	Male: 743 (10000 ppm) Female: 789 (10000 ppm)
Rat	Oral (in diet), 24 months	400, 2000, 7000, 15000 ppm	Male: 105 (2000 ppm) Female: 26.7 (400 ppm)
Mouse	Oral (in diet), 13 weeks	1750, 3500, 7000 ppm	Male: 807 (7000 ppm) Female: 1110 (7000 ppm)
Mouse	Oral (in diet), 18 months	700, 2000, 7000 ppm	Male: 824 (7000 ppm) Female: 994 (7000 ppm)
Dog	Oral (in diet), 13 weeks	4000, 12000, 40000 ppm	Male: 90.0 (4000 ppm) Female: 103 (4000 ppm)
Dog	Oral (in diet), 12 months	200, 800, 4000, 8000 ppm	Male: 19.2 (800 ppm) Female: 92.0 (4000 ppm)

Table 11 Developmental and reproductive toxicity summary of mandestrobin

Study	Species	Administration route and duration	Dose	NOAEL (mg/kg/day)	
Developmental toxicity	Rat	Oral (gavage) Days 6-19 of gestation	100, 300, 1000 mg/kg/day	Maternal Fetal	300 1000
	Rabbit	Oral (gavage) Days 7-28 of gestation	100, 300, 1000 mg/kg/day	Maternal Fetal	1000 1000
Two-generation reproductive toxicity	Rat	Oral (in diet)	1000, 3000, 10000 ppm	Parental	Systemic Male: 56.15 (1000 ppm) Female: 62.48 (1000 ppm) Reproductive Male: 559.1 (10000 ppm) Female: 628.5 (10000 ppm)
				Offsprings	Systemic Male: 56.15 (1000 ppm) Female: 62.48 (1000 ppm)

Table 12 Neurotoxicity and immunotoxicity summary of mandestrobin

Study	Species	Administration route and duration	Dose	NOAEL (mg/kg/day)
Neurotoxicity	Rat	Acute oral (gavage)	500, 1000, 2000 mg/kg/day	Male: 1000 Female: 1000
	Rat	Oral (in diet), 13 weeks	1500, 5000, 15000 ppm	Male: 338 (5000 ppm) Female: 1223 (15000 ppm)
Immunotoxicity	Rat (female)	Oral (in diet), 4 weeks	1500, 5000, 15000 ppm	1419 (15000 ppm)

(4) Neurotoxicity and immunotoxicity

In the acute neurotoxicity and subacute neurotoxicity tests using rats, no specific neurotoxic effect was observed in either. In addition, in an immunotoxicity test using rats, no effect on immune function was observed (**Table 12**).

(5) Mutagenicity

The results of the reverse mutation test using *Salmonella typhimurium* and *Escherichia coli*, the gene mutation tests using a Chinese hamster lung V79 cell line, the chromosomal aberration test using a Chinese hamster lung CHL/IU cell line and the micronucleus test in mice were all negative (**Table 13**).

Table 13 Mutagenicity summary of mandestrobin

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA100, TA1535 and TA1537 +S9 mix: 39.1-1250 μg/plate –S9 mix: 9.77-313 μg/plate *E.coli* WP2uvrA –/+S9 mix: 156-5000 μg/plate	Negative
Gene mutation	Chinese hamster V79 +S9 mix: 8.0-144 μg/mL (4 hours), 16.0-144 μg/mL (24 hours) –S9 mix: 1.0-10 μg/mL (4 hours), 7.5-50 μg/mL (24 hours)	Negative
In vitro chromosomal aberration	Chinese hamster CHL/IU +S9 mix: 100-150 μg/mL (6 hours) –S9 mix: 40-80 μg/mL (6 hours), 3.91-15.6 μg/mL (24 hours)	Negative
Micronucleus	CD-1 mice 500, 1000, 2000 mg/kg (gavage)	Negative

2. Metabolism in Animals and Plants

(1) Metabolism in animals

When ^{14}C labeled mandestrobin was orally administered to rats, it was absorbed rapidly into the body and distributed systemically. Thereafter, it was rapidly metabolized and mainly excreted in the feces, and there was no persistence or accumulation in the tissue. The principal metabolic reaction for mandestrobin was oxidation of the methyl groups and phenyl groups and subsequent glucuronidation and N-demethylation with further oxidation thereafter. From the results of a biliary excretion test, the oral absorption rate was estimated to be 90% or greater.

(2) Metabolism in plants

Plant metabolism studies using ^{14}C labeled mandestrobin with three different types of crops (wheat, lettuce and oilseed rape) demonstrated that the metabolism of mandestrobin is similar in all. Mandestrobin was metabolized *via* oxidation at the dimethylphenoxy ring to produce 4-hydroxyl, 2- and 5-hydroxymethyl, and 5-carboxyl derivatives with subsequent formation of the corresponding glycoside conjugates for the former three monooxidised compounds. In addition, it also underwent *O*-demethylation at the methoxy group and cleavage of the phenyl ether bonds. Finally, it was considered to be incorporated into the constituents of plants.

3. Behavior and residue in the environment

(1) Degradation in water

In the hydrolysis study, ^{14}C labeled mandestrobin was stable in buffer solutions at pH 4, 7 and 9, while its degradation in a buffer solution (pH 7) and natural water (pH 7 – 8) was significantly accelerated by exposure to light. The photodegradation half-lives (natural sunlight at Tokyo in spring) was 11.0 – 17.8 days and 12.1 – 20.5 days, respectively, *via* intermolecular adduction after radical cleavage of the phenyl ethyl bonds, and it further underwent a cyclization reaction, and was finally mineralized to carbon dioxide.

(2) Metabolism in soil

Mandestrobin labeled with ^{14}C was degraded in aerobic soil with a half-life of 50.6 – 323 days (20°C). Mandestrobin was metabolized *via* oxidation of the methyl groups at 2 or 5-position of the dimethylphenoxy ring to form carboxyl derivatives, *O*-demethylation at the methoxy group and cleavage of the phenyl ether bonds, then finally mineralized to carbon dioxide or firmly bound to the soil matrix. In addition, degradation of the mandestrobin on the soil surface was slightly accelerated by exposure to light, and the half-lives (20°C) were 63.9 – 75.7 days under light conditions (natural sunlight at Tokyo in spring) and 71.7 – 82.9 days in the dark controls.

(3) Field dissipation

Terrestrial field dissipation studies were conducted by applying a 2000-fold diluted solution of the mandestrobin 40% flowable three times with a 6/7-day interval at a rate of 300 L/10 a onto upland fields in Ibaraki, Kumamoto, Kagoshima, Saitama, Kochi and Miyazaki. The dissipation half-life was estimated to be 13.5 – 90.7 days with the maximum residual concentration at 0.99 – 3.79 mg/kg.

(4) Mobility in soil

The absorption coefficient $K_{Foc(ads)}$ and desorption coefficient $K_{Foc(dec)}$ of mandestrobin corrected with the soil organic carbon content were calculated using the Freundlich adsorption isotherm and were 287 – 797 and 340 – 1003, respectively.

(5) Residue in crops

Residue trials for 27 crops below were conducted with three applications of 2000-fold diluted formulation of mandestrobin 40% flowable with a seven-day interval at a rate of 150 – 460 L/10 a. The maximum mean residues were below the limit of quantification to 29.6 ppm.

Crops in residue trials: soybeans, cabbage, lettuce, eggplant, cucumber, string beans, watermelon, melon, peach, Japanese apricot, Japanese mustard, potherb mustard, takana, leaf lettuce, cherry tomato, snow pea, green bean, edible soybean, nectarine, plum, cherry, tea, apple, Japanese pear, grape and persimmon.

(6) Residue in succeeding crops

A field succeeding crop study was conducted by cultivating turnips and green peppers as succeeding crops in fields where tomatoes had been treated with a 2000-fold diluted solution of mandestrobin 40% flowable three times at seven-day interval at a rate of 300 L/10 a. The residues in both crops were less than the limit of quantification (0.01 ppm).

4. Effects on non-target species

The test results for aquatic plants and animals, honeybees, silkworms, natural enemy insects and birds are summarized in **Table 14**.

(1) Effects on aquatic plants and animals

The acute toxicity values for the mandestrobin technical product on carp, *Daphnia magna*, and freshwater green algae ($LC_{50}/EC_{50}/ErC_{50}$) were 1.4, 1.2 and 3.4 mg/L, respectively. In addition, the respective toxicity values for the formulation product mandestrobin 40%WG were 3.2, 3.0 and 12.0 mg/L. These values are sufficiently higher than the concentrations expected for water in the environment from practical use, and the effect of mandestrobin on aquatic plants and animals can be considered as low.

(2) Effects on honeybees, silkworms and natural enemy insects

The acute toxicity values (LD_{50}) for oral administration and contact administration of the mandestrobin technical product to western honeybees were >100.71 and >100 µg/bee, respectively. The mortality rate for oral administration of mandestrobin 40%WG to silkworms was 2.0%, and no effect was found on development. The mortality for natural enemy organisms was 2.0 – 4.0% with contact administration in *Amblyseius swirskii*, *Orius strigicollis* and *Aphelinus asychis*. From these facts, the effects of mandestrobin on honeybees, silkworms, natural enemy insects, *etc*. are low for practical use.

(3) Effects on birds

The acute oral toxicity of the mandestrobin technical product in bobwhite quail was low, and the LD_{50} value was greater than 2250 mg/kg. Thus, it can be assumed that the effects of mandestrobin on birds are low for practical use.

Table 14 Ecotoxicological summary of mandestrobin on non-target organisms

Test substance	Test species		Test type	Results
mandestrobin	Aquatic organisms	Carp	Acute (96 hrs)	LC_{50} = 1.4 mg/L
		Daphnia magna	Acute (48 hrs)	EC_{50} = 1.2 mg/L
		Green alga*	Acute (72 hrs)	ErC_{50} = 3.4 mg/L
	Honeybee	*Apis mellifera*	Acute oral (48 hrs)	LD_{50} > 110.71 µg/bee
		Apis mellifera	Acute contact (48 hrs)	LD_{50} > 100 µg/bee
	Bird	Bobwhite quail	Acute oral	LD_{50} > 2250 mg/kg
mandestrobin 40%WG	Aquatic organisms	Carp	Acute (96 hrs)	LC_{50} = 3.2 mg/L
		Daphnia magna	Acute (48 hrs)	EC_{50} = 3.0 mg/L
		Green alga*	Acute (72 hrs)	ErC_{50} = 12.0 mg/L
	Silkworm	*Bombyx mori*	Acute oral (7 days)	mortality 2.0% (at 200 mg a.i./L)
	Natural enemy	*Amblyseius swirskii* (adult)	Acute contact (72 hrs)	mortality 2.0% (at 200 mg a.i./L)
		Orius strigicollis (adult)	Acute contact (72 hrs)	mortality 2.0% (at 200 mg a.i./L)
		Aphelinus asychis (adult)	Acute contact (72 hrs)	mortality 4.0% (at 200 mg a.i./L)

* *Pseudokirchneriella subcapitata*

Based on the above, it is suggested that mandestrobin has low acute toxicity toward mammals, and does not have any carcinogenicity, teratogenicity or reproductive toxicity. In addition, it can be assumed safe use is possible based on the behavior in the environment and evaluation of effects on non-target organisms.

Conclusion

Mandestrobin shows inhibitory action in almost all stages of life cycle of *Sclerotinia sclerotiorum*. Additionally, in field trials, it exhibited highly practical effects against dry bean sclerotinia rot, pear scab and other fruit tree diseases. Furthermore, it can be used even in growth stages with a high risk of phytotoxicity (like flowering stage of pears). These kinds of features make mandestrobin a novel fungicide with excellent performance.

In the future, we think it is necessary to build up examples of the effects of mandestrobin in application programs and promote it, with careful thought about fungicide resistance management.

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References

1) FUNGICIDE RESISTANCE ACTION COMMITTEE, "Mode of Action of Fungicides-FRAC classification on mode of action 2016", http://www.frac.info/docs/default-source/publications/frac-mode-of-action-poster/frac-moa-poster-2016.pdf?sfvrsn=6 (Ref. 2016/4/25).
2) "Phillips McDougall-AgriService Products section-2014 market", Phillips McDougall Ltd. (2015), p.267.
3) i-map SIGMA database (version 3.9.3), Gfk Kynetec., Ltd.
4) S. Umemoto, JAPANESE JOURNAL OF PHYTOPATHOLOGY, **56**, 658 (1990).
5) T. Ogasawara, Y. Tomita and I. Hirosawa, Annual Report of the Kanto-Tosan Plant Protection Society, **48**, 57 (2001).
6) "PLANT DISEASES IN JAPAN (1st edition)", K. Kishi, Zenkoku Noson Kyoiku Kyokai (1988), p.673.

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