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ISOFETAMID, A UNIQUE NEW GENERATION OF FUNGICIDES FOR GRAPEVINE, STRAWBERRIES, STONE FRUITS AND OILSEED RAPE

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ABSTRACT

Isofetamid is a new fungicidal active substance developed by Ishihara Sangyo Kaisha Ltd (ISK, Japan). The compound belongs to the family of succinate dehydrogenase inhibitors (SDHI). However, it is the only substance based on the thiophene carboxamide moiety, which gives it a unique structure, allowing the control of pathogens (in particular *Botrytis cinerea*) carrying most of the mutations that cause SDHI resistance, thanks to its flexible bond.
Isofetamid is a translaminar compound, with a high preventive, curative and anti-sporulant activity, a good rainfastness, providing a long-lasting protection for the treated plants. It is a broad-spectrum fungicide, highly efficient against Botrytis, Monilia and Sclerotinia stem rot, developed first on grapevine (wine and table), strawberries, oil seed rape, cherries and apricots.

Keywords: isofetamid, thiophene carboxamide, flexible bond, long-lasting protection, Botrytis, Monilia, Sclerotinia.

RÉSUMÉ

ISOFETAMIDE, UNE NOUVELLE GENERATION UNIQUE DE FONGICIDE POUR VIGNE, FRAISIERS, FRUITS A NOYAU ET COLZA

L'isofétamide est une nouvelle substance active fongicide découverte par Ishihara Sangyo Kaisha Ltd (ISK, Japon). Cette substance active appartient à la famille des inhibiteurs de la succinate déshydrogénase (SDHI), mais est la seule substance basée sur la fraction thiophène carboxamide, ce qui lui confère une structure unique permettant le contrôle des pathogènes (notamment *Botrytis cinerea*) présentant la plupart des mutations associées aux SDHI, grâce à sa liaison chimique flexible.
L'isofétamide est un produit translaminaire, doté d'un haut niveau d'efficacité, tant en préventif qu'en curatif, anti-sporulant, présentant une bonne résistance au lessivage et apportant une longue protection aux plantes traitées. C'est un fongicide à large spectre, efficace contre Botrytis, Moniliose et Sclérotiniose, développé dans un premier temps sur vigne, fraises, colza, cerises et abricots.

Mots-clés : isofétamide, thiophène carboxamide, liaison chimique flexible, longue protection, Botrytis, Moniliose, Sclérotiniose.

INTRODUCTION

Isofetamid is a new fungicidal active substance discovered in 2005 and developed by Ishihara Sangyo Kaisha Ltd (ISK, Japan). The compound belongs to the FRAC mode of action group of succinate dehydrogenase inhibitors (SDHI). However, it is the only substance from this group based on a thiophene carboxamide moiety, which gives the substance a unique, more flexible molecular structure. This feature allows the compound to control pathogens carrying most of the mutations that cause SDHI resistance. Isofetamid is marketed in the European Union as a 400 g/L SC formulation of isofetamid, under the names KENJA®/ZENBY® (registered trademarks of Ishihara Sangyo Kaisha Ltd, distributed by Belchim Crop Protection). This formulation, coded IBE 4022, is authorised in France under AMM no. 2171010.

PHYSICO-CHEMICAL PROPERTIES AND RISK ASSESSMENT ASPECTS

GENERAL PHYSICO-CHEMICAL /DESCRIPTION

Isofetamid is a new fungicidal active ingredient based on a different chemical structure: phenyl-oxo-ethyl thiophene amide (Table I). The physico-chemical properties provide advantages for the behaviour in the environment (soil and water) and plants (uptake) (Table II).

Table I: chemical description and identification of the new active substance, isofetamid (présentation de la nouvelle substance active, isofétamide)

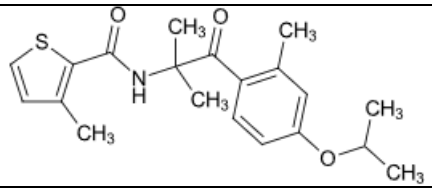
Common name	Isofetamid
Molecular formula	$C_{20}H_{25}NO_3S$
Structural formula	
IUPAC Name	N-[1,1-dimethyl-2-(4-isopropoxy-o-tolyl)-2-oxoethyl]-3-methylthiophene-2-carboxamide
Code numbers	CAS No 875915-78-9 CIPAC No 972
Chemical family	SDHI (succinate dehydrogenase inhibitors) – FRAC group C2
Chemical group	Phenyl-oxo-ethyl thiophene amide (isofetamid is the only registered fungicidal compound within this chemical group)

Table II: physical properties of the new active substance, isofetamid (données physiques sur la nouvelle substance active, isofétamide)

Molecular weight	359.48 g/mol
Appearance	white to light brown powder
Odour	none
Density	1.23 g/ml
Melting point	103.5 –105.0 °C (99.9% w/w)
Boiling point	n.a. (due to decomposition before boiling)
Solubility in water	5.33 mg/L at 20°C
Vapour pressure	4.2×10^{-7} Pa at 25°C
Log P_{ow}	2.5
Other	Isofetamid is not highly flammable and does not auto-ignite at environmental temperatures (no self-ignition observed up to 400°C). The compound is not explosive and is not considered an oxidiser.

TOXICITY

Isofetamid presents a low risk of acute toxicity for mammals when administered by oral, dermal or inhalation routes (Table III).

Table III: toxicological endpoints of the new active substance, isofetamid
(données toxicologiques sur la nouvelle substance active, isofétamide)

LD_{50} oral (rat)	> 2000 mg/kg bw → low toxicity
LD_{50} dermal (rat)	> 2000 mg/kg bw → low toxicity
LD_{50} inhalation (rat)	> 4.8 mg/L air/4 h → low toxicity
Eye irritation	non-irritant
Dermal irritation	non-irritant
Sensitization (LLNA)	not sensitising

Furthermore, the conclusions of the studies on possible mutagenicity, teratogenicity, carcinogenicity and reproductive toxicity properties on the active ingredient show that isofetamid is not likely to pose a toxicological risk to humans or other organisms in the environment.

ECOTOXICITY AND ENVIRONMENTAL FATE

The potential risks (acute and chronic) of an exposure of birds, aquatic organisms, bees, other non-target arthropods, earthworms, other soil micro- and macro-organisms and terrestrial plants were found to be acceptable for all registered uses.

Table IV: environmental fate endpoints of the new active substance, isofetamid
(données environnementales sur la nouvelle substance active, isofétamide)

DT_{50} in soil	37.1 days
DT_{50} in water	141.2 days
DT_{50} in air	1.38 hours

Due to the very acceptable degradation time in the soil and aquatic environment, a minimal and acceptable impact of the substance is anticipated on ecosystems and its very low volatility indicates that it is not expected to be found in any significant concentration in the air (Table IV).

LEGAL INFORMATION

According to criteria in relevant EU legislation isofetamid is subjected to the following risk phrases:

- H411: Chronic aquatic 2
- GHS09

EU MRLs (maximum residue levels) were obtained for all crops for which the compound isofetamid is launched in Europe (Table V). Following the Good Agricultural Practices (GAP) no residues were found in any trial for stone fruits (apricots and cherries when applied around flowering stage) or oilseed rape (application at flowering stage), therefore the MRL was fixed to the limit of quantification (LOQ = 0.01 mg/kg).

Table V: registered MRLs for the new active substance, isofetamid
(limites maximum de résidus pour la nouvelle substance active, isofétamide)

Uses	MRL (mg/kg)	Publication
Wine grapes	4	Reg. (EU) 2017/171
Table grapes	4	
Strawberries	4	Reg. (EU) 2018/687
Oilseed rape	0.01 (LOQ)	Reg. (EU) 2017/171
Cherries	0.01 (LOQ)	
Apricots	0.01 (LOQ)	

For humans, whilst considering a safety factor of 100, the acceptable daily intake (ADI) according to the risk assessment is 0.02 mg/kg bw/day and the acute reference dose (ARfD) is 1 mg/kg bw/day; therefore, intake of isofetamid residues via treated crops is unlikely to present a public health concern. A sufficient number of crop residue trials at the defined critical GAP were presented in support of the compound's registration. The effects of processing on the nature of isofetamid residues have also been investigated and no issues were found.
Furthermore, considering the specific circumstances of the critical GAP uses, it is highly unlikely that residues will be present in succeeding crops.

BIOLOGICAL PROPERTIES

Isofetamid is a broad-spectrum fungicide, highly effective against fungal pathogens belonging to Ascomycota (*Sclerotinia* sp., *Monilinia* sp., *Botrytis* sp., *Venturia* sp., *Cladosporium* sp. and many powdery mildew species) but not active against Basidiomycetes or Oomycetes. The formulated product is being developed first on grapevine (wine and table), strawberries, oilseed rape, cherries and apricots.

The compound has a translaminar property in plant tissues, with a high preventive, curative and anti-sporulant activity, resulting in a good rainfastness and providing a long-lasting protection for the treated plants.

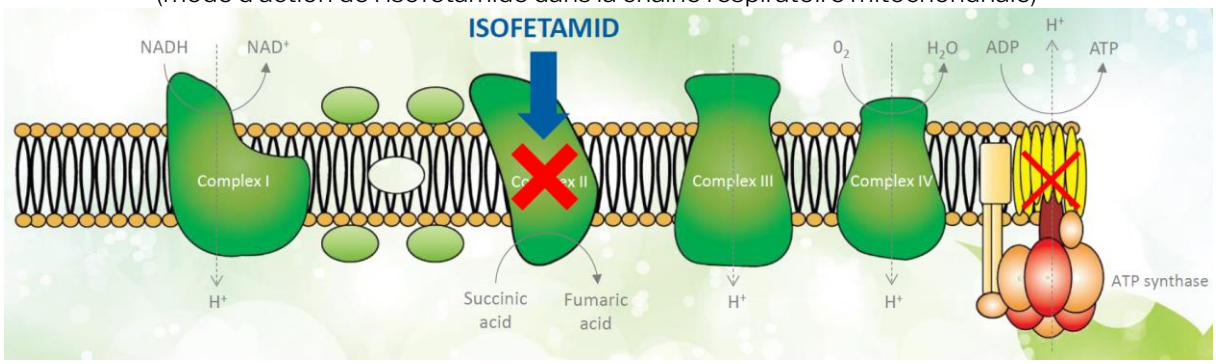
The recommended dose rates are varying according to the crop-disease combination and are of 0.8 to 1.5 L product/ha (320 – 600 g a.s./ha) per treatment. The interval between treatments (where relevant) is minimum 7-10 days, or related to specific growth stages of the crop.

MODE OF ACTION

Isofetamid acts by binding specifically on the succinate dehydrogenase (SDH) of complex II, a key enzyme of the mitochondrial respiratory chain which is at the crossroads of two metabolic pathways essential to fungal cell's life (Krebs cycle and electron transport phosphorylation).

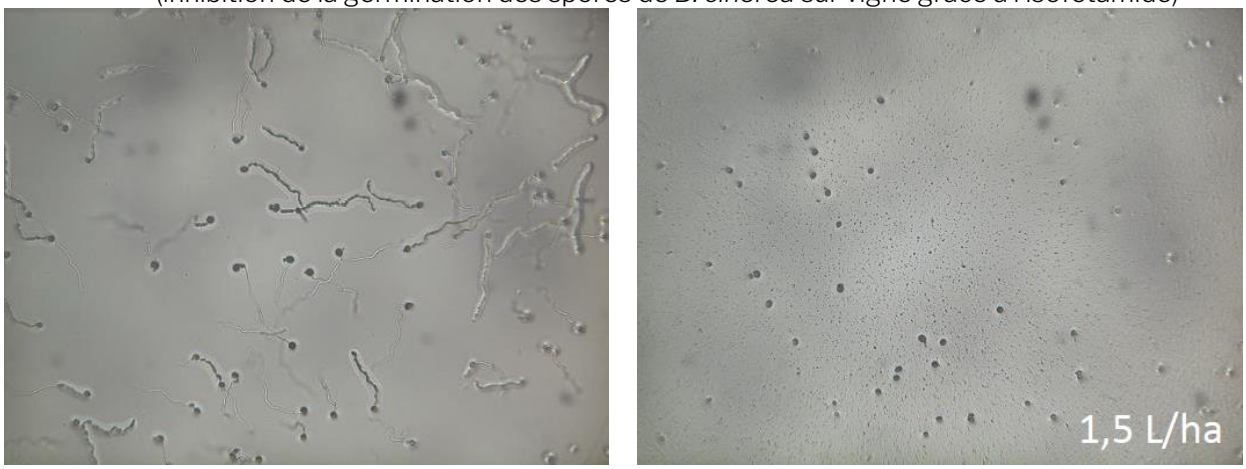
By binding on to this complex II, isofetamid blocks the production of molecules rich in energy at the Krebs cycle (FADH2 and also NADH). In addition, the electron transport chain (several complex) will be adversely affected and the conversion of ADP into ATP made by the ATP synthase will be reduced (Figure 1).

Figure 1: cellular mode of action of isofetamid in the mitochondrial respiratory chain
(mode d'action de l'isofétamide dans la chaîne respiratoire mitochondriale)



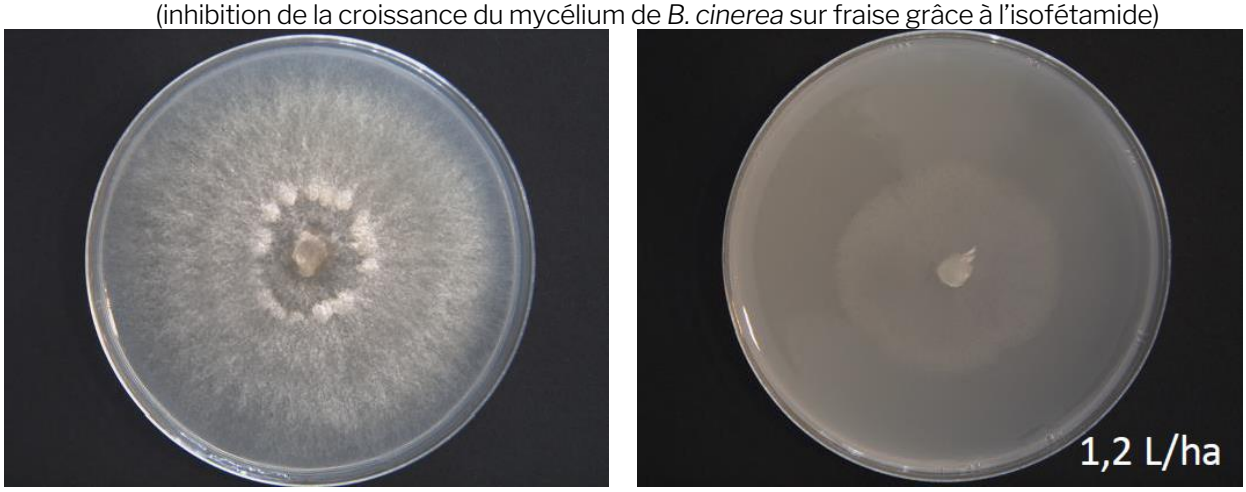
Due to this mode of action, isofetamid acts on all energy demanding life stages of the pathogen cycle: spore germination, germ tube growth, penetration into plant cells, mycelial growth and sporulation (Figures 2 and 3).

Figure 2: inhibition of the spore germination of *Botrytis cinerea* from grapevines with isofetamid (inhibition de la germination des spores de *B. cinerea* sur vigne grâce à l'isofétamide)



Untreated | Treated with isofetamid 600 g a.s./ha

Figure 3: inhibition of the mycelium growth of *Botrytis cinerea* from strawberry with isofetamid (inhibition de la croissance du mycélium de *B. cinerea* sur fraise grâce à l'isofétamide)



Untreated | Treated with isofetamid 480 g a.s./ha

Isofetamid has a good curative activity as well; however, it is always recommended to apply the compound preventively in order to implement a correct resistance management and to keep consistent high efficacy, preventing economical losses.

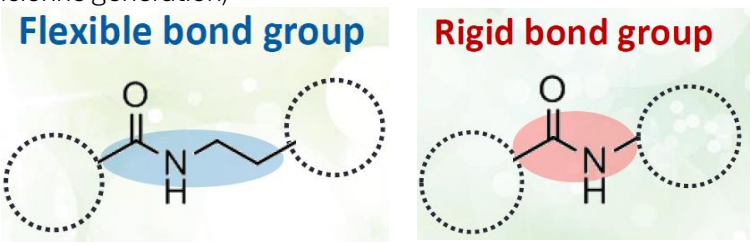
BEHAVIOUR IN THE PLANT

The isofetamid substance has translaminar properties. It can protect non-treated plant parts by going from one side of the leaf to the other side in less than 24 hours. In that way the substance is also less vulnerable from wash-off by rains following a treatment. The formulation IBE 4022 presents a good rainfastness even when confronted with 40 mm of rain, only 2 hours after treatment: the isofetamid specialty after a rainfall event has a better efficacy than a boscalid-based reference product without rain. Nonetheless, it is recommended to avoid rain 12 to 24 hours after treatment in order to keep the highest levels of efficacy. Isofetamid has a long-lasting effect. In laboratory conditions, it keeps a complete efficacy on vine berries until 4 weeks after application. Furthermore, despite the special attention paid during the development process, it can be confirmed that the selectivity of isofetamid at its registered rate is excellent, with no adverse symptoms ever recorded in the biological trials set up in the field, even in the second or third year of application.

RESISTANCE MANAGEMENT

An important innovation of isofetamid, compared to older generations of SDHI, is the different molecular structure, ethyl thiophene amide (Figure 4).

Figure 4: description of the flexible bond of isofetamid and comparison with an older generation SDHI (description de la liaison flexible de l'isofétamide et comparaison avec un SDHI d'une ancienne génération)



As a consequence, in a simulation modelling of SDH enzyme 3D structure and inhibitor binding, isofetamid can bind on both sensitive protein and H272Y protein with similar binding mode.
It is considered that flexibility of binding ability is caused of flexible bridge structure, ethyl thiophene amide. The isofetamid substance is able to continue to bind effectively to the altered action site of the SDH of complex II in some known mutant strains bearing resistances to most SDHI (Table VI).

Table VI: sensibility of various *B. cinerea* strains towards isofetamid and other SDHI (sensibilité de différentes souches de *B. cinerea* à l'isofétamide et autre SDHI)

	Isofetamid	Reference SDHI
Sensitive control	S	S
H132R	MR	R
P225F	R	R
P225T	R	R
N230I	S	MR
H272L	R	R
H272R	S	R
H272Y	S	R

S: Sensitive / MR: Moderately resistant / R: Resistant

AUTHORISED USES

IBE 4022, a straight formulation of isofetamid (400 g/l SC), is distributed by Belchim Crop Protection. At launch, it is authorised in France on grapevine (wine and table), strawberry, oilseed rape and at flowering stage of stone fruits (cherry and apricot). The target pathogens are *Botrytis cinerea*, *Sclerotinia* sp. and *Monilinia* sp. and the registered dose rates vary from 0.8 to 1.5 L/ha depending of the crop (Table VII).

Table VII: authorised uses in France for IBE 4022, based on the new active substance, isofetamid (usages autorisés en France pour IBE 4022, basés sur la nouvelle substance active, isofétamide)

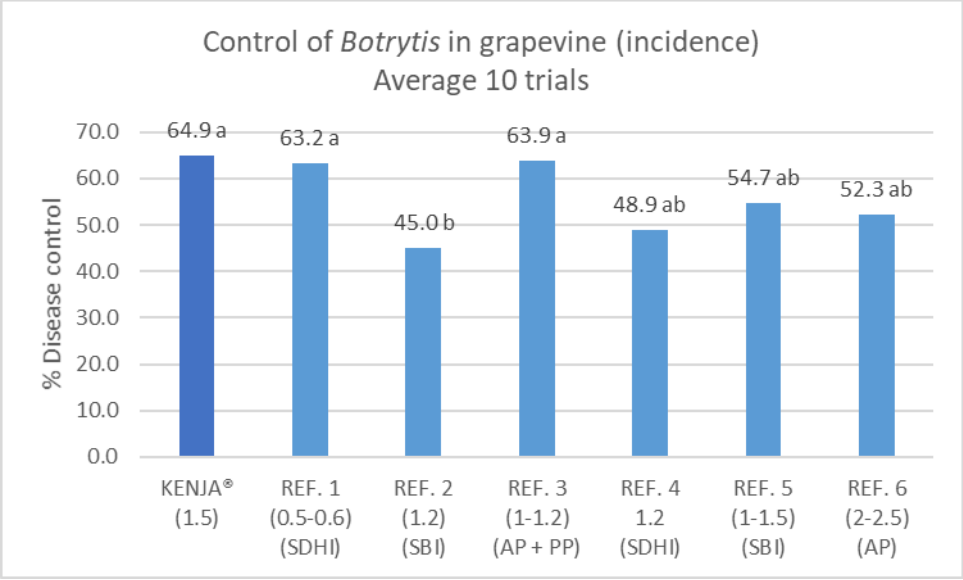
Crop	Pest	Dose rate	Number of Applications/season	Pre-harvest interval (PHI)	Buffer zone
Grapevine	*Botrytis*	1.5 L/ha	1	21 days	5 m
Strawberry	*Botrytis* and *Sclerotinia*	1.2 L/ha	2 (7-day interval)	1 day	5 m
Oilseed rape	*Sclerotinia*	0.8 L/ha	1	Until mid flowering	5 m
Cherry	*Monilia*	0.8 L/ha	2 (9-day interval)	Until end of flowering	5 m
Apricot	*Monilia*	0.8 L/ha	2 (9-day interval)	Until end of flowering	5 m

GRAPEVINE

In grapevine, the positioning of isofetamid is flexible: from BBCH 61 (stage A: flowering) up to BBCH 85 (stage D: 21 days before harvest). The recommendation is to apply IBE 4022, preferably in the A up to C stage, once per season at its maximum registered rate (1.5 L/ha) in alternation with active ingredients with a different mode of action for adequate resistance management.

The results of 10 trials conducted in Europe are presented in Figure 5, with IBE 4022 being compared to most relevant competitors. In these trials, 3 applications were carried out with each product (A, B and C at BBCH 65, 77 and 81). An application at timing D (BBCH 85) was not necessary in the conditions of these trials in France. The mean disease incidence was 48.8% in the untreated plots. The isofetamid based specialty was able to reach a level of efficacy comparable to those of the best products currently available on the market (statistical test: ANOVA followed by Student-Newman-Keuls test with a 5% risk).

Figure 5: average efficacy against *Botrytis* in grapevine - Pest incidence at harvest
(efficacité moyenne contre le botrytis sur vigne - Incidence à la date de récolte)

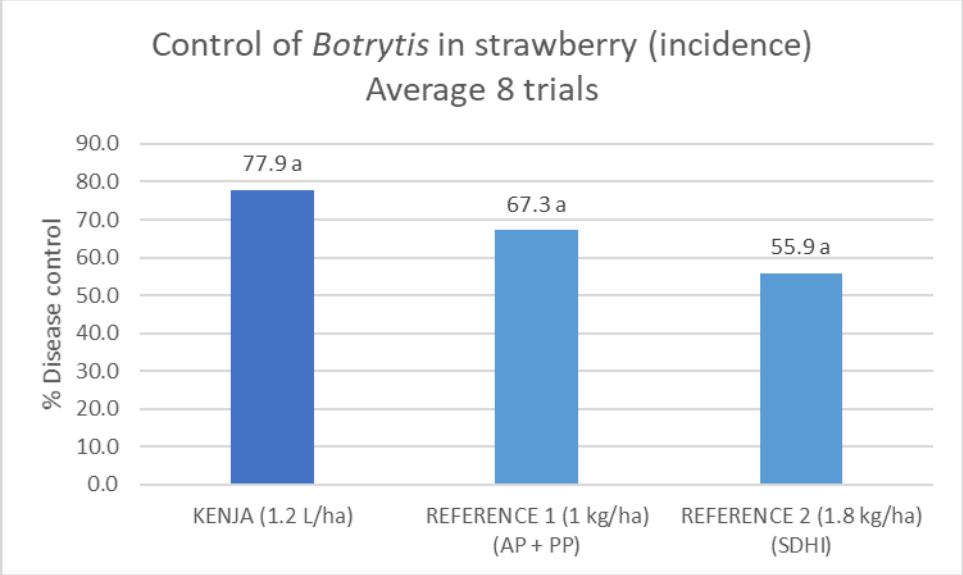


STRAWBERRY

In strawberry, isofetamid can be used from BBCH 60 (first flowers opened) to BBCH 87 (main harvest), with only 1-day PHI. It is recommended to apply IBE 4022 preventively at 1.2 L/ha, targeting the first applications in the season against botrytis for this crop. If possible, combine or alternate with another product having a different mode of action for adequate resistance management.

The results of 8 trials conducted in Europe (France and Spain, under protected conditions) are presented in Figure 6, with IBE 4022 being compared to most relevant competitors. In these trials, 3 preventive applications were carried out from flowering (BBCH 65) to harvest (BBCH 87) with each product, in 7-10 days intervals. The average disease incidence was 4.5 fruits per plant in the untreated plots. The isofetamid-based specialty was able to reach a level of efficacy comparable to or better than those of the most effective products currently available on the market. No statistical difference was observed between any of the treatments (ANOVA followed by Student-Newman-Keuls test with a 5% risk).

Figure 6: average efficacy (8 trials) against *Botrytis* in strawberry - Pest incidence at harvest (efficacité moyenne sur 8 essais contre le botrytis sur fraisier - Incidence à la date de récolte)



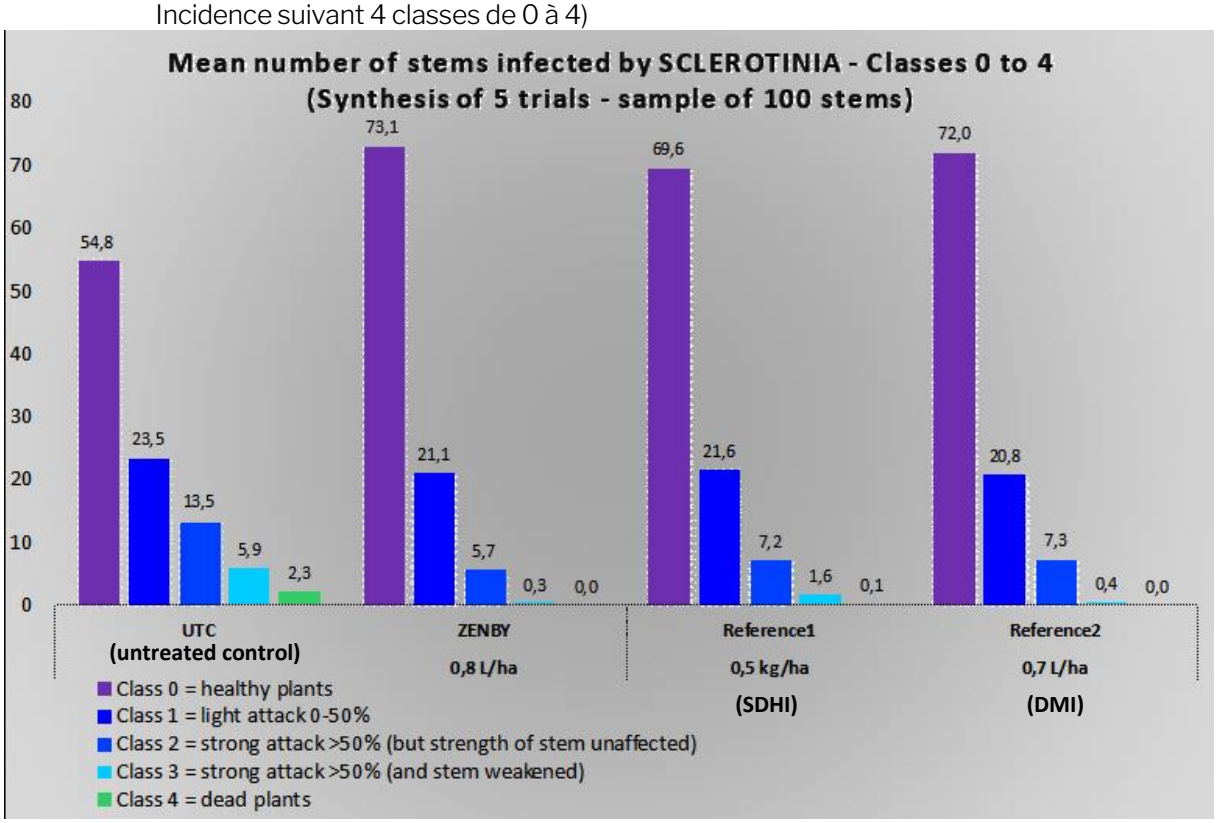
OILSEED RAPE

In oilseed rape, isofetamid is recommended to be applied at flowering (BBCH 60 to 65) at 0.8 L/ha to control *Sclerotinia sclerotiorum*.

The results from European internal development oilseed rape trials used for registration against *Sclerotinia* are displayed in Figure 7 (2 studies were located in France and 3 in Germany, experiments set up between 2009 and 2011); only one application was made at BBCH 61-65, generally 6 to 10 days after beginning of flowering, according to local temperatures. The mean incidence in the untreated plots was 45.2 stems infected on 100 stems with 23.5 stems in class 1 (light attacks), 13.5 stems in class 2 (stronger attacks >50%), 5.9 stems in class 3 (stronger attacks >50% with stems weakened) and 2.3 stems in class 4 (dead plants).

IBE 4022 gave similar control of *Sclerotinia* to the competitors (without significant statistical differences between the treatments, using a Newman-Keuls mean separation test with a 5% risk).

Figure 7: mean number of stems infected by *Sclerotinia* in oilseed rape - Pest incidence following classes from 0 to 4 (nombre moyen de tiges infectées par le *Sclerotinia* sur colza - Incidence suivant 4 classes de 0 à 4)

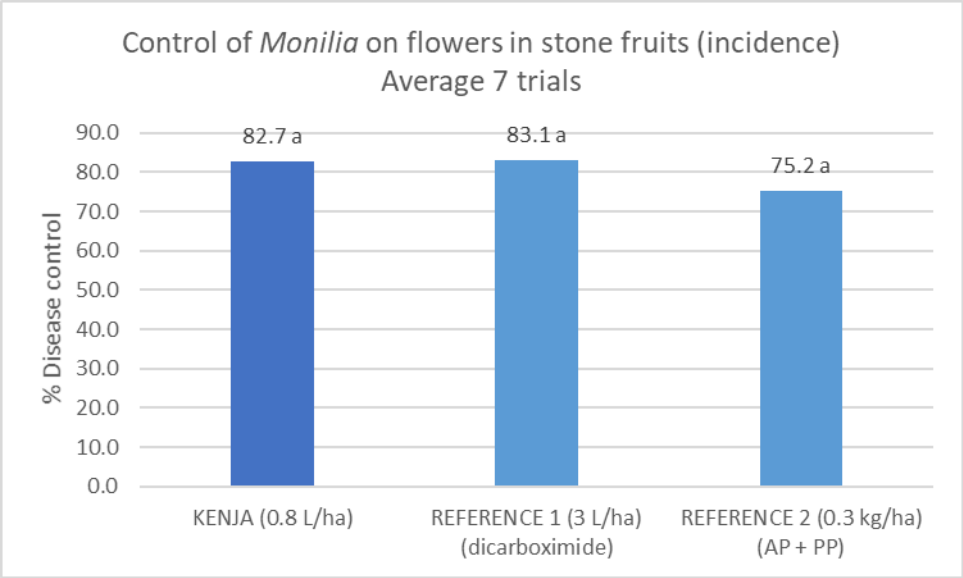


STONE FRUITS (CHERRY, APRICOT)

In cherry and apricot crops, it is recommended to apply isofetamid around flowering (BBCH 57-69) to control *Monilia* sp. attacks on flowers. Up to 2 applications at 0.8 L/ha are possible. If additional applications are needed against this disease, use a product with a different mode of action for adequate resistance management.

The shoots and flowers of cherry and apricot are attacked by the same *Monilia* species (*Monilia laxa* and *Monilia fructicola*). Therefore, and according to the EPPO extrapolation table, the efficacy results of these 2 crops are mixed together. In all trials, 250 flowers or flower clusters per plot were selected and assessed to determine the disease incidence on flower (PESINC). The application stages have been BBCH 56-65 for application A and 8-10 days later for application B. Studies were conducted in France, Spain and Italy. No statistical difference was observed between any of the treatments (ANOVA followed by Student-Newman-Keuls test with a 5% risk). Results are presented in Figure 8.

Figure 8: mean control of *Monilia* incidence on flowers in 7 trials on cherries and apricots (efficacité moyenne sur 7 essais contre la moniliose du cerisier et de l'abricotier à la fleur)



CONCLUSION

The new fungicidal active substance isofetamid, developed by Ishihara Sangyo Kaisha (ISK) and distributed by Belchim Crop Protection , represents a new generation of SDHI.

The compound's innovating properties deliver several advantages like the flexible molecular structure which allows to overcome part of the resistance phenomena encountered in the target pathogens. Its action on all the infective stages of the fungus cycle together with preventive, curative and anti-sporulant effects allow good flexibility of use and positioning the compound. Translaminarity, good rainfastness and long-lasting effects result in high consistency of diseases controls.

Thanks to all these characteristics, the formulated product provides an excellent control against *Botrytis* in grapevine and strawberries, *Sclerotinia* in oilseed rape and *Monilia* in stone fruits.

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