Végéphyl – 12e CONFÉRENCE INTERNATIONALE SUR LES MALADIES DES PLANTES
TOURS – 11 ET 12 DÉCEMBRE 2018

INATREQ™ ACTIVE (FENPICOXAMID), A NEW NATURAL FUNGICIDE FOR THE CONTROL OF *ZYMOSEPTORIA TRITICI* AND OTHER DISEASES IN CEREALS

M. BRILLAUD CAILLIAU [(1)], S. GATE [(1)], G. GUEPET [(2)], A. LEADER [(3)] and G. KEMMITT [(4)]

[(1)] DowDuPont Agriculture Division, 6 rue J.-P. Timbaud, 78067 Saint Quentin Yvelines, France
[(2)] DowDuPont Agriculture Division, 22 rue de Brunel, 75017 Paris, France
[(3)] DowDuPont Agriculture Division, CPC2 Capital Park, Fulbourn, Cambridge, CB21 5XE, UK
[(4)] DowDuPont Agriculture Division, 3B Park Square, Milton Park, Abingdon, OX14 4RN, UK
mcailliau@dow.com

ABSTRACT

INATREQ™[1] active (fenpicoxamid) is a novel fungicide discovered by Dow AgroSciences and the first member of a new class of picolinamide fungicides. It is derived from a natural product UK-2A which is obtained by fermentation of *Streptomyces sp. 517-02* - a naturally present soil borne Actinomycete. Post fermentation the UK-2A is modified by a simple one step conversion involving addition of a masking group to yield fenpicoxamid which has improved stability. Following uptake into the plant or when in contact with the pathogen, fenpicoxamid converts back to UK-2A which inhibits mitochondrial respiration at the complex III, cytochrome bc1 ubiquinone reductase (mode of action QiI). It has excellent activity against a key disease of wheat *Zymoseptoria tritici* and useful activity on cereal rusts. Fenpicoxamid has no target site cross-resistance with other current cereal fungicides and will be a key tool for the control of *Zymoseptoria tritici* and resistance risk management in cereals.

Keywords: INATREQ™ active, fenpicoxamid, picolinamide, foliar diseases, UK-2A.

RESUME

INATREQ™ ACTIVE (FENPICOXAMIDE), UN NOUVEAU FONGICIDE D'ORIGINE NATURELLE EFFICACE CONTRE *ZYMOSEPTORIA TRITICI* ET AUTRES MALADIES DES CEREALES

INATREQ™ active (fenpicoxamide) est un nouveau fongicide de la famille des picolinamides, issu de la recherche Dow AgroSciences. Il provient d'une molécule naturelle UK-2A obtenue par fermentation de *Streptomyces sp. 517-02* - Actinomycète naturellement présent dans le sol. Après fermentation, UK-2A est stabilisé par une simple transformation d'une étape. Une fois pénétré dans la plante ou en contact avec le pathogène, le fenpicoxamide retrouve sa forme naturelle UK-2A, qui inhibe la respiration mitochondriale au niveau du complexe III, l'ubiquinone réductase du cytochrome bc1 (mode d'action QiI). Il possède une excellente efficacité contre la principale maladie du blé *Zymoseptoria tritici* ainsi qu'une bonne activité contre les rouilles. Le fenpicoxamide ne présente aucune résistance croisée avec les autres fongicides disponibles et constitue un atout clé dans la lutte contre *Zymoseptoria tritici* et la gestion de résistances chez les céréales.

Mots-clés : INATREQ™ active, fenpicoxamide, picolinamide, maladies des céréales, UK-2A.

[1] Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

INTRODUCTION

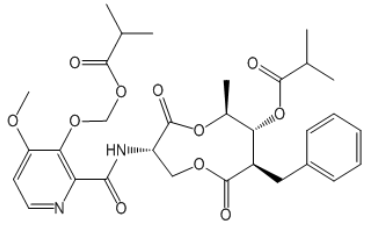
INATREQ™ active (fenpicoxamid), discovered by Dow AgroSciences and developed in cooperation with Meiji Seika Pharma, is a new fungicide belonging to the picolinamide chemical class. This molecule represents a new target site for disease control in cereals. Fenpicoxamid is derived from a natural substance UK-2A, produced by the soil microbe *Streptomyces sp. 517-02* (Ueki *et al*., 1996; Hanafi *et al*., 1996), and then stabilised by a minor synthetic modification post-fermentation. The original strain was isolated from a soil sample collected at Osaka City University in Japan. In the presence of fungi or in plant tissue, fenpicoxamid is converted back to UK-2A which inhibits fungal respiration at the Qi site of the respiratory cyt bc1 complex (ubiquinone reductase). The modification to form fenpicoxamid is completely reversible back to the natural product UK-2A. Fenpicoxamid will be used for the control of foliar diseases of cereals, delivering excellent efficacy against the major disease of wheat, *Zymoseptoria tritici*. Fenpicoxamid also has a useful level of control against both brown (*Puccinia triticina*) and yellow rust (*Puccinia striiformis)*. It will also be developed in banana against *Mycosphaerella fijiensis* (Black sigatoka).

This molecule will bring a new high value solution for cereal growers as a new active ingredient based on a molecule of natural origin and with no target site cross resistance to current chemistries. It will provide a new resistance management tool against *Z. tritici* and has both protectant and curative activity as well as translaminar mobility for the control of foliar diseases in cereals.

This article summarizes the biological characterization of fenpicoxamid and its key characteristics. Its efficacy against *Z. tritici* is covered, followed by its ability to provide effective control of isolates with reduced sensitivity to current key cereal chemistries including succinate dehydrogenase inhibitors (SDHIs), strobilurins and triazoles.

IDENTIFICATION OF THE MOLECULE

- Common name: fenpicoxamid (ISO approved)
- Active brand name: INATREQ™ active
- Chemical family: picolinamide
- Molecular formula: $C_{31}H_{38}N_2O_{11}$
- Structural formula:



- CAS number: 517875-34-2
- Mode of action (proposed): FRAC group C4#21, Inhibition of respiration at complex III (QiI fungicides)

BIOLOGICAL CONVERSION OF FENPICOXAMID BACK TO THE ORIGINAL NATURAL SUBSTANCE UK-2A

Fenpicoxamid is a fungicide obtained after a single step modification of the natural antifungal substance UK-2A (Figure 1). UK-2A is produced by fermentation of the Actinomycete bacteria *Streptomyces sp. 517-02* (Ueki *et al*., 1996; Hanafi *et al*., 1996). In early stage testing, it was found that *in vitro* fungal growth inhibition by UK-2A was not fully translated in field and greenhouse, due

to several inherent properties of the molecule and in particular UV instability. In order to improve UV stability, Dow AgroSciences dedicated considerable resources until arriving to a minor modification of UK-2A to form fenpicoxamid, which brings UV stability, increases efficacy at a lower dose and allows delivery in a novel formulation which improves retention and uptake of fenpicoxamid from the leaf surface (Owen *et al,* 2017).

In the manufacturing of fenpicoxamid, UK-2A is first produced through a conventional fermentation process within a contained bioreactor. Following fermentation, the natural substance UK-2A is isolated from the broth and purified. Afterwards, UK-2A is converted to fenpicoxamid through a single and reversible chemical modification (alkylation). This final product is then formulated to optimize storage, application delivery and product performance.

Metabolism studies in *Zymoseptoria tritici* and wheat cell suspension cultures have shown that fenpicoxamid is converted back to UK-2A by removal of the isopropylcarboxymethyl ether masking group that is added post fermentation for stability (Figure 1). Furthermore, biochemical studies indicate that UK-2A is the active moiety responsible for the fungicidal activity (Young *et al*, 2017; Owen *et al*, 2017). Preliminary laboratory studies conducted at Dow AgroSciences suggest that carboxylesterase (CE) enzymes may mediate the removal of the masking group, allowing the biological conversion of fenpicoxamid back to UK-2A (unpublished). CEs belong to the large serine hydrolase family of enzymes. Multiple CEs are present in both fungi and plants. Substrate specificity is poorly understood for most CEs but is known to differ greatly between enzymes (Satoh *et al.,* 2006). It is at present unclear if specific CEs in *Z. tritici* and/or in cereals are responsible for the conversion and further research is ongoing in this area.

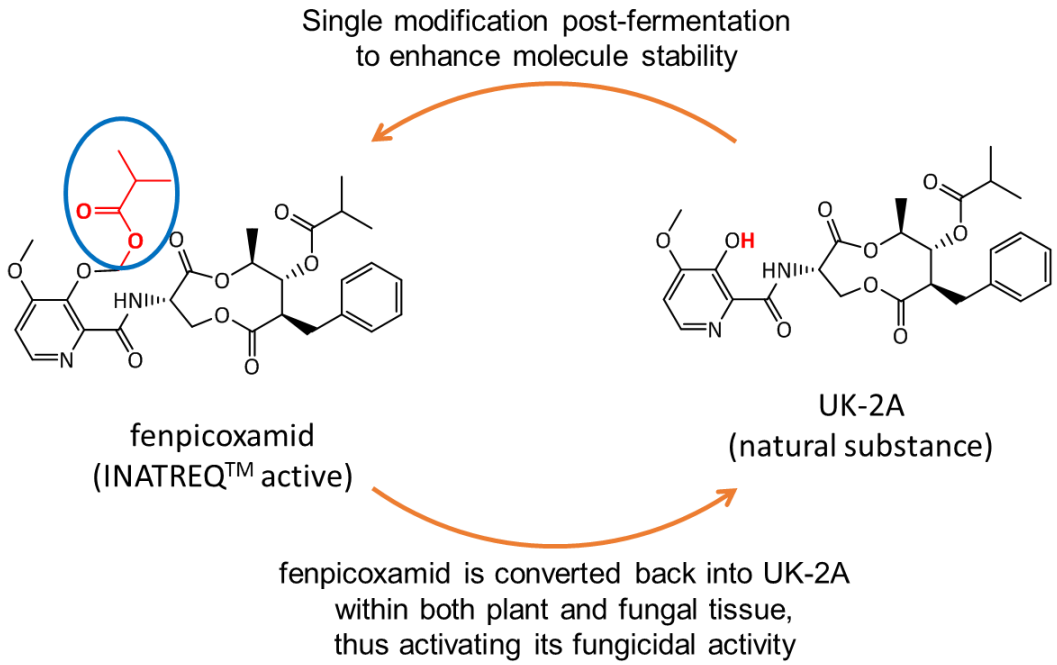


Figure 1 : Modification of UK-2A to fenpicoxamid post fermentation and conversion back to UK-2A in plants and fungi (Transformation de UK-2A en fenpicoxamide après fermentation puis reconversion en UK-2A au contact de la plante ou du pathogène)

Once sprayed onto leaf surface, fenpicoxamid forms a protective layer and binds itself to the cuticular waxes where it stays in the INATREQ™ form, thus forming a reservoir of material. This reservoir of fenpicoxamid is converted into UK-2A once in contact with germinating fungal spores, thus explaining the protectant properties of the product. In parallel, small amounts of fenpicoxamid penetrate into the leaf tissue where they are converted to UK-2A by the plant and/or when taken up by fungal tissue within the leaf, providing curative activity to the product.

MODE OF ACTION

Fenpicoxamid is the first member of a new class of cereal fungicides, the picolinamides. It is rapidly converted into UK-2A in both fungal and plant tissue which inhibits mitochondrial respiration in fungi by blocking electron transfer in the respiratory chain. Fenpicoxamid binds to the Qi site of the cytochrome *bc*1 (ubiquinone reductase) of complex III. It is a Quinone Inside Inhibitor (QiI) (Machida *et al*, 1999; Young *et al*, 2017). The mode of action of fenpicoxamid has recently been assigned to the group FRAC C4#21 (Fungicide Resistance Action Committee). The inhibition of mitochondrial respiration causes a reduction of ATP (Adenosin triphosphate) production. The lack of ATP production inhibits vital functions of the fungi cell. *In-vitro* studies have shown that fenpicoxamid is a potent inhibitor of spore germination and will also inhibit mycelia growth post germination thus explaining the outstanding protectant and strong curative properties observed when fenpicoxamid is applied pre or post infection.

MATERIALS AND METHODS

EFFICACY TRIALS

The field data presented in this article are based on a total of 21 trials conducted in France, United Kingdom and Germany from 2015 to 2018 on winter wheat, in areas with favourable agronomical and environmental conditions for the development of *Zymoseptoria tritici,* and known as areas where winter wheat is grown commercially and where *Z. tritici* is the primary disease. All trials were carried out by Dow AgroSciences or officially recognised contract research organisations in accordance with the principles of Good Experimental Practice and follow the guidelines and standards established and maintained by EPPO, especially PP 1/26. The trials were designed as randomized complete blocks with 4 replicates and a plot size ranging between 20 m^2 and 36 m^2. The applications were performed with compressed air sprayers using conventional or low drift flat fan nozzles delivering water volumes of 150-250 L/ha. The trials were carried out on commonly marketed varieties known to be susceptible to various diseases. Trials were set up on a wide geographic spread and on a range of soil types, in order to test the formulation efficacy and selectivity on a wide range of environmental conditions.

Fenpicoxamid was tested in all trials at 75 g ai/ha. The reference standards included were a straight triazole, a mixture of triazole + SDHI (Succinate DeHydrogenase Inhibitor) and a mixture of two triazoles. All trials received two applications of the tested products between growth stages BBCH 30-33 followed by BBCH 37-55 of winter wheat.

SENSITIVITY TEST

UK-2A is a potent inhibitor of mitochondrial electron transport (MET) binding to the Qi site of the cytochrome bc1 (ubiquinone reductase) complex III in the electron transport chain (Machida *et al*, 1999; Young *et al*, 2017). The MET III Qi binding site is distinct from the Qo site targeted by the strobilurin class of fungicides (Young *et al*, 2017). There is no target site-based cross resistance between fenpicoxamid and strobilurin, triazole and SDHI fungicides. This claim is supported from studies reported by Owen et al, 2017 (Figure 2) which investigated cross resistance. These cross-resistance studies were carried out on three isolates (UK-4, UK-7 and UK-12) of *Z. tritici* from United Kingdom. All three isolates are characterized as being resistant to both strobilurin and azole fungicide classes. Indeed, they were chosen for the presence of G143A site mutation conferring strobilurin resistance and of the CYP51 gene encoding the sterol C-14 demethylase target site for azole resistance. Isolate UK-12 also carries CYP51 overexpression genes, as well as the multi-drug resistance gene MgMfs1 (Omrane *et al*, 2015). The *Z. tritici* isolate ATCC 26518 was used as a control, due to still being highly sensitive to all substances, including triazole and strobilurin fungicides. Greenhouse tests were conducted on wheat plants which were inoculated with the *Z.tritici* isolates. As a comparison to fenpicoxamid, a representative molecule from 3 key cereal fungicide groups was chosen: a strobilurin, a triazole and a SDHI. Tested products were applied either one day before inoculation or three days after inoculation at 200 litres/ha using an automated track sprayer. Each fungicide was evaluated at doses of 100, 25, 6.25, 1.56 and 0.39 g a.i /ha. Inoculated plants were then moved to the greenhouse and assessed when symptoms first developed on untreated plants. Percentage disease control was calculated using the ratio of disease on treated plants relative to untreated. The effective concentration values to give 80 percent control (EC80) were calculated from the dose response curves.

RESULTS

EFFICACY TRIALS

In total, 21 trials were conducted in France, United Kingdom and Germany from 2015 to 2018 on winter wheat to evaluate the efficacy of fenpicoxamid against *Z. tritici.* Among these trials, the standard 1 (triazole A) was included in 17 trials, and the standard 2 (SDHI + triazole A) in 14 trials, whereas standard 3 (triazole A + triazole B) was included in 12 trials. The three standards were presented all together in 8 trials. Four summaries will be presented below in Table I in order to follow orthogonal data.

Trials were conducted under very high disease pressure situations. The table below presented the results based on the last assessment done on Flag Leaf, at 29 to 48 days after second application with an average of 39 days, and the yield. Mean percentage of *Z. tritici.* infection values of each individual trials were subjected to the analysis of variance, then compared by means of the Tukey HSD test to highlight treatments' differences $p<0.05$ level. The data from all trials were then subsequently combined to generate a mean control percentage values calculated with Abbott's formula.

Table I: Efficacy against Septoria tritici blotch on Flag leaf at 4-7weeks after T2 and yield impact of fenpicoxamid and standards (Efficacité sur Septoriose du blé sur F1 à T2 + 4-7 semaines et effet sur le rendement du fenpicoxamide en comparaison avec les produits de référence)

	Untreated		fenpicoxamid 75 gai /ha		Standard 1 (triazole A)		Standard 2 (SDHI + triazole A)		Standard 3 (triazole A + triazole B)	
Number of trials	(% disease infection Z. tritici on L1)	Yield T/ha	*Z. tritici* % *Control*	Yield T/ha (% untreated)	*Z. tritici* % *Control*	Yield T/ha (% untreated)	*Z. tritici* % *Control*	Yield T/ha (% untreated)	*Z. tritici* % *Control*	Yield T/ha (% untreated)
8	(53.7) a	5.8	88.7 c	7.8 (139.3)	76.4 b	7.8 (139.0)	88.3 c	8.4 (152.6)	78.1 b	7.9 (142.6)
12	(59.7) a	6.7	87.0 c	8.5 (133.0)	-		85.5 c	8.9 (141.8)	74.6 b	8.4 (132.4)
14	(62.5) a	6.4	87.5 b	8.3 (137.7)	-		86.6 b	8.7 (145.8)	-	
17*	(60.9) a	5.7	90.2 c	8.1 (149.9)	74.8 b	7.6 (140.2)	-		-	

(*) Only 15 trials harvested

SENSITIVITY TEST

The results of the greenhouse tests presented in Figure 2 clearly demonstrated that only the isolate ATCC 26518 is controlled by strobilurin and azole fungicides. At both application timings 1 day protectant (1DP) and 3 day curative (3DC), fenpicoxamid and SDHI reference delivered high control on the four isolates, including those with confirmed resistance to azoles and strobilurins. EC80 values are statistically lower for fenpicoxamid than for SDHI reference. Fenpicoxamid provided effective control of the isolates of *Z. tritici* resistant to azoles and strobilurins.

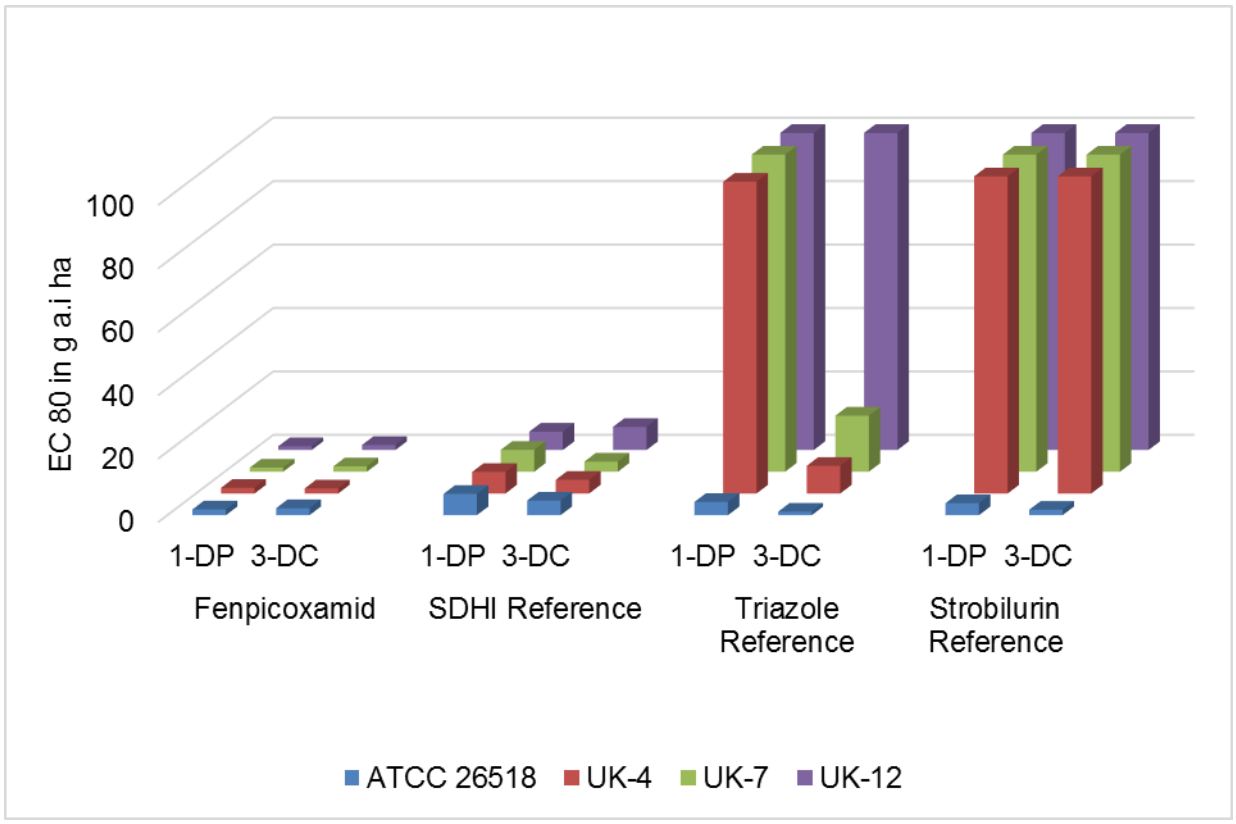


Figure 2. Comparative control of fungicide resistant isolates of *Z. tritici* by fenpicoxamid and current cereal fungicides in greenhouse tests as reported by Owen *et al*, 2017 (Comparaison de l'efficacité du fenpicoxamid à celle des autres fongicides céréales contre des souches résistantes de *Z. tritici* en essais sous serre, comme rapporté par Owen *et al*, 2017)

DISCUSSION

The data above showed that fenpicoxamid as a single active delivered high control of *Z. tritici* and clearly had a positive impact on the yield of winter wheat in the presence of diseases.

In terms of efficacy, when applied at 75 g ai/ha, straight fenpicoxamid was statistically superior to the straight triazole standard and to the co-formulation of two triazoles, and equivalent to mixture product containing SDHI + triazole. High level intrinsic activity of fenpicoxamid on *Z. tritici.* was demonstrated.

The yield measurement consistently reflected the outstanding performance of fenpicoxamid on *Z. tritici.* and justify its benefit for cereal growers.

In addition, the resistance studies suggested that fenpicoxamid will be a valuable tool to manage the resistance strategy against *Z. tritici.*

CONCLUSION

The biological studies presented in this review have highlighted the key attributes of fenpicoxamid. Under high disease pressure conditions and on very sensitive varieties, straight fenpicoxamid provided strong efficacy, and was seen to deliver control equivalent or superior to the high current products leading the cereal fungicide market.

Complementary studies demonstrate the flexibility in use of fenpicoxamid, in terms of positioning and also its strong performance when included in typical fungicide programs. Fenpicoxamid is a curative and protectant fungicide for control of foliar diseases in cereal crops with translaminar activity. Fenpicoxamid shows good crop safety and disease control which results in significant yield benefits. As first member of a new class of cereal fungicide with no cross resistance with current cereal chemistries, fenpicoxamid offers an additional fungicide solution for growers, and an important tool for resistance management.

For long-term use of this new active substance, it is important to follow Good Resistance Management Practices issued by the FRAC (Fungicide Resistance Action Committee). In particular, to minimize resistance risk, Dow AgroSciences recommends that fenpicoxamid should never be applied alone and applied only in mixture with fungicides having other effective modes of action against *Z. tritici*.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Meiji Seika Pharma Co. Ltd. for discovery of UK-2A and the assistance of Dow AgroSciences colleagues and external researchers.

BIBLIOGRAPHIE

Hanafi M., Shibata K., Ueki M. and Taniguchi M., 1996 - UK-2A, B, C and D, novel antifungal antibiotics from Streptomyces sp. 517-02. II. Structural elucidation. *Journal of Antibiotics*, 49, 1226-1231.

Machida K., Takimoto H., Miyoshi H. and Taniguchi M., 1999 - UK-2A, B, C and D, novel antifungal antibiotics from *Streptomyces sp. 517-02*. V. Inhibition mechanism of bovine heart mitochondrial cytochrome bc1 by the novel antibiotic UK-2A. *Journal of Antibiotics,* 52, 748-753.

Omrane S., Sghyer H., Audeon C., Lanen C., Duplaix C., Walker A-S. *et al.,* 2015 - Fungicide efflux and the MgMFS1 transporter contribute to the multidrug resistance phenotype in *Zymoseptoria tritici* field isolates - *Environmental Microbiology*, 17, 2805–2823.

Owen W J., Yao C., Myung K., Kemmitt G., Leader A., Meyer G K., Bowling J A., Slanec T. and Kramer J V., 2017 - Biological characterization of fenpicoxamid, a new fungicide with utility in cereals and other crops. *Pest Management Science*, Volume 73, Issue 10, 2005–2016.

Satoh T. and Hosokawa M., 2006 - Structure, function and regulation of carboxylesterases. *Chemico-Biological Interactions,* 162,195-211.

Ueki M., Abe K., Hanafi M., Shibata K., Tanaka T. and Taniguchi M., 1996 - UK-2A, B, C and D, novel antifungal antibiotics from Streptomyces sp. 517-02. I. Fermentation, isolation, and biological properties. *Journal of Antibiotics,* 49, 639-643.

Young H D., Wang X N., Meyer T S. and Avila-Adame C., 2017 - Characterization of the mechanism of action of the fungicide fenpicoxamid and its metabolite UK-2A. *Pest Management Science*, 74, 489–498.