

recorded, from the second year after the first application. This activity was mainly related with reduction in the spread of new infections; moreover, after the fourth year, a decrease in the number of dead plants was noticed, also on particularly susceptible varieties. Remedier<sup>®</sup>, applied especially after spring pruning at the sap bleeding stage, proved to be effective and of practical use. The best condition for *Trichoderma* colonization was achieved with applications at the sap bleeding stage. The product was fully compatible with all the main strategies for disease control in vineyards, and is the only microbiological agrochemical authorized in Italy to prevent one of the most serious diseases of grapevine.

**Phytoprotectors of grapevine: exploiting their potential to protect grapevine.** C. PINTO<sup>1,2</sup>, V. CUSTÓDIO<sup>1</sup>, A. SPAGNOLO<sup>2</sup>, C. RABENOELINA<sup>1</sup>, C. CLÉMENT<sup>2</sup>, A. GOMES<sup>1</sup> and F. FONTAINE<sup>2</sup>. <sup>1</sup>*Biocant, Centro de Inovação em Biotecnologia, Biocant-Park, Parque Tecnológico de Cantanhede, Núcleo 04, lote8, 3060-197 Cantanhede, Portugal.* <sup>2</sup>*Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. E-mail: catia.pinto@biocant.pt*

*Vitis vinifera* hosts a complex microbiome composed by neutral, beneficial or pathogenic microorganisms that are in close associations with this host. This microbiome or 'second plant genome' is essential for the plant health status, to promote plant growth, stress resistance or nutrient mobilization. Furthermore, these organisms play important roles in plant productivity and product quality. Unveiling the phytoprotector microorganisms from grapevine represents a challenge, to develop new sustainable strategies for control of grapevine diseases. The aims of this research were to isolate, identify and characterize potential phytoprotectors naturally present in vineyards and to characterize their interactions with grapevine. A total of 254 isolates (bacteria and yeasts) from Bairrada Appellation (Portugal) were isolated during 2010 and 2011 vine campaigns, and tested for their antagonistic potential against different trunk disease phytopathogens, including strains responsible for the *Botryosphaeria dieback*. Three promising phytoprotectors were selected and a significant decrease ( $P < 0.05$ ) of the normal pathogen growth was observed *in vitro*. Two of the potential phytoprotectors produced siderophores and solubilized phosphate. Their ability to colonize and to live within grapevine was also analyzed using *in vitro* plants. Two of the phytoprotectors were endophytic. In order to better understand the interactions between phytoprotectors and grapevine, a gene expression analysis of host defense responses is ongoing. These have identified potential grapevine

phytoprotectors. As these strains are naturally present at vineyards, they are likely to be well adapted and stable to the vineyard environment, which constitutes an advantage for future grapevine management.

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**New approaches for the reduction of foliar symptom expression in the esca complex of grapevine: nutrients and defense induction.** S. DI MARCO<sup>1</sup> and L. MUGNAI<sup>2</sup>. <sup>1</sup>*Institute of Biometeorology (IBIMET), National Research Council (CNR), Via P. Gobetti, 101, 40129 Bologna, Italy.* <sup>2</sup>*Department of the Agro-food Production and Environmental Sciences (DISPAA), Section of Plant pathology and Entomology, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy. E-mail: s.dimarco@ibimet.cnr.it*

The esca complex is the most important, widespread and destructive trunk disease in the grape growing areas of Europe, and is important to viticulture worldwide. The appearance of the typical foliar symptoms was, in the last decade, described as the Grapevine Leaf Stripe Disease (GLSD). Several studies agreed on the involvement of toxins produced by tracheomycotic fungi such as *Phaeoconiella chlamydospora* and *Phaeacremonium* spp. in determining the appearance of the typical leaf necrosis symptoms. Nevertheless, the occurrence and development of foliar symptoms is a complex process associated with different factors resulting in a decrease in chlorophyll, activation of defense responses and changes in metabolic patterns in leaves. No correlation was found between the severity of wood deterioration and the severity of leaf symptoms; leaf symptoms were found on vines that had only wood discoloration without any decay. Other studies stated that white rot in the cordons was the best predictor for the so called "chronic form" of esca. However, foliar symptom expression is strictly correlated with grape yield and quality reductions. Strategies aimed at reducing the incidence and/or severity of foliar symptoms would also limit losses in quality. The use of fungicides failed to provide satisfactory results, except for fosetyl-Al formulations (applied against downy mildew), which reduced the incidence of symptomatic plants and cumulative vine mortality. Recent studies suggest that host physiology and defense mechanisms, which in turn are correlated with climate, nutrition and agronomic conditions, play important roles in symptom development. Three-year

trials have demonstrated that foliar applications of a mixture of calcium chloride, magnesium nitrate and Fucales seaweed extract led to a significant reduction of foliar symptoms. Treated vines showed increased content of trans-resveratrol and flavonoids, and accumulation of calcium oxalate in crystal druses in the leaf mesophyll tissues. Ongoing trials with products based on copper, zinc and formulated with effective and/or innovative substances, such as hydracid of citric acid or hydroxyapatite crystals, also reduced foliar symptom expression and open new perspectives and opportunities for disease control. The potential of an approach that stimulates the host reaction in reducing interveinal leaf yellowing and necrosis is discussed.

**Synthesis and systemic activity of profungicides for controlling vascular diseases.** H. WU<sup>1</sup>, S. MARHADOUR<sup>1</sup>, J.-L. BONNEMAIN<sup>2</sup> and J.-F. CHOLLET<sup>1</sup>. <sup>1</sup>IC2MP (Institut de Chimie des Milieux et des Matériaux de Poitiers), UMR CNRS 7285, Université de Poitiers, 4 rue Michel Brunet, TSA 51106, F-86073 Poitiers cedex 9, France. <sup>2</sup>Laboratoire EBI (Écologie et Biologie des Interactions), UMR CNRS 7267, Équipe SEVE (Sucres, Échanges Végétaux, Environnement), Université de Poitiers, 3 rue Jacques Fort, TSA 51106, F-86073 Poitiers cedex 9, France. E-mail: jfcholle@univ-poitiers.fr

With the banning of sodium arsenite, it is necessary to find alternative fungicides with good activity against grapevine trunk disease pathogens. The uptake and distribution of fungicides in plants influence their activity on pathogens localized in vascular tissues. This study aimed to develop foliar-applied fungicides, which can be translocated to vascular tissues. We proposed two strategies to develop systemic profungicides, which involve modification of the non-systemic fungicide fenpiclonil by adding; 1) a group with a carboxylic acid function or 2) an amino acid or a sugar. The first strategy was based on the ion-trap mechanism. Three acidic derivatives and two ester derivatives of fenpiclonil were synthesized. The stability test in water and a systemicity test on castor bean (*Ricinus communis* L.) seedlings were carried out. The esters SM 26 and HW 34 were metabolized to the corresponding carboxylic acid compounds which move in plant sieve tube elements. The aim of the second strategy was to develop active transport of fungicide via nutrient carriers. The resulting molecules were amino acid and glucose conjugates. F 30-Lysine conjugate was detected in phloem sap of *Ricinus* seedlings. Our study suggested that modifying the fungicide fenpiclonil based on ion-trap and the active transport mechanism is a feasible approach to develop systemic fungicides.

**Grapevine trunk diseases: establishing a simplified model study for the evaluation of new control strat-**

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Considering the increasing economic impact of trunk diseases on viticulture worldwide, the identification of efficient and viable control strategies is urgently needed. However, understanding plant-pathogen interactions and plant physiological changes related to these diseases is essential for such accomplishment. A simplified study model which involved the use of greenhouse-grown 'Chardonnay' and 'Sauvignon' cuttings was set-up to: i) study the effect of artificial infection with the Botryosphaeria dieback agents *Neofusicoccum parvum* and *Diplodia seriata* on the plant physiology, and ii) evaluate the efficacy and feasibility of a control strategy involving dual of a potential biological control agent and a systemic profungicide. The plant growth-promoting rhizobacterium *Burkholderia phytofirmans* (strain PsJN: *gfp2x*) was tested as a biological control agent and applied to the soil 4 (T-28) and 3 weeks (T-21) before fungal inoculation. The ester derivative of the fungicide fenpiclonil "SM 26" was then applied as a foliar profungicide 2 d (T0-2) before fungal inoculation. The plants were each inoculated (at T0) on green stems under one of the SM 26-treated leaves, by applying a mycelial plug into a wound made by scratching the surface with a cork borer. One apical leaf was sampled 48h after the last profungicide application in order to study fungicide systemicity and metabolization in the plant, and leaf sampling for gene expression analysis was performed 3 (T0+3) and 7 d (T0+7) after inoculation. The possible effect of bacterial application, profungicide treatment or artificial fungal infection on photosynthetic activity was estimated by measuring the PSII activity once a week beginning from T0+4. Lesions associated to artificial infections were measured at T0+72. First results indicated that: i) no effect on photosynthetic activity was detected for any of the treatments and ii) no statistically significant effects of the PsJN or SM 26 treatments were recorded in relation to the lesion sizes associated to artificial infections. However, a synergistic effect of the two treatments was detected, especially for cuttings inoculated with *N. parvum*. This may have been due to the direct antifungal activity and/or to the eliciting effect of one or both treatments when applied together. Confirmation or disproof to this indication is expected from further ongoing experiments.

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