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ALD1901, AN INNOVATIVE BIOCONTROL SOLUTION TO MANAGE EFFICIENTLY TREATMENT STRATEGIES ON GRAPEVINE AND VEGETABLE CROPS AGAINST MAJOR FUNGAL DISEASES

P. LETOUSEY, A. MARTIN, J. DETOC, F. BOULISSET AND C. PROFIZI

AGRAUXINE LESAFFRE PLANT CARE, 2&4 Rue Henri Becquerel, 49070 BEAUCOUZE, France

ABSTRACT
AGRAUXINE Lesaffre Plant Care, a business unit of the LESAFFRE group, develops, produces and markets biocontrol products derived from micro-organisms to improve crop protection. Based on this expertise, AGRAUXINE Lesaffre Plant Care developed a natural active substance named cerevisane® (composed by cell walls of *S. cerevisiae* yeast strain LAS117) and the related product ALD1901. The mode of action of ALD1901 consists in the stimulation of plant natural defenses and the activation of several complementary defense pathways. In viticulture and vegetable crop production, ALD1901 demonstrated a significant efficacy against several major foliar fungal diseases such as powdery mildew, downy mildew and botrytis. Its innovative multi-target positioning allows a flexible integration into conventional or sustainable treatment programs. The regular use of ALD1901 optimises the applied quantities of conventional phytosanitary products and limits the risk of residues while ensuring a satisfactory efficacy level.

Keywords: crop protection, SDP, yeast, multi-target, low-risk substance.

RÉSUMÉ
AGRAUXINE Lesaffre Plant Care, filiale du groupe LESAFFRE, conçoit, fabrique et met en marché des produits de biocontrôle issus de micro-organismes pour améliorer la protection des cultures. Forte de cette expertise, AGRAUXINE Lesaffre Plant Care a mis au point une substance active naturelle nommée cerevisane® (composée de parois cellulaires de levures *S. cerevisiae* souche LAS117) qui a donné naissance au produit ALD1901. Son mode d'action consiste à stimuler les défenses naturelles de la plante et activer plusieurs voies de défense complémentaires. En viticulture et en cultures légumières, ALD1901 a démontré une efficacité significative vis-à-vis de maladies fongiques foliaires majeures telles que l'oïdium, le mildiou et le botrytis. Son positionnement innovant multi-cible permet une intégration flexible dans les itinéraires de traitement conventionnels ou raisonnés. L'utilisation régulière d'ALD1901 en programme optimise les quantités de produits phytosanitaires conventionnels appliquées et limite le risque « résidus » tout en assurant un niveau d'efficacité satisfaisant.

Mots-clés : protection des cultures, SDP, levure, multi-cible, substance à faible risque.

INTRODUCTION

Grapevine and vegetables crops are victims of several major fungal diseases such as powdery mildew, downy mildew and grey mold (Armijo *et al*., 2016), which require many phytosanitary treatments every year. In a context of sustainable agriculture and reduction of chemical inputs, the use of biocontrol products is relevant to reduce the applied quantities of conventional fungicides. Among biocontrol solutions, SDP (Stimulator of Plant Defenses, alternatively named elicitor) are a strategy under investigation to control plant pathogens (Benhamou & Rey, 2012).

The major components of yeast cell walls are polysaccharides, such as glucans, mannans and chitin (Klis *et al*., 2006). This type of molecules may act as plant elicitors by inducing defense mechanisms leading to plant protection against pathogens (Trouvelot *et al*., 2014). Glucans are known to protect grapevine, under controlled conditions, against downy mildew due to *Plasmopara viticola* and grey mold due to *Botrytis cinerea* (Aziz *et al*., 2003; Trouvelot *et al*., 2008). Similarly, yeast increases resistance against *Botrytis cinerea* in *Arabidopsis thaliana* (Raacke *et al*., 2006).

AGRAUXINE Lesaffre Plant Care developed the ALD1901 product, based on cerevisane®, for its potential to protect grapevine and vegetables against fungal pathogens and to stimulate plant defenses. The active substance cerevisane® (cell walls of *S. cerevisiae* strain LAS117) is a patented natural substance which is registered as a low risk active substance in accordance with Regulation (EC) No 1107/2009. Firstly, the efficacy of ALD1901 was investigated on powdery and downy mildew in grapevine under controlled conditions, with artificial inoculation of the pathogen. Then, GEP (Good Experimental Practices) field trials were carried out in grapevine on powdery and downy mildew as well as GEP greenhouse trials in lettuce on downy mildew. Grapevine trials were particularly focused on the integration of ALD1901 in a conventional treatment program, with reduced doses of chemical fungicides. Finally, molecular and histological studies were performed to precise the mode of action of ALD1901 and identify the defense pathways induced and the mechanisms of effective defense which are set up by the plant.

MATERIAL & METHODS

ALD1901 EFFICACY ON POWDERY AND DOWNY MILDEW IN GRAPEVINE UNDER CONTROLLED CONDITIONS

Studies have been conducted on *Vitis vinifera* cuttings (6 leaf stage = 2 month old plants, cultivar Marselan sensitive to powdery and downy mildew), cultivated in pots under greenhouse. Vine plants have been treated by spraying ALD1901 at 2,5 g/L on both sides of leaves, and then inoculated (72 h after treatment) by dusting *Erysiphe necator* conidia or by spraying a solution of *P. viticola* (10^4 sp/mL).
Microscopic developments of *E. necato*r and *P. viticola* have been observed respectively 15 and 7 days after contamination, according to this protocol: Foliar discs (0,7 cm) were picked and dipped into absolute methanol during 24h, and clarified in chloral hydrate (2,5 g/mL) during a night. Then chloral hydrate is eliminated by two successive baths of 15 min with a phosphate buffer 0,1M pH8. Finally, discs were placed in a basic solution of blue aniline (0,05 % in phosphate buffer) overnight, and placed between slides and coverslips. Observation is done on a minimum of 10 discs per treatment, using light microscopy for *E. necator* and epi-fluorescence microscopy with UV filter (excitation 340-380 nm, barrier filter LP 425nm) for *P. viticola*.

ALD1901 EFFICACY IN GRAPEVINE AND LETTUCE IN FIELD TRIALS

Field trials on grapevine

For downy mildew on grapevine, efficacy of ALD1901 was calculated based on % severity on bunches, compared to the Untreated Control (UTC). The mean was obtained from data of 8 GEP trials performed in France, Italy and Spain between 2009 and 2015. General guidelines were EPPO PP 1/152 (2/3), EPPO PP 1/181 (2/3) and EPPO PP 1/135 (2/3). Specific guidelines were EPPO PP 1/31 (3). Plot size was 13-63 m^2 and 4 replications were realized. Tested varieties were Grenache, Cabernet (3 trials), Chardonnay, Traminer, Barbera and Alfonso Lavalle. ALD1901 was applied preventively at 0.25 kg/ha via foliar spray (130-1000 L/ha water volume). 7 to 11 applications were realized between BBCH 15 and 85, every 7 to 12 days. Assessments were % of affected leaves or bunches (incidence, not shown) and % of affected leaf or bunch area (severity) on 50-100 leaves or bunches per plot. They were carried out 1-2 weeks after each application when infestation was visible (natural infestation).
The GEP trial showing the disease development kinetic (% severity on bunches) was achieved in Portugal in 2016 on Tinta Roriz cultivar. Eight preventive foliar applications were realized with an interval between applications of 7 days. ALD1901 was used alone or in tank-mix (0.25 kg/ha) with a copper hydroxide reference used at a reduced dose (1/2). Assessments were % of incidence (not shown) and % of severity on leaves (not shown) or bunches.

For powdery mildew on grapevine, efficacy of ALD1901 was calculated on % severity on bunches, compared to the UTC. The mean was obtained from data of 14 GEP trials performed in France, Italy and Spain between 2009 and 2015. General guidelines were EPPO PP 1/152 (2/3), EPPO PP 1/181 (2/3) and EPPO PP 1/135 (2/3). Specific guidelines were EPPO PP 1/4 (4). Plot size was 21-50 m^2 and 4 replications were realized. Tested varieties were Carignan (5 trials), Montepulcino, Local variety, Chardonnay (2 trials), Italia, Grenache, Moscato and Mazuelo. ALD1901 was applied preventively at 0.25 kg/ha via foliar spray (200-1000 L/ha water volume). 6 to 10 applications were realized between BBCH 13 and 83, every 7 to 14 days. Assessments were % of affected leaves or bunches (incidence, not shown) and % of affected leaf or bunch area (severity) on 50-100 leaves or bunches per plot. They were carried out 1-2 weeks after each application after infestation was visible (natural infestation for 13 trials, artificial infestation for 1 trial).
The GEP trial showing the disease development kinetic (% incidence on leaves) was achieved in France in 2016 on Carignan cultivar. Six preventive foliar applications were realized with an interval between applications of 14 days. ALD1901 was used in tank-mix (0.25 kg/ha) with all the fungicides of the reference conventional program used at a reduced dose (1/2) (tebuconazole+triadimenol / fluopyram+trifloxystrobin / metrafenone / proquinazide / metrafenone / spiroxamine). Assessments were severity on leaves (% of infected leaf area).

Greenhouse trials on lettuce

For downy mildew on lettuce, the average incidence was calculated from data of 9 GEP trials performed in France, Italy, Spain and Germany between 2010 and 2015. Tested varieties were Appia, Cocktail, Magenta, Modelo, Joviale, Adantia, Passepartout, Larissa, Romana and Chianti. ALD1901 was applied at 0.75 kg/ha as a preventive foliar application, 4 to 7 times, with an interval between applications of 7 to 14 days, in a spray volume of 400-1000 L/ha. Assessments were % of infected leaves per lettuce (incidence).

INVESTIGATION OF THE MODE OF ACTION OF ALD1901

Evaluation of the effects of ALD1901 in inducing defense responses in grapevine trough RNA-Seq analysis

RNA-Seq experiments was carried out in order to evaluate transcriptional changes associated with the induction of plant defence responses in ALD1901-treated grapevines one day after spraying. Rooted cuttings of table-grape cv. Italia were grown for six months in individual pots (7x7x10 cm) on sterilized peat under controlled greenhouse conditions at 21±2°C with a photoperiod of 16 h lightning. Periodically, 5 mL of 8% Blaich salt solution was added to each pot. ALD1901 was applied to fully expanded leaves of 30 plants using a handheld sprayer (up to dripping, about 30 mL for each vine). Untreated vines were used as control. Leaf samples were collected from both control vines and ALD1901-treated vines one day after treatment (one sample for treated vines and one sample for control vines). Each sample included 5 leaves (2 basal, 2 intermediate and 1 apical) taken each from five independant vines. The 2 samples were immediately frozen in liquid N_2 and stored at -80°C. Total RNA was extracted from 150 mg of leaf tissues using the CTAB-based method described by Gambino *et al.* (2008) and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). cDNA libraries were prepared from 4 µg total RNA using TruSeq RNA Sample Preparation Kit v2 according to Illumina's protocol. cDNA libraries were then multiplexed (3-6 libraries per lane), loaded onto a flow cell and used for cluster generation using a cBot System and TruSeq SR Cluster Kit v3 (Illumina). Short sequence reads were then generated using an Illumina HiScanSQ platform and TruSeq SBS kit v3 (Illumina) following manufacturer's instructions for Single Read sequencing (50 cycles). Sequenced reads were then mapped to the reference genome of grapevine using the Q-Seq module of the ArrayStar software v.5.0.0 (DNASTAR) to estimate gene expression. In detail, the abundance of each of the 37,962 transcripts annotated on grapevine genome was measured as Reads Per Kilobase per Million mapped reads (RPKM) (Mortazavi *et al.*, 2008) and differentially expressed genes were identified by comparing RPKM values in treated *vs* untreated leaves. Differential gene expression analysis was performed with the statistical R package edgeR. False Discovery Rate (FDR) was determined. Genes showing two-fold or greater differences in expression (Fold Change, FC) and FDR ≤ 0.05 were considered as differentially expressed genes (DEGs) and submitted for functional analysis.

Histological studies

Two typical tests demonstrating the induction of plant natural defenses have been performed:

- *Demonstration of production of phenolic compounds (phytoalexins)*

Tests have been done on vine cuttings (5-6 leaves, variety Marselan), cultivated in greenhouse. Three plants per treatment have been used. These plants have been treated with ALD1901 (2,5 g/L) on both sides of leaves, and then inoculated (48h after treatment) or not with a *Plasmopara viticola* solution (10^4 sp/mL). The production of phytoalexins has been observed on foliar disks 3 and 7 days after treatment (dpt). These discs were placed between slide and coverslip in a solution of Tween 0,1%, and abaxial face was examined with a fluorescence microscope equipped with a filter block A (λex : 340 à 380 nm, λem : 425 nm).

- *Demonstration of production of Active Forms of Oxygen (H_2O_2)*

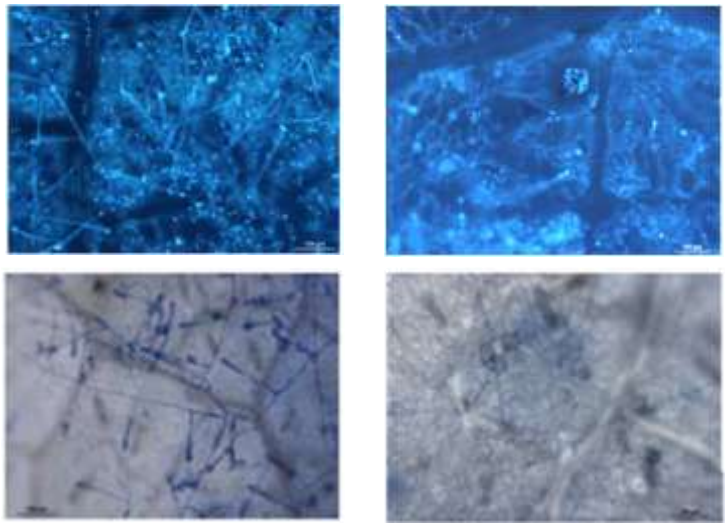
Tests have been done on vine cuttings (5-6 leaves, variety Marselan), cultivated in greenhouse. Six plants per treatment have been used. These plants have been treated with ALD1901 (2,5 g/L) on both sides of leaves, and then inoculated (48h after treatment) with a *Plasmopara viticola* solution (10^4 sp/mL). 4 days post-treatment, leaves were picked and placed into a solution of DAB (3,3'-diaminobenzidine) and placed under controlled light (80 µmol/m^2/s) during 5 hours. Then foliar discs (0,7 cm) were picked and dipped into absolute methanol, and clarified in chloral hydrate before being placed between slides and coverslips. Observations were done with a light microscope in white light. H_2O_2 was detected by the formation of brown precipitates.

RESULTS

ALD1901 PROTECTS EFFICIENTLY GRAPEVINE AGAINST POWDERY AND DOWNY MILDEW UNDER CONTROLLED CONDITIONS

The efficacy of ALD1901 was investigated on powdery mildew (*E. necator*) and downy mildew (*P. viticola*) in grapevine under controlled conditions, with artificial inoculation of the pathogen. For that, the effect of ALD1901 on the development of *E. necator* and *P. viticola* was observed with microscopic observations. ALD1901 strongly reduced the internal colonization, the mycelial growth and the sporulation of *E. necator* and *P. viticola* (Figure 1).

Figure 1 : Effect of ALD1901 on *P. viticola* (**A**) and *E. necator* (**B**) development in grapevine leaves.
Figure 1 : Effet d'ALD1901 sur le développement de *P. viticola* (**A**) et *E. necator* (**B**) dans des feuilles de vigne.



EFFICACY OF ALD1901 DEMONSTRATED IN GEP TRIALS ON GRAPEVINE AND LETTUCE

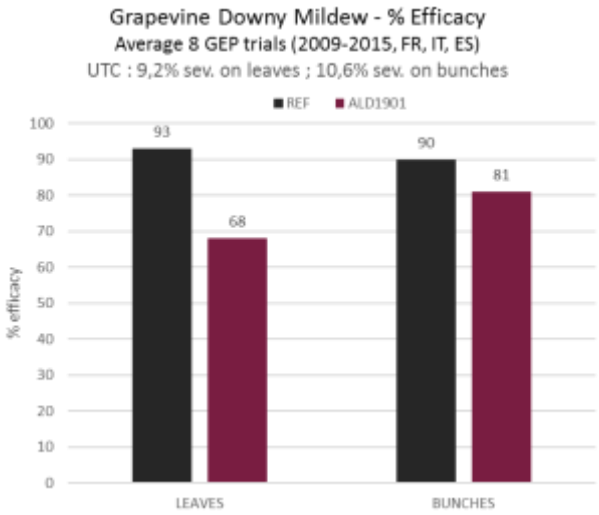
Field trials grapevine/downy mildew

Compared to UTC, the average efficacy of ALD1901 in 8 trials was very satisfactory with 68% on leaves and 81% on bunches (Figure 2A). For the program with conventional chemical treatments, results are slightly better with respectively 93% and 90% on leaves and bunches. When looking in details results obtained all along the disease development kinetic in one characteristic trial (Figure 2B), a good efficacy of ALD1901 used alone can be observed, but only at the beginning of attack under moderate pressure (60% efficacy at the 2nd assessment). However, when tank-mixed with reduced dose of copper (half-dose), ALD1901 allowed to improve the efficacy of the copper reduced dose (statistically different on last scoring) and to maintain the efficacy at the same level than with the full dose of copper (94% and 92% efficacy for the 3rd and the 4th assessment respectively).

Figure 2 : Efficacy of ALD1901 on downy mildew in grapevine. **A**, Average efficacy on leaves and bunches in 8 GEP trials from 2009 to 2015 (REF = conventional chemical treatment); **B**, Percentage of disease severity on bunches treated with ALD1901 alone or tank-mixed with a copper hydroxide reference at a reduced dose (1/2), obtained in one characteristic trial (Portugal, 2016). UTC = Untreated Control.

Figure 2 : Efficacité d'ALD1901 sur le mildiou de la vigne. **A**, Efficacité moyenne sur feuilles et grappes dans 8 essais BPE entre 2009 et 2015 (REF = traitement chimique conventionnel); **B**, Sévérité de la maladie sur des grappes traitées avec ALD1901 seul ou mélangé en tank-mix avec une référence chimique hydroxyde de cuivre en dose réduite (1/2), obtenu dans un essai caractéristique (Portugal, 2016). UTC = Témoin non Traité.

A



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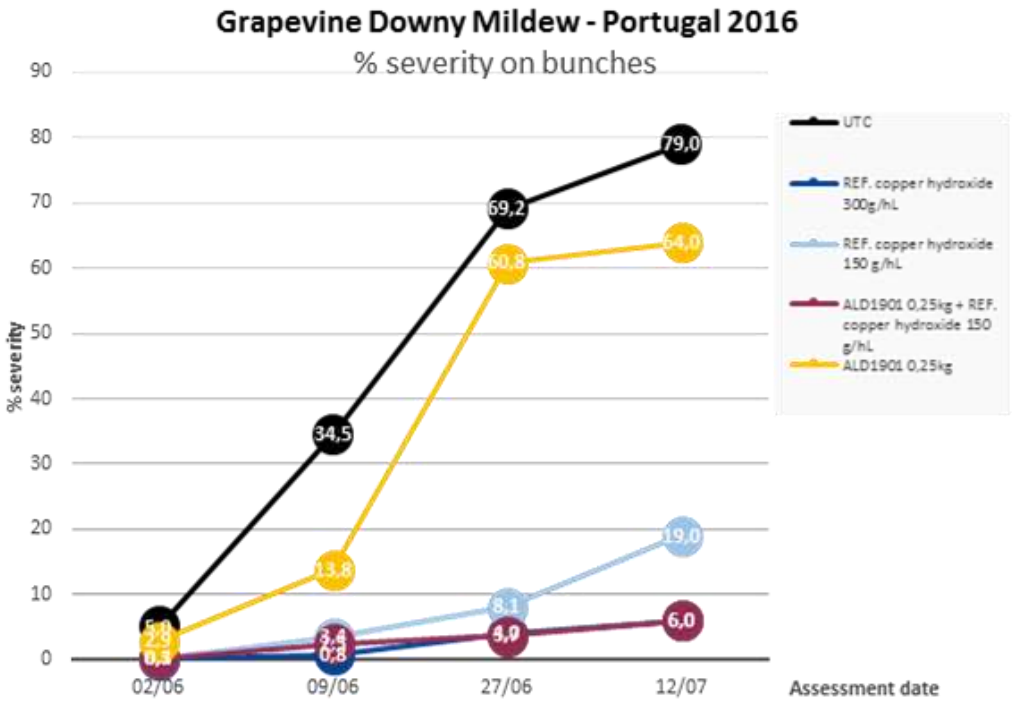
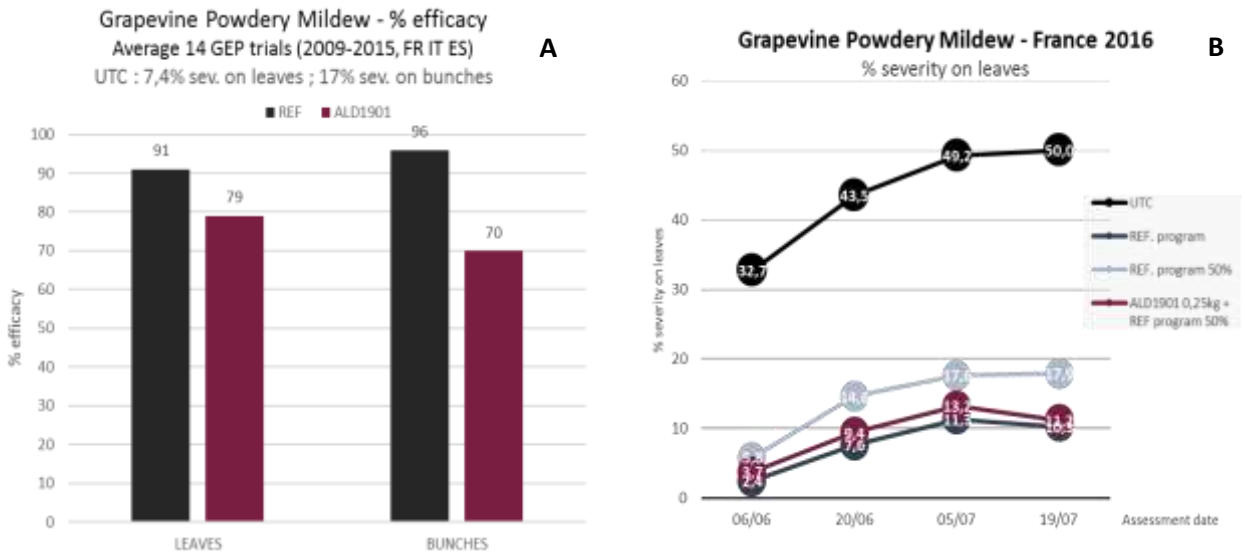


Field trials grapevine/powdery mildew

Compared to UTC, the average efficacy of ALD1901 in 14 trials was very satisfactory with 79% on leaves and 70% on bunches (Figure 3A). For the conventional chemical treatment, results are slightly better with respectively 91% and 96% on leaves and bunches. When looking in details results obtained all along the disease development kinetic in one characteristic trial (Figure 3B), we observed that the use of ALD1901 in tank mix with chemicals allowed 50% dose reduction of chemical program even under conditions of strong pressure and early attack.

Figure 3 : Efficacy of ALD1901 on powdery mildew in grapevine. **A**, Average efficacy on leaves and bunches in 14 GEP trials from 2009 to 2015 (REF = conventional chemical treatment); **B**, Disease severity on leaves treated with ALD1901 tank-mixed with the fungicides of reference (REF) conventional program used at a reduced dose (1/2), obtained in one characteristic trial (France, 2016). UTC = Untreated Control.

Figure 3 : Efficacité d'ALD1901 sur l'oïdium de la vigne. **A**, Efficacité moyenne sur feuilles et grappes dans 14 essais BPE entre 2009 et 2015 (REF = traitement chimique conventionnel); **B**, Sévérité de la maladie sur des feuilles traitées avec ALD1901 utilisé en tank-mix avec les fongicides d'un programme conventionnel de référence à dose réduite (1/2), obtenu dans un essai caractéristique (France, 2016). UTC = Témoin non Traité.

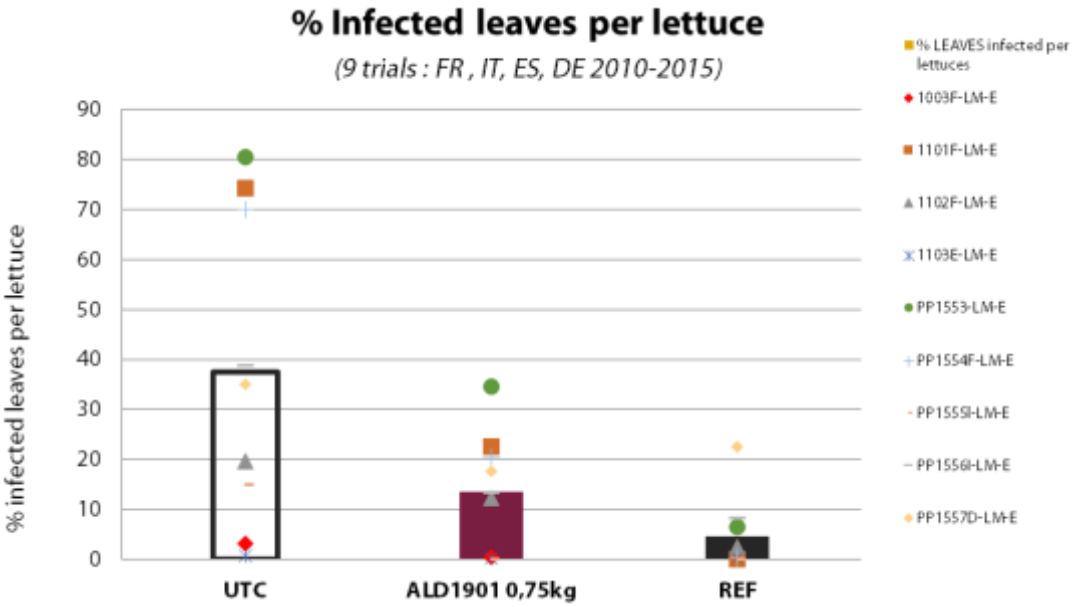


Greenhouse trials lettuce/downy mildew

The average disease incidence for the UTC in the nine compiled trials was 37%, which corresponds to a high pressure level (Figure 4). However, results showed a good efficacy of ALD1901 used alone (69% efficacy). The efficacy of the chemical reference was slightly higher (85%). Both efficacies were statistically different compared to untreated control in each individual trial.

Figure 4 : Efficacy of ALD1901 on downy mildew in lettuce: average disease incidence on leaves treated with ALD1901 or a conventional chemical treatment (REF) in 9 GEP trials from 2010 to 2015. Results obtained in each trial are also represented. UTC = Untreated Control.

Figure 4 : Efficacité d'ALD1901 sur le mildiou de la laitue : incidence moyenne de la maladie sur des feuilles traitées avec ALD1901 ou une référence chimique (REF) dans 9 essais BPE entre 2010 et 2015. Les résultats obtenus pour chaque essai sont également représentés. UTC = Témoin non Traité.



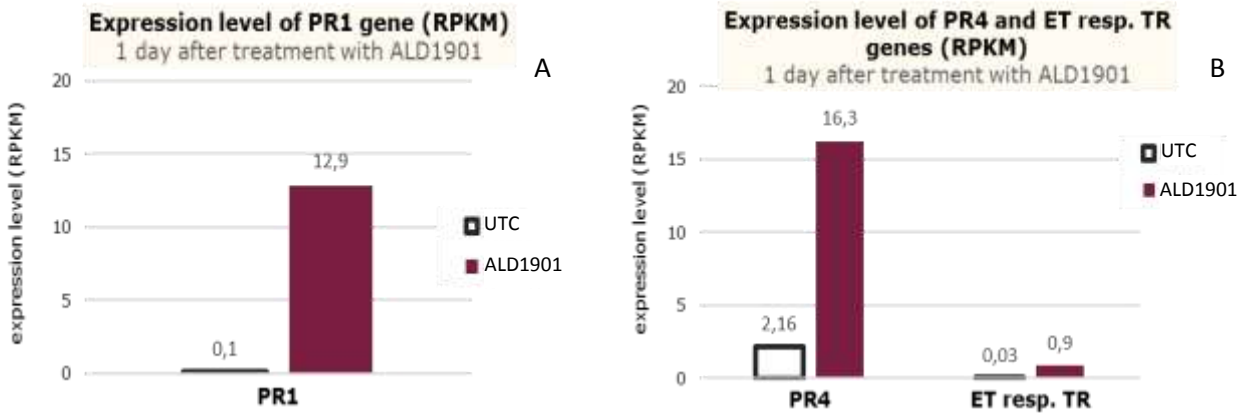
ALD1901 IS A STIMULATOR OF PLANT DEFENSES

ALD1901 induces both SA and JA/ET plant defense pathways

RNA-Seq analysis was carried out on grapevine leaves treated or not by ALD1901 since 1 day. This study revealed that ALD1901 stimulated the expression of many defense genes including genes linked to cell wall modification, lipid metabolism, ethylene response and PR proteins. In particular, PR1 gene, marker of the salicylic acid (SA) defense pathway, was strongly induced (Fold Change (FC)=140, Figure 5A). Similarly, marker genes for jasmonic acid (JA) defense pathway (PR4, FC=7.5) and ethylene (ET) pathway (ET resp. TR, FC=29) were also significantly induced (Figure 5B). These results showed that ALD1901 stimulated both SA and JA/ET defense pathways, as early as one day after application on leaves. Moreover, RNA-Seq experiment demonstrated that ALD1901 activated some defense processes known to be implicated in the reaction of grapevine genotypes resistant to downy mildew (data not shown).

Figure 5 : Molecular analysis of the grapevine leaf response to ALD1901 treatment showing stimulation of plant defenses one day after application of the product. Expression level of PR1 gene (**A**), marker of SA pathway, and of PR4 and ET resp. TR genes. (**B**), markers for JA and ET pathway respectively, in untreated (UTC) or ALD1901-treated leaves.

Figure 5 : Analyse moléculaire de la réponse foliaire de la vigne à un traitement par ALD1901 démontrant la simulation des défenses un jour après application du produit. Taux d'expression du gène PR1 (**A**), marqueur de la voie du SA, et des gènes PR4 et ET resp. TR. (**B**), marqueurs de la voie du JA et de l'ET respectivement, dans des feuilles traitées avec ALD1901 ou non (UTC).



ALD1901 activates the setting up of chemical barriers in plant tissues

Histological studies were performed to investigate effective defenses established in plant tissues in response to ALD1901 treatment. Production of phenolic compounds, probably phytoalexins, was observed in foliar tissues of ALD1901-treated grapevine plants at each assessment (3 and 7 days after treatment), while no production was observed in the untreated control (Figure 6). Similarly, production of H_2O_2 was observed in foliar cells of plants treated with ALD1901 while no production was observed in the untreated (Figure 7). Thus, ALD1901 elicits the setting up of chemical barriers in plant tissues few days after treatment (ROS and antimicrobial phytoalexins).

Figure 6 : Visualization under UV of the accumulation of autofluorescent phenolic compounds (phytoalexins) in grapevine leaf tissues treated with ALD1901 or not (UTC), 3 and 7 days post treatment (dpt).

Figure 6 : Visualisation sous UV de l'accumulation de composés phénoliques autofluorescents (phytoalexines) dans les tissus foliaires de la vigne traitée avec ALD1901 ou non (UTC) , 3 et 7 jours après traitement (dpt).

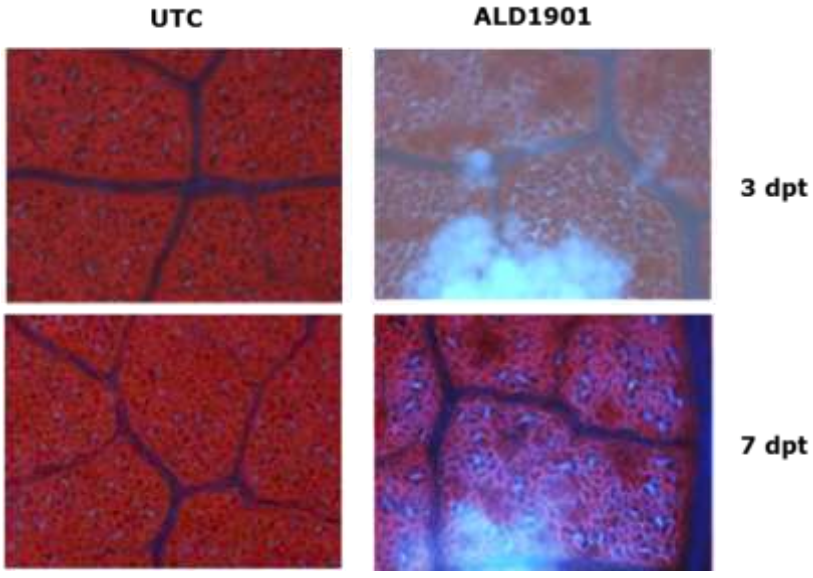
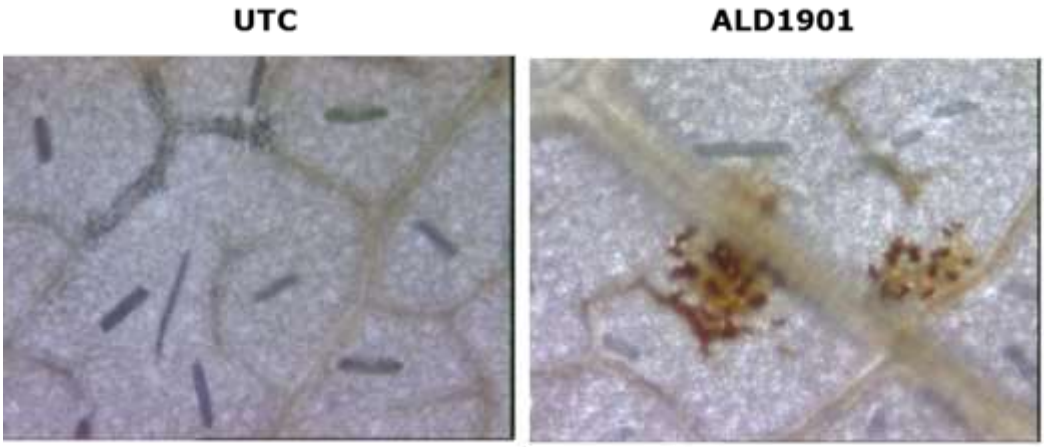


Figure 7 : Visualization of the accumulation of H_2O_2 (brown precipitates) in grapevine leaf tissues treated with ALD1901 or not (UTC), 5 days post treatment (dpt).
Figure 7 : Visualisation de l'accumulation de H_2O_2 (précipités bruns) dans les tissus foliaires de la vigne traitée avec ALD1901 ou non (UTC) ; 5 jours après traitement (dpt).



DISCUSSION

Results obtained in the laboratory, field and greenhouse demonstrated that ALD1901 protects efficiently grapevine against powdery and downy mildew and lettuce against downy mildew. Furthermore, ALD1901 was tested for its potential to control grey mold and results were satisfactory (data not shown). Thus, ALD1901 is a natural product with a broad spectrum of action, efficient on biotrophic pathogens (powdery and downy mildew) but also necrotrophic pathogens (*Botrytis cinerea*). This multi-target characteristic may be explained by the mode of action of ALD1901. Indeed, molecular studies by RNA-Seq were performed to precise the mode of action of ALD1901 and showed that both SA and JA/ET defense pathways were early induced in plant tissues after treatment with ALD1901. It is generally considered that JA and ethylene mediate necrotrophic pathogens defense, while SA is involved against the biotrophic ones (Pieterse *et al*., 2009).
Histological studies demonstrated that treatment by ALD1901 lead to the production by the plant of ROS (Reactive Oxygen Species) such as H_2O_2 and phenolic compounds which are probably antimicrobial phytoalexins. This kind of molecules have been reported as produced in response to carbohydrate elicitors (Trouvelot *et al*., 2014). The setting up of these chemical barriers by ALD1901 confers a partial resistance state to the plant which is ready to control further pathogen attacks.

CONCLUSION
The active substance of ALD1901, cerevisane® (cell walls of *S. cerevisiae* strain LAS117), is a patented natural substance, which is registered as a low-risk active substance in accordance with Regulation (EC) No 1107/2009. Cerevisane® is the first registered biofungicide elicitor coming from microbial origin fraction.
ALD1901 was demonstrated to protect efficiently grapevine against powdery and downy mildew and lettuce against downy mildew in the laboratory, but also in GEP field and greenhouse trials. The efficient dose was 250 g/ha for grapevine and 750 g/ha for lettuce, and ALD1901 was used via preventive foliar treatment. When tank-mixed with conventional fungicides, ALD1901 allowed to

improve the efficacy of the fungicide reduced dose and to maintain the efficacy at the same level than the full dose of fungicide.
Molecular and histological investigations demonstrated that ALD1901 is a stimulator of plant natural defenses, which induces both SA and JA/ET defense pathways as well as the setting up of chemical barriers in plant tissues. The multi-target disease efficacy and the broad spectrum of ALD1901 may thus be explained by the early co-activation of SA and JA/ET pathways. Studies are in progress to further investigate and understand the mode of action of ALD1901.
It is essential for a biocontrol product to be able to be integrated into conventional or sustainable treatment programs. ALD1901 showed clearly a flexibility of integration into protection programs. Moreover, the safe profile of ALD1901 is an undeniable advantage for agriculture today: active substance low-risk, not classified, no MRL, low PHI. Finally, ALD1901 takes advantage of the industrial solidity of the Lesaffre group (capacity of production) and is a very stable product with 2-year shelf-life.

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BIBLIOGRAPHY
Armijo G., Schlechter R., Agurto M., Munoz D., Nunez C., Arce-Johnson P., 2016 - Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios. Frontiers in Plant Science, 7, 382 doi: 10.3389/fpls.2016.00382.

Aziz A., Poinssot B., Daire X., Adrian M., Bézier A., Lambert B., Joubert J.-M., Pugin A., 2003 - Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Molecular Plant-Microbe Interactions*, 16, 12, 1118-1128.

Benhamou N., Rey P., 2012 - Stimulateurs des défenses naturelles des plantes: une nouvelle stratégie phytosanitaire dans un contexte d’écoproduction durable. II : Intérêt des SDN en protection des cultures. *Phytoprotection*, 92, 1, 24-35.

Gambino G., Perrone I., Gribaudo I., 2008 - A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis*, 19, 520-525.

Klis F.M., Boorsma A., De Groot P.W.J., 2006 - Cell wall construction in *Saccharomyces cerevisiae*. *Yeast*, 23, 182-202.

Mortazavi A., Williams B.A., Mc Cue K., Schaeffer L, Wold B., 2008 - Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods*, 5, 621-628.

Pieterse C.M., Leon-Reyes A., Van der Ent S., Van Weed S.C., 2009 - Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, 5, 308-316.

Raacke I.C., Von Rad U., Mueller M.J., Berger S., 2006 - Yeast increases resistance in *Arabidopsis* against *Pseudomonas syringae* and *Botrytis cinerea* by salicylic acid-dependent as well as-independent mechanisms. *Molecular Plant-Microbe Interactions*, 19, 10, 1138-1146.

Trouvelot S., Héloir M.-C., Poinssot B., Gauthier A., Paris F., Guillier C., Combier M., Trda L., Daire X., Adrian M., 2014 - Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Frontiers in Plant Science*, 5, 592 doi:10.3389/fpls.2014.00592.

Trouvelot S., Varnier A.-L., Allègre M., Mercier L., Baillieul F., Arnould C., Gianinazzi-Pearson V., Klarzynski O., Joubert J.-M., Pugin A., Daire X., 2008 - A β-1.3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. *Molecular Plant-Microbe Interactions*, 21, 2, 232-243.