spread in other Mediterranean countries, it is important to improve oak disease monitoring and to understand the pathways by which this pathogen can be spread.

NOVEL HIGH-THROUGHPUT DIAGNOSTIC TOOLS FOR THE SIMULTANEOUS DETECTION OF INVASIVE CITRUS PATHOGENS. G. Loconsole[1], V. Savino[1], R.K. Yokomi[2] and M. Saponari[3]. *[1]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy. [2]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Parlier, CA 93648, USA. [3]Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: giuliana.loconsole@uniba.it*

A number of important citrus pathogens are spread by graft propagation, arthropod vector transmission and inadvertent import and dissemination of infected plants. For these reasons, citrus disease management and clean stock programs require economical and sensitive pathogen detection systems apt to maintaining a healthy industry. To this end, multiplex quantitative real-time PCR (qPCR) assays were developed allowing high-throughput and simultaneous detection of major invasive citrus pathogens. Automated high-throughput extraction comparing several bead-based commercial extraction kits were tested and compared with tissue print and manual extraction to obtain nucleic acids. Then two one-step TaqMan-based multiplex RT-qPCR assays were developed. The first assay included primers and probes for broad spectrum detection and genotype differentiation of '*Candidatus* Liberibacter asiaticus' (*C*Las) and *Citrus tristeza virus* (CTV). In the second assay primers and probes were used for *Hop stunt viroid* (HSVd), *Citrus exocortis viroid* (CEVd) and the mitochondrial NADH dehydrogenase (nad5) mRNA as an internal citrus host control. The assays were validated using infected tissues from our pathogen collection (HSVd, CEVd and CTV) or non-infectious *C*Las infected tissues obtained from China. Based on quantitation cycle values, automated high-throughput extraction of samples proved to be as suitable as manual extraction. The multiplex RT-qPCR assays detected both RNA and DNA pathogens in the same dilution series as singleplex assays and yielded similar quantitation cycle values. Taken together, high throughput extraction and multiplex RT-qPCR assays reported in this study provided a rapid and standardized method for routine and simultaneous diagnosis of different RNA and DNA invasive citrus pathogens.

PLANTS CRY FOR HELP: BIOTIC AND ABIOTIC STRESSES ATTRACT BENEFICIAL MICROBES TO THE ROOTS. N. Lombardi[1], A. Pascale[1], M. Ruocco[2], S. Woo[1,2], G. Manganiello[1], F. Vinale[2], R. Marra[1], S. Lanzuise[1], V. Matteoli[1] and M. Lorito[1,2]. *[1]Dipartimento di Agraria, Università di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. [2]Istituto di Protezione delle Piante del CNR, UOS Napoli, Via Università 133, 80055 Portici (NA), Italy. E mail: lorito@unina.it*

Trichoderma spp. are ubiquitous saprophytic fungi, known for their ability to colonize a variety of niches, antagonize and control plant pathogenic microorganisms and establish a direct beneficial interaction with plants, resulting in the enhancement of growth, nutrient uptake and systemic resistance to diseases. It has been recently demonstrated that the root colonizing fungus *Trichoderma longibrachiatum* strain MK1 positively affects the production of volatile compounds that attract both predators and antagonists of the aphid *Macrosiphum euphorbiae* (Battaglia *et al*., 2013. *Molecular Plant-Microbe Interaction* DOI: 10.1094/MPMI-02-13-0059-R). Therefore, we hypothesize that a similar mechanism is used by the plant to promote the growth and the activity of beneficial root-colonizing microbes (RCM) upon the exposure to pathogen attack or certain abiotic stresses. This may occur by the release of compounds that, more or less specifically, modify the root microbiome in favour of species that help the plant to overcome the incoming stresses. We observed in a split root experiment that the contact with *Pythium ultimum* on one side stimulates the development of *Trichoderma* colonies located in a separated compartment, also increasing the chemiotactic growth of the mycelium toward the plant. This suggests the release of specific compounds through the root system that attract and stimulate beneficial root-colonizing microbes, which helps the plant to overcome biotic and/or abiotic stresses.

DEVELOPMENT OF A NEW VIRUS-INDUCED GENE SILENCING VECTOR FOR GRAPEVINE, BASED ON *GRAPEVINE ALGERIAN LATENT VIRUS*. A. Lovato[1], L. Santi[2], C. Malvezzi[1] and A. Polverari[1]. *[1]Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy. [2]Department of Science and Technology for Agriculture, Forestry, Nature and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: annalisa.polverari@univr.it*

Establishment of functional genomics in *Vitis* sp. is still challenging, due to the lack of reliable high-throughput tools for grapevine transformation. Virus-induced gene silencing (VIGS) makes use of a plant virus-based vector carrying a sequence of an endogenous plant gene. By delivery of the vector and replication of the recombinant virus within the plant, the plant defence mechanism known as post-transcriptional gene silencing (PTGS) is activated against the virus resulting in silencing of the plant gene. This system has been recently applied on *Vitis vinifera* plants with vectors based on the *Grapevine Virus A* (GVA) and *Grapevine leafroll-associated virus 2* (GLRaV-2). We developed a new VIGS system using a vector based on *Grapevine Algerian latent virus* (GALV), a member of the genus *Tombusvirus*. The whole GALV genomic sequence was cloned into the binary vector pK7WG2 and modified to carry a polylinker site in which different sequences could be easily inserted to induce silencing against any gene of interest. Preliminary results of *Agrobacterium*-mediated GALV infections in grapevine have been obtained. GALV could replicate and spread systemically in several grapevine varieties. The presence of a ribozyme sequence immediately downstream of the viral sequence was crucial for viral replication. The silencing-inducing ability of the GALV-based vector was confirmed by downregulation of the phytoene desaturase gene in *N. benthamiana*, associated to the expected bleaching phenotype. Availability of an efficient GALV-based VIGS vector for grapevine could be precious for functional genomics as it is a systemic and latent grapevine virus.

WIDESPREAD OCCURRENCE OF APPLE PROLIFERATION DISEASE IN LOW-INTENSITY ORCHARDS OF BASILICATA. C. Marcone[1] and E. Seemüller[2]. *[1]Dipartimento di Farmacia, Università degli Studi di Salerno, Via Giovanni Paolo II, 134, 84084 Fisciano (SA), Italy. [2]Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, 69221 Dossenheim, Germany. E-mail: cmarcone@unisa.it*

Visual symptom assessment and PCR amplification were used to survey the occurrence of apple proliferation (AP) disease in low-intensity orchards in the Agri valley, a major cultivation area of

Basilicata (southern Italy). The apple trees examined, whose cultivars were not determined as they consisted mostly of local types, were more than 20-year-old. Therefore, these plants had been exposed to insect vectors for a long time. The survey revealed that a high percentage of trees were infected reaching more than 50% in some locations. The symptoms of diseased trees were generally mild and consisted of enlarged stipules, rosettes, witches'-brooms as well as subterraneous witches'-broom-like growth arising from large roots. However, the incidence and severity of symptoms in the aerial parts of affected trees were more pronounced in trees which had been heavily pruned in the previous dormant season. Specificity of the primers used and RFLP analysis of PCR-amplified 16S rDNA sequences employing *Ssp*I and *BsaA*I restriction endonucleases showed that the trees testing positive by PCR were infected by the AP agent '*Candidatus* Phytoplasma mali'. The high incidence of AP infections in low-intensity orchards of the Agri valley is likely due to inappropriate vector control. The trees examined were not or rarely treated with insecticides. Although a few AP-affected apple trees grown in a low-intensity orchard in the Agri valley had previously been observed, our survey shows that the distribution of AP disease in Europe extends further south than previously thought and that the climatic conditions of southern Italy are not unsuitable for this quarantine disease.

BIOACTIVE METABOLITES PRODUCED BY *TRICHODERMA*: NOVEL EFFECTORS FOR PLANT BIOSTIMULATION. **R. Marra[1], S. Lanzuise[1], F. Vinale[2], S. Woo[1], M. Ruocco[2], N. Lombardi[1], G. Manganiello[1], A. Pascale[1] and M. Lorito[1].** *[1]Dipartimento di Agraria, Sezione di Patologia Vegetale, Università degli Studi di Napoli 'Federico II', 80055 Portici (NA), Italy. [2]Istituto per la Protezione delle Piante del CNR, UOS Portici, 80055 Portici (NA), Italy. E-mail: lorito@unina.it*

Fungi of the genus *Trichoderma* are successfully applied worldwide in the form of live inocula as biofungicides and biofertilizers due to their ability to protect crops from various diseases, as well as to increase plant growth, development and yield under different conditions. These microbes are capable of producing a plethora of chemically different compounds, i.e. lytic enzymes, antibiotics, resistance inducers, etc. that have beneficial effects on the plant and a negative activity on pathogens. Treatment with *Trichoderma* metabolites produces significant modifications of the plant expressome, proteome and metabolome, acting on specific pathways involved in the defence response to biotic/abiotic stresses and nutrient uptake. The direct use of bioactive metabolites may provide various advantages over the application of the living microbes. For instance, the semi-purified compounds are less susceptible to losses of activity due to storage or environmental changes, thus allowing to overcome some of the performance inconsistencies often observed in the field. We have conducted a large *in vivo* study by testing isolated metabolites from different *Trichoderma* species, both singly and in a variety of combinations also with the living microbes, on many different crops, pathogens and applications. Selection of the compounds to be used was based on the actual knowledge of the mechanisms of action, in order to complementing different beneficial effects. New biopesticides based on secreted proteins and/or secondary metabolites related to the biocontrol/plant-growth-promoting-activity of *Trichoderma* spp. are being patented, registered and included in the pipeline for commercialization.

PREFORMED ANTIFUNGAL COMPOUNDS IN PEACH PEEL FRUIT AT DIFFERENT DEVELOPMENTAL STAGES AGAINST *MONILINIA* spp. **C. Martini[1], L. Ugolini[2], C. Sabattini[1] and M. Mari[1]** *[1]CRIOF, Università degli Studi di Bologna, Via Gandolfi, 19, 40057 Cadriano (BO), Italy. [2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per le Colture Industriali, Via di Corticella 133, 40128 Bologna, Italy. E-mail: camilla.martini2@unibo.it*

The aim of this study was to relate peach fruit susceptibility to brown rot, the main disease on stone fruits caused by *Monilinia* spp., to the presence of secondary metabolites with antifungal properties, that are mostly concentrated in the peel at the immature stage. The susceptibility to pathogens was evaluated weekly as the percentage of infected fruits after artificial inoculation in wounded and unwounded fruits incubated for seven days at 20°C. The susceptibility presented the same seasonal pattern for the three *Monilinia* species and a high resistance to *Monilinia* rots was found 6-8 weeks after full bloom. In this phase, the pit hardening occurred (S2). The presence of antifungal compounds ethanol extracts from peach peels of cvs Maycrest, Red Heaven, and Tardibelle at different developmental stages was evaluated by 1D TLC biossays. Using a solvent system 60:40:30 (hexane:ethylacetate:methanol), inhibition spots with R_f values of 73.3, 53.3 were found in S1 (4 weeks after full bloom), S2 (pit hardening) and S4 (harvest) stages. Spots with R_f values of 26.6, 13.3 were also observed in S2. No inhibition spots were detected on cvs Maycrest and Tardibelle in S4, while very feeble inhibition spots were found in cv. Red Heaven with R_f 73.3 and 53.3. These results suggest that the presence of preformed compounds in peach peel could be considered partially responsible for the resistance to brown rot observed in fruit 6-8 weeks after full bloom. However, further investigations are required to identify these compounds and understand their role in brown rot susceptibility.

TEMPERATURE INFLUENCE ON THE RESISTANCE AND SENSITIVITY OF *MONILINIA LAXA*, *MONILINIA FRUCTICOLA* AND *MONILINIA FRUCTIGENA* ISOLATES TO TEBUCONAZOLE AND THIOPHANATE METHYL FUNGICIDES: *IN VITRO* AND *IN VIVO* TRIALS. **C. Martini, A. Di Francesco and M. Marta.** *[1]CRIOF, Università degli Studi di Bologna, Via Gandolfi, 19, 40057 Cadriano (BO), Italy. E-mail: camilla.martini2@unibo.it*

The effect of environmental factors such as temperature should be studied to better assess the resistance of *Monilinia* spp. to chemical fungicides used in the orchards. For *in vitro* assay, PDA plates amended with tebuconazole or thiophanate methyl at a concentration corresponding to EC_{50} value for each isolate were inoculated with both sensitive (S) and resistant (R) isolates. Thirteen *M. laxa*, seven *M. fructicola*, and three *M. fructigena* isolates were tested. For *in vivo* assay peaches were wounded and inoculated with a spore suspension (10^3 conidia ml^{-1}) of *M. laxa* or *M. fructicola* six hour after treating the fruits with the fungicides at a concentration of half the label rate. To determine the effect of the temperature, the plates and the fruits were incubated at 15, 20, 25, 30°C for seven days in the dark. The percentage of infected fruits were calculated in the *in vivo* assay. *In vitro* results showed that the growth of *M. laxa* on tebuconazole-amended substrates incubated at 20°C was significantly reduced (-78%) while in the case of *M. fructigena* isolates the treatment was more effective at 15°C (-90%). *In vitro* assays with thiophanate methyl showed that the largest pathogen reduction was obtained when *M. laxa* and *M. fructicola* isolates were incubated at 30°C (with a reduction of -62% and -73%, respectively). Similar results were observed in *in vivo* tests with both fungicides, demonstrating that the temperature affects the efficacy of the fungicide treatments against *Monilinia* isolates.