RNA HYDROLYSIS AND CYTOKININ BINDING ACTIVITIES OF PR-10 PROTEINS ARE DIFFERENTLY PERFORMED BY TWO ISOFORMS OF THE PRU P 1 PEACH MAJOR ALLERGEN AND ARE POSSIBLY FUNCTIONALLY RELATED. E. Baraldi1, P. Zubini1, P. Bertolini1, B. Zambelli2, F. Musiani2 and S. Ciurli2. 1CRIOF, Dipartimento di Protezione e Valorizzazione Agroalimentare, Laboratorio di Biotecnologie, Università degli Studi, Via Fanin 46, 40127 Bologna Italy. 2Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Laboratorio di Chimica Bioinorganica, Università degli Studi, Via Fanin 46, 40127 Bologna, Italy. E-mail: elena.baraldi@unibo.it

PR-10 proteins are a family of pathogenesis related (PR) allergenic proteins playing multifunctional roles. The peach (Prupe persica) major allergen, Pru p 1.01, and its isoform Pru p 1.06D were highly expressed in the fruit skin at the pit hardening stage, when fruits transiently lose their susceptibility to Monilinia spp. To investigate the possible role of the two Pru p 1 isoforms in plant defense, the recombinant proteins were expressed in E. coli and purified. Light scattering experiments and circular dichroism spectroscopy showed that both proteins are monomers in solution with secondary structures typical of PR-10 proteins. Even though the proteins do not display direct antimicrobial activity, they both act as RNases, a function possibly related to defense. The RNase activity is different for the two proteins, and only that of Pru p 1.01 is affected in the presence of the cytokinin zeatin, suggesting a physiological correlation between Pru p 1.01 ligand binding and enzymatic activity. The binding of zeatin to Pru p 1.01 was evaluated using isothermal titration calorimetry, which provided information on the stoichiometry and on the thermodynamic parameters of the interaction. The structural architecture of Pru p 1.01 and Pru p 1.06D was obtained by homology modeling and the differences in the binding pockets, possibly accounting for the observed difference in binding activity, were evaluated.

PREDICTING SUPPRESSIVENESS TO DISEASE BY ORGANIC AMENDMENTS. G. Bonanomi, V. Antignani, M. Capodillupo and F. Scala. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. E-mail: giuliano.bonanomi@unina.it

Organic matter (OM) amendments have been proposed to control diseases caused by soil-borne pathogens. However, the inconsistent results reported in previous works seriously hindered their practical application. In this work we analyzed the behaviours of different OM amendments (compost, crop residues, peat and organic wastes) reported in literature to assess: (i) the capability of a specific organic amendment to control different diseases; (ii) the influence of OM decomposition on disease suppressiveness, and (iii) the parameters that are better correlated with suppressiveness. OM was consistently suppressive on different pathogens only in few cases, and only when a limited number of pathogens were tested, suggesting that suppressiveness is often pathogen-specific. During OM decomposition, disease suppressiveness gave very variable responses. Peat suppressiveness generally decreased during decomposition, while responses of composts and crop residues were much more complex. Among the parameters analyzed (181 with 643 correlations), only few showed consistent relationship with disease suppression. The response of pathogen populations to OM amendments was reliable only for OM with low C-to-N ratio and for pathogens with limited saprophytic attitude (Thielaviopsis basicola and Verticillium dahliae). Enzymatic and microbiological parameters were much more informative to predict suppressiveness than the chemical ones. The most useful parameters were hydrolysis of fluorescein diacetate, substrate respiration, microbial biomass, total culturable bacteria, fluorescent Pseudomonads and Trichoderma populations. We conclude that the integration of different parameters could be a promising approach to characterize suppressive amendments.

A POSSIBLE NOVEL STRATEGY OF RNA SILENCING SUPPRESSION IN THE GEMINIVIRIDAE FAMILY. A.V. Carluccio1,2, P.V. Shivaprasad1 and L. Stavolone2,1Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 163/A, 70126 Bari, Italy. 2Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK. E-mail: lstavolone@ba.ivv.cnr.it

Plants have developed small RNA-mediated post-transcriptional gene silencing as a defense mechanism against viruses. In response, plant viruses encode proteins that can interfere with various steps of the silencing pathway with modes of action not yet completely understood. Bipartite geminiviruses encode a small protein, AC4, with controversial biological functions which, depending on the virus species, include virus movement, symptom expression and silencing suppression. In this study, we examined the role of AC4 encoded by Mungbean yellow mosaic virus (MYMV, genus Begomovirus) showing that AC4 is essential for MYMV pathogenesis and suppresses exogenous GFP-induced silencing in transgenic Nicotiana benthamiana 16c lines. The same protein fused with GFP, when transiently expressed in N. benthamiana protoplasts, is targeted to the plasma membrane (PM) and the nucleus. Mutation of a single cysteine in the AC4 sequence abolishes protein localization to PM. Such cysteine was predicted to bind a fatty acid that mediates AC4 binding to PM by a process of location regulation (S-palmitoylation) previously unreported for any plant virus protein. In addition, replacement of cysteine with alanine hinders AC4 silencing suppression activity, indicating a direct relation between AC4 localization and function. Preliminary results indicate that MYMV AC4 suppresses silencing a step downstream of siRNA production and support the hypothesis that the interference with the spread of silencing signal depends on plasma membrane binding.

MULTIPLE GENE SEQUENCE ANALYSES REVEAL THE COMPLEX POPULATION STRUCTURE OF ‘CANDIDATUS PHYTOPLASMA MALI’ IN ITALY. P. Casati1, F. Quaglini1, A. Stern1, R. Tedeschi2, A. Alm2 and P.A. Bianco1. 1Dipartimento di Produzione Vegetale, Sezione Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Entomologia e Zoologia Applicate all’Ambiente “Carlo Vidano”, Facoltà di Agraria, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10093 Grugliasco (TO), Italy. E-mail: paola.casati@unimib.it

Apple proliferation (AP) is an important disease present in several European countries. The causal agents of AP are ‘Candidatus Phytoplasma mali’ (‘Ca. P. mali’)-related strains. In the present study we evaluated the genetic diversity among isolates of ‘Ca. P. mali’ populations in orchards of north-western Italy where apple proliferation (AP) disease is widespread and causes severe economical losses. ‘Ca. P. mali’ has been detected, by PCR/RFLP analysis of phytoplasmal 16S rDNA, in 89% (101/114) samples from apples and insect vectors. Collective RFLP patterns, obtained by multiple gene (16S rRNA, rpv-rpsC, secY, and nitrore-
ductase) sequence analyses, revealed the presence of 12 distinct genetic lineages among 60 selected representative ‘Ca. P. mali’ isolates. Multiple gene sequence analyses underscored an unexpected high degree of genetic heterogeneity among the ‘Ca. P. mali’ populations in north-western Italy. The prevalence of distinct ‘Ca. P. mali’ genetic lineages in diverse geographic regions opens new interesting avenues for studying the epidemiology of AP disease. Molecular markers determining different ‘Ca. P. mali’ genetic lineages could be useful for investigating the life cycle of the AP phytoplasma, with the perspective of developing new strategies for the control of AP epidemics.

CONTROL OF VERTICILLIUM WILT OF ARTICHOKE USING RESISTANT CARDOON AS ROOTSTOCK. F. Ciccarese1, P. Crino2, C. Fumara1 and M. Gallo1. 1Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. 2ENEA, C.R. Casaccia, Dipartimento Biotecnologie, Agrindustrie e Protezione della Salute, Via Anguillarese 301, 00123 Roma, Italy. Email: fcccare@agr.uniba.it

Grafting with resistant rootstock represents an effective control method for soil-borne pathogens. The control of Verticillium dahliae in artichoke is particularly difficult due to the lack of effective chemicals and the absence of resistant cultivars. Recent investigations have shown that artichoke has grafting affinity with cardoon. Therefore, in this work the results are reported of screening of cardoon cultivars and accessions for resistance to Verticillium wilt. Tests were carried out in a greenhouse at 24±2°C on 15 cultivars, each with relative accessions, totalling 53 lines. Plants were inoculated at the 2-3 leaf stage with a conidial suspension at the concentration of 1×10⁸ CFU ml⁻¹. Disease development was recorded 60 days after inoculation, estimating the severity of external symptoms and of vascular discoloration with an empirical scale of 5 classes (from 0 to 4). Data were used for calculating the Mc Kinney index. A wide range of reactions was observed both within the cardoon population and some of the accessions. Of 15 genotypes tested, 6 proved to be highly susceptible to Verticillium wilt, 7 showed disease of intermediate severity and 2 accessions of Cynara cardunculus var. sylvestris were resistant.

COMPLETE CONTROL OF VERTICILLIUM WILT OF OLIVE IS OBTAINED WITH RESISTANT ROOTSTOCKS. M. Giruli and G. Bubici. Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: giosabub@email.it

The potential of grafting susceptible cultivars on resistant rootstocks was evaluated for the control of Verticillium wilt of olive. Cultivars Frantoio (highly resistant clone), Coratina (partially resistant clone) and Leccino (highly resistant clone) were used as scions and rootstocks in all combinations. Two years old self-rooted plants were used for grafting, and one year later they were inoculated by root-dipping with the defoliating pathotypes of Verticillium dahliae. Plants were pot-grown and maintained in greenhouse. Severity of external disease symptom was evaluated weekly up to 90 days after inoculation, when vascular browning was assessed and V. dahliae re-isolation was attempted. The reactions to Verticillium wilt of the three tested cultivars were confirmed in this study, and were not affected by grafting each of the cultivars on itself. Based on external symptoms, cv. Frantoio rootstock protected partially Coratina and completely Leccino, while Coratina and Leccino rootstocks did not provide any protection to the scions. These results were also confirmed by vascular browning reactions. An increase of Verticillium wilt occurred in Frantoio scions grafted on Leccino and less markedly on Coratina rootstocks, compared with Frantoio/Frantoio graft combinations or self-rooted Frantoio plants. Such disease increase was observed regardless of disease parameters considered, such as severity of external symptoms, vascular browning and V. dahliae re-isolation. Finally, to investigate V. dahliae colonization progress in the rootstock/scion combinations, quantitative estimations of xylem vessel plugging were carried out on transverse micro-sections (20 µm thick) of rootstocks and scions.

NEW ASPECTS OF THE INTERACTION BETWEEN THRIPS AND TOSPOVIRUSES. M. Ciuffo1, P. Margaria1, G. Maurino2, L. Bosco2, L. Tavella2 and M. Turina1. 1Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Torino, Strada delle Cacce 73, 10135 Torino, Italy. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Entomologia e Zoologia Applicate all’Ambiente, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: m.ciuffo@ivv.cnr.it

Western flowers thrips (Frankliniella occidentalis) is the main vector of Tomato spotted wilt virus (TSWV), the type member of the genus Tospovirus. The virus is transmitted in a persistent propagative manner. In order to characterize NSs-defective strains, we forced the obtainment from a wild-type strain (p202/3WT) of a NSs-truncated strain by mechanical inoculation on resistant pepper. This strain, named p202/3RB, carried a single nucleotide mutation (G deletion) in the NSs coding region, resulting in a frame-shift and a truncated NSs protein of 443aa (compared to 467aa of p202/3WT). Transmission experiments (leaf disk assay) using both strains yielded a percentage of transmission higher than 50% for p202/3WT, whereas there was no transmission of p202/3RB. Quantitative RT-PCR assays on adult thrips immediately after the third inoculation access period (IAP) detected a very low titer of viral RNA for p202/3RB compared to a much more abundant p202/3WT viral RNA. Sequence comparison of the genomic segments of the two related strains showed 100% homology for the S segment with the exception of the G deletion and 100% homology for the M segment. Taken together, our data provide genetic evidence for the involvement of the S segment in the TSWV-thrips relationship and for a role of NSs in efficient vector infection. In a different experiment the number of thrips able to transmit wild type TSWV after each of four subsequent IAPs was evaluated. Among 200 adults analyzed, ca. 70% could transmit at the first passage and, surprisingly, 5% of the individuals stopped to transmit after the first passage, indicating a possible individual recovery from viral infection.

PEPINO MOSAIC VIRUS OUTBREAKS IN SICILY. S. Davino1, A. Tiberini2, L. Tommassi2, A. Colombo5, S. Carataldi7, V. Mondello1, G.E. Agosteo8 and M. Davino2 1Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. 2Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via Santa Sofia 100, 95123, Catania, Italy. 3CRA, Centro di Ricerca di Patologia Vegetale, Via C.G. Beretta 22, 00136 Roma, Italy. 4Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Fco di Vito, 89122 Reggio Calabria, Italy. 5Osservatorio delle Malattie delle Piante, Via Sclafani 32, 95024 Acireale, Italy. E-mail: davino@unipa.it

Pepino mosaic virus (PepMV), genus Potexvirus, family Flex-
Viroids are infectious non-protein-coding RNAs that may cause plant diseases by a still unknown mechanism. A recent hypothesis has linked viroid pathogenesis to RNA silencing, a sequence-specific degradation mechanism mediated by small RNAs of 21-24 nt (sRNAs). This hypothesis, which has not been conclusively proved, relies on the detection of viroid-derived 21-24 nt sRNAs (vd-sRNAs) in infected tissues and predicts that some of them drive down-regulation of host gene(s) eliciting the pathogenic process. Here, we exploited the experimental system formed by the chloroplast-replicating Peach latent mosaic viroid infecting its natural host peach to further dissect the biogenesis of vd-sRNAs and the role that they may have in viroid pathogenesis. To this aim, vd-sRNAs accumulating in peach leaves infected by two PLMVd variants, which induce an albino phenotype (peach calico, PC) and a typical mosaic, were characterized by deep sequencing (Sensex- Illumina). Results suggest the involvement of cytoplasmatic DICER-like enzymes in the genesis of the vd-sRNAs of 21 and 22 nt, using as templates the genomic PLMVd RNA of both polarities or the dsRNAs synthesized by a host RNA-dependent RNA polymerase. Moreover, since vd-sRNAs from the insertion of 12-14 nt in which the PC domain is reduced in FVG. Molecular identification and characterization was carried out on symptomatic kiwifruits produced in Italy. E-mail: f.diserio@ba.ivv.cnr.it

DEEP SEQUENCING OF VIROID-DERIVED SMALL RNAs ACCUMULATING IN PEACH PLANTS INFECTED BY PEACH LATENT MOSAIC VIROID. F. Di Serio1, A. Gisels2, B. Navarro3, S. Delgado1, A.E. Martinez de Alba1, G. Donvito3 and R. Flores3. 1Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. 2Istituto di Tecnologie Biomediche del CNR, Via G. Amendola 122/D, 70126 Bari, Italy. 3Istituto di Biologia Molecular y Celular de Plantas (UPV-CSIC), Universidad Politecnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain. 4Istituto Nazionale di Fisica Nucleare, Via Oraobona 4, 70126 Bari, Italy. E-mail: f.diserio@ba.ivv.cnr.it

DNA-BASED METHODS FOR THE CHARACTERIZATION AND DETECTION OF CADOHPHORA LUTEO-OLIVACEA STRAINS IN KIWIFRUIT WITH SKIN-PITTING. S. Di Lenarda, M. Martini1, S. Borselli1, A. Pittana2 and R. Osler1. 1Dipartimento di Biologia e Protezione delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. 2FRULIKWI S.C.Agr, Via Poligono, 33090 Rauscedo (PN), Italy. E-mail: serena.dilenarda@unitrd.it

The skin pitting disease of kiwifruit became important in Friuli Venezia Giulia (FVG, northern Italy) since 2003. Skin pitting symptoms on kiwi fruit normally appear in February-March after about three months of cold storage. In 2005-2008, a survey with traditional (isolation on PDA, microscope observation) and molecular techniques was carried out on symptomatic kiwifruits produced in FVG. Molecular identification and characterization was based on DNA extraction from fungal mycelium, followed by PCR amplification with primers ITS1/ITS4 and sequencing of ITS regions. Sequence analysis disclosed the presence in FVG of two different strains of the fungus Cadophora luteo-olivacea (= Phialophora luteo-olivacea) denoted as type A and B. Isolation assays resulted in a very high frequency of C. luteo-olivacea in typically pitted fruits (62% in 2005 and 72% in 2006). Pathogenicity tests using spores of C. luteo-olivacea strains A and B proved that both are able to produce skin-pitting. A DNA-based diagnostic method was also devised using C. luteo-olivacea specific primers directly on total genomic DNA extracted from infected fruit tissues following a CTAB method. Species-specific primers were designed on alignment of the ITS region sequences of different strains of C. luteo-olivacea and other closely related species. This diagnostic tool showed that, in average, 95% of symptomatic fruits produced in 2005, 2006 and 2007 were positive for the presence of C. luteo-olivacea. RFLP analysis performed on specie-specific PCR products clearly differentiated the two C. luteo-olivacea type A and B strains.
MOLECULAR PROPERTIES OF FIG MOSAIC VIRUS. T. El-beaino1, M. Digiaro1, A. Alabdullah2, A. Mintra2 and G.P. Martelli2,3. 1Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. 2Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. 3Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: elbeaino@iamb.it

The agent of Fig mosaic disease has remained undetermined for long time. The consistent presence in symptomatic plants of enveloped round to ovoid bodies (DMBs) 90-200 nm in diameter, their absence in symptomless trees, and their successful transmission by the eriophyid mitte Aceria fuscus followed by reproduction of disease symptoms, now strongly support the notion that DMBs are the particles of Fig mosaic virus (FMV). Four single-stranded (+)RNA segments were recovered from symptomatic figs and sequenced. Each segment comprises a single open reading frame (ORF) 7093, 2252, 1490 and 1472 nucleotides in size, encoding the RdRp (264 kDa), a putative glycoprotein (73 kDa), a putative nucleocapsid protein (35 kDa) and a protein with unknown function (40.9 kDa), respectively. In phyllogenic trees constructed with the whole amino acid sequences of RNA-1 and RNA-2 FMV clustered consistently with European mountain ash ringspot-associated virus (EMARaV), the type strain of the proposed novel genus Emaravirus, in a clade close to those comprising members of the genera Hantavirus, Orthobunyavirus and Tospovirus, family Bunyaviridae. The amino acid sequence of the putative FMV nucleocapsid protein encoded by RNA-3 shared identity with comparable sequences of EMARaV and the unclassified viruses Pegeonpea sterility mosaic virus (PPSMV) and Maize red stripe virus (MRSV). Based on molecular, morphological and epidemiological features, FMV is related to PPSMV and MRSV. Since these viruses are also phylogenetically related to EMARaV, they are eligible for classification in the genus Emaravirus, which, in turn, may find a taxonomic allocation in the family Bunyaviridae.

A STRAIN-SPECIFIC PROBE DETECTS THE BIOCONTROL AGENT BACILLUS LICHENIFORMIS MBBL1 IN THE OLIVE RHIZOSPHERE. M. Ferrara, V. Rivelli, A. Ippolito and F. Nigro. Dipartimento di Protezione delle Pianta e Microbiologia Applicata, Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigrof@agr.uniba.it

Bacillus licheniformis, a Gram-positive, spore-forming bacterium, is used in the biotechnology industry to manufacture various bioproducts. The species is best known as producer of a wide range of extracellular substances (enzymes, antibiotics, surfactants) that may contribute to nutrient cycling in nature. There is now clear evidence that several bacteria and their products play a key role in the suppression of various soilborne plant pathogens. Among the most promising biocontrol agent, Bacillus licheniformis, strain MBBL1, proved to be effective in reducing the inoculum density of Verticillium dahliae Kleb. in the rhizosphere of olive trees, under controlled condition. However, the introduction of a large amount of the antagonist in the soil requires the necessity of monitoring its dispersal and interactions with the natural soil microbiota. In this study a specific DNA probe, based on AFLP analysis, to detect the strain MBBL1 of B. licheniformis, was developed. The probe specific of ubiquitous Bacillus spp. isolates and international reference strains of B. licheniformis. The detection limit was 10 CFU mL⁻¹, thus providing a reliable tool to track the strain specifically. Moreover, the probe was able to detect MBBL1 strain in olive rhizosphere up to 2 months after application. No signal was detected after 8 months, suggesting a limited persistence and a low environmental impact of the strain in the soil.

RHIZOBACTERIA FOR THE BIOLOGICAL CONTROL OF COMMON BACTERIAL BLIGHT OF BEAN. N.S. Iacobellis, V. Shanmugaiyah and P. Lo Cantore. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale Ateneo Lucano 10, 83100 Potenza, Italy. E-mail: iacobel-lis@uniba.it

Common bacterial blight caused by the varieties fusans and no fusans of Xanthomonas campestris pv. phaseoli remains an important bean disease worldwide, irrespective of the recent AFLP characterization of a collection of the two varieties that confirmed the proposal to reclassify the varieties as distinct species. Although resistant/tolerant cultivars are available, the disease can cause important crop losses so that, because of the limited availability and efficacy of chemical measures, new and alternative control measures appear necessary. The aim of this study was to assess the potential of bacteria isolated from bean rhizosphere to control the disease. Sixty of 162 bacterial isolates recovered from bean rhizosphere inhibited in vitro the growth of target strains of common bean bacterial and fungal pathogens including the varieties of X. campestris pv. phaseoli. The majority of antagonist isolates were shown to produce lytic enzymes. Among the 60 antagonist bacteria, evaluated for their interference with the pathogenicity and/or virulence of a strain of X. campestris pv. phaseoli var. fusans in in vitro and in greenhouse cotyledon and trifoliate pathogenicity leaf assays, seven (2 G+ and 5 G-) protected plant tissues in both pathogenicity assays. In particular, in vitro and greenhouse assays a lesion reduction ranging from 30 to 66% when compared to control (100%) were observed. A positive correlation between the assays was also observed, suggesting that in vitro assay are useful for screening a high number of rhizobacteria isolates.

THE ROLE OF ETHYLENE AND ABScisSIC ACID IN CHITOSAN-INDUCED RESISTANCE TO TOBACCO NECROSIS VIRUS IN PHASEOLES VULGARIS. M. Iriti1,3, G. Castorina2, V. Picchi1, L. Mignani1, S. Vitalini1 and F. Faoro1,2. 1Dipartimento di Produzione Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. 2Dipartimento di Biologia, Università degli Studi, Via Celoria 26, 20133 Milano, Italy. 3Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: marcello.iriti@unimi.it

Besides regulating many physiological process, ethylene (Et) and abscisic acid (ABA) are also involved in tolerance to abiotic stresses and in defence mechanisms against some pathogens. In particular, Et is involved in resistance to necrotrophyc pathogens, while ABA plays opposite roles depending on the considered pathosystem. Resistance inducers able to activate plant innate immunity often utilize the plant hormonal system for signal transduction leading to defence gene transcription. Among inducers, chitosans (CHTs), deacetylated chitin derivatives, deserve particular attention, being able to induce resistance also against viruses. We have previously reported that a foliar spray with 0.15% CHT enhances ABA level in treated tissues, and this response is associated with callose deposition and resistance to TNV. Using a pharmacological approach, here we show that Et does not seem to be involved in CHT-induced resistance. In fact, pre-treatments with 0.1-0.2 mM aminooethoxyvinylglycine (AVG) and 1-5 mM isobutryic acid (AIB), inhibitors of 1-aminoacyclopropane-1-carboxilic
acid (ACC) synthase and ACC oxidase, respectively, do not influence CHI antiviral activity, as well as the application of 0.05-0.5 mM AgNO₃, inhibitor of Et receptors. Gas chromatography has confirmed these results, as an Et peak was detectable only in untreated plants inoculated with TNV alone.

THE LUXR FAMILY TRANSCRIPTIONAL REGULATOR RFIA PLAY AN INDISPENSABLE ROLE IN PSEUDOMONAS CORRUGATA VIRULENCE ON TOMATO. G. Licciardello1,2, I. Bertani2, L. Steindler2, P. Bella3, C. Strano3, V. Venturi2 and V. Catara1. 1Parco Scientifico e Tecnologico della Sicilia, z.i. Blocco Palma I, 95131 Catania, Italy. 2Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano 99, 34149 Trieste, Italy. 3Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: vcatara@unitct.it.

Molecular studies on Pseudomonas corrugata, causal agent of tomato pith necrosis, are limited only to a few aspects. An important role is attributed to phytotoxic and antimicrobial cationic lipopeptidoplipopeptides. In a previous study we isolated the pcoI/pcoR gene, which, as assessed by mutant derivatives, has an important role in virulence and in vitro antagonistic activity. Here we describe a gene, rfia, coding for a transcriptional regulator belonging to the LuxR family located downstream the pcoI gene. RT-PCR analysis revealed that rfia is directly linked to QS by co-transcription with pcoI. In the downstream region of pcoI-rfia, a RND (Resistance Nodulation System) transport system which is directly regulated by Rfia was identified. We found that pcoI, pcoR and rfia knock-out mutant culture filtrates are unable to inhibit the growth of Rhodotorula palmena and Bacillus megaterium, usually used to detect the LDP production. Moreover, time course monitoring revealed that both AHLs and LDPs are produced at high bacterial population cell densities. The role of Rfia in the development of disease symptoms in tomato was demonstrated by the absence of necrosis in stem pith tissues of plants inoculated with its mutant, as compared to those inoculated with the wild-type strain. Our data strongly suggest that P. corrugata QS regulates LDPs production through the transcriptional activator Rfia which then regulates the transcription of genes responsible for toxin production and secretion.

MOLECULAR CHARACTERIZATION OF COLLETOTRICHUM POPULATIONS ASSOCIATED WITH OLIVE ANTHRACNOSE IN EUROPE. M.A. Mammella1, L. Schena1, S.O. Caccio1, R. Faeld1, G.E. Agosteo1, S. Frisullo2 and G. Magnano di San Lio2. 1Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Fos di Vito, 89122 Reggio Calabria, Italy. 2Dipartimento di Chimica Biologica, Chimica Medica e Biologia Molecolare, Università degli Studi, Viale Andrea 6, 95125 Catania, Italy. 3Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 4Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Università degli Studi, Via Napoli 25, 71100 Foggia, Italy. E-mail: g.magnano@unict.it.

The identity of Colletotrichum spp. causing olive anthracnose in Italy and Portugal was investigated by amplifying and sequencing two genomic regions of approximately 600 and 1000 bp, respectively, from the β-tubulin genes 1 and 2. Isolates of C. gloeosporioides and C. acutatum from citrus, camellia, basil, strawberry, azalea and cherry were included in this study as well as isolates of Colletotrichum spp. from almond and oleander and a C. musae isolate from banana. Sequences of Glomerella cingulata, C. gloeosporioides and C. graminis (accessions Nos. AB273716, U14138 and M34492) were used as references. While sequences of Vorticillium dahliae var. longisporum, V. dahiae, Fusarium luneatum and Fusarium spp. (accessions Nos. DQ166861, DQ166894, EU926357 and EU926418) were chosen as outgroup sequences. Both β-tubulin genes were amplified and sequenced using degenerate primers designed by aligning and comparing more than 1000 available sequences. Sequences were aligned using ClustalX and introduced to TOPALi for phylogenetic analysis with the Neighbor-Joining method based on Jukes-Cantor distances. Olive isolates from Portugal were identified as C. acutatum and grouped with isolates from strawberry. A second molecular group included C. gloeosporioides isolates from citrus and olive while a third group comprised isolates from citrus, olive and camellia. Isolates responsible for olive anthracnose in southern Italy clustered with azalea isolates and formed a genetically uniform group distinct from both C. gloeosporioides and C. acutatum, suggesting that this disease is caused by a separate Colletotrichum species.

ANALYSIS OF THE EFFECTS OF FLAVESCENCE DORÉE PHYTOPLASMA INFECTION ON THE PROTEOME OF VITIS VINIFERA cvS NEBBIOLO AND BARBERA BY 2-DE AND MASS SPECTROMETRY. P. Margaria and S. Palmano. Istituto di Virologia Vegetale del CNR,, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: s.palmano@ivv.cnr.it.

Flavescence dorée (FD) is a serious phytoplasma disease of grapevine. Phytoplasmas are cellular wall-less prokaryotes belonging to the class Mollicutes and phloem restricted plant pathogens, unable to grow on axenic conditions. In this work, we studied the effects of FD phytoplasma in grapevine at the proteomic level. The proteome profile of the red berried wine Vitis vinifera cvs Nebbiolo and Barbera, naturally infected by FD phytoplasma, was evaluated using two-dimensional electrophoresis and the differential spots were processed by mass spectrometry. Responses of both cultivars were analyzed and compared with the correspondent healthy plant proteomic profile. Numerous proteins were differentially expressed in phytoplasma-infected grapevine midrib tissues; changes in protein expression involved a wide spectrum of biological functions, including cell defence, metabolism and the Reactive Oxygen Species (ROS) pathway. Our data provide an interesting picture of the response of grapevine to FD phytoplasma infection and allowed the selection of several proteins as targets for functional and expression analysis. This is the first study of global protein profile analysis in different grapevine cultivars in response to phytoplasma infection.

EFFECT OF CHANGING CLIMATIC CONDITIONS ON CRYSANTHEMUM YELLOWS PHYTOPLASMA INFECTION. C. Marzachi1, F. Maggi2, C. Roggia3 and D. Bosco4. 1Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. 2Berkeley Water Center, University of California, 413 B O’Brian Hall Berkeley, CA 94720-1718, USA. 3Università degli Studi di Torino, Via Leonardo da Vinci 44, Grugliasco (TO), Italy. E-mail: c.marzachi@ivv.cnr.it.

Phytoplasma infection of both plant and insect hosts was studied in climatic chambers under different constant temperatures ranging from 15°C to 30°C. Chrysanthemum carpinitum Scouboe, Macrosteles quadrirunctatus Kirschbaum and “Candidatus Phytoplasma asteris”, chrysanthemum yellows (CY) isolate
were used. To describe temperature-dependent phytoplasma multiplication in the plant, at each temperature apical leaves of daisy test plants were sampled at different times post inoculation (dpi) and phytoplasma concentration was measured by quantitative real-time PCR. Similarly, we described temperature-dependent phytoplasma multiplication in the insect by measuring phytoplasma titre in CY-infected leafhoppers at different times post acquisition. To describe temperature-dependent latency of CY in the insects, at each temperature leafhopper nymphs were allowed to feed on CY-infected daisies, then they were serially moved to one healthy daisy for successive inoculation access periods (IAP). To describe the temperature-dependent time gap between CY inoculation and symptom appearance in the plant, at each temperature 30 leafhopper nymphs were caged for an IAP of 48 h on healthy daisies, and plants were observed daily for symptom evaluation. To describe the temperature-dependent developmental time of the insect, batches of M. quadrifurcatus females were allowed to lay eggs on oat plants, insects were then removed and oats were transferred to climatic chambers at different temperatures and newly emerged adults were counted daily. The number of plants that became infected following the exposure to the vector over time was also studied to provide an estimate of the disease spread under different temperature conditions.

**TOMATO TRANSCRIPTOME ANALYSIS UNDER VIRAL INFECTION: LOOKING FOR A STABLY EXPRESSED REFERENCE GENE.** T. Mascia¹, E. Santovito², F. Cillo¹ and D. Gallitelli¹², ¹Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 165/A, 70126 Bari, Italy. ²Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: tizianamascia@email.it

Plant transcriptome analysis is being approached under a variety of experimental conditions. In the area of plant-virus interactions, transcript and miRNA profiling is providing new perceptions into the mechanisms underlying pathogenesis, disease symptoms development and basal defence. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is being largely used in transcriptome analyses also as a mean to validate results obtained by wider analytical systems like microarrays. The reproducibility of the results obtained by qRT-PCR strongly depends on accurate transcript normalization using stably expressed genes, known as housekeeping or reference genes. We have evaluated the robustness of six usually employed housekeeping transcripts β-tubulin (TUB), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), elongation factor 1α (EF1α), ubiquitin 3 (UBI3), actin (ACT) and 18S ribosomal RNA (18S), in addition to uridylic kinase (UK) and cyclophilin (CYP) that were characterized these isolates as well as five race 1 and nine race 0 Pto strains collected in Italy and California for the presence of the avrPto effector gene, whose product was found to interact with the Pto protein. All race 1 strains lacked the avrPto gene, while all race 0 strain contained it, except for two strain. We can therefore suppose a complete deletion of avrPto gene in race 1 strains. Since also avrPtoB was found to interact with Pto, further investigations are in progress to determine the presence of the avrPtoB gene in all strains studied.

**ULTRASTRUCTURAL MODIFICATIONS INDUCED BY THE FUNGAL ENDOPHYTE EPICOCCUM NIGRUM TO CANDIDATUS PHYTOPLASMA MALI IN PERIWINKLE PLANTS.** R. Musetti, S. Grisan, C. Paduano, M. Martini, R. Poliizotto and R. Osler, Dipartimento di Biologia e Protezione delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine. E-mail: rita.musetti@unitrd.it

Apple proliferation (AP), associated with the phytoplasma ‘Candidatus Phytoplasma mali’ (‘Ca. P. mali’) affects all apple cultivars in Europe. Since curative methods are not available, the possibility of using endophytes as biocontrol-agents or resistance-inducers has been emphasized. We investigated the interactions, at the ultrastructural and molecular level, between ‘Ca. P. mali’ and the apple endophyte Epicoccum nigrum in the experimental host Catharanthus roseus. AP symptom severity was evaluated in E. nigrum-treated plants and compared with untreated controls and phytoplasmas and were quantified by SYBR® Green I real-time PCR. Ultrastructural observations revealed that the endophyte treatment caused important modifications to phytoplasma cells, such as irregular shape, cytoplasm confinement at the periphery, and little distinguishable cell membrane. Cytological modifications of plant cells, such as abundant callose depositions and P-protein aggregates in sieve elements, were also observed, suggesting the activation of defense responses. Endophyte treatment induced a reduction of symptom severity. In particular, in treated-phytoplasma infected periwinkles, flowers had normal shape and size, being not different from those of uninfected controls. Real-time PCR showed that ‘Ca. P. mali’ concentration in E. nigrum-treated plants was about 2.3 times lower than in untreated ones. These results suggest that inoculation with E. nigrum influences phytoplasma infection in C. roseus plants, while the ultrastructural modifications of host cells are indicative of an enhancement of
host defense response. Expression analyses of some genes involved in defense mechanisms (i.e. PR 1, callose synthases, P-proteins) are in progress.

DEEP SEQUENCING ANALYSIS OF VIRAL SHORT RNAs FROM PINOT NOIR CLONE ENTAV 115. V. Pantaleo1, P. Sal- darelli2, L. Miozzi1, A. Giampetruzzi1, A. Gisel1, S. Moxon1, T. Dalmary4 and J. Burgyan1. 1Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. 2Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 163/A, 70126 Bari, Italy. 3Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, 70126 Bari, Italy. 4Istituto di Tecnologie Biomediche del CNR. Unità Organizzativa di Bari, Via Amendola 122/O, 70126 Bari, Italy. 5School of Computing Sciences, University of East Anglia, NR4 7TT, Norwich, UK. 6School of Biological Sciences, University of East Anglia, NR4 7TT, Norwich, UK. E-mail: j.burgyan@icc.cnr.it

Total small RNAs from tissues of Vitis vinifera cv. Pinot noir clone ENTAV 115 were sequenced by a Solexa high throughput technology. A subset of short interfering RNAs (siRNAs) of viral origin was identified by BLASTN analysis of the source library. Viral (v)-siRNAs were homologous to genomic sequences of the foceavirus Grapevine rupesstris stem-pitting associated virus (GRSPaV), the maculavirus Grapevine fleck virus (GFkV) and Grapevine red globe virus (GRGV), the marafiviruses and Grapevine asteroid mosaic associated virus (GAMaV), and the nepovirus Grapevine fanleaf virus (GFLV). The presence of all these viruses was confirmed by RT-PCR analysis and by direct sequencing of virus-specific amplicons. v-siRNAs were dominated by 21 and 22 nt species spanning the entire length of GRSPaV genome or being discontinuously distributed throughout the GFkV genome. v-siRNAs derived from positive and negative viral RNA strands of RSPaV or, abundantly, from negative viral RNA strand of GFkV.

HARMONIZATION AND VALIDATION OF DIAGNOSTIC PROTOCOLS FOR THE DETECTION OF PLUM POX VIRUS. G. Pasquini1, P.A. Bianco2, D. Boscia3, P. Casati2, M. Digiaro4, F. Faggioni5, F. Palmisano5, C. Poggi-Pollini5, C. Rubies5 and M. Barbara1. 1Centro di Ricerca per la Patologia Vegetale, CRA, Via C.G. Bertero 22, 00156 Roma, Italy. 2Dipartimento di Produzione Vegetale, Sezione di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. 3Istituto di Virologia Vegetale del CNR Unità Organizzativa di Bari, Via Amendola 163/A, 70126 Bari, Italy. 4Istituto Agronomico Mediterraneo, Via Ceglie 9 70010 Valenzano (BA), Italy. 5Dipartimento di Scienze e Tecnologie Agroalimentari, Università degli Studi, Viale Faini 40, 40127 Bologna, Italy. E-mail: gvezziella.pasquini@entecra.it

Plum pox virus is one of the most detrimental pathogens of stone fruit trees, largely spread in many areas throughout the world. It is included in the EPPO A2 quarantine pathogens list and must be submitted to the international and national quarantine requirements. The high importance of the harmonization of the diagnostic procedures, the contribution to greater transparency during the diagnosis of regulated pests and the need of the resolution of disputes among trading partners suggested, in the framework of the Project ARNADIA financed by the Italian Ministry of Agriculture, to set up a validated diagnostic protocol, officially approved and published at the national level. Different diagnostic methods, protocols and reagents were compared in five different laboratories, using the same target and non-target reference samples. ELISA, RT-PCR and real time RT-PCR protocols were selected and applied to determine their performance characteristics for validation under the standard ISO 17025. Sensitivity was determined performing experiments with seven serial dilutions of sample extracts for serological analysis and of total RNA extracts for molecular analysis. Specificity was determined assaying different infected samples, representative of the genomic and geographical variability of PPV and non-target samples. Reproducibility was assessed through performance of the experiments by different laboratories. Repeatability will be established in the frame of a ring test among Italian phytosanitary laboratories. Results showed that the accuracy and sensitivity of ELISA and RT-PCR are comparable, whereas real time RT-PCR is recommended for testing symptomless samples.

INTERACTION AMONG MYCOTOXIN PRODUCING FUNGI INVOLVED IN FUSARIUM HEAD BLIGHT IN EMILIA ROMAGNA. A. Prodi1, S. Toni1, P. Nipoti2, D. Pancaldi2, U. Hetwer3, P. Karlovsky4 and A. Pisi5. 1Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum, Università degli Studi, Viale Faini 40, 40127 Bologna, Italy. 2Dipartimento di Protezione e Valorizzazione Agro-alimentare, Alma Mater Studiorum, Università degli Studi, Viale Faini 40, 40127 Bologna, Italy. 3Department of Crop Sciences, Molecular Phytopathology and Mycotoxin Research Unit, University of Goettingen, Grisebachstrasse 6, 37077 Goettingen, Germany. E-mail: antonio.prodi@unibo.it

Fusarium head blight (FHB) of wheat is a very insidious disease caused by several fungal species prevalently belonging to the genus Fusarium. In Italy it has been consistently present since 1995. The most frequent species of Fusarium in wheat ears are F. graminearum and F. culmorum, but the composition of Fusarium population associated with this disease in the Emilia Romagna (northern Italy) has changed during the years. Our study has revealed the increased frequency of other Fusaria such as F. poae. Fusarium species involved are responsible for yield losses and decrease in grain quality, including the accumulation of mycotoxins detrimental to humans and animals. F. graminearum strains were examined using specific primers for chemotypes based on the production of the trichothecenes, deoxynivalenol (DON) (15ADON and 3ADON) and nivalenol (NIV). F. poae strains are also able to produce an extensive range of mycotoxins. In this work we found F. poae strains able to produce T-2 toxin, which is the most dangerous among all trichothecenes of A and B classes, and its derivative HT-2. The aim of the present work was to characterize Fusarium strains for their ability to produce different mycotoxins and to test their interaction with experimental trials, for controlling FHB and limiting mycotoxin contamination of grains.

c-TERMINAL DOMAIN OF HEL PROTEIN FROM ARABIDOPSIS HAS POTENT ANTIMICROBIAL AND RIBONUCLEASE ACTIVITIES. S. Proietti1, M.P. Aleandri2, L. Bertini3, G. Chilosi2, C. Caporale1 and C. Caruso1. 1Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 2Dipartimento di Protezione delle Piante, Università della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: s.proietti@unitus.it

The ability of plants to cope with abiotic and biotic stresses is essential for their survival. During millions of years, plant evolved defensive strategies to protect themselves against invading pathogens. Disease resistance often depends on whether the plant is able to recognize the pathogen early in the infection process.
Recognition of pathogen triggers a coordinate activation of a specific set of genes encoding pathogenesis-related proteins (PRs), the final shot in the plant’s arsenal against the invader. At present, a large number of PR proteins have been characterized and grouped into 17 families but, although the key role of PR proteins on plant defence responses is clear, more intriguing is the elucidation of their mechanism of action. We have focussed our attention on the functional characterization of a PR4 protein in wheat (Triticum aestivum) and in Arabidopsis thaliana. In wheat, we isolated a PR4 protein of class II (wheatin1) and recently we correlated its antifungal properties with ribonuclease activity producing mutants affecting specific amino acids of the active site. We also isolated the ortholog gene in Arabidopsis thaliana, coding a PR4 protein of class I named HEL that is overexpressed after biotic and abiotic stresses. The recombinant C-terminal domain, expressed in E. coli showed stronger antifungal and ribonuclease activity than wheatin1. Functional proteomic studies are in progress to identify putative partners of HEL protein whose involvement in a protein complex would be strongly suggestive of its biological function.

PEROXISOME FUNCTIONALITY, LIPID METABOLISM AND OXIDATIVE STRESS AFFECT AFLATOXIN BIOSYNTHESIS AND DEVELOPMENT IN ASPERGILLUS FLAVUS.

M. Punelli1, C.A. Smith2, A. Ricelli3, F. Pinzari4, G. Cardinali5, N. Aspite6, S. Russo1, G.A. Payne6, A.A. Fabbri1, C. Fanelli1 and M. Reverberi1, 1Dipartimento di Biologia Vegetale, Università “Sapienza”, Largo Cristina di Svezia 24, Roma, Italy. 2Oklahoma State University, 900 N Portland Ave, Oklahoma City, OK 73107, USA. 3Istituto di Chimica Biomolecolare del CNR, Piazzale Aldo Moro 5, 00185, Roma, Italy. 4Ministero dei Beni Culturali, Via Micanio, 00184 Roma, Italy. 5Istituto Festoterapeutico Ospitaliero S. Gallicano, Via Elio Chianesi 53, 00144 Roma, Italy. 6North Carolina State University, Raleigh, NC 27695, USA. E-mail: marta.punelli@uniroma1.it

Oxidative stress occurs in all the organisms during metabolic pathways such as β-oxidation of fatty acids. In aflatoxicogen fungi, this process is able to create the two main pre-requisites for aflatoxin synthesis, i.e. acetyl-CoA and an hyper oxidant status of the cell. The β-oxidation of long chain fatty acids in fungi occurs in peroxisomes, which also have a role in the scavenging abilities of the fungal cell. In order to demonstrate a correlation between peroxisome functionality, lipid metabolism, cell redox balance and aflatoxin synthesis, a gene of Cymbidium ringspot virus encoding a protein, p33, able to induce peroxisome proliferation, was inserted in A. flavus NRRL 3357. Afp33GFpΔRRED presents an up-regulation of the lipid metabolism and of the TCA cycle which probably leads to a hyper oxidant status (higher ROS and 9-oxypins formation) in the cell. These events trigger aflatoxin biosynthesis both in vitro and also when Afp33GFpΔRRED was inoculated on its natural host, i.e. in maize seeds. How peroxisome proliferation, lipid oxidation and oxidative stress, are able to modulate aflatoxin biosynthesis? In silico N_SITE software analysis of the promoter region of the aflatoxin regulator aflR indicates the presence of regulatory elements (RE) responsive to SREBP-ADD1, AP1, and PPARα binding factors which are involved in the modulation of lipid metabolism, oxidative stress and peroxisome proliferation in animals. A scenario emerges in which the creation of an oxidative cell environment and the generation of signal molecules (e.g. oxypins), related to peroxisomes activity, are able to modulate aflatoxin synthesis by affecting aflR transcription.

APPLICATION OF CAPILLARY ELECTROPHORESIS-SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS FOR THE CHARACTERIZATION OF CITRUS TRISTEA VIRUS STRAINS. D. Raspagliesi1, S. Rizza1, A. Lombardo2 and A. Catar1, 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Laboratorio di Diagnosi e Biotecnologie Fitosanitarie, Parco Scientifico e Tecnologico della Sicilia, Blocco Palma I, Z1, 95030 Catania, Italy. E-mail: srizza@unict.it

A new fluorescence-based Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) protocol was developed for characterizing Citrus tristeza virus (CTV) strains. The migration in the capillary is dependent on DNA folding. Thus, abnormal folding due to a mutation in the tested fragment is detected as a different peak pattern in the electropherogram. The protocol was set up on three single SY-CTV isolates found in Sicily (S29, TDV, Tapi), and a fourth one (a mixture of two isolates) from abroad (C3), and validated with several field isolates from different citrus areas of Sicily. All isolates were previously indexed on a standard panel of citrus indicators, showing a different biological response as well as different profiles in conventional SSCP electrophoresis. CE-SSCP analysis of the PCR-amplified p18 gene, allowed to clearly distinguish the four isolates by their characteristic peak patterns: i.e. two distinctive profiles for S29 and C3, and the same profile for TDV and Tapi. Moreover, when separately analyzed, the two C3 components showed two different profiles, one of which close to S29. According to these results the method, never applied to plant viruses, appears to be a very powerful tool for CTV characterization. Simple to use and highly sensitive, this technique gives reproducible results and permits to process a large number of samples at once. Furthermore, it allows to analyze fragments of over 300 bp, to test different temperatures, to have an internal control, to identify very small mutations, and to distinguish each strand of the DNA fragment thanks to the labelling with different fluorophores.

CONSTRUCTION AND ANALYSIS OF GENOMIC, FULL-LENGTH CLONES FOR THE STUDY OF THE MECHANISMS INVOLVED IN THE PLANT-BENYVIRUS INTERACTION. C. Ratti1, M. D’Alonzo2, M. Nàger Grifo1, E. Uberegui Bernad1, D. Gilmer2 and C. Rubies Autonell3, 1Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Università degli Studi, Viale G. Fanin, 40, 40127 Bologna, Italy. 2Institut de Biologie Moleculaire des Plantes du CNRS, Université de Strasbourg, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France. E-mail: claudio.ratti@unibo.it; david.gilmer@bnp-ulp.u-strasbg.fr

Beet soil-borne mosaic virus (BSBMV) and Beet necrotic yellow vein virus (BNYVV) are members of the genus Benyvirinae. BSBMV has been reported only from the United States while BNYVV has a worldwide distribution. Both viruses are vectored by Polygonum betae, possess similar host ranges and particles morphology. These viruses are not serologically related but have similar genomic organizations. Field isolates possess four RNA species but some BNYVV isolates contain a fifth RNA. RNA-1 and -2 are essential for infection and replication while RNA-3 and -4 play important roles in plant and vector interactions, respectively. In mixed infections, BNYVV reduces BSBMV accumulation in both susceptible and resistant cultivars. BNYVV and BSBMV cross-study, exploiting their similarities and divergences, can improve investigation of molecular interactions between sugar beets and benyviruses. cDNA copies from all BSBMV RNAs and BNYVV (type P) RNA-1 and -2 were successfully synthe-
sized and infectivity of the in vitro transcribed RNAs evaluated through rub-inoculation onto Chenopodium quinoa. Recombination experiments demonstrated that BNYVV RNA-1 and -2 or BSBMV RNA-1/BNYVV RNA-2 viral machineries are able to replicate and to encapsidate BSBMV RNA-3 and RNA-4 in planta. The capability of BSBMV RNA-4 to promote BNYVV RNA-1, -2 and -3 transmission through the vector P betae in Beta vulgaris plants, was also assayed. Finally a new form of BSBMV RNA-4 1,733 nt long has been detected and characterised. Two putative ORFs have been identified which encode two proteins of 52 and 13 kDa, respectively.

**PRIMARY INFECTIONS OF PLASMOPARA VITICOLA: FROM BASIC STUDIES TO A MODEL-BASED DECISION SUPPORT SYSTEM.** V. Rossi1, T. Caffi1, S. Giosue2 and R. Bugiani3. 1Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Parnasone 84, 29100 Piacenza, Italy. 2Horta Srl, spin off company of Università Cattolica del Sacro Cuore, Via Parnasone 84, 29100 Piacenza, Italy. 3Servizio fitosanitario regionale, Via di Corticella 133, 40100 Bologna, Italy. E-mail: vittorio.rossi@unicatt.it

Downy mildew of grapevine, caused by Plasmopara viticola, can cause severe yield losses under favourable environmental conditions. To control the disease, growers apply multiple chemical treatments in a preventative manner, some of which are unjustified. Models have been proposed to reduce unnecessary sprays, but none of them is used by growers because of the low accuracy and robustness of the model output. Recently, a weather-driven, mechanistic, dynamic model was developed in Italy. The model, which overcomes problems of the previous models, simulates the development of any oospore cohort during the primary inoculum season, from oospore germination to the appearance of lesions. Developmental steps of this this model include: (i) analysis of the P. viticola-grapevine system; (ii) definition of the lack knowledge in the mechanisms underlying oospore maturation and germination; (iii) execution of experiments to acquire the necessary biological data; (iv) development of the mathematical structure of the model; (v) validation of the model under different epidemiological conditions, i.e., comparison of model output with real data; and (vi) use of the model for scheduling fungicide sprays. The model showed high sensitivity, specificity, and accuracy. Because of a certain proportion of false positive predictions, confidence is higher in predicting non-infections than infections. Use of the model resulted in high levels of disease control in vineyards when the potential for disease was very high (in 2008) or moderate (in 2006). The average reduction in the number of sprays was 54%.

**THE MOLECULAR BIOLOGY OF TWO TOMBUSVIRUS-ASSOCIATED SATELLITE RNAs.** L. Rubino and M. Russo. Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: l.rubino@ba.ivv.cnr.it

Plant virus infections are often associated with subviral RNA molecules, constituted by defective interfering (DI) or satellite (sat) RNAs, which are both totally dependent on helper virus-encoded factors for their replication. DI RNAs are shortened forms of the viral genome and by discontinuities of the viral replicase and deprived of all viral genes required for replication. SatRNAs share little or none sequence homology with the helper virus genome and are dispensable for virus propagation. Three satRNAs associated with members of the genus Tombusvirus have been described so far. They are linear, single-stranded non-coding RNAs c. 600-800 nt in size, with no sequence homology with the helper virus, except for a 50 nt stretch present in the 5' non-coding region of all tombusviral genomes and DI RNAs. The well characterized satRNA associated with Cymbidium ringspot virus (CyRSV) does not attenuate virus-induced symptom expression, is not replicated in protoplasts from transgenic Nicotiana benthamiana plants expressing the viral replicase, and is the target of virus-induced RNA-silencing. By contrast, satB10, a different satRNA associated with natural Tomato bushy stunt virus (TBSV) infections, attenuates symptom expression. We show that the expression of viral replicase proteins in transgenic protoplasts is sufficient for satB10 replication, suggesting that different strategies are deployed for the replication of different satRNAs. We also describe the biological properties of satRNA L, a novel satRNA associated with TBSV. In particular, we show that satRNA L and B10 are both initiators and targets of RNA silencing.

**PLANT GROWTH AND ISR PROMOTION BY TRICHODERMA HARZIANUM T22.** M. Ruocco1, S. Lanzuise1, S.L. Woo2, M. Reverberi2, R. Marra1, V. Aloni2 and M. Lorito3. 1Istituto di Protezione delle Piante del CNR, Unità Organizzativa di Napoli, Via Università 133, 80055 Portici (NA), Italy. 2Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università di Napoli Federico II, Via Università 100 80055 Portici (NA), Italy. 3Università “Sapienza”, Largo Cristoforo di Svezia 24, 00165 Roma, Italy. E-mail: mirucco@unicatt.it

Trichoderma harzianum T22, a fungal antagonist, is one of the most widely used active ingredients in commercial bio-fungicides and bio-fertilizers. It has not only mycoparasitic activity, but can also activate extensive metabolic changes in treated plants, resulting in a systemic resistance to a pathogen attack, thus indirectly altering plant-pathogen interactions. By analyzing the T. harzianum T22 “secretome” a few key “effectors” involved in Trichoderma-plant cross-talk were identified. One of these effectors secreted was a class II hydrophobin HYTRA1. This protein was tested for its antimicrobial activity and its ability to induce a defense response in tomato plants. In in vitro and in vivo assays, HYTRA1 directly inhibited pathogen development. In tomato plants, depending upon the concentration, it induced a multiplicity of effects, HYTRA1 activated oxidative burst, the antioxidant system, and ISR with the accumulation of defence-related compounds important in plant defence. Further physiological effects included the induction of de novo rhizogenesis, and an epinastic phenotype in HYTRA1-transformed tomato. The involvement of HYTRA1 in the T. harzianum molecular cross-talk with the plant was confirmed when HYTRA1 disruptants proved unable to induce root growth promotion and ISR in tomato.

**SPECTORADIOOMETRIC DISCRIMINATION OF CITRUS TRISTEZA VIRUS INFECTED TREES.** F. Santoro, S. Gualano, M. Bouneb, K. Djelouah and A.M. D’Onghia. Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: donghia@iamb.it

The implementation of a large-scale CTV monitoring programme poses major difficulties in terms of result reliability and sustainability. Recent developments in proximal and remote sensing provide opportunities for pest/disease identification in some crops by introducing variation of spectral, biochemical and biophysical properties on a different scale. The main objective of this study was to collect, process and analyze spectral signatures of CTV-infected and CTV-free citrus species grown in Apulia...
(southern Italy). Two trials were made under controlled and field conditions, respectively. In the first, a plant probe and a spectro-radiometer (325-1075 nm) were used to detect the leaf spectral signatures of a population of CTV-free and CTV-infected plants (Mexican lime on Troyer citrange), inoculated with an isolate of CTV-quick decline (IAMB-Q 109), and grown under a climatized greenhouse or a screenhouse. The second trial was conducted in spring-summer in two commercial groves (‘Precoce di Massafra’ clementine and ‘Navelina’ orange), located in a CTV outbreak area, where all plants had been preliminarily tested for virus presence by serological and molecular assays. In both groves, the canopy spectral signatures of 30 plants were collected, half of which were CTV-infected. In the two trials, spectral characterization highlighted a reflectance difference between CTV-positive and CTV-negative plants, thus allowing the development of some vegetation indices (produced by the algebraic combinations of reflected or emitted energy values measured in the different bands of the electromagnetic spectrum) to check the monitoring ability of optical satellite sensors.

**Quercetin and Umbelliferone Modulate the Expression of Penicillium expansum Genes Involved in Patulin Biosynthesis and Reduce Accumulation of the Mycotoxin.** S.M. Sanzani, L. Schena, F. Nigro, A. De Girolamo, and A. Ippolito. 1Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. 2Dipartimento di Scienze Agrarie e Forestali, Università Mediterranea, Località Feo di Vito, 89124 Reggio Calabria, Italy. 3Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. E-mail: ippolito@agr.uniba.it

Infection of pome fruits by *Penicillium expansum* Link, has potential public health significance, since the pathogen produces the mycotoxin patulin, which has immunotoxic and neurotoxic effects in vertebrated. The activity of two phenolic compounds, quercetin and umbelliferone, as single or mixed treatment on *P. expansum* growth and patulin accumulation was assessed by in vitro trials. Although tested phenolics did not consistently affect mycelial growth, they reduced toxin accumulation, particularly when applied in combination (68% reduction). To determine quercetin and umbelliferone mode of action, their effect on gene expression of five enzymes related to patulin biosynthesis was evaluated using quantitative real-time PCR. The involvement of the selected genes in toxin biosynthesis was preliminarily confirmed by amplifying cDNA from a toxigenic and a non-toxigenic strain of *P. expansum*. When the toxigenic isolate was grown on a medium supplemented with quercetin or umbelliferone, the expression of two cytochrome P450 monoxygenases (p450-1 and p450-2) was reduced. Furthermore, higher reduction of gene expression was provided by the combination of the two phenolics, which down-regulated also genes coding for an isoepoxydon dehydrogenase (IDH), a 6-methylsalicylic acid synthase (msas) and an ATP-binding cassette transporter (peab1). Although, other control points may occur in the biosynthetic pathway of patulin, on the whole, these results showed with a reasonable degree of certainty that both quercetin and umbelliferone reduce *P. expansum* patulin accumulation by acting on genes transcription.

**Horizontal Transfer of the Ophiostoma Gene Encoding Cerato-Ulmin into Unrelated Species of the Genus Geosmithia.** A. Scala, L. Bazzichi, R. Bernardi, G. Cappugi, L. Carresi, D.O. Cicero, C. Comparini, F. Martelli, L. Pazzaglia, T.A. Pertinhez, and A. Spisni. 1Dipartimento di Biotecnologie Agrarie, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 12, 50019 Sesto Fiorentino (FI), Italy. 2Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università degli Studi, Via Matteotti 1/B, 56124 Pisa, Italy. 3Dipartimento di Scienze Biologiche, Università degli Studi di Firenze, Via della Lastruccia 12, 50019 Sesto Fiorentino (FI), Italy. 4Dipartimento di Scienze Chimiche, Università “Tor Vergata”, Via della Ricerca Scientifica 1, 00133 Roma, Italy. 5Dipartimento di Medicina Sperimentale, Università degli Studi, Via Valturio 39, 43100 Parma, Italy. E-mail: anielo.scala@unifi.it

Horizontal gene transfer (HGT) is a relevant evolutionary mechanism by which plant pathogens have emerged in agro-ecosystems over different time scales. In prokaryotes, HGT is generally considered a major factor for the evolution of genomes, whereas in multicellular eucaryotes HGT is assumed to play a minor role. In fungi, HGT has been invoked to justify unusual features of genetic elements such as single genes or gene clusters. Among the few examples of horizontally transferred genes, we can mention those for the biosynthesis of host-selective toxins clustered in *Alternaria alternata*, *Cochliobolus carbonum* and *C. heterosporus*, the pea pathogenicity (PEP) cluster in *Nectria haematococa* and the *toxA* gene from *Phaeosphaeria nodorum* in *Pyrenobora tritici-repent.* In the present work we showed that strains of *Geosmithia pallida* and *G. langdonii* possess and express the *cu* gene coding for the cerato-ulmin (CU) hydrophobin. CU is produced by various *Ophiostoma* species (a taxon distant from the genus *Geosmithia*), and gives the Ophiostomas causing Dutch elm disease key advantages in parasitic fitness and virulence. We are working to understand how and why a portion of the genome of *Ophiostoma novo-ulmi*, including the *cu* gene, has been transferred to *Geosmithia* strains, and which are the advantages for the *Geosmithias* of acquiring this gene, in terms of fungal fitness. Moreover, we are characterizing the CUs produced by the *Geosmithias* and investigating how they behave towards the elms.

**Cerato-Platanins: Non-Catalytic Proteins with Functions in the Relational Life of Fungi.** S.M. Sanzani, I. Bazzichi, R. Bernardi, G. Cappugi, L. Carresi, D.O. Cicero, C. Comparini, F. Martelli, L. Pazzaglia, T.A. Pertinhez, and A. Spisni. 1Dipartimento di Biotecnologie Agrarie, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 12, 50019 Sesto Fiorentino (FI), Italy. 2Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università degli Studi, Via Matteotti 1/B, 56124 Pisa, Italy. 3Dipartimento di Scienze Biologiche, Università degli Studi di Firenze, Via della Lastruccia 12, 50019 Sesto Fiorentino (FI), Italy. 4Dipartimento di Scienze Chimiche, Università “Tor Vergata”, Via della Ricerca Scientifica 1, 00133 Roma, Italy. 5Dipartimento di Medicina Sperimentale, Università degli Studi, Via Valturio 39, 43100 Parma, Italy. E-mail: anielo.scala@unifi.it

Plant pathogenic fungi produce a lot of proteins without catalytic activity, which are involved in various aspects of parasitism and in the development of disease, such as the dissemination of fungi by vectors, the attachment of the fungus to the surface of plant organs, the expression of symptoms, and the elicitation of defense responses. Currently, seven non-catalytic fungal protein families have been identified, including the cerato-platanin family (PF07249), the class I hydrophobins (PF01185), the class II hydrophobins (PF06766), the elicitors (PF00964), the PcF family (PF09461), and the NIP-1 protein family (PF08999). The CFEM family (PF05730) contains a cysteine-rich domain present into
some other fungal proteins for which a role in pathogenesis has been proposed. Since different families may contain proteins with similar functions in plant pathogenesis, and a single family contains proteins with different functions, we wanted to investigate why this happens. To this aim, a study model was developed, represented by two proteins belonging to the cerato-platanin family: cerato-platanin (CP), the founder protein of the family produced by Ceratocystis platani, and cerato-polypin (Pop1) produced by C. populi. Both CP and Pop1 have been purified, cloned in Pichia pastoris and characterized biochemically and functionally. They are well-structured α/β proteins (but with different percentages of the α-helix), and have only 70.1% of identical or highly conserved amino acids. Moreover, they behave as PAMPs, stimulating plants to activate defense responses able to reduce consistently the fungal growth. Their capability to elicit differently defense events is under study.

A FUSARIUM GRAMINEARUM POLYGALACTURONASE GENE IS REQUIRED FOR FULL VIRULENCE DURING WHEAT INFECTION. L. Sella1, F. Giacomelli1, W. Schäfer2 and F. Favaron1. 1Dipartimento Territorio e Sistemi Agro-Forestali, Gruppo di Ricerca in Patologia Vegetale, Università di Pavia, Via dell’Università 16, 35020 Legnaro, Italy. 2Department of Molecular Phytopathology and Genetics, University of Hamburg, Biocenter Klein Flottbek, Hamburg, Germany. E-mail: luca.sella@unipd.it

Fusarium graminearum is the main causal agent of Fusarium head blight (FHB) in cereals. In wheat, FHB causes remarkable yield and quality losses because of mycotoxins accumulation in the infected kernels. F. graminearum is known to produce two endo-polygalacturonases (PGs) in liquid culture and during infection of wheat plants. The role played by these PGs during the infection process has not yet been ascertained and it has been often neglected because graminaceous plant tissues have a cell wall consisting mainly of cellulose and xylan. In order to establish the role of these PGs in pathogenesis, we have disrupted by targeted homologous recombination the pg encoding genes of this fungus. When grown in liquid culture containing pectin as the sole carbon source, the PG activity produced by the ΔPG1 mutant resulted negligible compared to that produced by wild-type and ΔPG2 mutant strains. However, the dry weight of wild-type and of both mutant strains was comparable. The virulence of each mutant has been evaluated by infecting wheat plants. Results indicate that both pg knock-out mutants maintain the capability to infect wheat, although the ΔPG1 mutant shows a significant reduction of virulence compared to the wild-type strain (about 50% less infected spikelets), while no reduction of virulence is observed with the ΔPG2 mutant. Therefore, PG1 can be considered a virulence factor of F. graminearum during spike infection.

GENETIC DIVERSITY OF FUSARIUM OXYSPORUM POPULATIONS ISOLATED FROM WHEAT FIELDS. S. Somma1, S. Sarrocco2, F. Rossi2, A. Moretti2 and G. Vannacci2. 1Istituto di Scienza delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. 2Istituto di Coltivazione e Difesa delle Specie Legnose “G. Scaramuzzo”, Università degli Studi, Via del Borgoletto 80, 56124 Pisa, Italy. E-mail: stefania.somma@ispacnr.it

Fusarium head blight (FHB) is one of the most important wheat diseases causing yield and quality losses as well as grain contamination with deoxynivalenol (DON). Different approaches can be devised to prevent pathogen growth and mycotoxins contamination. Biological control using microorganisms able to reduce the initial inoculum of the pathogen, by competition for cultural debris, seems to be a promising strategy. Fusarium oxysporum complex (FOC) includes both pathogenic and non-pathogenic isolates, the latter often proposed for biocontrol programmes. Although non-pathogenic populations are ubiquitous and represent one of the major components of soil microflora, little is known about the size and genetic variation of their populations. Aim of this work was to study the genetic variability of 71 F. oxysporum isolated from three different soils, all with a previous history of wheat cultivation, using as baits wheat straw treated or not with DON. Based on the translation elongation factor (TEF) and Amplified Fragment Length Polymorphism (AFLP), strains grouped into four main clades, independently of the soil of origin and of the presence of DON in the baits. AFLP analysis showed a high degree of genetic variability and clustering (bootstrap >81%) was confirmed by the TEF sequence-based tree. AFLP similarity coefficient among the four clusters was around 50%, suggesting that the four sub-populations could represent different taxa. These results offer interesting phylogenetic and evolutionary information about these populations and put the basis for further research aimed at detecting potential antagonists for FHB biocontrol.

MOLECULAR STRATEGIES FOR THE IDENTIFICATION OF ASPERGILLUS SPECIES IN VINEYARD. D. Spadaro1, S. Patharajan2, M. Karthikeyan2, A. Lore2, A. Garibaldi2 and M.L. Gullino2. 1Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia Vegetale, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. 2Centro di Competenza per l’Innovazione in Campo Agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it

Ochratoxin A (OTA), produced by different species of Aspergillus, is a mycotoxin particularly dangerous for its nephrotoxic activity. The presence of black aspergilla, producing OTA in the grape bunches, is a key factor in the period between veraison and harvest. Different molecular strategies have been set up to identify the ochratoxigenic fungi. Internal transcribed spacers (ITS) and translation elongation factor (TEF) and Amplified Fragment Length Polymorphism (AFLP) based restriction fragment length polymorphism (ITS-RFLP) was carried out on different Aspergillus species. Restriction endonuclease digestion of ITS products distinguished five different RFLP patterns corresponding to A. niger, A. carbonarius, A. tubingensis, A. japonicus and A. aculeatus. Black aspergilla were tested for their ability to produce OTA. Most of the OTA-producing isolates were A. carbonarius. In addition, species-specific primers CARBO1/CARBO2 were used to confirm the A. carbonarius isolates. A polyketide synthase (PKS) gene of A. carbonarius was isolated, cloned and sequenced. The nucleotide sequence data showed high homology to PKS domain of other A. carbonarius strains. The sequence data were used to design a new set of primers in order to identify the OTA producers of A. carbonarius. The PCR results showed that the primers specifically detected A. carbonarius strains and did not amplify strains belonging to other species of Aspergillus spp. The methods described in this study represent rapid and reliable procedures to identify black Aspergillus species isolated from grapes and, in particular, OTA producers of A. carbonarius.

MICROSATELLITE MARKERS FROM GENOME SEQUENCES FOR PHYTOPHTHORA INFESTANS POPULA-
TION ANALYSIS. A. Testa1, Y. Li2,4, F. Govers3, O. Mendes1 and T.A.J. van der Lee4. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Scuola di Scienze Biotecnologiche, Università degli Studi “Federico II”, Via Università 100, 80055 Portici (NA), Italy. 2Institute of Vegetables and Flowers, China Academy of Agricultural Sciences, Beijing, PR China. 3University of Wageningen University, P.O. Box 9101, 6700 HB Wageningen, The Netherlands. 4Plant Research International, P.O. Box 16,6700 AA Wageningen, The Netherlands. E-mail: antonino.testa@unina.it

Over 300 non redundant SSR markers were identified by bioinformatic tools. Simple sequence repeats were identified in genomic and EST sequences of Phytophthora infestans, the causal agent of potato late blight. Primers were developed and tested on a reference set of P. infestans isolates. Finally, eight most polymorphic and robust scoring SSR markers were assembled into two multiplex sets using fluorescent primers. These microsatellite markers were used for monitoring population diversity of P. infestans isolates in several parts of the world. In a survey of Dutch late blight populations from 2004 to 2007, nearly 500 fungal isolates were collected from commercial potato crops. Analysis of these eight polymorphic SSRs among Dutch isolates revealed clonal lineages. Multilocus analysis confirmed the clonality of this pathogen in the Netherlands. The genetic structure of Dutch isolates was of intermediate complexity, which included multiple, closely related genotypes. The analysis of population migration in diverse continents, emphasized the role of commercial plant trade in the movement of this pathogen. Objective of this work was to generate SSR markers that would allow efficient monitoring of worldwide populations of P. infestans.

MULTIPLE DETECTION AND GENOTYPING OF TOMATO VIRUSES BY MICROARRAY TECHNOLOGY. A. Tiberini1,3, L. Tomassoli1, A. Ferrari2 and M. Barba1. 1CRA. Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. 2Dipartimento Biotecnologie, Università degli Studi, Unità LATEMAR, Strada Le Grazie 15, 37134 Verona, Italy. 3Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. E-mail: antonio.tiberini@entecra.it

Tomato (Solanum lycopersicum L.) is affected by several pathogens that induce significant economical losses. Italy is the most important producer of this crop among the European Union countries with a total cultivated area of 118,224 ha. Therefore, the detection and identification of viruses and their strains affecting tomato crop is of critical importance for quarantine and certification programs. We have developed a genomic strategy, based on a DNA microarray chip using “CombiMatrix” platform 40-mer oligonucleotide probes, for screening the most economically important tomato-infecting viruses from plant tissue or biological samples, so as to facilitate their detection and genotyping. A total of 539 probes were designed for the following viruses, including their strains: Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Pepino mosaic virus (PepMV), Tomato virus Y (TYV), Tomato infectious chlorosis virus (TICV), Tomato chlorosis virus (ToCV), Tobacco mosaic virus (TMV) and Tomato mosaic virus (ToMV), Tomato yellow leaf curl virus (TYLCV) and Tomato yellow leaf curl Sardinia virus (TYLCV-S). Each sample, in single and/or in mixed infection, was tested using two different target preparation protocols. Most of the specific 40-mer oligonucleotide probes used were able to detect and genotype the considered virus, regardless of the use of different target preparation protocols. This microarray-based detection method, besides allowing simultaneous virus detection and genotyping, proved to be easy to handle and economically convenient because the chip can be re-used at least 8 times.

ROLE OF SOME EFFECTOR GENES IN DISCRIMINATING PSEUDOMONAS SYRINGAE pv. TOMATO STRAINS WITH WIDE AND NARROW HOST RANGE. M. Zaccardelli1, F. Campanile1 and B.A. Vinatzer2. 1CRA, Centro di Ricerca per l’Orticoltura, Azienda e Laboratori di Battipaglia, Strada Statale 18 n° 204, 84091 Battipaglia (SA), Italy. 2Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA, USA. E-mail: massimo.zaccardelli@entecra.it

Effector genes encode proteins secreted into plant host cells through the Type III secretion system. Their role is the suppression of host defenses. DNA analyses performed on Pseudomonas syringae pv. tomato (Pto) strains, able to infect only tomato (Pto “narrow host range”) and on Pto strains able to infect also crucifers (Pto “wide host range”), showed some differences in presence/absence or in the sequence of the effector genes avrPto, hop A1, hop AM1, hop A1, hop B1 and hop M1. Fourteen Pto isolates with narrow or wide host range were analysed for these effector genes, in comparison with isolates of Pseudomonas syringae pv. maculicola (Pma), able to infect crucifers. AvrPto was present in all Pto and Pma strains, except for one strain of Pma, whereas avrPto2 was absent in a Pto narrow host range strain and in all Pma strains. Sequence analyses showed stop codons in the sequence of hop A1, hop B1 and hop M1, only in Pto narrow host range strains, whereas no mutations were observed in the same effector genes in wide host range Pto and Pma strains. Further investigations regarding the role of the mutations (presence of stop codon) in hop A1, hop B1 and hop M1, are in progress.