**LAMP-BASED DETECTION OF *XANTHOMONAS CAMPESTRIS* pv. *CAMPESTRIS* IN BRASSICA PLANTS AND SEEDS.** **G.R. Quintero Macías[1], G. Stampone[1], S. Panno[2], S. Davino[2], S. Drago[1], V. Catara[3], P. Bella[2].** *[1]Enbiotech S.r.l., Via Aquileia 34, 90144 Palermo, Italy. [2]Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze Ed. 5, 90128 Palermo, Italy. [3]Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. E-mail: patrizia.bella@unipa.it*

*Xanthomonas campestris* pv. *campestris* (*Xcc*) is the causal agent of black rot, a severe seed-borne disease of *Brassicaceae*. Other two closely related pathovars, *X. campestris* pv. *raphani* and *X. campestris* pv. *incanae* are responsible for a leaf spot and bacterial blight disease on brassica species. In this study, a new LAMP protocol was developed for the identification and detection of *X. campestris* pv. *campestris* and related pathovars based on the ICGENE technology (Enbiotech, Palermo) characterized by ready to use and temperature stable reagents and the portable ICGENE mini device. A set of six primers were designed on the *ndvB* gene which encodes a glycosyltransferase required for cyclic glucan synthesis and involved in the virulence of *Xcc*. Positive amplifications were obtained from a collection of *Xanthomonas campestris* pathovars isolated from different brassica species in different geographical location and including strains of different *Xcc* races. No target DNA amplification was obtained from other xanthomonads. A rapid DNA extraction protocol at 65°C for 10 min coupled with the LAMP assay in the ICGENE mini device allowed to detect the bacterium from artificially inoculated plant tissues and spiked seed extracts in about 40 min. The detection limit of the LAMP assay was the same both for pure bacteria culture and spiked seed extracts indicating that the assay is not affected by plant inhibitors. The ICGENE technology represents a rapid, sensitive and cost-effective tool for detecting *Xcc* by LAMP from cultures and from plants. In addition, results suggest its application in the seed testing protocol.

**PATHOGENICITY ASSESSMENT OF DIFFERENT *PLECTOSPHAERELLA* SPECIES ON BASIL, PEPPER AND TOMATO CROPS.** **M.L. Raimondo, A. Carlucci.** *Università degli Studi di Foggia SAFE, Via Napoli 25, 71122 Foggia, Italy. E-mail: antonia.carlucci@unifg.it*

*Plectosphaerella* species have been isolated in many countries from different hosts such as tomato, sunflower, soybean, melon, pumpkin and other cucurbits, endive and rocket, and Lamb lettuce. The most common and known species of *Plectosphaerella* is *Pa. cucumerina*, which was reported as a pathogen and endophyte from different horticultural crops as well as a biological agent to control of *Galium spurium*, *Sagittaria trifolia* and nematodes of potato. To date *Plectosphaerella* genus consists of 11 species such as *Pa. alismatis*, *Pa. citrulli*, *Pa. cucumerina*, *Pa. delsorboi*, *Pa. melonis*, *Pa. oligotrophica*, *Pa. oratosquillae*, *Pa. pauciseptata*, *Pa. plurivora*, *Pa. populi* and *Pa. ramiseptata*. To ascertain the role that these fungi play in diseases of horticultural crops, nine *Plectosphaerella* species were artificially inoculated on three different hosts (basil, pepper and tomato) to perform pathogenicity tests *in vitro* (detached lives) and *in vivo* (young 30-day-old plants). These tests were carried out in a greenhouse with an experimental design consisting of two independent batches. Each host per isolate combination was replicated five times. The individual disease severity was assessed 15-30 days post inoculation on leaves, roots and collars showing symptoms. Pathogenicity tests demonstrated that except for *Pa. oratosquillae*, all *Plectosphaerella* species tested are able to cause symptoms on all hosts essayed with different levels of disease severity. *Pa. paucisepatata* and *Pa. plurivora* showed a vascular behaviour while the others seven species had a parenchymatous behaviour. *Pa. ramiseptata* proved to be the most pathogenic species to the three hosts.

**ACTIVITY OF POLYSACCHARIDES EXTRACTED FROM *ECKLONIA* sp. AND *JANIA* sp. AGAINST *BOTRYTIS CINEREA*.** **H. Righini[1], E. Baraldi[1], A. Martel-Quintana[2], Y. García-Fernández[2], C. Pérez-Reyes[2], R. Roberti[1].** *[1]Dipartimento di Scienze Agrarie, Alma Mater Studiorum, Università di Bologna. [2]BEA-Banco Español de Algas, Universidad de Las Palmas de Gran Canaria (ULPGC), Las Palmas, Canary Islands, Spain. E-mail: roberta.roberti@unibo.it*

Algae extracts contain several bioactive compounds, such as polysaccharides, well known to be elicitors of plant defence responses, but few information are available on their antifungal activity. The aim of this research was to investigate the effect of cationic polysaccharides extracted from two macroalgae, *Ecklonia* sp. (*Ochrophyta*) and *Jania* sp. (*Rhodophyta*) against *Botrytis cinerea in vitro* and *in vivo* on strawberry. For the *in vitro* assay, fungal colony portions were treated by immersion in polysaccharides aqueous concentrations, 1.65, 0.82 and 0.41 mg/ml for *Ecklonia* sp. and 0.18, 0.09 and 0.045 mg/ml for *Jania* sp. After 6 h of incubation, the colony growth was measured daily. *Ecklonia* sp. polysaccharides significantly inhibited *B. cinerea* growth by 21% (1.65 mg/ml) and 23% (0.82 mg/ml) two days after treatment. *Jania* sp. polysaccharides did not inhibit fungal growth. For the biological assay, strawberry ripe fruits, cv. Cristal, were treated before or after harvesting, by immersion in polysaccharide aqueous solutions of the two algae (0.82 and 0.41 mg/ml for *Ecklonia* sp. and 0.09 and 0.045 mg/ml for *Jania* sp.). *Botrytis cinerea* was inoculated by spraying fruits with spore suspension ($1 \times 10^5$ spore/ml) 24 h after treatment. Disease symptoms were scored as percentage of infected area. Pre-harvest treatment with *Jania* sp. reduced disease symptoms by 100% at 0.09 mg/ml and of 50% at 0.045 mg/ml and with *Ecklonia* sp. by 17% (0.82 mg/ml) and 11% (0.41 mg/ml). No inhibition of disease symptoms was obtained in post-harvest treatment.

**ELUCIDATION OF THE MECHANISM OF ACTION OF ESSENTIAL OILS TO CONTROL POSTHARVEST DISEASES OF APPLES AND PEACHES.** **K. Santoro[1,2], D. Spadaro[1,2], A. Garibaldi[2], M.L. Gullino[1,2].** *[1]DISAFA - Dipartimento di Scienze Agrarie, Forestali ed Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italy. [2]Centro di competenza per l'Innovazione in campo agro-ambientale (AGROINNOVA), Università degli Studi di Torino, Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Essential oils are considered a powerful and natural resource to control postharvest pathogens of pome and stone fruits. The efficacy of these natural products has been deeply investigated *in vitro* but only few of them are applied *in vivo*. Essential oils can be applied in different ways, by dipping or spraying the fruit surface. Their high volatility permits the application also by fumigation, which is preferable because of the lack of contact with the fruit. Thyme and savory essential oils were successfully applied through biofumigation at 0.5% and 0.1% against brown rots on nectarines and peaches. The most effective components of thyme and savory essential oils are thymol and carvacrol, respectively. The antimicrobial activity of essential oils, useful to control fungal pathogens, could be due to a synergy of chemical components. In addition to direct inhibition of pathogen growth, essential oils can induce resistance in the fruit host. Thyme essential oil can promote the expression of the pathogenesis related gene PR-8 in apple, which is involved in host defense response. Moreover, essential oils showed a positive role in slowing down senescence processes reducing

weight loss and preserving vitamin C and carotenoid content during storage.

**MICROCANTILEVER RESONATORS FOR OCHRATOXIN A DETECTION IN FOOD SAMPLES.** **K. Santoro[1,3], D. Spadaro[1,2], M.L. Gullino[1,2], C. Ricciardi[3].** *[1]Università degli Studi di Torino, DISAFA, Largo Braccini 2, 10095 Grugliasco (TO), Italy. [2]Università degli Studi di Torino, Centro AGROINNOVA, Largo Braccini 2, 10095 Grugliasco (TO), Italy. [3]Politecnico di Torino, DISAT, Corso Duca degli Abruzzi 24, 10129 Torino (TO), Italy. E-mail: davide.spadaro@unito.it*

An innovative and rapid detection method based on microcantilever resonators for ochratoxin A (OTA) detection in food matrix was developed. The harmful effects of OTA on human and animal health lead to develop and optimize highly sensitive, fast and accurate methods for OTA detection. Ochratoxin A can contaminate a wide number of foodstuffs during postharvest representing a serious threat to human health. Microcantilever resonator arrays could effectively identify OTA at low concentrations (less than 6 ng/ml), with relatively low uncertainty (about 10%) and good reproducibility for the same target concentration. Furthermore, the developed immunosensing method showed limited cross-reactivity to different mycotoxins, paving the way to a highly specific technique, able to identify different mycotoxins in the sample. The microcantilever technology was tested in different food matrices, to detect OTA in grape juice, green coffee and red wine with high sensitivity and reproducibility. This work demonstrates the possibility to apply microcantilever technology in food safety field, developing an innovative biosensing platform able to detect OTA with high sensitivity and reproducibility.

**IMPROVEMENT OF QUADRUPLEX TAQMAN REAL TIME METHOD TO SCREEN THE PRESENCE OF *XANTHOMONAS VESICATORIA*, *X. EUVESICATORIA*, *X. PERFORANS*, *X. GARDNERI* IN TOMATO SEEDS.** **A. L'Aurora, V. Scala, N. Pucci, S. Loreti.** *Council for Agricultural Research and Economics, Research Center for Plant Protection and Certification, Roma, Italy. E-mail: stefania.loreti@crea.gov.it*

Bacterial spot of tomato, a major problem in many tomato production areas, is caused by *Xanthomonas vesicatoria*, *X. euvesicatoria*, *X. perforans* and *X. gardneri*. In the frame of the project ASPROPI financed by the Italian Ministry of Agricolture and Forestry, we investigated the possibility to validate a new protocol for the preliminary screening of *Xanthomonas vesicatoria*, *X. euvesicatoria*, *X. perforans* and *X. gardneri* in tomato seeds. In order to detect the bacterial spot pathogens, the region of *hrpB* operon was evaluated as target for a quadruplex real-time polymerase chain reaction (PCR). The PCR products are highly conserved within each species, with a single-nucleotide polymorphism (SNP) among bacterial spot of tomato agents. Four probes and two primers were employed to detect the four bacterial spot pathogens simultaneously. The optimized quadruplex assay was assessed for analytical specificity and sensitivity showing good performance criteria. The new protocol was validated within a test performance study (TPS) and compared with the already available diagnostic methods. Seven different laboratories of the Plant Protection Services participated to the TPS to verify the reproducibility of the tested method. The obtained results showed that this method holds great potential as a diagnostic tool for the detection of each bacterial spot pathogen from seed tomato matrix, and also for the identification of *Xanthomonas*-like pure cultures.

**NEW CLASS OF LIPID COMPOUNDS IN *XYLELLA FASTIDIOSA* STRAIN CoDiRO.** **V. Scala[1], N. Pucci[1], S. Lucchesi[1], A. L'Aurora[1], M. Ludovici[2], M. Reverberi[3], S. Loreti[1].** *[1]Council for Agricultural Research and Economics, Research Center for Plant Protection and Certification, Roma, Italy. [2]Laboratory of Cutaneous Physiopathology and Integrated Center of Metabolomics, San Gallicano Dermatologic Institute (IRCCS), Rome, Italy. [3]Department of Environmental biology, Sapienza University of Rome, Italy. E-mail: valeria.scala@crea.gov.it*

Modulating signals involved in plant-pathogen interaction represent a powerful mean to develop innovative and sustainable approaches to control plant pathogens. We investigated some of these signals, *i.e.* oxylipins in *Xylella fastidiosa* CoDiRO strain (variant "sequence type 53"), associated with the olive quick decline syndrome. During plant-pathogen interactions, lipids have different roles, as pathogen perception, signal transduction and downstream defence responses. The composition of the bacterial membrane is not constant but depends on the environmental conditions to which the cells are exposed. Oxidized fatty acids are an important class of signalling molecule especially related to stress responses. Recently, other authors reported that oxylipins have a regulation activity in motility, biofilm formation and virulence of *Pseudomonas aeruginosa*. In the frame of the Xf-actors project we explored the oxylipin signals of *X. fastidiosa* subsp. *pauca* CoDiRO strain CFBP8402 in pure culture and during the interaction with the model plant *Nicotiana tabacum* "Petite Havana SR1". The analyses were performed by LC-MS/MS in dynamic MRM modality allowing quantification of oleic, linoleic and linolenic acid-derived oxylipins. The results showed the presence of oxidized fatty acids in *X. fastidiosa* CoDiRO strain in pure culture and in inoculated tobacco plants.

**DEEP SEQUENCING OF TWO *CITRUS TRISTEZA VIRUS* ISOLATES CROSS PROTECTIVE AGAINST HOMOLOGOUS SEEDLING YELLOWS-VT STRAIN.** **G. Scuderi[1], M. Russo[1], R. Ferraro[2], M.C. Bazzano[1], O.F. Giarrusso[1], A. Catara[2], G. Licciardello[1].** *[1]Agrobiotech, ZI Blocco Palma I, Via V. Lancia 57, 95121 Catania, Italy. [2]Parco Scientifico e Tecnologico della Sicilia, ZI Blocco Palma I, Via V. Lancia 57, 95121 Catania, Italy. E-mail: glicciardello@agrobiotech.it*

A wide indexing and genotyping of VT-like *Citrus tristeza virus* (CTV) in Sicily has identified some isolates that are potentially cross-protective (CP) on sour orange against the prevalent seedling yellows (SY) isolate SG29, but retain the stem pitting (SP) phenotype on grapefruit. As a result of bioindexing they have a biotype 10 instead of severe SY associated to biotype 4. Deep sequencing of two candidate CP isolates and alignment of their full genomes against the challenger SG29 (KC748392) showed 13 and 14 point mutations, respectively, including 5 and 6 silent mutations. No evidence of recombination and/or additional strains was present. Interestingly, eight changes were shared in the same position and three of them were located within *p33* (positions 11490, 11585, 11756 nt), and one in *p23* (18508 nt). The two isolates differed by nine nucleotides, three within *orf1A* (position 486, 3208, 4780 nt), one in *p33* (positions 11721 nt), three in the intergenic region *p33-p6* (11790, 11791, 11792 nt), one in *p25* (position 16316 nt) and one in *p18* (position 16970). While the precise genomic events that have led to the mutation changes remain to be established, the results show that: (i) the "superinfection exclusion" conditions for cross protection predicted for T36 strains attain also to VT strain; (ii) it may occur "naturally" in the field, eventually from recovered plants and aphid spread; (iii) the genetic determinants of SP phenotype are different from those of SY; (iv) the search for cross protective mutants needs a mandatory genotyping and a phenotype analysis on specific indicators.