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ZORVEC™ (OXATHIAPIPROLIN), A NEW FUNGICIDE FROM DUPONT DE NEMOURS AGAINST OOMYCETES

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ABSTRACT
DuPont™ Zorvec™ (oxathiapiprolin) is a novel fungicide discovered by DuPont 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 reliable disease control. This product is being developed on crops where oomycete pathogens limit agricultural productivity and profitability including potatoes, grapes and vegetables. Its new mode of action makes oxathiapiprolin a valuable option for fungicide resistance management strategies, while safety to key beneficial organisms confer a strong fit within integrated pest management programs. Finally, its toxicological and environmental profile, combined with low use rates, provides large margins of safety for consumers, agricultural workers and the environment.

Keywords: oxathiapiprolin, oomycetes, curative activity, translocation, wash-off.

RÉSUMÉ
ZORVEC™ (OXATHIAPIPROLINE), UN NOUVEAU FONGICIDE DE DUPONT DE NEMOURS CONTRE LES OOMYCETES
DuPont™ Zorvec™ (oxathiapiproline) est un nouveau fongicide découvert par DuPont 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 attributs biologiques se traduisent par une très bonne performance. Ce fongicide est développé contre les mildious des principales cultures d'importance économique comme la pomme de terre, la vigne, les cultures légumières. De par son nouveau mode d'action, cette substance active constitue un outil supplémentaire pour la gestion de la résistance. Sa sélectivité vis-à-vis des organismes auxiliaires permet son utilisation dans des programmes de lutte intégrée. Enfin, son profil toxicologique et écotoxicologique et ses doses d'utilisation très faibles assurent des marges de sécurité importantes pour l'utilisateur, le consommateur et l'environnement.

Mots-clés : oxathiapiproline, oomycètes, activité curative, redistribution, lessivage.

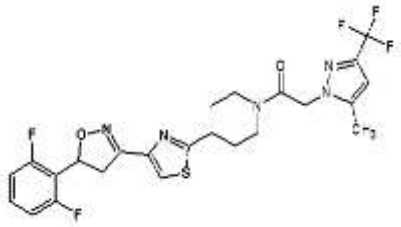
INTRODUCTION

DuPont™ Zorvec™ (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. It will provide a new biochemical mode of action in farmer's pesticide toolbox and has very interesting biological properties. The class of chemistry that includes oxathiapiprolin received in 2012 an Agrow Award for the most innovative chemistry.

This article summarizes physical, toxicological and ecotoxicological properties of the molecule oxathiapiprolin. Its biochemical mode of action is then described. Finally, its main biological attributes (activity on life cycle, movement in the plant, resistance to wash-off) are presented.

Identification of the molecule:

- Common chemical name : (ISO) : oxathiapiprolin
- Chemical class : piperidinyl thiazole isoxazoline
- Company development code : DPX-QGU42
- Molecular formula : $C_{24}H_{22}F_5N_5O_2S$
- Chemical structure :



- CAS number: 1003318-67-9

Physical properties:

- Water solubility : 0.175 mg/L
- Log Kow : 3.67
- Kfoc : 8368 g/kg
- Vapor pressure (20°C) : 1.14 x 10^{-6} PA
- Hydrolysis : Stable
- Aqueous photolysis: DT_{50} - 15 days
- Degradation in soil : DT_{50} – 90 days

Toxicity and ecotoxicity:

While oxathiapiprolin is active on oomycetes at very low rates, its toxicity on non-targeted organisms is extremely low. Mammalian oral and dermal toxicity, neurotoxicity and developmental toxicity is low, as is toxicity on birds, bees and aquatic organisms.

Biochemical mode of action:

The mode of action of oxathiapiprolin is novel and is currently listed by FRAC (Fungicide Resistance Action Committee) as U15. Its target site has been determined to be the oxysterol-binding protein (OSBP) (Patent WO 2013/009971 A1). The function of OSBP is under investigation.

Disease control spectrum:

Oxathiapiprolin is being developed on crops where pathogens limit agricultural productivity and profitability including potatoes, grapes, cucurbits and other vegetable crops. Table I summarizes main uses on which this fungicide is currently being developed worldwide.

Table I: Main uses where oxathiapiprolin is being developed worldwide
(Principaux usages sur lesquels oxathiapiprolin est développé dans le monde)

Crops	Disease caused by	Common name
Potato	*Phytophthora infestans*	Late blight
Grape	*Plasmopara viticola*	Downy mildew
Cucurbits	*Pseudoperonospora cubensis* *Phytophthora capsici*	Downy mildew Phytophthora crown rot
Tomato and related crops	*Phytophthora infestans*	Late blight
Peppers	*Phytophthora capsici*	Phytophthora crown rot
Tobacco	*Phytophthora nicotiane*	Tobacco black shank
Leafy vegetables	*Bremia lactucae* *Peronospora species*	Downy mildew
Bulb vegetables	*Peronospora destructor*	Downy mildew
Other vegetables	*Phytophtora species* *Peronospora species*	Various blights, rots and downy mildews

EFFECTS ON LIFE CYCLE OF TARGETED PATHOGENS

Microscopy studies (inverted microscopy, fluorescence microscopy or scanning electronic microscopy) have been carried out on *P. infestans*, *P. viticola* and *P. cubensis* in order to characterize the effect of oxathiapiprolin at different stages of pathogen life cycles. These studies reveal that the fungicide is active at very low rates on several stages of fungi life cycle (Table II). It inhibits zoospore release (effect demonstrated with *P. infestans* and *P. cubensis*), their motility and their germination. In the specific case of *P. infestans*, oxathiapiprolin also inhibits direct germination of sporangia.

Table II: Intrinsic activity on *P. infestans* compared with two standards
(Activité intrinsèque sur *P. infestans* comparée à deux références)

☑ Highly active ☐ Active ☒ Inactive

In-vitro tests	Oxathiapiprolin EC_{50}, ppm	Standard A EC_{50}, ppm	Standard B EC_{50}, ppm
Zoospore release	☑ <0.01	☐ 0.03	☒ > 1.0
Zoospore germination	☑ < 0.00001	☐ 0.00002	☑ < 0.00001
Sporangia direct germination	☑ < 0.00001	☐ 0.0002	☒ > 0.1
Mycelial growth	☑ 0.0002	☐ 0.004	☒ > 0.1

EC_{50} = concentration needed to inhibit 50 % of the population

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).

This fungicide demonstrates excellent activity at various stages of development of targeted pathogens. It is however important to remind people that according to resistance management guidelines, the product will be recommended only under preventative situations.

Photo 1: Effect of oxathiapiprolin on zoospore release and germination
Scanning Electronic Microscopy with the example of *P. infestans* on potato leaves.
Preventive application, photo taken 4-6 hours after inoculation
Photo 1a: untreated; Photo 1b: oxathiapiprolin at 30 ppm

(Effet d'oxathiapiprolin sur le relargage et la germination des zoospores
Microscope Electronique à Balayage, exemple de *P. infestans* sur feuilles de pomme de terre
Application préventive, photo prise 4-6 heures après inoculation
Photo 1a : non traité ; Photo 1b : oxathiapiprolin à 30 ppm)

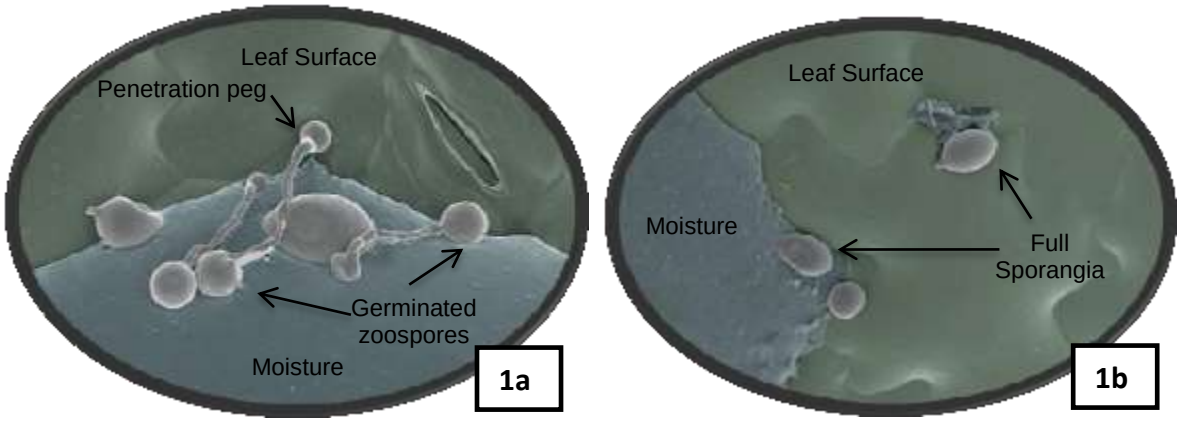
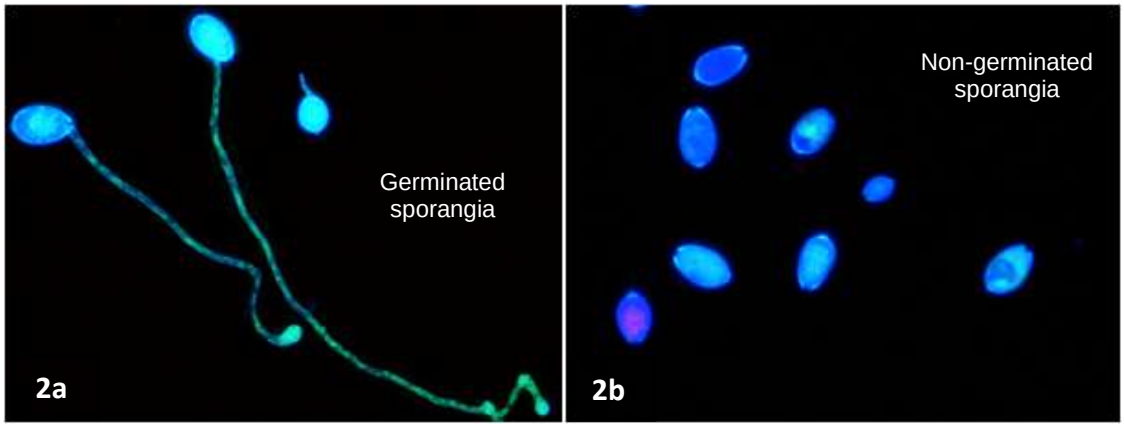


Photo 2: Effect of oxathiapiprolin on sporangia germination. Fluorescence microscopy with *P. infestans.* 2a: untreated; 2b: oxathiapiprolin at 5 ppm. Photos taken 24 hours after inoculation

(Effet d'oxathiapiprolin sur la germination des sporocystes. Microscopie par épifluorescence avec *P. infestans*. 2a : non traité ; 2b : oxathiapiprolin à 5 ppm. Photos prises 24 heures après inoculation)



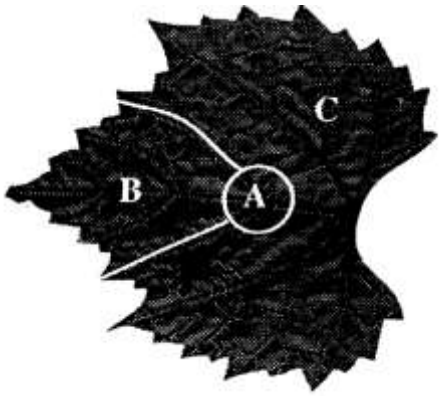
BIOLOGICAL ATTRIBUTES

Materials and Methods:

Xylem systemic and translaminar activity

Grape seedlings (Chardonnay) were used in this experiment. 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. After these two hours, the felt pads were removed and 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 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 locations (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 xylem)
- Activity in area C shows an basipetal movement via the phloem

Curative activity:

Curative activity of oxathiapiprolin has been evaluated under controlled conditions on both potato plants (Bintje *in-vitro* culture) and grapes seedlings (Chardonnay). Following an inoculation at 3-4 true leaf stage, plants were stored in a dew chamber for one day then in a growth chamber prior to the treatment with tested fungicides.

Fungicide application was carried out 2 days after inoculation in a laboratory tunnel sprayer. Plants were then stored in a growth chamber until they were rated (evaluation of leaf infected area). In the case of grape downy mildew, seedlings were previously stored in a dew chamber during 24 hours to favor *P. viticola* sporulation.

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. In this article, only the work on potato is presented.

Potato plants (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:

- Fully expanded growth: leaves fully developed at the time of application
- Expanding growth: leaves having 20 to 30 % of their full size when fungicide was applied
- 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)
- Axillary growth: total of new growth emerging from leaf axils following application that were not present at the time of the treatment.

Resistance to wash-off

Young potato plants and grape seedlings were sprayed in a laboratory tunnel sprayer at two rates, N/4 and N/16, where N corresponds to the registered rate of the tested products (targeted registered rate in the case of oxathiapiprolin). Twenty minutes after application, half of the plants were subjected to an artificial rain of 60 mm (30 mm per hour during two 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. In the case of grape downy mildew, seedlings were previously stored in a dew chamber for 24 hours to promote fungal sporulation.

Results:

Xylem systemic and translaminar movement

Systemic movement of oxathiapiprolin in xylem tissues gives very good protection outward to the leaf tip (95.3% control) while its translaminar movement protects the underside of the plant (100% control). These levels of protection are much better than tested standards even under high disease pressure (see Table III). However, oxathiapiprolin does not provide basipetal protection, with 4.4 % efficacy on *P. viticola.*

Table III: Translaminar activity and local systemic movement of oxathiapiprolin in grapes (4 tests)
(Activité translaminaire et de systémie locale d'oxathiapiprolin sur vigne (4 tests))

	% Control on *P. viticola*				
	Oxathiapiprolin 20-30 ppm	**Standard A**	**Standard B**	**Standard C**	**Untreated (% disease)**
Translaminar activity	100.0	51.9	72.9	23.5	92.3
Local acropetal control	95.3	29.7	25.0	7.9	87.1
Local basipetal control	4.4	3.2	1.3	3.1	86.6

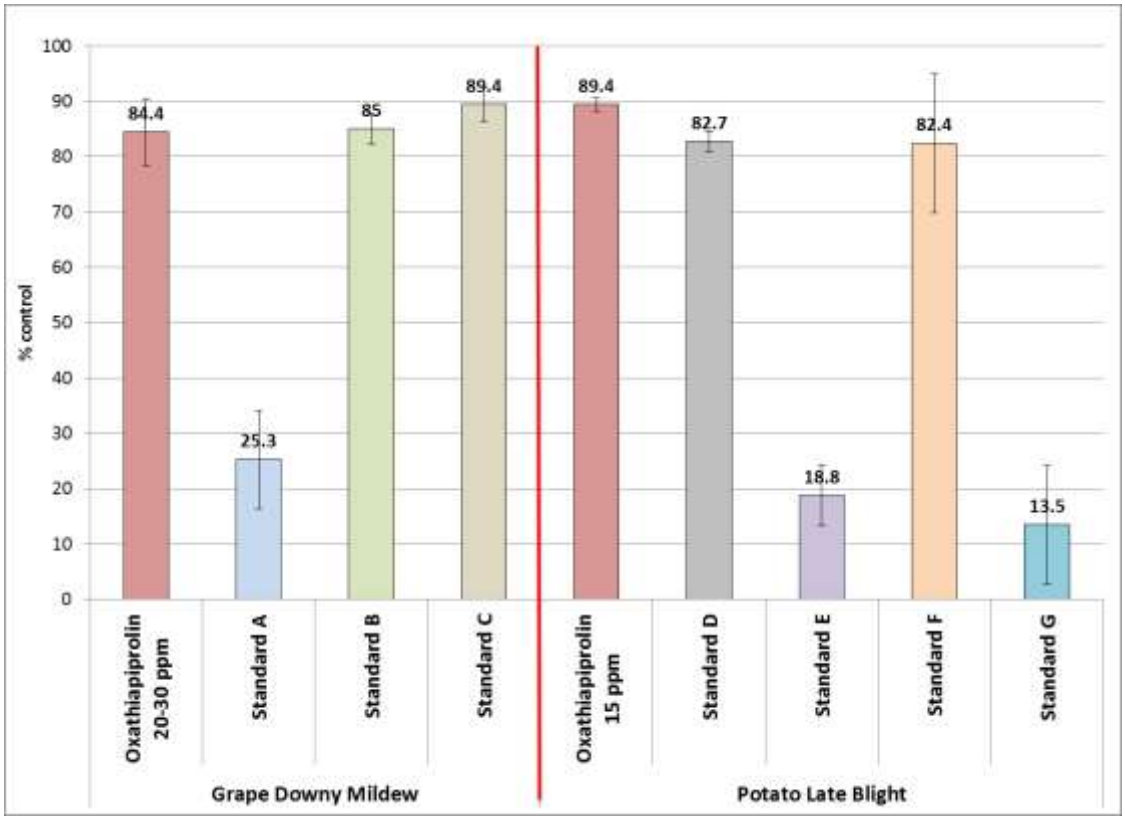
Curative activity

When oxathiapiprolin is applied 2 days after an infection event, it demonstrates excellent curative activity on grape downy mildew at a level close to the best current curative standards (84.4% efficacy for oxathiapiprolin treated plants compared to 85.0 % for standard B and 89.4% for standard C).

On potato late blight, very good curative effect of the fungicide is also seen, with 89.4 % efficacy. This level is close to the best reference (standards D and F which provide respectively 82.7% and 82.4% efficacy) and much better than other tested standards (see Figure 2).

Figure 2: 2 day curative activity of oxathiapiprolin on *P. viticola* and *P. infestans*
Mean of 4 tests on grape downy mildew (89.3 % infection in untreated)
Mean of 3 tests on potato late blight (90.1 % infection in untreated)

(Activité curative 2 jours d'oxathiapiprolin sur *P. viticola* et *P. infestans*
Moyenne de 4 tests sur mildiou de la vigne (89.3 % attaque dans le témoin)
Moyenne de 3 tests sur mildiou de la pomme de terre (90.1 % attaque dans le témoin))



Protection of new growth

Oxathiapiprolin demonstrates excellent activity on leaves at several developmental stages, and higher activity than that observed for the two tested standards which are recognized as providing good protection of new growth. The level of efficacy on fully expanded growth was approximately 93.8%, compared to 87.3% for standard A and 58.5% for standard B (see Table IV).

On expanding growth, oxathiapiprolin provides outstanding activity, with 96.0% control against potato late blight. This level was statistically higher than one of the two tested references which provided 84.0 % and 62.0% respectively.

The efficacy of the fungicide on emerging growth (leaf having 5-20 % of their final size at the time of the application), was also excellent and statistically better than standards A and B, which did not provide very good activity on this leaf type.

Finally, effect on axillary growth (leaves not exposed at all by fungicides) was very good for oxathiapiprolin treated plants (75.0% efficacy). This level of protection was also statistically higher than that observed for the standards (38.0 % and 20.0% efficacy).

Table IV: Effect of oxathiapiprolin on protection of new growth in potato (1 experiment)
Effet d'oxathiapiprolin sur la protection des nouvelles pousses en pomme de terre (1 test)

	% Control on *P. infestans*				
	Oxathiapiprolin 15 gai/ha	**Standard A**	**Standard B**	**Untreated (% disease)**	**p- value (Fisher)**
Fully expanded growth	93.8 (a)	87.3 (a)	58.5 (b)	100.0	< 0.05
Expanding growth	96.0 (a)	84.0 (b)	62.0 (c)	100.0	< 0.05
Emerging new growth	72.0 (a)	27.0 (b)	25.0 (b)	98.0	< 0.05
Axillary growth	75.0 (a)	38.0 (b)	20.0 (c)	98.0	< 0.05

The statistical group using Fisher test at alpha = 0.05 is presented between brackets

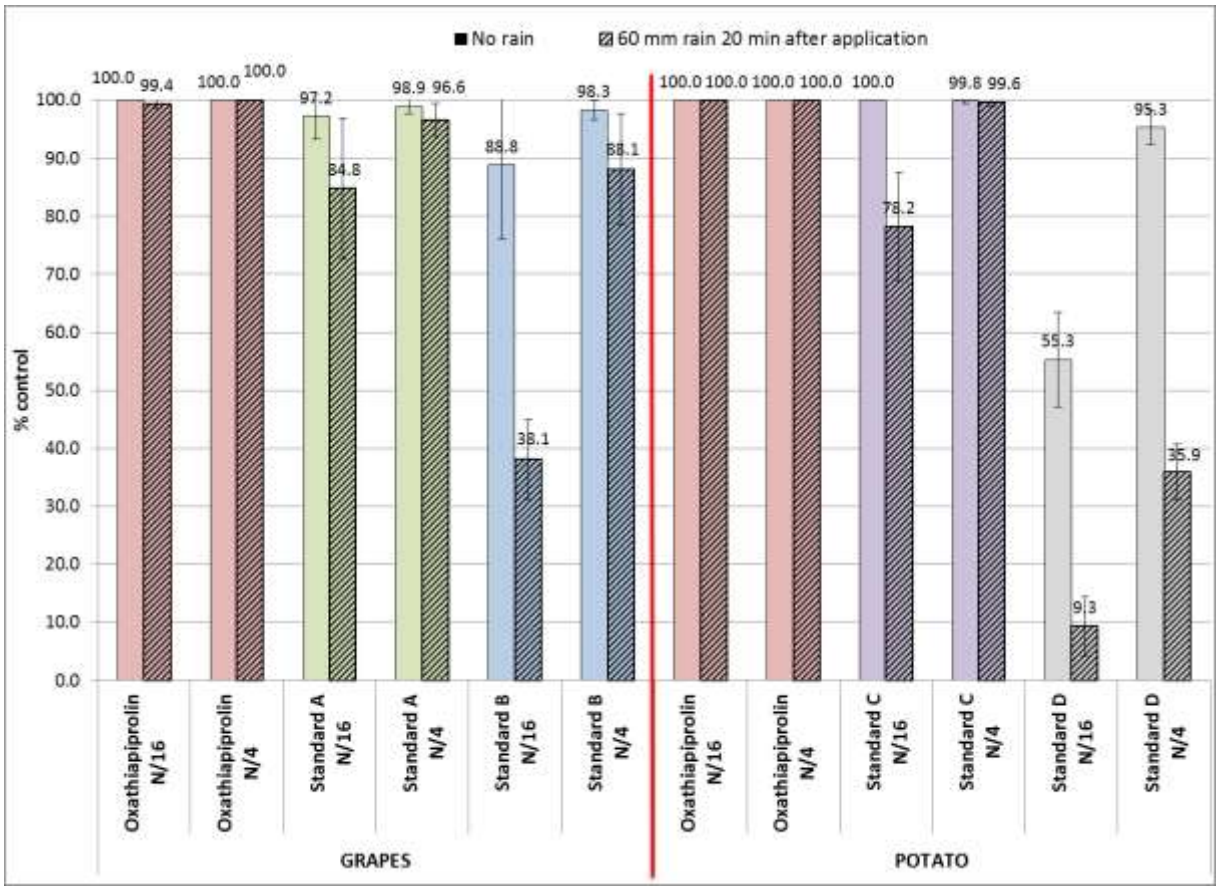
Resistance to wash-off

Figure 3 shows the level of control provided by tested products, either in absence of rain or when an artificial event occurred 20 minutes after treatment. Standards B and D, known to be sensitive to rain conditions, did not maintain good efficacy especially at N/16, which validates the methodology of the bioassay. Standards A and C maintain their excellent rainfastness on grapes and on potato, respectively. At N/4, their performance was not affected by the 60 mm of rain. At N/16, a shift of efficacy was seen (reduction of approximately 13% control on grapes and 22% on potato).

Oxathiapiprolin maintained more than 99 % of its activity after severe rain conditions occurring soon after application, even at the lowest tested dilution, which demonstrates its superior rainfastness 20 minutes after application.

Figure 3: Rainfastness of oxathiapiprolin 20 minutes after application (60 mm, 30 mm/hour)
Mean of two tests on grapes and 1 test on potato
Percentage of severity in untreated check: 83.8-86.4% (grapes); 94.1-99.0% (potato)

(Résistance au lessivage d'oxathiapiprolin après 20 minutes (60 mm, 30 mm/heure)
Moyenne de 2 tests sur vigne et 1 test sur pomme de terre
Pourcentage d'attaque dans le témoin : 83.5-86.4 % (vigne) ; 94.1-99.0 % (pomme de terre))



DISCUSSION:

The field or laboratory studies carried out either on grapes or on potato identify key benefits linked with the use of oxathiapiprolin.

When the fungicide is applied before infection, it acts at multiple stages of fungus life cycle: inhibition of zoospore release (demonstrated for *P. infestans* and *P. cubensis*), strong effect on zoospore germination and in the specific case of potato, reduction of sporangia direct germination. The strong *in-vitro* activity observed in microscopic studies is directly translated into very robust efficacy on plants even at low rates.

If oxathiapiprolin is applied after infection, it stops mycelium growth inside the leaves. Data generated on whole plants demonstrate that the fungicide provides excellent curative activity, equivalent to best current curative standards. However, despite this excellent curative effect, it is important to mention that according to current resistance management guidelines developed by

DuPont, the use of oxathiapiprolin will be recommended only under preventive conditions. In addition, oxathiapiprolin will have to be mixed with a fungicide with another mode of action that is effective on the targeted pathogen. The curative activity of oxathiapiprolin should not be used as a reason for application after disease develops in a crop. However, this attribute provides a greater flexibility for the user, especially when consecutive rain events prevent the crops from being sprayed preventatively.

This flexibility of use is reinforced by the excellent redistribution of the product on leaves (xylem systemic and translaminar activity) which compensates for potential imperfections in coverage or partial overlapping of leaves during the application.

The outstanding rainfastness of oxathiapiprolin, even 20 minutes after a strong rain event avoids the need to respray when such scenarios occur, providing additional peace of mind for the user.

Finally, the excellent protection of new growth that oxathiapiprolin demonstrates (expanding growth, emerging new growth and new untreated growth) allows its positioning at rapid growth stages and with extended spray intervals.

CONCLUSION

The *in-vitro*, *in-planta* and field experiments carried out with oxathiapiprolin on both potato late blight and grape downy mildew demonstrate that the fungicide has excellent activity at very low use rates on both diseases, at multiple stages on their life cycle, and provides biological attributes which contribute to a very good flexibility of use and excellent robustness of efficacy despite severe infection conditions.
It has been demonstrated that oxathiapiprolin demonstrates excellent curative activity at least as good as the best curative standards. Redistributed by the xylem, the fungicide also provides very good acropetal and translaminar activity. It is protected from rain within 20 minutes of an application which will avoid the need to respray if such rain scenarios occur. Finally, oxathiapiprolin demonstrates superior protection of new growth, which will allow its positioning at rapid growth stages of treated crops.

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