

THE FUNGAL MAMP CERATO-PLATANIN TRIGGERS RESISTANCE AGAINST *BOTRYTIS CINEREA* IN ARABIDOPSIS LEAVES. I. Baccelli¹, S. Luti², L. Lombardi³, R. Bernardi⁴, P. Picciarelli⁴, F. Faoro⁵, L. Pazzagli² and A. Scala¹. ¹Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino (FI), Italy. ²Dipartimento di Scienze Biomediche, Sperimentali e Cliniche, Università degli Studi, Viale Morgagni 50, 50134 Firenze, Italy. ³Dipartimento di Biologia, Università degli Studi, Via Luca Ghini 13, 56124 Pisa, Italy. ⁴Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università degli Studi, Via Matteotti 1/B, 56124 Pisa, Italy. ⁵Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università degli Studi, Via G. Colombo 42, 20133 Milano, Italy. E-mail: ivan.baccelli@unifi.it

Cerato-platanin (CP) is a non-catalytic protein with a double- α -barrel fold located in the cell wall of the phytopathogenic fungus *Ceratocystis platani*. CP is the first member of a protein family named the "cerato-platanin family" (Pfam PF07249), which comprises proteins produced by plant pathogenic and non-pathogenic fungi. Some of these proteins show resistance-inducing activity in plants. Their biological role in fungal life remains unknown, however concerning CP we have recently hypothesised a dual role: a structural role in the fungal cell wall due to the ability to bind chitin, and a role in the fungus-plant interaction due to the ability to weaken cellulosic materials. CP induces defence-related responses when applied on host and non-host plants. In plane tree (*Platanus acerifolia*) leaves these responses have been extensively studied. In the present study we show that CP triggers resistance in *Arabidopsis* leaves: CP quickly induced the synthesis of hydrogen peroxide at the level of stomata, the synthesis of nitric oxide, it caused stomatal closure and overexpression of salicylic acid- and ethylene-dependent genes and it induced the synthesis of camalexin. After 24h of surface treatment with CP, *Arabidopsis* leaves showed reduced susceptibility to *Botrytis cinerea* similarly to that occurred by using chitosan. This is the first report of a resistance-inducing activity by CP against a pathogen able to colonise leaves. We are currently investigating the mechanism by which CP is perceived by the plant leaf surface and the ability of this protein to protect *Arabidopsis* leaves against *Pseudomonas syringae* pv. *tomato*.

CHARACTERIZATION OF BENEFICIAL ENDOPHYTIC BACTERIA ISOLATED FROM HEALTHY AND 'CANDIDATUS PHYTOPLASMA MALI'-INFECTED PLANTS. D. Bulgari¹, P. Casati¹, F. Quaglino¹ and P.A. Bianco¹. ¹Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: piero.bianco@unimi.it

Apple proliferation (AP), caused by 'Candidatus Phytoplasma mali', is one of the most important phytoplasma diseases in Europe. So far, due to the absence of AP-resistant cultivars, the management of AP mainly consists in insecticide treatment against the insect vectors and in the eradication of diseased plants. These treatments have a strong economic and environmental effect, representing a risk for both operators and consumers. One of the most innovative solutions to develop sustainable approaches is the use of endophytes as inducers of the natural plant defense responses. In this work, we describe and characterize the endophytic bacterial community associated with healthy and AP phytoplasma-infected apple roots. Endophytic bacterial diversity associated with these plants was analyzed by cultivation-dependent and -independent methods. Sequence analysis of 16S rRNA gene libraries showed the presence of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chlamydiae*, and *Firmicutes*. The endophytic bacteria isolated in culture media were characterized for five beneficial traits related to mineral

nutrition (phosphate solubilization, siderophores, nitrogen fixation), development (indolacetic acid synthesis), stress relief (catalase activity), disease control (siderophores). On the basis of these data some putative biocontrol strains (e.g. *Burkholderia* sp. and *Pantoea* sp.) were transformed for the production of fluorescent proteins in order to study the colonization pattern and their life cycle inside apple plants. *Pantoea* sp. pRL67511prfp successfully colonized the interior part of the plant and survived at least on month inside the tissues. Future studies will be carried out to investigate the ability of this endophyte to control apple proliferation.

GENOME SEQUENCING AND ANNOTATION OF THE PLANT-ASSOCIATED BACTERIUM *PSEUDOMONAS CORRUGATA*. V. Catara¹, G. Licciardello², P. Bella¹, C.P. Strano¹, A.F. Catara², D.L. Arnold³, V. Venturi⁴, M.W. Silby⁵ and R.W. Jackson⁶. ¹Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Università degli Studi, Via Santa Sofia 100, 95131 Catania, Italy. ²Parco Scientifico e Tecnologico della Sicilia, z.i. Blocco Palma I, 95121 Catania, Italy. ³Centre for Research in Plant Science, University of the West of England, Bristol BS16 1QY, UK. ⁴International Centre for Genetic Engineering and Biotechnology (ICGEB), Area Science Park, Padriciano, Trieste, Italy. ⁵Department of Biology, University of Massachusetts Dartmouth, N. Dartmouth, MA 02747-2300, USA. ⁶School of Biological Sciences, University of Reading, Reading RG6 6AJ, UK. E-mail: vcatara@unict.it

Pseudomonas corrugata is a ubiquitous bacterium in the *P. fluorescens* group isolated from a wide variety of plant sources. It was described as the causal agent of tomato pith necrosis yet it is also known for biological control abilities against plant pathogens as well for being a polyhydroxycarboxylate producer. To gain more knowledge on the genetic mechanisms of this highly versatile bacterium, we sequenced the genome of the strain *P. corrugata* CFBP 5454, extensively studied in our laboratory as a model strain. Its draft genome sequence yielded 157 contigs with a total length of approximately 6.2 Mb and a 60.5% G+C. The genome analysis revealed clusters of genes coding for secondary metabolites, which may contribute to bacterial fitness, competitiveness and phytopathogenicity. A total of 121,730 base pairs belonged to putative non ribosomal peptide synthetase genes, corresponding to 1.9% of the total genome nucleotide composition. They belong to at least three biosynthetic clusters one of which may be associated with the production of the siderophore corrugatine. The other two showed high homologies in domain structures and sequences to clusters associated with the production of syringomycin and syringopeptins in *P. syringae*. We therefore predict these to be involved in the biosynthesis of the cyclic lipopeptides cormycin and corpeptins. In addition, a number of other secondary metabolite biosynthetic clusters including hitherto unknown putative toxic metabolites and cyanide were revealed. Another major discovery was that although functional annotation provided us with genes of several ABC transporters, type IV and type VI secretion systems, no type III secretion system was detected.

RELATIONSHIP BETWEEN FUMONISIN PRODUCTION AND FUM GENE EXPRESSION IN *FUSARIUM VERTICILLIODES* AND *FUSARIUM PROLIFERATUM* UNDER DIFFERENT ENVIRONMENTAL CONDITIONS. F. Fanelli, M. Haidukowski, A. Logrieco and G. Mulè. Institute of Sciences of Food Production, CNR, Bari, Italy. E-mail: giuseppina.mule@ispa.cnr.it

Fusarium verticillioides and *Fusarium proliferatum* are the main source of fumonisins, a group of mycotoxins that can contaminate

maize-based food and feed and cause diseases in humans and animals. We analyzed the effect of temperature (15-35°C), water activity (aw: 0.999-0.93), salinity (0-125 g/l NaCl), pH (5-8) and light of different wavelength (650-390 nm) on the growth, the production of fumonisins B (FB) and the expression of FUM1 and FUM21. For *F. verticillioides* the highest growth rate was measured at 25°C, aw of 0.998-0.99, 0-25 g/l of NaCl and white light. Optimal conditions for fumonisin production were 30°C, aw of 0.99, 25 g/l of NaCl, pH 5, red and blue light. For *Fusarium proliferatum* the highest growth rate was measured at 25°C, aw of 0.99 and 0-25 g/l of NaCl, pH 7 and green light. Optimal conditions for fumonisin production were 25°C, aw of 0.998, 0 g/l of NaCl, pH 6, red and blue light. *F. verticillioides* showed a better adaptability compared to *F. proliferatum* and was able to produce moderate levels of fumonisins under a wide range of conditions. FUM gene expression not always mirrored FB production, indicating a post-transcriptional mechanism regulating fumonisin production. The study of the effect of different environmental conditions on toxin production should provide information that can be used to develop strategies to minimize the risk.

FACTS AND ARTIFACTS OF INDUCED RESISTANCE TO PLANT VIRUSES. F. Faoro^{1,2}, D. Abbati¹ and D. Maffi¹. ¹Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. ²Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: franco.faoro@unimi.it

HR-inducing plant viruses have been of paramount importance in the discovering of systemic acquired resistance (SAR) and to unravel its mechanisms. Unfortunately, in nature true virus diseases are induced by viruses able to systematize in the plant and SAR is scarcely effective against them. Being these viruses mostly transmitted by animal vectors, a more effective approach to control them could be based on the induction of resistance to both virus and vector. By using the pathosystem *Bean common mosaic virus* (BCMV)-*Phaseolus vulgaris* we tried to induce resistance to the pathogen and the aphid vector *Myzus persicae* separately, in order to dissect the two resistance levels achievable with the most used chemical elicitors. Results showed that BTH and chitosan were able to reduce infection severity in BCMV mechanically inoculated plants, but were unable to prevent infection. On the other hand, chitosan and 2-isobutyric acid (IBA), applied as root-drench, could reduce aphid population by half. Therefore, combining the two effects and using chitosan, partially effective against both the virus and the vector, it could be possible to obtain an acceptable resistance level in the field, where BCMV is actively spread by aphids. To verify this hypothesis, experimental transmission with viruliferous aphids in chitosan and IBA-treated plants are now in progress.

INDUCED SYSTEMIC RESISTANCE IN ARABIDOPSIS THALIANA ELICITED BY BEAN (PHASEOLUS VULGARIS) RHIZOBACTERIA AND THEIR VOLATILES AGAINST XANTHOMONAS CAMPESTRIS pv. ARMORACIAE. A. Giorgio¹, P.A.H.M. Bakker² and N.S. Iacobellis¹. ¹Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. ²Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail: iacobellis@unibas.it

Bacteria that are naturally associated to the rhizosphere of *Phaseolus vulgaris* were isolated and evaluated for the production of

diffusible and volatile antimicrobial metabolites and hydrolytic enzymes. Six strains of rhizobacteria that inhibited the growth of bean pathogens, were identified as *Pseudomonas* and *Bacillus* spp. When applied to bean seeds, these strains reduced the disease caused by *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* inoculated by injection in the leaves, suggesting the involvement of induced systemic resistance (ISR). *Arabidopsis thaliana* is a model plant for which ISR has been well documented, including signal transduction pathways involved, and we assessed whether three selected rhizobacteria were able to elicit ISR in the pathosystem *A. thaliana*/*Xanthomonas campestris* pv. *armoraciae*. *Pseudomonas fluorescens* WCS417 was used as a positive control for ISR in *A. thaliana*. Inducing plants by root colonization or exposing *A. thaliana* to volatiles produced by three selected rhizobacteria reduced *Xanthomonas campestris* pv. *armoraciae* growth in plant tissues and disease symptoms after inoculation of the leaves by dipping. Expression analysis of the defense marker genes PR1, ERF1, PDF1.2, and VSP2 in *A. thaliana* Col-0 after pathogen infection showed that exposure to the volatiles resulted in priming. The involvement of specific defense-related pathways in ISR was investigated using *A. thaliana* mutants affected in salicylic acid, ethylene, or jasmonic acid signaling. These results provide new insights on plant defense responses through different signal transduction pathways, depending on the rhizobacterium used and on the involvement of rhizobacterial volatiles in eliciting ISR.

ROLE OF A LECTIN GENE IN THE SUSCEPTIBILITY OF STRAWBERRY FRUITS TO COLLETOTRICHUM ACUTATUM. M. Guidarelli, L. Zoli, A. Orlandini, P. Bertolini and E. Baraldi. Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. E-mail: elena.baraldi@unibo.it

The fungal pathogen *Colletotrichum acutatum*, the causal agent of strawberry (*Fragaria × ananassa*) anthracnose, interacts with fruit hosts at pre-harvest unripe stages and becomes quiescent as melanized appressoria on white unripe strawberry until fruits ripens, causing anthracnose symptoms only on ripe strawberry fruits. In order to understand the molecular basis of the low susceptibility of white unripe fruits, the role of a *lectin* gene, which becomes overexpressed in white fruits upon *C. acutatum* inoculation, was investigated using Agrobacterium-mediated transient transformation, for silencing and overexpressing the *lectin* gene in white and red strawberry fruits respectively. *Lectin*-silenced unripe strawberry fruits appeared more susceptible to *C. acutatum* with respect to controls. On the other hand, overexpression of *lectin* in ripe strawberry fruits resulted in low susceptibility to *C. acutatum*, and to pathogen arrest at the stage of melanized appressoria. Moreover, overexpression of a GFP-lectin protein allowed to localize the protein on the external cell layers, either plasma membrane or apoplast. Together, these data suggest that this lectin could play important role in regulating the different level of susceptibility of ripe and unripe strawberry fruits to *C. acutatum*.

DEVELOPMENT OF A REAL-TIME QUANTITATIVE ASSAY APPLIED TO THE STUDY OF FUSARIUM OXYSPORUM f. sp. MELONIS COLONIZATION IN GRAFTED MELON PLANTS. A. Haegi¹, V. Catalano¹, L. Luongo¹, S. Vitale¹, M. Scotton², N. Ficcadenti³ and A. Belisario¹. ¹Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy; ²Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy; ³Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per l'Orticoltura, Via Salaria 1, 63077 Monsampolo del

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Fusarium oxysporum f. sp. *melonis* (FOM) is the causal agent of a devastating disease of melon. The fungus evolves in new races with different infection strategies to overcome resistance and grafting represents a widely used tool to confer resistance against new virulent races of FOM. We developed a real-time quantitative PCR (qPCR) assay for the detection of *Fusarium oxysporum*. The method is reliable, species-specific and sensitive (1 pg of fungal DNA) and suitable for the detection and quantification of FOM in grafted melon plants. qPCR was used to study disease development in Charentais-T (susceptible) and Nad-1 (resistant) melon cultivars, both used either as rootstock and scion inoculated with FOM race 1 and race 1,2. Results highlighted the effects of grafting on fungal development *in planta*: the principal significant effect on fungal development was due to the melon genotype used as rootstock. Moreover, in both combinations the resistant genotype (Nad-1) had an evident influence on both fungal races by reducing their development. A general characteristic in FOM/melon interaction is the ability of *F. oxysporum* to colonize melon plants independently of symptom induction. The work underlines the different infection pattern of the two FOM races: race 1,2 has the highest ability to grow in melon stems in the presence of host resistance, whereas race 1 is generally faster in colonizing melon plants in the absence of resistance. The different behavior observed between FOM race 1 and 1,2, in colonizing melon plants, suggests a different genetic background as a probable result of independent evolutionary processes.

RAPID DIFFERENTIATION OF CITRUS HOP STUNT VIROID VARIANTS BY REAL-TIME RT-PCR AND HIGH RESOLUTION MELTING ANALYSIS. G. Loconsole¹, N. Önelge², R.K. Yokomi³, R. Abou Kubaa⁴, V. Savino¹ and M. Saponari⁵. ¹Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Bari, Italy. ²Çukurova University Agriculture Faculty, Plant Protection Department, 01330 Balcali Adana, Turkey. ³United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Parlier CA, USA. ⁴Department of Plant Protection, Ministry of Agriculture and Agrarian Reform, Damascus, Syria. ⁵Istituto di Virologia Vegetale del CNR, UOS Bari, Italy. E-mail: giuliana.loconsole@uniba.it

The RNA genome of pathogenic and non-pathogenic variants of Hop stunt viroid (HSVd) in citrus differ by five to six nucleotides located within the variable (V) domain and referred to as the “cachexia expression motif”. Sensitive hosts such as mandarin and its hybrids are seriously affected by cachexia disease. Current methods to differentiate HSVd variants rely on lengthy greenhouse biological indexing on Parson’s Special mandarin and/or direct nucleotide sequence analysis of HSVd amplicons from infected plants. In order to develop a rapid and high throughput assay to segregate HSVd variants, two independent real-time RT-PCR assays, one based on EVAGreen and the other on TaqMan chemistry, were developed and coupled with High-Resolution Melting Temperature (HRM) analysis for simultaneous detection and rapid differentiation of cachexia and non-cachexia HSVd variants. The molecular assays targeted several single nucleotide polymorphisms in the V domain which discriminated HSVd variants into three clusters by distinct melting temperatures with a confidence level higher than 98%. The specificity of the HRM assays was validated by nucleotide sequencing of representative samples. To our knowledge, this is the first report of a rapid and sensitive approach to detect and differentiate HSVd variants associated with different biological behaviors. Although HSVd is found in several crops including citrus, cachexia variants are restricted to some citrus-growing areas, particularly the Mediterranean region. Rapid diagnosis for cachexia

and non-cachexia variants is, thus, important for the management of HSVd in citrus and reduces the need for bioindexing and sequence analysis.

EFFICACY OF TWO ATOXIGENIC ITALIAN *ASPERGILLUS FLAVUS* STRAINS USED AS BIOCONTROL AGENTS IN MAIZE. A. Mauro¹, E. Garcia², M. Piombino³, M. Marzi³, P.J. Cotty⁴ and P. Battilani¹. ¹Istituto di Patologia Vegetale ed Entomologia, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy. ²Food Technology Department, Lleida University, UTPV-XaRTA-Agrotecnio, Rovira Roure 191, 25198 Lleida, Spain. ³Pioneer Hi-Bred Italia, Via Pari Opportunità 2, 26030 Gadesco Pieve Delmona (CR), Italy. ⁴USDA-ARS, School of Plant Sciences, University of Arizona, 85721 Tucson (AZ), USA. E-mail: paola.battilani@unicatt.it

Aflatoxin B₁ (AFB₁), a cancerogenic toxin, is produced by *Aspergillus flavus* in economical important crops like maize. Use of atoxigenic *A. flavus* to reduce aflatoxins contamination has been shown as a powerful strategy. Two Italian atoxigenic strains of *A. flavus* were selected and tested in maize for their ability to reduce AFB₁ contamination. They were applied separately on the ears, with the pin-bar technique, and jointly on the soil in maize crops. The percentage of AFB₁ reduction achieved, when biocontrol agents were applied through the pin-bar technique, was of 2.4 and 93.2%, respectively for the two strains. In the soil application, the percentage of AFB₁ reduction was greater than 80% although the *A. flavus* CFU did not show significant differences between the control and the treated thesis. The 2 strains were monitored in maize crops through the VCG analysis; one strain was isolated more frequently than the other and the percentage of the two strains in the treated thesis was at least 20% higher compared to the control. Amplification of 19 microsatellite loci showed three null alleles in both strains and seven polymorphic loci. Analyses of mating-type genes revealed the presence of the allele MAT1-2 and MAT1-1, respectively in the two strains. These studies report for the first time the efficacy of Italian atoxigenic strains to reduce AFB₁ contamination in *in vivo* experiment.

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EXPRESSION OF DEFENCE-RELATED GENES AND PRODUCTION OF PHYTOALEXINS IN TWO RICE CULTIVARS WITH DIFFERENT LEVEL OF RESISTANCE TO *FUSARIUM FUJIKUROI*. S. Matic^{1,2}, A. Prella¹, M.L. Gullino^{1,2}, A. Garibaldi¹ and D. Spadaro^{1,2}. ¹Centre for Agro-Environmental Innovation (AGROINNOVA), Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. ²Dipartimento di Scienze Agrarie, Forestali ed Alimentari Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Commercial cultivars of rice (*Oryza sativa*), one of the world’s most important crop, show different levels of resistance to *Fusarium fujikuroi*, the causal agent of the bakanae disease. Twelve rice cultivars were evaluated for their resistance/susceptibility to *F. fujikuroi*. Based on independent evaluation trials, the cv. Selenio was found to be the most resistant, while cv. Dorella behaved as the most susceptible. Based on these results, the aim of this study was to investigate the differential expression of defence genes and phytoalexin production in the resistant cultivar (Selenio) and in the susceptible cultivar (Dorella). The GF14e gene that negatively affects

rice disease resistance, the defence-related genes belonging to the groups of MAP kinases and glycosyl hydrolases, and thaumatin and WRKY50 genes were differentially expressed in cvs Selenio and Dorella. Furthermore, differential productions of two phytoalexins, momilactone and naringenin, were measured in the resistant and susceptible cultivars. Both, defence-related genes and phytoalexins, might be involved in rice resistance to *F. fujikuroi*.

PHAGE THERAPY FOR FIRE BLIGHT CONTROL IN POMACEOUS PLANTS. A. Mazzucchi², C. Lucchese¹ and U. Mazzucchi¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Via Fanin 44, 40127 Bologna, Italy. ²Consulenze Fitopatologiche V.P.S. srl, 40024 Castel San Pietro Terme (BO), Italy. E-mail: carla.lucchese@unibo.it; antonio.mazzucchi@fastwebnet.it

Fire blight caused by *Erwinia amylovora* (*Ea*) is a serious disease of pomaceous plants, causing significant damages. The chemical control of this disease is problematic for phytoiatric reasons and environmental issues. Resistant cultivars are difficult to manage because the susceptibility of flowers and shoots is not always positively correlated. The use of bacteriophages for the control of a number of bacterial disease management is increasing as it shows a great potential for replacing the currently prevailing chemical control methods and can be used effectively in integrated disease management strategies. Twenty-nine phages were isolated from 207 soil samples and infected tissues collected in Po valley orchards with a fire blight story. *In vitro* assays allowed the selection of a phage (M9) with high lytic activity on *Ea*, which was purified and propagated. *In vivo* assays on immature pears and on blooming corymbs of young pear trees showed that phage M9 afforded a statistically significant protection from *Ea* in both fruits and flowers. Genomic assays and electron microscope observations identified phage M9 as a member of the family *Tectiviridae*. Phage M9 can be propagated on *Pantoea vagans*, an enterobacterium living in the phyllosphere of pomaceous plants. For its ability to infect other phyllosphere enterobacteria M9, could offer permanent protection against *Ea* infections when released in the orchard.

The University of Bologna applied for a patent application concerning phage M9 treatment (patent application No. MO2013A000117).

MOLECULAR CHARACTERIZATION AND *IN VIVO* ACTIVITY OF *PSEUDOMONAS FLUORESCENS* STRAIN 4, A POTENTIAL BIOCONTROL AGENT AGAINST *RHIZOCTONIA SOLANI* ROOT ROT ON LAMB'S LETTUCE. S. Moruzzi and M. Martini. Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. E-mail: marta.martini@uniud.it

Biocontrol microorganisms can play an important role in suppressing root pathogens (i.e. genera *Pythium* and *Rhizoctonia*) in soilless systems. In the present work, *Pseudomonas fluorescens* strain 4 (Pf4), isolated from roots of healthy lamb's lettuce (*Valerianella locusta*) plants grown in hydroponics in Friuli Venezia Giulia (north-eastern Italy), and selected for its ability to inhibit the *in vitro* growth of *Rhizoctonia solani*, was molecularly characterized using multilocus sequence typing (MLST) based on 16S rRNA, *rpoD*, *secY*, *gyrB* and *rpsC-rplV* genes. Sequences obtained from Pf4 and other 11 closely related *Pseudomonas* spp. strains isolated from lamb's lettuce roots, were aligned and compared. The *gyrB* gene turned out to be the most variable locus, allowing to differentiate Pf4 from the other strains. On the basis of *gyrB* gene sequence, strain-specific primers for Pf4 were designed, and an EvaGreen

real-time PCR assay was developed, followed by high-resolution melting (HRM) analysis. Pf4 was used for preliminary *in vivo* treatments on lamb's lettuce against *R. solani* root rot. Lamb's lettuce was planted in small-scale hydroponics, treated with bacterial inoculation on seeds, seedlings and in the nutrient solution (10⁶ CFU/ml), and artificially infected with the fungal pathogen. Untreated plants were also infected and used as control. The presence and quantity of Pf4 bacterial cells on treated roots were checked by real-time PCR. Symptoms of wilting occurred 8 days post inoculation. After 2 weeks, 81.23% of untreated plants and only 34.36% of treated plants became wilted demonstrating a protective effect of Pf4 against *R. solani*.

DISSECTION OF VIROID-HOST INTERPLAY BY DEEP SEQUENCING OF SMALL RNA LIBRARIES AND DEGRADOME ANALYSES. B. Navarro¹, A. Gisel², R. Flores³ and F. Di Serio¹. ¹Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto di Tecnologie Biomediche del CNR, UOS Bari, Via Amendola 122/D, 70126 Bari, Italy. ³Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Campus Universidad Politécnica, Avenida de los Naranjos, 46022 Valencia, Spain. E-mail: f.diserio@ba.ivv.cnr.it

The involvement in viroid-host interactions of RNA silencing, an RNA-based network regulating gene expression and defense against invasive nucleic acids in most eukaryotes, is supported by the accumulation in tissues infected by nucleus- and chloroplast-replicating viroids of viroid-derived small RNAs (vd-sRNAs) of 21-24 nt, structurally similar to host microRNAs (miRNAs) and small-interfering RNAs (siRNAs). Based on these findings it was proposed that vd-sRNAs, similarly to miRNAs, might target host mRNAs for degradation (or translation inhibition), thus leading to symptom expression in the infected plants. In the last few years, using high-throughput technologies, we have characterized the vd-sRNAs derived from a chloroplast-replicating viroid [*Peach latent mosaic viroid* (PLMVd)]. Moreover, by semi-quantitative RT-PCR and RNA ligase-mediated rapid amplification of cDNA ends, we have shown that two vd-sRNAs (containing the pathogenicity determinant strictly associated with an albino phenotype) target for degradation a host mRNA, thus providing the first experimental evidence that, indeed, vd-sRNAs function like miRNAs. Interestingly, the targeted mRNA codes for a protein (cHSP90) involved in chloroplast biogenesis, which is the developmental pathway specifically compromised in the albino tissues infected by PLMVd variants generating the two vd-sRNAs (Navarro *et al.*, 2012. *The Plant Journal* 70: 991-1003). Altogether these data strongly support the involvement of RNA silencing in PLMVd pathogenesis and, possibly, a more general role of vd-sRNAs in modulating host gene expression during viroid infection. For a deeper insight into this question, we have integrated data from high-throughput sequencing of vd-sRNAs accumulating in tissues infected by chloroplast- and nucleus-replicating viroids with the respective degradome analyses. Based on experimental data, the implications will be discussed of the RNA degradation patterns potentially elicited by vd-sRNAs during viroid infection.

NATURAL AND NATURAL-LIKE PHENOLIC COMPOUNDS INHIBIT TRICHOHECENE BIOSYNTHESIS IN *FUSARIUM CULMORUM*. G. Pani¹, V. Balmas¹, B. Scherm¹, A. Marcello¹, D. Fabbri², M.A. Dettori², E. Azara², A. Dessì², R. Dalocchio², A. Fadda³, G. Delogu² and Q. Migheli¹. ¹Dipartimento di Agraria, Sezione di Patologia Vegetale ed Entomologia, and Unità di Ricerca dell'Istituto Nazionale di Biostrutture e Biosistemi,

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Fusarium culmorum is a major fungal pathogen of wheat, causing foot and root rot (FRR) and fusarium head blight (FHB). Yield losses are reported as the grain becomes contaminated by mycotoxins. Among the most bioactive compounds are trichothecenes, sesquiterpene epoxides which are able to inhibit eukaryotic protein synthesis and may cause toxicoses to humans or animals consuming contaminated food or feed. Trichothecenes induce apoptosis and may play an important role in the aggressiveness of phytopathogenic *Fusarium* species towards plant hosts. The aim of this project was to design, prepare and study new natural and natural-like compounds to be applied in the control of *F. culmorum* mycotoxin production. Particular attention was paid to the selection and preparation of compounds with selective trichothecene B inhibitory activity compared to compounds showing both mycotoxin inhibitory and fungitoxic activities. The first inhibition experiments were performed using compounds belonging to the family of gallic acid, phenylpropanoids and cinnamic-derived acids. *In vivo* and *in vitro* test and molecular modeling with computational studies were carried out. A straightforward thin layer chromatography (TLC) method and a quantitative LC-MS analysis were used to identify the presence of B trichothecenes and to evaluate the influence of each compound on different *F. culmorum* culture extracts. Preliminary results indicate that several molecules are able to inhibit the severity of *F. culmorum* *in planta* and its growth, as well as trichothecene production *in vitro*. The level of inhibition of 3AcDON range from 67 to 100% under inducing conditions. Fast and effective methodologies for seed dressing were developed using a natural matrix.

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PRELIMINARY INVESTIGATIONS ON THE INTERACTIONS BETWEEN PEACH ROOTSTOCKS AND *ARMILLARIA MELLEA* BY USING A HIGH-THROUGHPUT RNA SEQUENCING APPROACH. S. Pollastro, R.M. De Miccolis Angelini, G. Botalico, C. Rotolo and F. Faretra. Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: stefania.pollastro@uniba.it

RNA-Seq experiments have been carried out to study the interactions between peach rootstocks and the root rot fungus *Armillaria mellea* by exploring gene expression changes in both host plant and pathogen during the infection process. cDNA libraries suitable for high-throughput sequencing (Illumina technology) were prepared from mRNA isolated from the following samples: (i) non inoculated control and inoculated roots of *Prunus domestica* cv Tetra and *Prunus persica* GF305, six days post inoculation (dpi, early stages of infection) and 28 dpi (roots fully colonized by the pathogen); (ii) *A. mellea* mycelium grown on artificial medium and from roots of host plants 28 dpi. About 15 million sequences per library were generated through a Single Read sequencing run (50 cycles) and analysed to identify plant genes potentially involved in defense responses of the host plant as well as the fungal genes involved in infection. Genes encoding β -glucosidases, recognized as activators of plant defenses, were up-regulated (FC > 20) in infected roots. Several other functional categories of plant genes, including

PR-proteins, thaumatin-like proteins, chitinases, protease inhibitors, disease resistance proteins, a tumor-related protein, phospholipases, peroxidases, lipoxigenases also appeared to be induced by *A. mellea* challenge. A first *de novo* assembly and characterization of the *A. mellea* transcriptome allowed the identification of fungal genes potentially involved in pathogenicity, including cell-wall degrading enzymes, such as glycoside hydrolases and carbohydrate esterases, laccases, peroxidases, aryl-alcohol oxidases and dehydrogenases, transmembrane proteins functioning as transporters, and a FUN34 protein which could play a role in the host-surface recognition.

EVIDENCE FOR BIRTH-AND-DEATH EVOLUTION AND HORIZONTAL TRANSFER OF A MYCOTOXIN BIOSYNTHETIC GENE CLUSTER IN PATHOGENIC *FUSARIUM* SPECIES. R.H. Proctor¹, F. Van Hove², A. Susca³, G. Stea³, M. Busman¹, T. Van der Lee⁴, C. Waalwijk⁴, T.J. Ward¹ and A. Moretti³. ¹National Center for Agricultural Utilization Research, USDA, Peoria, USA; ²Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium; ³Institute of Sciences of Food Production, National Research Council, Bari, Italy; ⁴Plant Research International B.V., Wageningen, The Netherlands. E-mail: antonio.moretti@ispa.cnr.it

In fungi, genes required for synthesis of secondary metabolites are often clustered. The *FUM* gene cluster is required for synthesis of fumonisins, a family of toxic secondary metabolites produced by species in the *Fusarium* (syn. *Gibberella*) *fujikuroi* species complex (FFSC). Fumonisins are a health and agricultural concern because their consumption is associated with cancer and neural tube defects in humans and other animals. Among FFSC species, the *FUM* cluster is discontinuously distributed but uniform in gene order and orientation. Here, phylogenetic analyses indicated discord between species phylogenies and *FUM* gene-based phylogenies. Subsequent constraint analyses confirmed the discord, and analyses of variation in synonymous sites indicated that cluster divergence predated, in some cases, and postdated, in one case, divergence of lineages of *Fusarium* in which the cluster occurs. The results are not consistent with the discord resulting from trans-species evolution of ancestral cluster alleles, or with interspecies hybridization, but are consistent with duplication of the cluster within an FFSC ancestor and subsequent loss and sorting of paralogous clusters in a manner consistent with birth-and-death evolution of multigene families. Although the results are also consistent with horizontal transfer of the cluster, such a model is less parsimonious because it requires multiple transfer events from unknown but related donors to multiple FFSC recipients. However, the analyses do provide strong support for horizontal transfer of the cluster from FFSC to another *Fusarium* lineage. Thus, despite conservation of gene organization within it, the *Fusarium FUM* cluster has had a complex evolutionary history.

COMBINATION OF COPPER WITH *LYSOBACTER CAPSICI* AZ78: TOWARDS NEW STRATEGIES FOR THE BIOLOGICAL CONTROL OF *PLASMOPARA VITICOLA*. G. Puopolo, O. Giovannini and I. Pertot. Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. E-mail: gerardo.puopolo@fmach.it

Several members of the bacterial genus *Lysobacter* can protect plants against pathogenic oomycetes. In this work, *Lysobacter capsici* strain AZ78 has been evaluated for the biological control of *Plasmopara viticola*, causal agent of grapevine downy mildew. Prophylactic application of AZ78 to grapevine leaves reduced downy mildew as

a copper-based fungicide. AZ78 can survive in presence of copper ions. Its resistance to this metal is associated with genes coding for copper oxidase (*copA*) and copper exporting P_{1B}-type ATPases (*ctpA*). These genes have been detected also in other *Lysobacter* members, showing that resistance to copper is a biological trait conserved in this bacterial genus. Resistance to this metal allowed to combine AZ78 with a low-dose copper-based fungicide, leading to a more effective control of grapevine downy mildew. Furthermore, AZ78 persists in the phyllosphere of grapevine plants and tolerates environmental stresses such as starvation, freezing, mild heat shock and UV light irradiation. All these characteristics, make *L. capsici* AZ78 a suitable candidate for developing new sustainable strategies for controlling grapevine downy mildew in vineyards on its own or in combination with low doses of copper-based fungicides.

EFFECT OF PATHOGEN-INDUCED VOLATILE ORGANIC COMPOUNDS ON POWDERY MILDEW INFECTION, APHID INFESTATION AND ON GENE INDUCTION IN TOBACCO. M. Quaglia¹, M. Fabrizi, A. Zizzerini and C. Zadra.

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Stress factors can alter the blend of volatile organic compounds (VOCs) released by plants, inducing an higher amounts of phytochemicals synthesized *de novo* or emitted. These novel compounds are referred as induced VOCs (IVOCs). We previously reported that *Golovinomyces cichoracearum*, a powdery mildew agent, induced an increased emission of methyl-jasmonate (MeJA), (*E*)-2-hexenal and (*E*)- β -ocimene in the susceptible *Nicotiana tabacum* cv. Havana 425. We have now tested these compounds for their ability to protect tobacco from *G. cichoracearum* and for accumulation of *lipoxygenase* (*LOX*), *allene oxide cyclase* (*AOC*) and *defensin* genes transcripts, markers of JA-defence pathway. MeJA 2 mM and (*E*)-2-hexenal 5 mM reduced the disease severity, compared to the control. Protection conferred by MeJA could be due to the activation of the JA-defence pathway, as it induced in tobacco the accumulation of *LOX*, *AOC* and *defensin* genes transcripts and did not inhibit fungal germ tube growth. Protection conferred by (*E*)-2-hexenal could be mainly attributable to its antimicrobial activity, as it did not induce the accumulation of genes transcripts but inhibited fungal germ tube growth. The increased disease severity caused by (*E*)- β -ocimene 0.18 mM could be due to the *in vitro* stimulation of conidial germination. At the above tested concentrations, the three compounds significantly reduced *Myzus persicae* infestation with respect to the control. Thus, the defence responses induced by the pathogen were able to protect the plant also from aphid attacks emphasizing the importance of studying plant responses also in a multitrophic interaction system.

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APPLICATION OF RESISTANCE INDUCERS IN GRAPEVINES AFFECTED BY BOIS NOIR: EFFECTS ON QUALITATIVE AND QUANTITATIVE PRODUCTION PARAMETERS.

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Bois noir (BN) can be considered the main phytoplasma disease of the grapevine in Europe and Mediterranean area. It induces deep changes in the plant physiology and causes heavy crop losses.

Because there are no effective means for controlling the disease once established, a way to increase recovery rate can reside in the application of resistance inducers. Five commercial compounds based on chitosan, Phosetyl-Al, benzothiadiazole, and glutathione + oligosaccharines (two formulations) were applied weekly to the canopy of grapevines cv. Chardonnay from the beginning of May to the end of July. Plants sprayed with a formulation of glutathione+oligosaccharines did not show any reduction of shoot length in May and June. The yield of plants treated with resistance inducers where BN symptoms persisted was about half of that observed in recovered plants and control. The amount of dried clusters in plants that showed BN symptoms after application of resistance inducers did not differ among these vines and from the control (around 30%). Overall, recovered plants following application of resistance inducers showed qualitative and quantitative production parameters not different as compared to healthy vines. Among tested inducers, the best results in increasing recovery rate were obtained with the compounds based on benzothiadiazole (Bion), and the two formulations containing glutathione+ oligosaccharines (Olivis and Kendal). However, further investigations are needed for the elaboration of a protocol to be proposed to growers for BN infection management in the vineyard.

TRICHODERMA LONGIBRACHIATUM AFFECTS RELEASED VOLATILE ORGANIC COMPOUND PROFILE AND TOMATO-INSECT INTERACTION.

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Fungi belonging to the genus *Trichoderma* are known for their antagonistic activity against other microbes, ability to colonize roots, establish chemical communication with the plant and systematically alter the expression of many host genes. The changes caused by *Trichoderma* spp. to plant physiology often result in an improved resistance to abiotic stresses, nutrient uptake, response to pathogens and photosynthetic efficiency. Mechanisms responsible of increased ISR (induced systemic resistance) mediated by selected *Trichoderma* strains have been actively investigated, but the effects of these plant growth promoting fungi (PGPFs) on plant response to insects have been poorly studied so far. When compared to the un-colonized controls, plants whose roots were colonized by *T. longibrachiatum* strain MK1 showed: (i) quantitative differences in the release of specific VOCs; (ii) better aphid (*Macrosiphum euphorbiae*) population growth indices; (iii) a higher attractiveness towards an aphid parasitoid (*Aphidius ervi*) and an aphid predator (*Macrolophus pygmaeus*); (iv) a faster development of the aphid predator. The results of this study show for the first time that root colonization by a biocontrol strain of *Trichoderma* modifies the outcome of the complex, multiplayer interaction between plants, aphids and their natural enemies, and suggests novel and integrated strategies for the control of both phytophagous pests and microbial pathogens.

HOST INFLUENCE ON PATULIN PRODUCTION BY *PENICILLIUM EXPANSUM* STRAINS CAUSING BLUE MOULD AND CONSEQUENT EFFECT ON THEIR AGGRESSIVENESS.

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Penicillium expansum, the main causal agent of blue mould of pome fruits, affects also several other hosts including sweet cherries and table grapes. Although the role of mycotoxins in plant pathogenesis is being increasingly studied, few reports have addressed their influence on the variation in host preference amongst producing isolates. In the present investigation the host influence on *P. expansum* pathogenicity/virulence was investigated, with particular emphasis on the relationship with patulin production. Three *P. expansum* strain groups, originating from apples, sweet cherries, and table grapes (7 strains/host), were grown on their hosts of isolation and derived artificial media. Each group proved to be more aggressive and produced more patulin than the other two when grown on the host of origin. On the whole, table grape strains were the most aggressive (81% disease incidence) and strongest patulin producers (up to 554 µg/g). The different aggressiveness was appreciable only in the presence of a living host, suggesting a significant influence of the host on *P. expansum* ability to cause the disease. Disease incidence/severity and patulin production proved to be positively correlated, supporting the role of patulin as virulence/pathogenicity factor. The existence of a genetic variation amongst isolates was confirmed by a high resolution melting assay which permitted not only to discriminate *P. expansum* from other species (*P. chrysogenum* and *P. crustosum*) but also, within the same species, amongst the host of origin. Host effect on toxin production was exerted at a transcriptional level.

THE LINOLEATE SYNTHASE GENE *LDS1* IS INVOLVED IN FUMONISINS SYNTHESIS AND REPRODUCTION OF *FUSARIUM VERTICILLIOIDES*. V. Scala¹, E. Camera², M. Ludovici², A.M. Emili¹, C. Dall'Asta³, M. Cirilini², P. Giorni⁴, R. Gregori⁴, P. Battilani⁴, C. Fanelli¹ and M. Reverberi¹. ¹Department of Environmental Biology, University Sapienza, Rome, Italy; ²Laboratorio di Fisiopatologia Cutanea e Centro Integrato di Metabolomica, Istituto Dermatologico San Gallicano, Roma, Italy; ³Department of Organic and Industrial Chemistry, Food Chemistry and Natural Substances Unit, University of Parma, Viale G.P. Usberti 17/A, 43100 Parma, Italy; ⁴Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. E-mail: valeria.scala@uniroma1.it

Fusarium verticillioides is a fungal pathogen that causes ear and stalk rot in maize and produces the harmful series of mycotoxin fumonisins. Oxylipins are compounds derived from fatty acids and factors able to modulate the plant-mycotoxigenic fungi interaction and mycotoxins synthesis. In this study we inactivated a copy of the linoleate diol synthase 1 (*LDS1*) gene (*lds1*-acc. N. FVEG_09294.3) of *Fusarium verticillioides* strain ITEM 10027. *LDS1* produces mainly 8-HPODE and, subsequently, different di-HODEs which are involved in the control of sexual/asexual reproduction, secondary metabolism and pathogenicity in *Aspergillus*. This study was aimed at investigating the role played by this gene in *F. verticillioides*. All the mutant strains showed an altered asexual and sexual reproduction, produced a higher amount of conidia compared with the wild type (WT), whereas the sexual reproduction was variable among the mutants tested. Moreover, mutant strains presented a higher fumonisin accumulation with respect to the WT. LC-MS/MS oxylipins profile was carried out on mutant strains and significant differences with the WT were found, also related to the unsaturated fatty acids profile. These results indicate that also in *F. verticillioides*, oxylipins play a role in the modulation of reproduction, mycotoxin accumulation and, probably, in the control of the lipid profile.

HEAT TREATMENT APPLICATION TO CONTROL BROWN ROT OF PEACH AND NECTARINE FRUITS. A. Spadoni¹, M. Guidarelli¹, S.M. Sanzani², A. Ippolito² and M. Mari¹. ¹Dipartimento di Scienze Agrarie, Università degli Studi, Viale Fanin 44, 40127 Bologna, Italy. ²Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. E-mail: alice.spadoni3@unibo.it

Thermotherapy is one of the safest methods for controlling postharvest fruit pathogens. In the present study the effect of hot water treatment (HWT) (60°C for 30 and 60 sec) on brown rot of peaches and nectarines was investigated. More specifically the influence of HWT was determined *in vitro* on conidial germination of *M. laxa*, *M. fructicola* and *M. fructigena* and *in vivo* on artificially and naturally infected peach and nectarine fruits, during laboratory, semi-commercial and commercial-scale trials. *M. fructicola* showed greater resistance to heat than *M. laxa* and *M. fructigena*. In *in vivo* trials, the highest reduction of brown rot was obtained on cv. Royal Summer peaches artificially infected by *M. laxa* and dipped for 20 sec in water at 60°C (-94.1% with respect to the control). HWT showed a good activity also on naturally infected fruits; in fact, after 6 days at 0°C and 3 days at 20°C, in both semi-commercial and commercial trials, the inhibition of decay was higher than 78% in four trials out of six. A gene expression analysis was also carried out by qRT-PCR for scavenging genes, cell wall genes and the phenylalanine ammonia lyase gene. An up-regulation of gene expression in HWT fruits was shown after 15 and 60 min from treatment for PAL and scavenging genes, respectively, whereas a down-regulation of cell wall gene expression was recorded after 15 and 180 min. The results demonstrated that HWT is a promising method to control Monilinia rots of peaches and nectarines.

VIRUS INDUCED GENE SILENCING IN TOMATO PLANTS SUGGESTS A STRONG RNAi-BASED RESPONSE OF A LOCAL TOMATO VARIETY TO TOMATO SPOTTED WILT VIRUS-RB INFECTION. R. Spanò^{1,2}, T. Mascia^{1,2} and D. Galitelli^{1,2}. ¹Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: roberta.spano@uniba.it

Plant RNAi operates as a potent defence system where RNA-dependent RNA polymerases (RDRs) enzymes are core components of the pathways involved in siRNA biogenesis and responsible for the spread of the silencing signal on short and long distance. We used a Tobacco rattle virus (TRV) based virus-induced gene silencing (VIGS) system to investigate the role of RDR1 and RDR6 genes in response to viral infection supported by RB strains of Tomato spotted wilt virus (TSWV) in two tomato cultivars, LV and UC82, characterized by a different response to the disease. The RDR silencing resulted in an increased susceptibility to viral infection, in both cultivars, suggesting that the tolerance to TSWV observed in LV but not in UC82 involves a stronger RNAi-based response. Consistent with previous findings, RDR1 and RDR6 cooperated to limit primary infections and to prevent systemic invasion, indicating a synergistic interaction through the RNA silencing-based antiviral system. Transcriptome analysis suggests the propensity of both varieties to compensate the lower expression of the silenced gene through the over-expression of the other. Interesting, in plants with one RDR silenced gene, cv. LV exhibited the potential to counteract viral infection by an increased transcription level of the other non-silenced gene compared with cv. UC82.