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ZORVEC™ (oxathiapiprolin), SOME NEWS PREMIXES AGAINST OOMYCETES

O. VINCENT [1]
et B. PEROTIN, S. PIERRE, C. SHEPHERD, S. SALAS, G. MAJEAU
1 DuPont de Nemours (France) S.A.S., Centre Européen de Recherche et de Développement, 24 rue du Moulin, F-68740 Nambsheim - CORTEVA agriscience™ Agriculture Division of DowDuPont
Contact : Olivier.vincent@dupont.com

ABSTRACT
Zorvec™ (oxathiapiprolin) is a novel fungicide and the first member of a new class of piperidinyl-thiazole-isoxazoline fungicides. Its high intrinsic efficacy, an effect on multiple stages of pathogen development and its biological attributes allow oxathiapiprolin to provide consistent and provide reliable control of most of blights and downy mildew. With a new mode of action, this active will be an additional tool for fungicide resistance management strategies.
Therefore oxathiapiprolin will be recommended systematically in mixture with an oomycete fungicide having a different mode of action in order to limit or delay resistance development.
New premixtures are currently developed with folpet or zoxamide on grapes and famoxadone, benthiavalicarbe or amisulbrom on potato and vegetables.

Keywords: oxathiapiprolin, oomycete, protection of new growth, redistribution, wash-off.

RÉSUMÉ
ZORVEC™ (oxathiapiproline), DE NOUVEAUX MELANGES CONTRE LES OOMYCETES
Zorvec™ (oxathiapiproline) est un nouveau fongicide et le premier membre de la famille des piperydinyl-thiazoles isoxazolines. Sa remarquable activité intrinsèque, son efficacité sur tous les stades de développement des agents pathogènes et ses caractéristques biologiques se traduisent par une performance exceptionnelle contre les mildious. De par son nouveau mode d'action, cette substance active constitue un outil supplémentaire pour la gestion de la résistance. Ainsi l'oxathiapiproline sera systématiquement recommandé en mélange avec un fongicide efficace sur mildiou et à mode d'action différent permettant de limiter ou retarder le développement de la résistance. Des nouveaux mélanges sont actuellement en cours de développement avec le folpel et la zoxamide sur vigne ainsi que la famoxadone, le benthiavalicarbe ou l'amisulbrom sur pomme de terre et cultures légumières.

Mots-clés : oxathiapiproline, oomycète, protection of new growth, redistribution, lessivage.

INTRODUCTION

Oxathiapiprolin, discovered and developed by DuPont, is the first member of a new chemical class of fungicides, piperidinyl thiazole isoxazoline. This molecule has excellent efficacy on oomycetes and is being developed for crops like potato, grapes, cucurbits and other vegetable crops. The fungicide acts at various stages of the life cycle of targeted pathogens such as inhibits zoospore release, affect their mobility and germination (*Phytophthora infestans* and *Pseudoperonospora cubensis*).When the product is applied after an infection event, it blocks mycelial growth inside infected plant tissues. If applied on visible lesions, the fungicide stops the growth of these lesions (eradicant activity) and inhibits spore formation and spore viability (antisporulant activity) (Bruhn et al., 2013).
It belongs to FRAC group 49 (OSBPI) and it provides a new biochemical mode of action in farmer's pesticide toolbox with very interesting biological properties.

This article summarizes some key biological attributes of each new premixe formulation such as movement in the plant, protection of new growth or resistance to wash-off. Specific lab or field studies were conducted on grape downy mildew (*Plasmopara viticola*) or on potato late blight (*P.infestans*) to charaterize those biological attributes. (Table II).

In order to limit or delay resistance development, oxathiapiprolin is recommended for use in a preventive manner and systematically in mixtures with a fungicide having a different mode of action and a good efficacy on the target diseases.

Currently DPX-QGU42 100 g/L OD (oxathiapiprolin straight) is registered in some European countries, under trade name of Zorvec Zelavin® (Italy and France) on grapes and Zorvec Enicade® (Ireland, U.K, Belgium, Netherland, Italy and France) on potatoes and onions.

The different formulations currently developed are listed on Table I.

TABLE I: Oxathiapiprolin premixture partners (partenaires des prémélanges de l'oxathiapiproline)

Oxathiapiprolin premixtures (partner)	Composition g/L (oxathiapiprolin + partner)	CROP
DPX-UBL47 340 g/L SE (zoxamide)	40 + 300	Grapes
DPX-S5K22 510 g/L SC (folpet)	10 + 500	
DPX-UKH70 100 g/L OD (benthiavalicarb)	30 + 70	Potatoes & vegetables
DPX-TAH88 330 g/L SE (famoxadone)	30 + 300	
DPX-WVG35 288g/L SE (amisulbrom)	48 + 240	

KEY BIOLOGICAL ATTRIBUTES

MATERIAL AND METHODS:

TABLE II: Experiments and premixtures tested. (Tests et prémélanges étudiés)

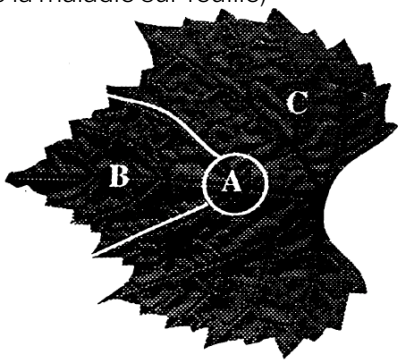
	DPX-S5K22 510 SC	DPX-UBL47 340SE	DPX-UKH70 100OD	DPX-TAH88 330SE	DPX-WVG35 288SE
Xylem systemic and translaminar movement	X	X	X	X	X
Protection of new growth	X	X	X	X	
Resistance to wash-off	X	X	X	X	

XYLEM SYSTEMIC AND TRANSLAMINAR MOVEMENT

- Grapes:

Grape seedlings (variety Chardonnay) were used in this experiment and conducted on 2 tests with 4 replicates/leaves per test. Felt pads dipped into fungicide solutions were deposited on the upper side of leaves, at their center. Plants were then stored in a dew chamber at 20°C for 1 hour then in a growth chamber at 20°C for 1 hour. Subsequently, the felt pads were removed and the plants were placed in a growth chamber.

Figure 1: Leaf areas considered during disease rating (Zones prises en compte lors de la notation de la maladie sur feuille)



Inoculation was carried out on the lower side of leaves 24 hours after having removed the felt pads. Plants were then stored in a dew chamber for 1 day, and in a growth chamber for 5 days. Before disease rating, plants were placed in a dew chamber for 24 hours to favor pathogen sporulation.
The control on downy mildew was evaluated in three leaf sections (Figure 1):
- Activity on the disease in area A (directly opposed to area of application) reveals translaminar activity
- Activity in area B demonstrates a local acropetal movement (via the xylem)
- Activity in area C shows an basipetal movement via the phloem

- Potatoes:

Potato variety “Bintje” seedlings were used for this experiment and conducted on 1 test with 8 replicates/leaves per test..
Before treatment application, aluminum foil was placed on the outer half portion of the first true leaf of each plant to protect that portion of the leaf from the fungicide spray (one leaf per plant). Then fungicide treatments were applied on the plants. The protective foil from each leaf was removed once the spray was completely dry. The plants were then moved into a growth chamber set at 20°C/ 70%RH and 16-hour photo-period/per day until inoculation 48h after (see figure 2).
Evaluation of percent leaf area with visible disease symptoms (disease severity) was made five days after inoculation based on a 0-100% rating scale. Only the leaf used to demonstrate local acropetal systemic movement was assessed. Two separate portions of the leaf were evaluated:

Category 1: protected, tip portion, outer half of the leaf.
Category 2: exposed (treated), back portion of the leaf, closest to the main stem of the plant.

Figure 2: Potato plant with aluminum foil placed on the outer haft portion of the first true leaf of each plant to protect that portion of the leaf from the fungicide. (première feuille entièrement développée et protégée partiellement d'une feuille d'aluminium au moment de l'application du fongicide)

PROTECTION OF NEW GROWTH:
The capacity of oxathiapiprolin to protect new growth has been evaluated under field or semi-field conditions, on both potato and grapes.

- Potatoes:

Potato plants (var. Red Pontiac) were sprayed at 300 L/ha 5 weeks after planting, at the beginning of the rapid growth phase of the crop. Leaves present at the time of fungicide application were tagged. Eleven days after treatment, 12 plants per replicate were sampled and inoculated in the laboratory. They were stored 24 hours in a dew chamber and 4 days in a growth chamber until they were rated (evaluation of the surface of leaves colonized by the fungus). The rating was done separately on different leaf types:
o Fully expanded growth: leaves fully developed at the time of application
o Expanding growth: leaves having 20 to 30 % of their full size when fungicide was applied
o Emerging new growth which represents the top part of the plant constituted by terminal bud and 1-3 very small folded leaflets (5-20 % of final size at the time of the application)
o Axillary growth: total of new growth emerging from leaf axils following application that were not present at the time of the treatment.

- Grapes:

Two field trials (variety Auxerrois blanc), were carried out over 2 years in Nambsheim (68) using the same methodology, in order to evaluate the capacity of DPX-UBL47 340SE and DPX-S5K22 510SC to protect new growth in the field. Each trial included 4 replicates of 5 vines, where the three central vines were used for efficacy assessments. A single application at their target rates of DPX-UBL47 340SE (0,5L/ha) and DPX-S5K22 510SC (2L/ha), was made at the time that each shoot had at least three fully expanded and three growing leaves.
Both premixtures were compared to two systemic standards, known to have either acropetal or basipetal activity and therefore good protection of new growth, were applied at their respective field dose.
After at least three new leaves had grown, 8-9 days after treatment, the vines were inoculated with *P. viticola*. Subsequent misting was carried out to encourage disease development. Assessments were made 12 days after inoculation on leaves of four different stages at the time of treatment either:

- Fully expanded growth: leaves fully developed and exposed to the application

- Expanding growth: leaves having between 20 and 80 % of their final size at the time of the fungicide treatment
- Emerging new growth: small leaves having between 5 and 20 % of their final size at the time of the application
- New untreated growth: leaves formed only after the treatment

RESISTANCE TO WASH-OFF:

Young potato plants and grape seedlings were sprayed in a laboratory tunnel sprayer, with 4 replications/plants per test.

On potatoes, a 0,5L/ha rate was tested as a N rate for both DPX UKH70 & DPX- TAH88.

As crop canopy and plant structure often do not allow leaves to receive a full dose of a fungicide, N/4 and N/16 rates were sprayed which reflected potential amount of fungicide that may contact shadowed leaves. On grape only a N rate was applied.Twenty minutes after application, half of the plants were subjected to an artificial rain of 100 mm (25 mm per hour during four hours). The other half were kept without rain for comparison.

One day after fungicide application, plants were inoculated and stored in a dew chamber for one day then in a growth chamber up to disease assessment on the whole plant. In the case of grape downy mildew, seedlings were previously stored in a dew chamber for 24 hours to promote fungal sporulation before assessment.

RESULTS

XYLEM SYSTEMIC AND TRANSLAMINAR MOVEMENT

- Grapes:

Local systemic movement of either DPX-S5K22 510SC or DPX-UBL47 340SE, in xylem tissues exhibited very good protection outward to the leaf tip while its translaminar movement protected the underside of the plant. These levels of protection are significantly higher than those provided by the standards (excepted standard F) even under high disease pressure. However, never premixes provided basipetal protection, on *P. viticola* (Tables III and IV).

Table III: Translaminar, local acropetal & basipetal activities of DPX-S5K22 510SC on *P. viticola*. (Activités translaminaire, acropétale et basipétale locales de DPX-S5K22 510SC sur *P. viticola*)

Average of 2 lab tests	% control of *P. viticola*				Untreated (% disease)
	DPX-S5K22 510SC - 2 L/ha (oxathiapiprolin +folpet)	Standard A	Standard B	Standard C	
Translaminar activity (area A)	100 (e)	72.9 (d)	28.8 (b)	54.2 (c)	(92.2) (a)
Local acropetal control (area B)	88.8 (g)	25 (de)	3.8 (ab)	30.8 €	(81.3) (a)
Local basipetal control (area C)	4.2 (a)	0 (a)	0 (a)	19.2 (b)	(83.8) (a)

Results in the same row sharing the same letter are not significantly different, Student-Newman-Keuls (SNK) procedure, $\alpha = 5\%$

Table IV: Translaminar, local acropetal & basipetal activities of DPX-UBL47 340SE on *P. viticola*. (Activités translaminaire, acropétale, basipétale locales de DPX-UBL47 340SE sur *P. viticola*)

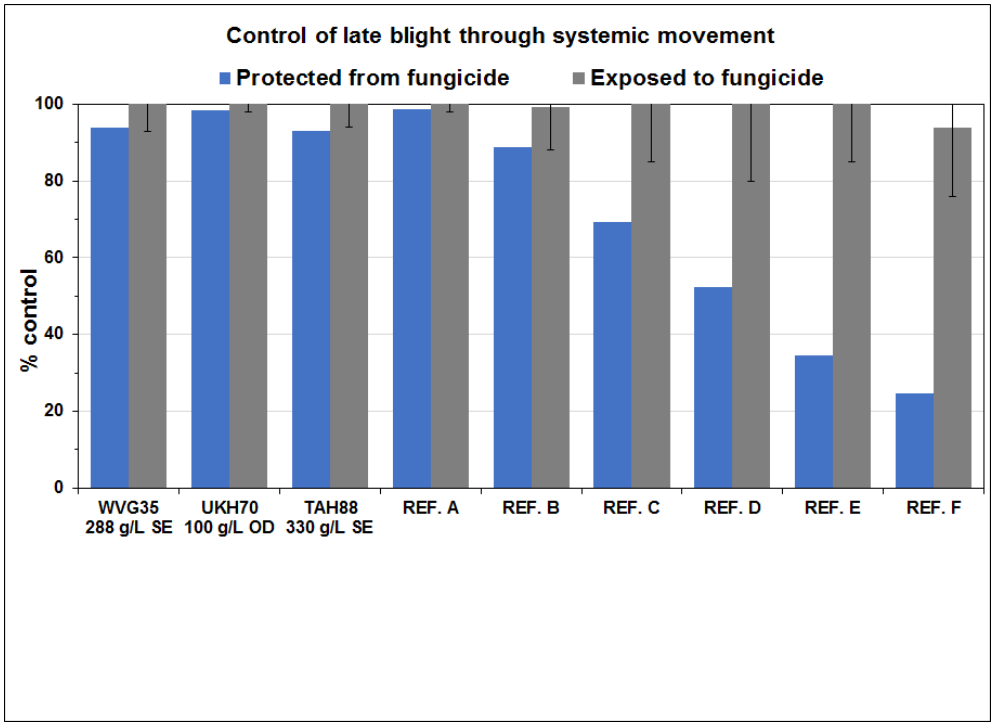
Average of 2 lab tests	% control of *P. viticola*							
	DPX-UBL47 340SE(oxathiapiprolin + zoxamide) 0,5 L/ha	Standard A	Standard B	Standard C	Standard D	Standard E	Standard F	Untreated (% disease)
Translaminar activity (area A)	100 (a)	81.3 (d)	37.9 (b)	22.4 (b)	62.2 (c)	31.9 (b)	100 (e)	(98.7) (a)
Local acropetal control (area B)	84.8 (c)	31 (b)	25.1 (b)	19 (b)	27.5 (b)	20.8 (b)	100 (c)	(96.3) (a)
Local basipetal control (area C)	17.1 (c)	17.1 (c)	31.5 (c)	21.9 (bc)	7.8 (ab)	10.2 (bc)	92.5 (d)	(97.3) (a)

Results in the same row sharing the same letter are not significantly different, Student-Newman-Keuls (SNK) procedure, $\alpha = 5\%$

- Potatoes :

All three oxathiapiprolin premixtures as well as standard products A & B, demonstrated outstanding protection against a US-23 isolate of *P. infestans* (A1 mating type, metalaxyl/mefenoxam sensitive) on the non-treated portion of a potato leaf through acropetal (local) systemic movement (Figure 3)

Figure 3: Control of late blight on plant tissue exposed or protected from a preventive fungicide application. (% efficacité sur mildiou de la pomme de terre de plantes exposées ou non à une application préventive de fongicide)



PROTECTION OF NEW GROWTH:

- Grapes:

Studies confirmed the capacity of DPX-UBL47 340SE and DPX-S5K22 510SC to protect new growth in grapes following one application under field conditions even under strong disease pressure.

Oxathiapiprolin premixtures showed similar efficacy to the systemic standards on expanding, emerging and new untreated growth (Tables V and VI)

Table V: Efficacy on *P. viticola* of DPX-UBL47 340SE on either fully expanded, expanding, and emerging leaves or new growth (Efficacité sur *P. viticola* de DPX-UBL47 340SE sur des feuilles soit complétement ou partiellement développées, émergeantes ou nouvelles pousses)

Trial number: F-15-021 (2015)				
Treatment	% Control on disease severity			
	Fully expanded leaves	Expanding leaves	Emerging growth	New growth
DPX-UBL47 340 g/L SE 0. 5 L/ha (170 g a.s/ha)	97.7 (b)	97.7 (b)	80.9 (c)	60.1 (b)
Standard A	94.4 (b)	94.4 (b)	56.4 (bc)	30.2 (ab)
Standard B	89.7 (ab)	89.7 (ab)	37.6 (ab)	27.9 (ab)
Untreated control (% infection)	(10.9%)	(40.8%)	(70.2%)	(54.8%)

Results in the same column sharing the same letter are not significantly different, Student-Newman-Keuls (SNK) procedure, $\alpha = 5\%$

Trial number: F-16-23 (2016)				
Treatment	% Control on disease severity			
	Fully expanded leaves	Expanding leaves	Emerging growth	New growth
DPX-UBL47 340 g/L SE 0. 5 L/ha (170 g a.s/ha)	85.4 (a)	96.6 (c)	92.8 (b)	58.2 (b)
Standard A	84.4 (a)	92.3 (bc)	87.9 (b)	79.1 (b)
Standard B	76.8 (a)	85.9 (b)	85.5 (b)	61.7 (b)
Untreated control (% infection)	(44.8%)	(64.9%)	(76.2%)	(25.8%)

Results in the same column sharing the same letter are not significantly different, Student-Newman-Keuls (SNK) procedure, $\alpha = 5\%$

Table VI: Efficacy of tested fungicides on either fully expanded, expanding, emerging or new growth on *P. viticola* (Efficacité sur *P. viticola* de S5K22 510SC sur des feuilles soit complétement ou partiellement développées, émergeantes ou sur de nouvelles pousses)

Trial number: F-15-014 (2015)				
Treatment	% Control on disease severity			
	Fully expanded leaves	Expanding leaves	Emerging growth	New growth
DPX-S5K22 510 g/L SC 2.0 L/ha (1020 g a.s./ha)	98 (b)	97 (cd)	68 (d)	34 (b)
Standard A	95 (b)	88 (bc)	45 (c)	9 (a)
Standard B	97 (b)	82 (b)	36 (c)	35 (b)
Untreated control (% infection)	(44 a)	(64 a)	(77 a)	(54 a)

Results in the same column sharing the same letter are not significantly different, Student-Newman-Keuls (SNK) procedure, $\alpha = 5\%$

- Potatoes:

Both oxathiapiprolin premixtures DPX-UKH70 100OD and DPX-TAH88 330SE provided outstanding preventive late blight control on expanding leaves that were approximately 20-30% full size at the time of application, at least equal or superior to reference products. These

premixtures provided also a good control on emerging leaves that were approximately 5-20% full size at the time of fungicide application (Table VII).

Table VII: Efficacy of tested fungicides on either expanding, emerging or new growth on *P. infestans* (Efficacité sur *P. infestans* des fongicides sur des feuilles soit partiellement développées, émergeantes ou nouvelles pousses).

Trial number: F-17-08			
Treatment	% control on disease severity		
	L1	L2	L3
DPX-UKH70 100OD 0.4 L/ha (40 gai/ha)	**99,2**	**98,5**	**64,7**
DPX-TAH88 330SE 0.4L/ha (132 gai/ha)	**94,3**	**97,1**	**55,8**
Standard A	**82,2**	**67,3**	**11,3**
Standard B	**93,9**	**84,1**	**19,2**
Standard C	**97,4**	**91,2**	**51,7**
Untreated check % disease severity	(92,3)	(95,4)	(99,5)

L1 (Expanding Growth): leaves present at the time of fungicide application (20-30% of full leaf size)
L2 (Emerging Growth): terminal bud and 1-3 small, folded leaflets present at time of fungicide application (5-20% full leaf size)
L3 (New Untreated Growth) total of new growth emerging after fungicide application

RESISTANCE TO WASH-OFF:

- Grapes:

The rainfastness of DPX-UBL47 340SE and DPX-S5K22 510SC on grapes was evaluated in two tests carried out, under controlled conditions in comparison of two tandards A & B, reported as being very rainfast products, versus Standard C known as having poor rainfastness (Tables VIII and IX).

Table VIII: Efficacy on grape downy mildew of DPX-UBL47 340SE and reference products, following an artificial rain of 100 mm, percentage relative to plants kept dry. (Efficacité sur mildiou de la vigne d'UBL47 340 g/L SE et des produits de référence, après une pluie artificielle de 100 mm, en comparaison avec des plantes non lessivées.)

Treatment	% Efficacy relative to unwashed control (average of 2 tests)	Stat group SNK, α=0.05
DPX-UBL47 340 g/L SE 0,5L/ha	100	a
Standard A	93.50	ab
Standard B	76.20	bc
Standard C	52.70	de
Untreated (% severity)	(84.9-87.7)	

Table IX: Efficacy on grape downy mildew of DPX-S5K22 510 SC and reference products, following an artificial rain of 100 mm, percentage relative to plants kept dry. (Efficacité sur mildiou de la vigne du DPX-S5K22 510SC et des produits de référence, après une pluie artificielle de 100 mm, en comparaison avec des plantes non lessivées.)

Treatment	% Efficacy relative to unwashed control	Stat group SNK, α=0.05
DPX-S5K22 510 g/L SC 2L/ha	93.2	ab
Standard A	67	bcd
Standard B	65.5	bcd
Standard C	45	cdef
Untreated (% severity)	(98.6)	

- Potatoes:

DPX-TAH88 330SE and DPX-UKH70 100OD were compared to standards A and B recognized as being a very rainfast products and a standard C tested as a positive standard (sensitive to wash-off). For easier reading, only the N & N/16 rates are presented here. (Tables X and XI).

Table X: Efficacy on potato late blight of DPX-TAH88 330SE, and reference standards, percentage relative to unwashed plants – following a artificial rain of 100 mm. (Efficacité sur mildiou de la pomme de terre du DPX-TAH88 330 SE et des produits de référence, en comparaison avec des plantes non lessivées après une pluie artificielle de 100 mm)

Treatment	Rate	% efficacy relative to unwashed control	Stat group Fischer, α=0.05
DPX-TAH88 330 g/L SE	N = 0,5L/ha	100	A
	N/16	99,4	A
Standard A	N	100	A
	N/16	49,1	BC
Standard B	N	97,9	A
	N/16	32,7	C
Standard C	N	41	C
	N/16	6,6	D
Untreated (% severity)		(98,9%)	

Table XI: Efficacy on potato late blight of DPX-UKH70 100OD, and reference products, percentage relative to unwashed plants, following a artificial rain of 100 mm (Efficacité sur mildiou de la pomme de terre du DPX-UKH70 100OD et des produits de référence, en comparaison des plantes non lessivées, après une pluie artificielle de 100 mm)

Treatment	Rate	% efficacy relative to unwashed control	Stat group Tukey, α=0.05
DPX-UKH70 100 g/L OD	N = 0,5 L/ha	100	A
	N/16	99.4	A
Standard A	N	100	A
	N/16	64	B
Standard B	N	100	A
	N/16	65	B
Standard C	N	4,7	C
	N/16	0	C
Untreated (% severity)		100%	

DISCUSSION

XYLEM SYSTEMIC AND TRANSLAMINAR MOVEMENT

- Grapes:

Both oxathiapiprolin premixes exhibited a very good redistribution in grape leaves, with excellent translaminar and local acropetal activity.

- Potatoes:

The acropetal (local) systemic movement from a treated to non-treated area on the treated leaf was confirmed for all 3 oxathiapiprolin premixes DPX-WVG35 288 SE, DPX-TAH88 330 SE, and DPX-UKH70 100 OD.

PROTECTION OF NEW GROWTH

- Grapes:

Results show that both DPX-UBL47 340SE or DPX-S5K22 510SC can achieve excellent control of *P. viticola* on existing foliage, including expanding and emerging growth (leaves partially exposed at the time of the application). Leaves not emmerged at premixtures applications, were also partially protected. A degree of protection may also be given to leaves that emerge shortly after treatment.

- Potatoes:

Both oxathiapiprolin premixtures, DPX-UKH70 100 OD & DPX-TAH88 330SE provided excellent protection of expanding leaf growth that was approximately 20-30% full size at the time of application. These products demonstrate significant late blight protection either on emerging leaves that were approximately 5-20% full size at the time of application or on leaves not present at the time of application.

RESISTANCE TO WASH-OFF

- Grapes

Oxathiapiprolin premixtures with folpet and zoxamide are highly resistant to an artificial rain applied after 20 minutes of fungicide application on grapes. Their rainfastness was stronger versus both reference products recognized as being very resistant to wash-off. These tests support a recommendation not to re-apply oxathiapiprolin based products after such rain conditions, i.e. 100 mm occurring 20 minutes or more after application.

- Potatoes:

Even under very severe rain scenario, DPX-UKH70 100OD and DPX-TAH88 330SE conserved perfect performance. Oxathiapiprolin based products were statistically more rainfast than standard products at their lowest tested rate. This demonstrates the outstanding rainfastness of those premixtures.

CONCLUSION

Those results, clearly demonstrates the ability of oxathiapiprolin premixtures to move within the xylem tissues at a high enough concentration to provide significant protection of new growth in the plant. The ability to protect new growth, will provide growers with a valuable tool and help prevent downy mildew and late blight infections possibly occuring between fungicides applications, particularly during periods of rapid plant growth. In addition, the rainfastness attributes will offer growers more flexibility respraying case of a rain event occurring within 20 minutes of oxathiapiprolin premixtures application.

All those studies, either *in-vitro, in planta* and field experiments demonstrated that all Zorvec based formulations have key differenting biological attributes which results in a consistent high level of performance and persistence, even in condition of high disease pressure.

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