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Fungal biological control agents (BCAs) are a sustainable alternative for crops protection. Several BCAs are reported to produce metabolites that have antibiotic, fungicidal, insecticidal or antiviral properties. However, some of these metabolites, such as gliotoxin (GT), can be toxic to animals, humans included. Gliotoxin is a toxin produced by the "Q" strains of *Trichoderma virens*, with a role in the mycoparasitism of other fungi. Beside its potential use in biocontrol applications, GT is also involved in the virulence of the opportunistic human pathogen *A. fumigatus*. Thus, apparently, GT can represent either a beneficial or a deleterious fungal metabolite depending on the situation. More information about GT production by BCAs and its environmental role should be gained in order to minimize the GT 'load' in the environment and for the safe use of *Trichoderma* spp. as a biofungicide. Three different *Trichoderma* species have been analyzed and the presence of the gliotoxin biosynthetic genes cluster has been ascertained in the isolates *T. virens* Gv29-8, known to be a GT producing strain, *T. reesei* RUT-C30 and QM6a, known to be GT non-producing strains and *T. afrobarzianum* T6776 and B97, for which no information about GT production was available. A time course of their GT production *in vitro* has been produced and GT quantified by HPLC analysis. Moreover, the induction of GT biosynthesis by plants or other fungi has been evaluated either by HPLC and *gli* cluster gene expressions analysis.

DEVELOPMENT OF REAL TIME RT-qPCR FOR THE DETECTION OF OLIVE LEAF YELLOWING ASSOCIATED VIRUS. A. Fontana, A. Tiberini, G. Albanese. Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di AGRARIA, Località Feo di Vito, 89122 Reggio Calabria (RC) Italy. Email: anna.fontana@unirc.it

Olive leaf yellowing associated virus (OLYaV, an unassigned virus in the family *Closteroviridae*) after the first detection in Italy was found in many orchards of southern Italy as well as in other Mediterranean countries. Detection protocol of olive viruses needs repeated analyses for several subsequent spring and autumn seasons due to the erratic distribution in olive plants. In the frame of PON3PE00090_2 OLIVO research project, a Real-Time RT-qPCR assay specific for OLYaV was developed. Primer sets were designed on published nucleotide sequences of HSP70 by Primer Express software (Applied Biosystems) and applied in a Real-Time RT-qPCR assay using a Power SYBR® Green RNA-to-CT™ 1-Step Kit. Reactions were performed in a StepOnePlus™ Real-Time PCR System with a standard amplification cycle including melting curves analysis to exclude aspecific amplicons. Analytical specificity was assessed *versus* other viruses phylogenetically related to OLYaV (i.e. *Citrus tristeza virus*, genus *Closterovirus*) and/or other viruses reported on olive, as *Strawberry latent ringspot virus* (genus *Nepovirus*). No amplification products were detected for the non-target viruses analyzed. Moreover, analytical sensitivity was determined using 5-fold dilution series of OLYaV-infected olive leaf extracts. The analytical sensitivity has been directly compared with end point RT-PCR. Moreover, to assess a relative quantification method based on a $\Delta\Delta C_t$ method, different reference genes, commonly used and reported in literature, were compared and validated to be successfully applied to normalize the relative quantification data allowing to investigate the modulation of virus titer in the pathosystem OLYaV-olive over the project research.

NEW FUNGI ATTACKING POMEGRANATE (*PUNICA GRANATUM*) IN ITALY. S. Frisullo¹, S.M. Mang², H.S. Elshafie², I. Camele². ¹Department of Agricultural Sciences, Food and Environment, University of Foggia, Via Napoli 25, 71121 Foggia, Italy. ²School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: ippolito.camele@unibas.it

Symptoms of chlorosis, decay, leaves falling and plant death were observed in autumn 2015/16 in commercial orchards on pomegranate (*Punica granatum* L.) in Apulia and Basilicata regions (southern Italy). In some cases the disease incidence reached 20%. Sections of the trunks taken from symptomatic plants revealed vessels browning. In order to isolate the likely pathogens, sterilized pieces of symptomatic tissues were plated in Petri dishes containing potato dextrose agar (PDA). Three fungal species were isolated consistently on PDA. Total DNA of three isolates for each species was extracted and the ITS1-5.8S ITS2 region was amplified with ITS5/ITS4 primers and sequenced. On the basis of morphological features and ITS sequence, isolates were identified as *Xenoacremonium falcatum*, *Sporotrix variecibatus* and *S. mexicana*. The ITS sequences of one isolate for each species were deposited in GenBank under the accession Nos. LT799728-LT799729-LT799730. Thirty healthy twigs of pomegranate (10 cm length) for each fungal species, previously surface sterilized, were wound-inoculated with a single fungal pure culture on agar (0.3 × 0.3 cm) and then placed in a moist chamber at 25°C. Ten controls were agar-inoculated using the same procedure. After 15 days the pathogen inoculated twigs showed vessels browning until 3-4 cm length from the inoculation zone. *X. falcatum*, *S. variecibatus* and *S. mexicana* were always reisolated only from twigs individually inoculated with the above fungi. The occurrence of *X. falcatum*, *S. variecibatus* and *S. mexicana* is reported for the first time on *P. granatum* plants in Italy.

OCCURRENCE OF COLLETOTRICHUM ACUTATUM ON ACCA SELLOWIANA IN ITALY. S. Frisullo¹, S.M. Mang², H.S. Elshafie², I. Camele². ¹Department of Agricultural Sciences, Food and Environment, University of Foggia, Via Napoli 25, 71121 Foggia, Italy. ²School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: ippolito.camele@unibas.it

In summer-autumn 2015/16, a severe typical symptoms of anthracnose with an incidence of 10 to 20% were observed on feijoa [*Acca sellowiana* (O. Berg) Burret] mature fruits and leaves in gardens and commercial orchards (about 35 hectares) located in Bari and Matera Provinces (southern Italy). The symptoms were dark sunken necrotic lesions on mature fruits which may become covered by pink conidial masses in the center with successively fruit rot. On the leaves, chlorotic lesions that evolved to brown spots and sunken necrotic zones were observed. In order to isolate the likely pathogen, small pieces of symptomatic plant tissues were plated in Petri dishes containing potato dextrose agar (PDA). A *Colletotrichum* species was isolated consistently on PDA. Conidia produced by the pure isolates were hyaline, smooth-walled, aseptate and cylindrical to fusiform. On the basis of colony and conidia morphology, isolates were identified as *Colletotrichum acutatum* J.H. Simmonds. Ten *C. acutatum* isolates were identified through both morphological and molecular analysis based on a multilocus investigation of four genes: the rDNA-ITS region (ITS), partial actin (ACT), β -tubulin (TUB-2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Phylogeny inferred from combined datasets of ACT, ITS, β -tubulin and GAPDH revealed its belonging to *C. acutatum*. Pathogenicity tests were carried out twice. Symptoms identical to those observed in the field appeared within 10 to 15 days. *C. acutatum* was always isolated only from inoculated leaves and fruits. The occurrence of

C. acutatum is reported for the first time on *A. sellowiana* plants in Italy.

PRELIMINARY INVESTIGATIONS ON PRESENCE OF FUNGI ON OLIVE TREES INFECTED AND APPARENTLY UNINFECTED BY XYLELLA FASTIDIOSA STRAIN CoDiRO IN LECCE PROVINCE (SOUTHERN ITALY). S. Frisullo¹, S.M. Mang², H.S. Elshafie², L. Prudente¹, I. Camele². ¹Department of Agricultural Sciences, Food and Environment, University of Foggia, Via Napoli 25, 71121 Foggia, Italy. ²School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: ippolito.camele@unibas.it

In order to verify the presence and frequency of fungi on olive trees infected and apparently not infected by *Xylella fastidiosa* strain CoDiRO, a survey was carried out in 20 farms located in Lecce province (southern Italy) in spring/summer 2015/2016. For this scope, 20 two-year-old twigs were collected from apparently uninfected and *X. fastidiosa* infected olive trees, respectively. Three sections were performed on each twig taken from different height lengths. Both under bark and woody cylinder parts were used for fungal isolation on Potato Dextrose Agar (PDA). Other 10 branches, three- to five-year old, from infected and from apparently non infected olive trees, respectively, were used for fungal isolation only from woody cylinder part (3 sections/branch) on PDA. Outcomes of this investigation showed that a higher number of genera and fungal isolates were present on apparently uninfected olive trees, from both twigs and branches, compared with the *X. fastidiosa* infected samples. The fungal genera more frequently isolated from apparently healthy olive trees were *Botryosphaeria*, *Colletotrichum*, *Alternaria*, *Phaeoacremonium*, *Phaeomoniliella* and *Phialophora*. The *X. fastidiosa* role in the bacteria/fungi pathosystem will require further investigations.

This study was supported by Apulia Region under the project "EZIOCONTROL".

INVESTIGATION OF THE ROLE OF HISTIDINE KINASE 1 IN STEMPHYLIUM VESICARIUM. K. Gazzetti, A. Ciriani, M. Collina. University of Bologna, Department of Agricultural Science, Viale G. Fanin 46, 40127 Bologna (Italy). E-mail: marina.collina@unibo.it

Brown spot of pear (BSP), a fungal disease caused by *Stemphylium vesicarium* (Wallr.) Simmons, is the most important pear pathogen in Italy and in other European countries. Preventive applications of fungicides are needed from petal fall to fruit ripening to control the disease. A previous molecular analysis conducted by our group on field isolates of *S. vesicarium*, established a correlation between dicarboximide and phenylpyrroles fungicide resistance and single amino acid substitutions, corresponding to single nucleotide polymorphisms (SNPs) in the nucleotide sequence of *SvHK1*. This gene is predicted to encode a 1,329 amino acid protein, putatively belonging to the class III of two-component histidine kinases, homologous to Nik1/OS1 from *Neurospora crassa*. *SvHK1* orthologues are involved in fungicides resistance, osmotic and oxidative stress response, hyphal growth, development and differentiation, nutrient-sensing pathways, pathogenicity mechanisms. Gene deletion mutants were generated by gene replacement from protoplasts obtained from a reference sensitive strain (WT), and monoconidial mutant strains were characterized in order to collect insights on the role of HK1 in *S. vesicarium*. The whole genome of WT strain has been *de novo* sequenced and is currently available on our private

Online Personal BLAST Server, together with an *ab initio* gene prediction. The database is very helpful to identify the components of signaling pathway to which SvHK1 belongs, and his relationship with other signal cascades.

PRODUCTION OF ACTIVE PROPAGULES OF BENEFICIAL MICROBES USING AGRO-FOOD WASTES. C. Gigliotti¹, M. Consales¹, G. Murolo¹, F.L. Fedele¹, M. Pascale², S. Lanzuise², E. Comite², M. Napolitano², A. Sicari¹, S. Bolletti Censi¹, M. Lorigio². ¹Linfa S.C.A.R.L., Zona Industriale Snc-89900 Porto Salvo Vibo Valentia (VV), Italy. ²Università degli Studi di Napoli Federico II, Dipartimento di Agraria, Via Università 100, 80055 Portici (NA), Italy. E-mail: info@laboratoriolinfa.it

Annually, the agro-food industry produces considerable amounts of by-products and waste materials that can cause serious environmental problems, with storage and disposal having an elevated cost. Bioactive molecules, such as antioxidants, vitamins, pigments, etc., are often present in high concentrations in industrial wastes, and they can be recovered for use in the formulation of functional foods. Extraction of valuable nutrients or recycling of the industrial by-products represent a valid alternative to simply discarding the waste. This work proposes an innovative application for undesirable material from industrial processing. Tomato or olive by-products were used as a growth medium for the cultivation of beneficial microbes, such as fungi belonging to the genus *Trichoderma*, that are used as the active ingredient of biofertilizers and biopesticides marketed worldwide for agricultural applications. Tomato or olive by-products, obtained from the food processing companies involved in the "Linfa" project, were treated in four different manners (sterile or nonsterile, with or without the addition of 1% sucrose) then inoculated with *Trichoderma* spp. Solid state fermentations were performed in air strip bags, incubated at 25°C for one month in the dark. Fungal growth and spore production were monitored every seven days. Generally, the fungus developed and sporulated abundantly on both tomato and olive residues, with the highest spore concentrations obtained when the substrate was sterilized and amended with sucrose. Further investigations using different microbes or growth conditions are in progress.

Work was supported by LINFA project (PON03PE_00026).

BEHAVIOR OF FUSARIUM EQUISETI ON LETTUCE AND WILD ROCKET: RESULTS FROM EPIDEMIOLOGY STUDIES UNDER CONTROLLED CONDITIONS. G. Gilardi¹, A. Garibaldi¹, M.L. Gullino^{1,2}. ¹Università degli Studi di Torino, Centro AGROINNOVA, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy. ²Università degli Studi di Torino, DISAFA, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy. E-mail: giovanna.gilardi@unito.it

Fusarium equiseti is spreading quickly in Italy on different leafy vegetables, such as lettuce, wild and cultivated rocket, which are grown intensively in monoculture. In order to have a better understanding of the effect of temperature and relative humidity (RH) exposure on the severity of *F. equiseti*, trials have been carried out in growth chambers at 10, 15, 20, 25, 30 and 35°C at 100% RH. Twenty-five-day-old plants of lettuce and wild rocket were sprayed with 1×10^6 conidia/ml of the pathogen isolated from affected plants in field. Plants were kept at the different temperatures and covered with a transparent polyethylene film for 0, 1, 3, 6, 12, 24 and 48 hours. Disease incidence (DI) and severity (DS) were recorded 8 days after inoculation counting the number of affected leaves out of 100 and estimating the percentage of leaf area affected (DS). Results showed that *F. equiseti* severely affected lettuce quality