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GENERAL CHARACTERISTICS OF MANDESTROBIN, A NOVEL FUNGICIDAL COMPOUND FOR CONTROLLING *SCLEROTINIA* ROT AND *MONILINIA* DISEASES

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RÉSUMÉ

La mandestrobine est un nouveau fongicide mis au point par Sumitomo Chemical Co., Ltd. Il possède une activité anti-fongique élevée contre les agents pathogènes tels que *Sclerotinia* ou *Botrytis*, en inhibant différents stades de leur cycle de vie - germination des ascospores, élongation des tubes germinatifs, formation des sclérotes et des apothécies - dans le cas de la sclérotiniose. Après pénétration dans la plante, la mandestrobine se déplace par systémie. Un effet physiologique est observé sur le colza et participe à l'augmentation du rendement en grains. Une excellente efficacité est également observée contre les Monilia des fruits à noyau, dont trois sous-espèces (*M. laxa*, *M. fructicola*, *M. fructigena*) sont bien contrôlées par la mandestrobine. Sans effet indésirable significatif sur les auxiliaires, la mandestrobine est une solution utile pour le contrôle des maladies telles que mentionnées ci-dessus.
Mots-clés : mandestrobine / QoI / *Sclerotinia sclerotiorum* / *Monilia spp.*

ABSTRACT

Mandestrobin is a new fungicide developed by Sumitomo Chemical Co., Ltd. It has high antifungal activity, against the pathogens like *Sclerotinia* or *Botrytis*, by inhibiting various stages of their lifecycle – ascospore germination, germ tube elongation, formation of Sclerotia and apothecia – in the case of *Sclerotinia*. Mandestrobin can penetrate and translocate in plants to give systemic activity. Physiological effect on oilseed rape is observed, and considered to be one factor of increase in grain yield. Excellent efficacy is observed also against stone fruits *Monilinia*, of which three subspecies (*M. laxa*, *M. fructicola*, *M. fructigena*) are consistently controlled by mandestrobin. With no significant adverse effect on beneficials, mandestrobin is a reasonable option for the control of diseases such as above.
Keywords: mandestrobin / QoI / *Sclerotinia sclerotiorum* / *Monilinia spp.*

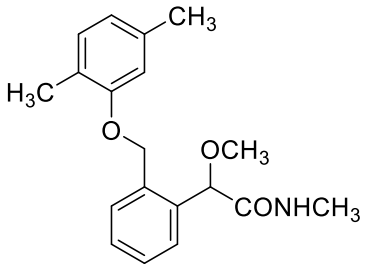
Introduction

Mandestrobin is a novel fungicidal compound discovered and developed by Sumitomo Chemical Co., Ltd. This compound controls a wide range of phytopathogenic fungi, especially the ones belonging to Leotiomycetes like *Sclerotinia* sp., *Monilinia* sp., and so on. Sclerotinia rot, caused by *Sclerotinia sclerotiorum*, is a destructive disease in various crops especially oilseed rape, which can cause significant yield loss. Practically, fungicide is applied preventively at flowering stage, the infection timing of *S. sclerotiorum*, knowing no solutions in the market can be used on a curative way without having significant loss of yield/quality. However, Sclerotinia rot may not occur at significant level depending on climate condition, and return on investment by fungicide application is sometimes questionable.

In this article, physical and chemical properties, toxicological profiles, mode of action and efficacy characteristics of mandestrobin are described. Additionally, physiological effect of mandestrobin to oilseed rape is also presented as a potential contributor of yield enhancement.

Physical and chemical properties of mandestrobin are presented in Table I

Table I : Physical and chemical properties of mandestrobin
(Propriétés physico-chimiques de la mandestrobine)

Code name	S-2200
Chemical name (IUPAC)	(*RS*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl] acetamide
Chemical structure	
Molecular formula	$C_{19}H_{23}NO_3$
Molecular weight	313.39 g/mol
Physical state	Solid (at 20 °C)
Melting point	102 °C (375.2 K)
Vapor pressure	3.36 x 10^{-8} Pa (at 20°C)
Octanol-water partition coefficient	3.51 (at 25°C and pH 5)
Water solubility	15.8 mg/L (at 20 °C)
Adsorption coefficient	$K_{Foc(ads)}$ 287 - 797 mL/g

Toxicological profiles of mandestrobin (Table II, III and IV)

Table II : Toxicological profile of mandestrobin (Profil toxicologique de la mandestrobine)

Acute oral LD_{50}	Rat	> 2000 mg/kg
Acute dermal LD_{50}	Rat	> 2000 mg/kg
Acute inhalation LD_{50}	Rat	> 4.96 mg/L
Eye irritation	Rabbit	Mild transient irritation (not triggering classification)
Skin irritation	Rabbit	Not irritating
Genotoxicity		Not genotoxic
Carcinogenicity	Rat, Mice	Not carcinogenic
Multigeneration reproduction	Rat	Negative
Developmental toxicity	Rat, Rabbit	Negative

Table III : Eco-toxicological profile of mandestrobin (Profil éco-toxicologique de la mandestrobine)

Acute single oral LD_{50}	Bobwhite quail (*C. virginianus*)	> 2250 mg/kg
Short-term (8-day) dietary LC_{50}	Bobwhite quail (*C. virginianus*)	> 5620 mg/kg feed
Short-term (8-day) dietary LC_{50}	Mallard Duck (*A. platyrhynchos*)	> 5620 mg/kg feed
Acute toxicity (14 day) LC_{50}	Earthworm (*Eisenia fetida*)	84 mg/kg soil
Acute oral LD_{50}	Honey bee (*Apis mellifera*)	> 110.71 µg/bee

Table IV : Toxicity to beneficials of mandestrobin (Toxicité de la mandestrobine sur les auxiliaires)

Acute oral toxicity 48 h LD50	mandestrobin Technical	Apis mellifera	> 110.71 µ g a.s./bee
Acute contact toxicity 48 h LD50	mandestrobin Technical	Apis mellifera	> 100 µ g a.s./bee
Acute oral toxicity 48 h LD50	mandestrobin 25 SC	Apis mellifera	> 109 µ g a.s./bee
Acute contact toxicity 72 h LD50	mandestrobin 25 SC	Apis mellifera	> 100 µ g a.s./bee
Laboratory glass plates (2D) LR50	mandestrobin 25 SC	Aphidius rhopalosiphi (adults)	> 1000 g a.s./ha
Laboratory glass plates (2D) LR50	mandestrobin 25 SC	Typhlodromus pyri (protonymphs)	> 1000 g a.s./ha

a.s. : active substance

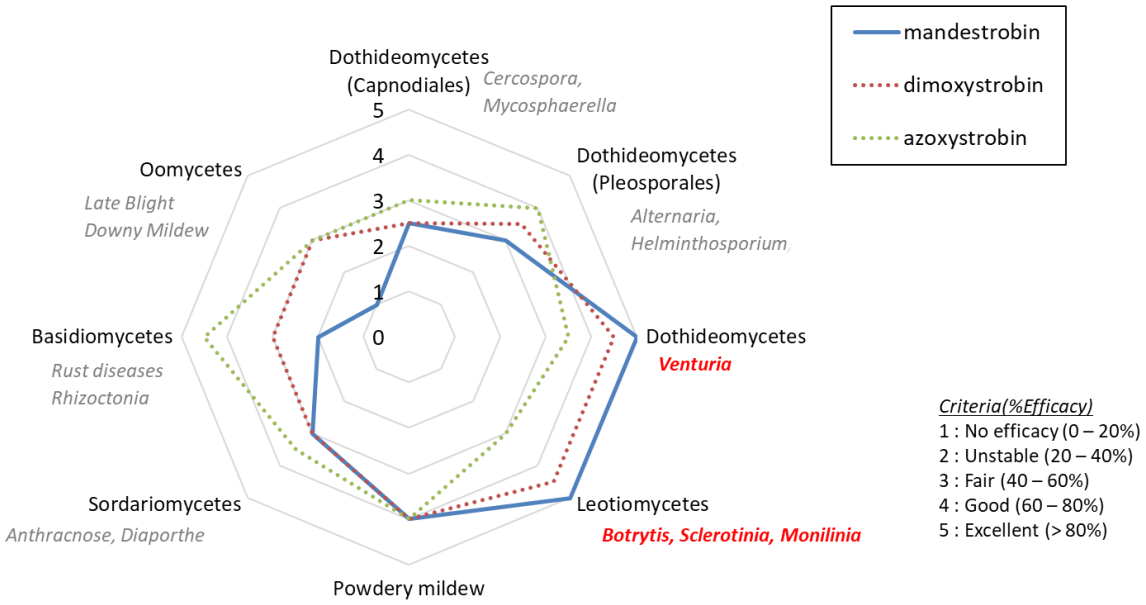
Antifungal activity of mandestrobin

Inhibition of mycelial growth of plant pathogenic fungi was tested. Mandestrobin has antifungal activity against wide range of plant pathogenic fungi. Amongst the tested pathogens, Mandestrobin showed high antifungal activity against *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Monilinia* sp., *Phomopsis* sp. and *Venturia inaequalis* (Table V). The radar chart based on antifungal tests and pot tests which indicates the fungicidal spectrum of mandestrobin in comparison with dimoxystrobin and azoxystrobin is shown in Figure 1.

Table V : Antifungal activity of mandestrobin against plant pathogens
(Activité antifongique de la mandestrobine contre des agents pathogènes des plantes)

Tested pathogen (Disease name)	EC_{50} (ppm)	Tested pathogen (Disease name)	EC_{50} (ppm)
Sclerotinia sclerotiorum (Sclerotinia stem rot)	0.022	*Botrytis cinerea* (Gray mold)	0.024
Monilinia fructicola (Stone fruit blossom blight / brown rot)	0.034	*Monilinia laxa* (Stone fruit blossom blight / brown rot)	0.016
Monilinia fructigena (Stone fruit blossom blight / brown rot)	0.075	*Phomopsis sp.* (Stone fluit Phomopsis fruit rot)	0.019
Glomerella cingulata (Apple anthracnose)	1.0	*Venturia inaequalis* (Apple scab)	0.0082
Diplocarpon mali (Apple Marssonina blotch)	0.062	*Diaporthe citri* (Citrus melanose)	0.18
Cercospora beticola (Sugar beet leaf spot)	0.50	*Rhizoctonia solani* (Damping off)	0.45
Ustilago maydis (Corn smut)	1.0	*Fusarium graminearum* (Wheat head blight)	25
Microdochium nivale (Wheat head blight)	0.043	*Pyrenophora tritici-repentis* (Wheat tan spot)	0.42
Colletotrichum lagenarium (Cucumber anthracnose)	5.4	*Alternaria solani* (Patato early blight)	15

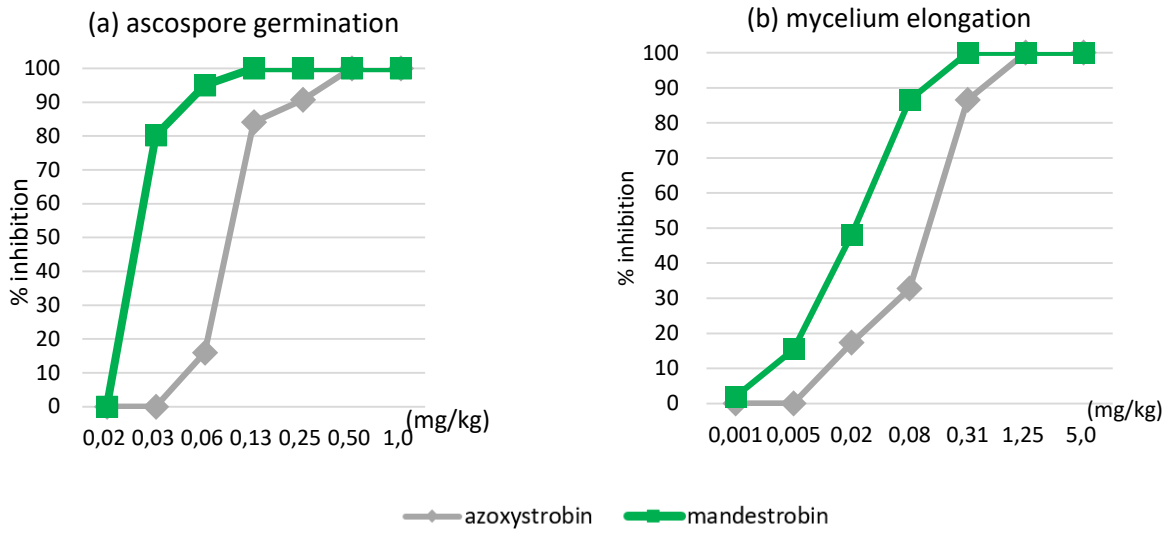
Figure 1 : Fungicidal spectrum of mandestrobin in comparison with dimoxystrobin and azoxystrobin (Spectre antifongique de la mandestrobine comparé à ceux de la dimoxystrobine et de l'azoxystrobine).



Inhibitory activity on ascospore germination and mycelium elongation against *Sclerotinia sclerotiorum*

Inhibitory activities of mandestrobin and azoxystrobin on *S. sclerotiorum* ascospore germination were evaluated. Ascospores are put on 2% agar media containing fungicides and incubated for one day at 23°C in the dark. Germination of ascospores was checked and germination rate was calculated. Additionally, inhibitory activity on *S. sclerotiorum* mycelium elongation was also evaluated. Potato Dextrose Agar (PDA) media incubated with *S. sclerotiorum* was clipped by cork borer and put on other PDA media containing fungicides and 100 ppm SHAM (salicylhydroxamic acid). Two days after incubation at 18°C in the dark, mycelial growth was measured. As shown in Figure 2, mandestrobin showed significantly higher Inhibitory activity on both ascospore germination and mycelium elongation against *S. sclerotiorum* than azoxystrobin.

Figure 2 : Inhibitory activity of fungicides on *S. sclerotiorum* ascospore germination and mycelium elongation (Activité inhibitrice des fongicides sur la germination des ascospores et sur l'élongation mycélienne de *S. sclerotiorum*)



Mode of action

Due to the similarity of chemical structure, mandestrobin was expected to have the same mode of action as other strobilurin fungicides, which act as quinone outside inhibitor (QoI). In order to check the inhibitory activity at quinone outside of complex III, inhibition rate at *in vitro* electron transport chain was measured by using mitochondria derived from *Botrytis cinerea*. Inhibition of electron transfer was measured by two experiments. Inhibition from Complex I to III was measured by the reduction of NADH, while that from Complex II to III was done by the reduction of cytocrome c. Mandestrobin showed similar tendency to azoxystrobin, a known QoI, which inhibited electron transfer in both experiments. The result was in contrast to that of known inhibitors of Complex I or Complex II, tolfenpyrad and boscalid respectively. IC_{50} values of tested molecules are described in Table VI.

Therefore it is suggested that mandestrobin acts as QoI (quinone outside inhibitor) fungicide. Mandestrobin is classified as QoI fungicide by FRAC (Code No. C3).

Table VI : 50% inhibitory concentration (IC_{50}) of fungicides at Complex I to III and Complex II to III (Concentration inhibitrice 50% (IC50) des fongicides sur les complexes I à III et II à III)

Compound	IC_{50} (mg/L)		Target site
	Complex I to III (NADH)	Complex II to III (Succinate)	
mandestrobin	0.011	0.0024	Complex III
azoxystrobin	0.016	0.0065	Complex III
boscalid	>0.04 no efficacy	0.003	Complex II
tolfenpyrad	0.012	>1 no efficacy	Complex I

Laboratory tests against *Sclerotina sclerotiorum* (Cucumber)

To demonstrate the basic profile of mandestrobin, we conducted pot tests under four different conditions such as 'Preventive', 'Residual', 'Curative' and 'Translaminar' using cucumber as a model plant.

Chemical spray was done as the designated dosages with the water volume of 200 L/ha. (Test dosages X = 200 g a.s./ha (mandestrobin), 250 g a.s./ha (azoxystrobin, tebuconazole)). Inoculum of *S. sclerotiorum* was prepared as a hyphal fragment mixed with 0.8% PDA. After or before chemical treatment of the fungicidal solution 50 μL of inoculum was placed onto the designated surface of tested plant then incubated at 23°C with saturated moisture condition for infection.

Preventive condition: Chemical spray was made a day before inoculation. Then the test plants were incubated for 4 days. Assessment was done at four days after inoculation.

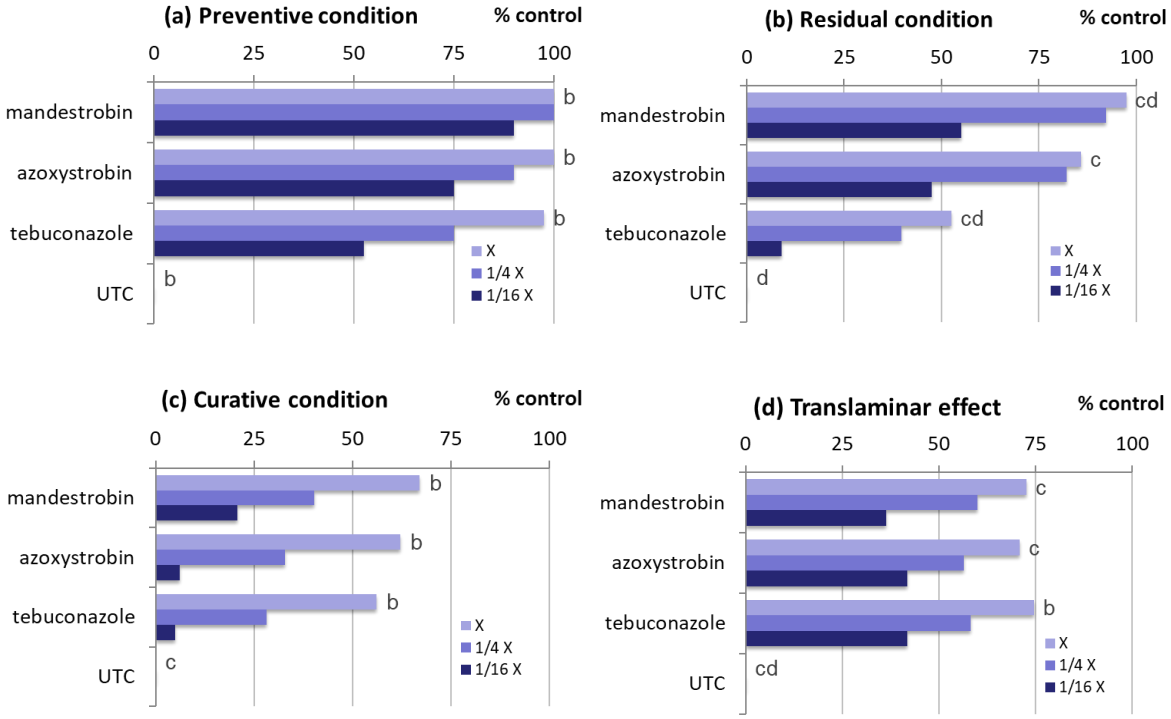
Residual condition: Chemical spray was done 14 days before inoculation. Test plants were grown in green house until inoculation, then incubated at 23°C with saturated moisture for four days. Assessment was done at four days after inoculation.

Curative condition: Inoculation was done a day before chemical spray. Before and after chemical spray, the plants were incubated at 23°C with saturated moisture. Assessment was done at three days after chemical spray.

Translaminar test: Chemical spray was done on the upper surface of leaves of the tested plants a day before inoculation. After making inoculation on the lower surface of the leaves, the plants were incubated at 23°C with saturated moisture for four days. Assessment was done at four days after inoculation.

Mandestrobin showed efficacy at a level which is equal to superior to the reference molecules under these four conditions (see figure 3)

Figure 3 : Efficacy results of laboratory tests for mandestrobin and two reference molecules under four different conditions (Résultats d'efficacité des tests au laboratoire pour la mandestrobine et deux molécules de référence sous quatre conditions)



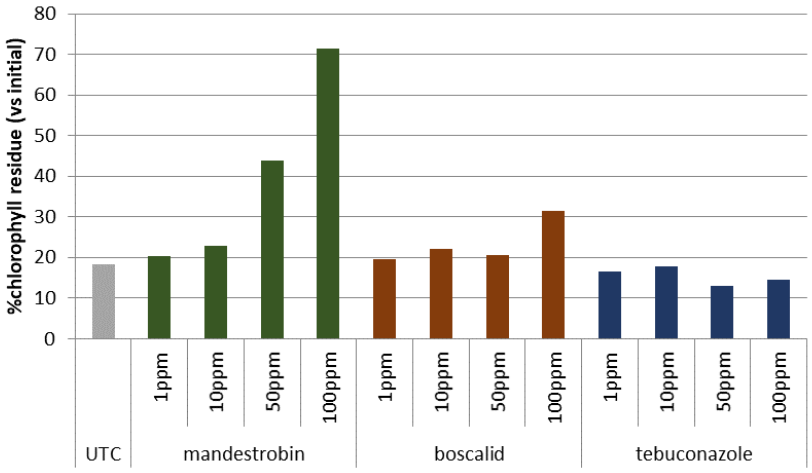
Physiological effect of mandestrobin – greening effects – on oilseed rape

In the field trials, some physiological effects, so called 'greening effects' on the treated plants were observed. To confirm the 'greening effects' of mandestrobin, chlorophyll content of treated oilseed rape leaves which were healthy was evaluated according to the following formula by measuring the optical absorbance of chlorophyll extracts of treated leaves at 645 nm (A_{645}) and 663 nm (A_{663}) with spectrophotometer.

Total chlorophyll (µg/mL) = $8.02A_{663} + 20.20A_{645}$

The chlorophyll content after mandestrobin treatment in % of initial content is shown in Figure 4. Mandestrobin significantly improved the amount of chlorophyll preserved. By increasing the chlorophyll content in the leaves, mandestrobin is assumed to promote 'greening effects' on oilseed rape. This may further result in yield enhancement.

Figure 4 : Chlorophyll content of oilseed rape treated with fungicides in comparison with untreated control (UTC) (Teneur en chlorophylle des feuilles de colza traitées en comparaison au témoin non traité)

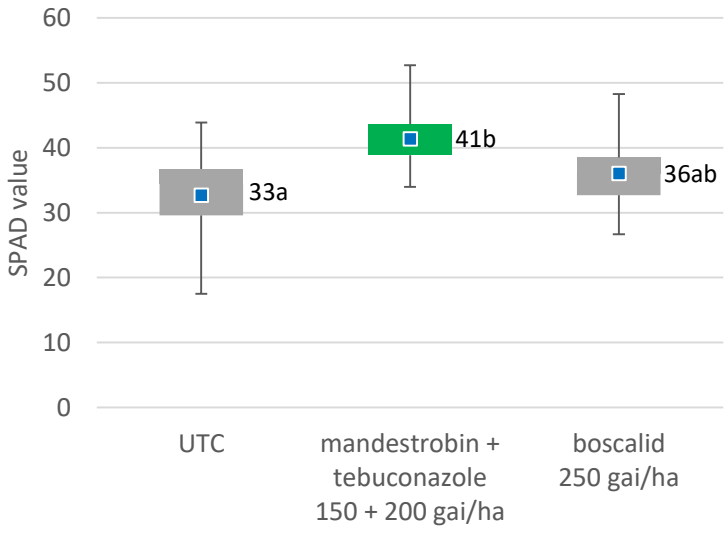


ppm = mg/kg

Also, chlorophyll content represented by the measured SPAD value of treated oilseed rape leaves was evaluated in field without disease. SPAD is indication value measured by chlorophyll meter and is correlated with chlorophyll content.

Product associate mandestrobin and tebuconazole provided significantly bigger SPAD value than UTC and boscalid (Figure 5), indicating that mandestrobin containing product increases chlorophyll content of oilseed rape in field level condition.

Figure 5 : SPAD value of oilseed rape in field without disease (Valeur de SPAD au champ sur Colza en l'absence de maladie, pour les parcelles traitées fongicides en comparaison avec la modalité non traitée)



Statistical analysis: 5% Tukey's HSD mean separation on average

To estimate the mechanism of the 'greening effects' of mandestorobin, we focused on the effect of phytohormone in plant. Since it is known that two phytohormones, salicylate (SA) and jasmonate (JA), regulate plant growth including senescence, the effect of mandestrobin on the gene expression of three SA-responsive genes (*GRX480, PR1*, and *PR5*) and four JA-responsive genes (*MYC4, LOX2, VSP1*, and *SAG29*) were evaluated in *Arabidopsis thaliana*, a model plant of the family *Brassicacecae* same as oilseed rape. *GRX480* encodes glutaredoxin family protein which plays a role in the repression of JA signaling pathway. *PR1* and *PR5* are SA-inducible pathogenesis-related genes. *MYC4* encodes a transcription factor that positively regulates JA signaling pathway. *LOX2* is one of the JA biosynthesis genes. *VSP1* encodes an acid phosphatase that acts against herbivore attack. *SAG29* is one of senescence-associated genes, which is a member of the SWEET sucrose efflux transporter family proteins. In addition to the SA- and JA-responsive genes, the expression of the three genes related to chlorophyll degradation (*NYE1*, *NYE2*, and *PAO*) was also analyzed.

Excised leaves of *Arabidopsis* were treated with 100 μM mandestrobin solution for 48 hours in the dark before RNA extraction, and the expression of the genes was quantified by qRT-PCR. Mandestrobin significantly up-regulated the SA-responsive genes and down-regulated the JA-responsive genes (Figures 6 and 7). Simultaneously, the genes related to chlorophyll degradation were down-regulated by mandestrobin treatment (Figure 8). These data suggest that mandestrobin represses plant senescence by modulating the SA and JA signaling pathways, leading to the 'greening effects'.

Figure 6 : Expression of SA-responsive genes after treatment with mandestrobin, in comparison with DMSO (Expression du gène SA dépendant après traitement par la mandestrobine en comparaison au DMSO)

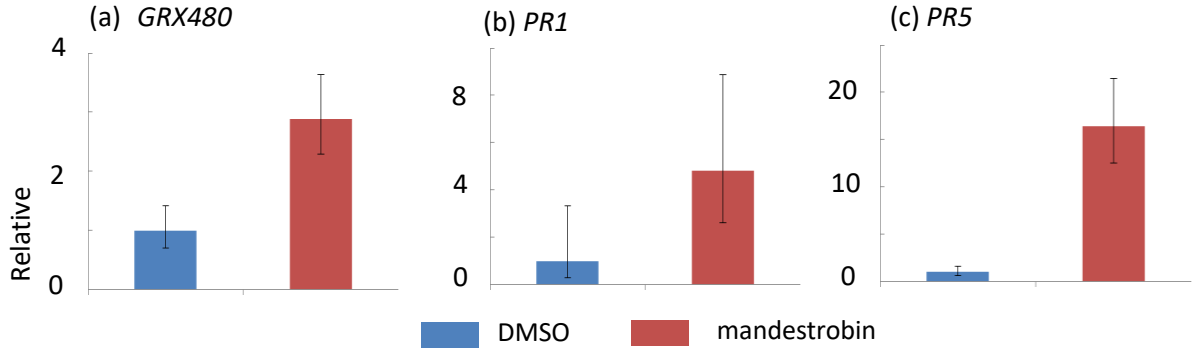


Figure 7 : Expression of JA-responsive genes after treatment with mandestrobin, in comparison with DMSO (Expression du gène JA dépendant après traitement par la mandestrobine en comparaison au DMSO)

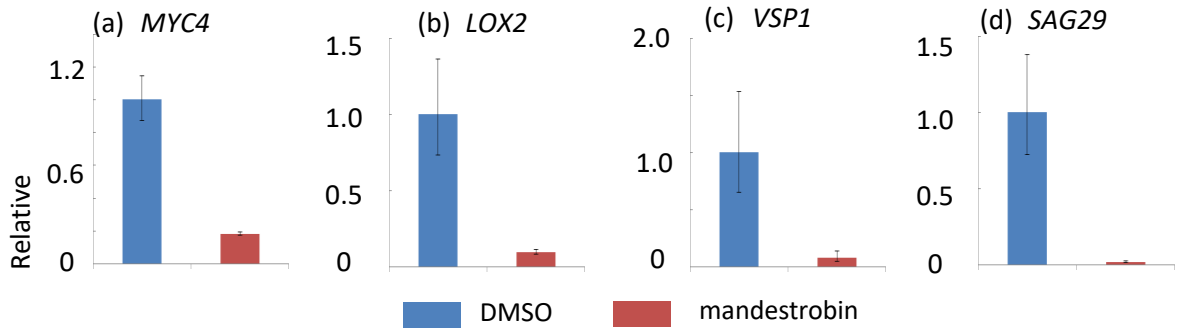
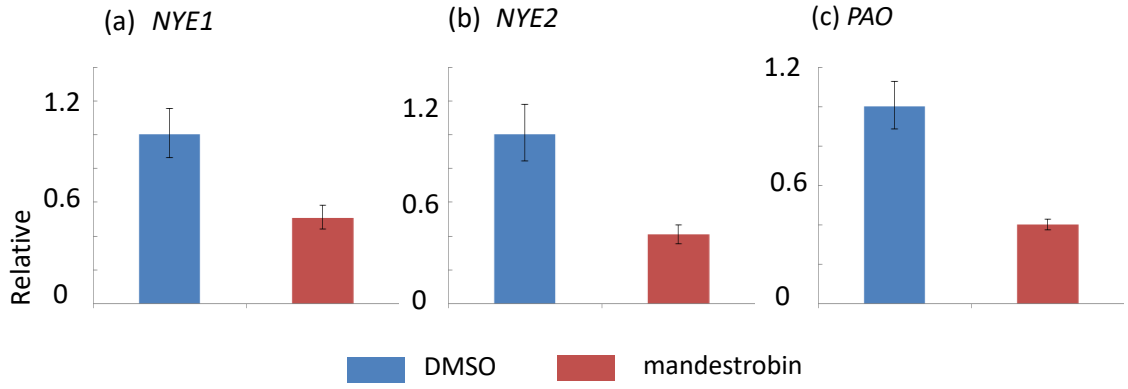


Figure 8 : Expression of genes related to chlorophyll degradation after treatment with mandestrobin, in comparison with DMSO (Expression des gènes liés à la dégradation de la chlorophylle après traitement par la mandestrobine en comparaison à DMSO)



Conclusion

Mandestrobin is a new QoI fungicide which has antifungal spectrum against wide range of plant pathogens. In this report, favorable profile of mandestrobin was confirmed as a fungicide for oilseed rape production. Both *Sclerotinia* control and physiological effect can be expected in the use to realize improved yield of oilseed rape. In the case of stone fruits, mandestrobin could be used as a new tool to control *Monilinia* sp.