

OPENING LECTURE

PLANT PATHOLOGY IN LOMBARDY: INITIAL STEPS AND SUBSEQUENT DEVELOPMENTS. G. Belli¹, A.M. Picco², S. Quaroni¹. ¹Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano. ²Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Via S. Epifanio 14, Pavia. Fax: +39.02.70631287; e-mail: giuseppe.belli@unimi.it.

We can say that Plant Pathology was initiated in Lombardy (northern Italy) by a small group of mycologists which used to meet, during the years 1830-1837, in Milano to discuss their studies (mainly microscope observations) on new fungal species. Among those mycologists it is worth mentioning Giuseppe De Notaris, Giuseppe Balsamo Crivelli, Carlo Vittadini, Vincenzo Cesati, Antonio Venturi and, especially, Agostino Bassi who, in 1835, provided experimental evidence that a disease of the silkworm ("Mal del calcino") was caused by a fungus (*Bauveria bassiana*). This finding was of great significance because, at that time, many scientists maintained that diseases originated always inside the organism. In 1858, De Notaris founded the "Società Crittogamologica" that can be regarded as the first society of Italian mycologists. In 1871, Santo Garovaglio, professor of Botany, founded in Pavia the "Laboratorio Crittogamico" that, for about 100 years, has been an active center for research and an outstanding school for plant pathologists. In 1892, Augusto Napoleone Berlese, together with his brother Antonio, initiated in Padova the publication of the "Rivista di Patologia Vegetale", the oldest Italian phytopathological journal. In 1901 A.N. Berlese became Professor of Plant Pathology in the College of Agriculture of Milano. This is recognized as the first university chair of Plant Pathology in Italy. Since then, the "Rivista di Patologia Vegetale" was mostly published in Milano (except, for a few years, in Pavia) until 1995, when it became the official journal of the Italian Society for Plant Pathology (SIPaV) under the name of "Journal of Plant Pathology". Unfortunately, A.N. Berlese died soon after his appointment in Milano and was substituted for in 1903 by Ugo Brizi, who pioneered investigations on the phytotoxic effects of atmospheric pollutants coming from industrial plants and, a few years later (1919), published a book on "Le malattie delle piante agrarie". Meanwhile, in Pavia, the "Laboratorio Crittogamico" was expanding his research activity under the guidance first of Giovanni Briosi and then of Luigi Montemartini and Gino Pollacci, who chaired it from 1927 to 1942, when he retired. His place was taken by Raffaele Ciferri (1897-1964), one of his scholars, who had operated in Pavia, the Dominican Republic, and the University of Florence. R. Ciferri was very active both in Botany and in Plant Pathology, with special reference to mycology and to disease control. He founded the "Società Italiana di Fitoiatria" (SIF) and the journal "Notiziario sulle malattie delle piante". The chair of Plant Pathology in Milano was held by Giovan Battista Traverso (1878-1955), then (since 1948) by Elio Baldacci (1909-1987), who continued R. Ciferri's work as President of the SIF and expanded very much the research activities of the Plant Pathology Institute of Milano, establishing connections with other European and American research institutions. Many of his scholars are still active in Plant Pathology today.

PECTOLYTIC ENZYMES PRODUCED BY *FUSARIUM CULMORUM* IN VITRO AND DURING COLONISATION OF WHEAT SEEDLINGS. M.P. Aleandri, G. Chilosi, P. Magro. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. Fax: +39.0761.357473; e-mail: magro@unitus.it.

Foot rot caused by *Fusarium culmorum* represents one of the most serious seed- and soil-borne diseases of wheat worldwide. During an ongoing research addressing wheat-*F. culmorum* inter-

actions, pectolytic enzymes produced by this pathogen in a liquid medium containing pectin as sole carbon source and in inoculated wheat seedlings were studied quantitatively and qualitatively. The fungus was capable of producing *in vitro* both polygalacturonase (PG) and pectin-lyase (PNL). Total PNL was found to be active also on polygalacturonic acid and in the absence of Ca⁺⁺; however, increasing doses of this cation consistently augmented the catalytic activity. Total PNL and PG activity was represented by an isoenzymatic complex. During early *in vitro* growing, only PG was produced whereas in later phases the pathogen produced mainly PNL accompanied by an increase of pH of the medium close to the PNL optimum activity. PNL isoenzymes were differentially active at diverse pH values, thus indicating a high degree of flexibility of this enzyme complex. *F. culmorum* induced a pectolytic isoenzymatic pattern similar to that observed *in vitro* also during wheat seedlings colonisation and enhanced the pH level of tissues even if less pronounced than that observed *in vitro*. These results suggest that alkalisation process may represent a co-factor in *F. culmorum* pathogenicity.

A PHYSIOLOGICAL AND MOLECULAR APPROACH REVEALS A POSSIBLE MODE OF ACTION OF *PHOMA TRACHEIPHILA* IN ITS INTERACTION WITH LEMON. C. Betti¹, A.A. Fabbri¹, A. Ricelli², C. Fanelli¹, M. Reverberi¹. ¹Dipartimento di Biologia Vegetale, Università "La Sapienza" di Roma, Largo Cristina di Svezia 24, 00165 Roma, Italy. ²Istituto di Scienze delle Produzioni Alimentari, CNR, Via G. Amendola 122, 70126 Bari, Italy. Fax +39.06.6833878; e-mail: massimo.reverberi@uniroma1.it.

The mitosporic ascomycete *Phoma tracheiphila* causes "mal secco" disease of lemon (*Citrus limon*) and produces a 60 KDa toxic glycoprotein, denoted Pt60, responsible for most of the disease symptoms. These effects are enhanced when lemon leaves are illuminated, suggesting that the fungus may interfere with the photosynthetic process. Since ROS are hugely produced during photosynthesis, for disease induction *P. tracheiphila* has to cope with a highly oxidative environment. *In vitro* experiments were carried out inoculating *P. tracheiphila* in a culture medium supplemented with lyophilised lemon leaves (2% w/v). Antioxidative enzymatic activity [superoxide dismutase (SOD) and catalase (CAT)] was enhanced under these cultural conditions as compared with the control, suggesting that *P. tracheiphila* was under oxidative stress. To verify this hypothesis two lemon cultivars (Monachello and Femminello, kindly supplied by Dr. Germanà), with a different susceptibility to the pathogen, were tested. Enzymatic activities of *P. tracheiphila* [SOD, CAT, glutathione peroxidase (GPX), laccase, Mn peroxidase, and polygalacturonase] and lemon [(lipoxygenase, polygalacturonase inhibiting protein (PGIP), SOD, CAT, GPX)] were analysed. A molecular approach was also followed to the aim of determining the complete sequence of the Pt60 gene, starting from six peptides previously identified (Fogliano *et al.*, 1998). Three of the clones obtained showed some 50-60% similarity with proteins involved in redox reactions, i.e. a putative NADH-flavin reductase and a NADPH-dependent reductase, supporting our hypothesis.

EFFECT OF *PHAEACREMONIUM ALEOPHILUM*, *PHAEOMONIELLA CHLAMYDOSPORA* AND *FOMITIPORIA MEDITERRANEA* ON ACCUMULATION AND CHANGES OF POLYPHENOLS AND PHYTOTOXINS IN GRAPEVINE CALLI AND IN PLANTA. G. Bruno, L. Sparapano. Dipartimento di Biologia e Patologia vegetale, Sezione di Patologia vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442906; e-mail: sparlor@agr.uniba.it.

Calli of grapevine cvs Italia and Matilde were grown in the

presence of *Phaeoemoniella chlamydospora* (*Pcb*), *Phaeoacremonium aleophilum* (*Pal*) and *Fomitiporia mediterranea* (*Fme*). Xylem sap was collected in early spring from cv Sangiovese vines showing symptoms of "esca" for five consecutive years. Accumulation and changes in calli or in xylem sap of 17 phenolic compounds including acids, phenolic aldehydes, flavonols, flavonol-3-*O*-glycosides, flavan-3-ols, and stilbenes and two phytotoxic polyketides, scytalone and isosclerone were analysed by high-performance liquid chromatography. Polyphenol concentration in calli proved to be strongly related to the grapevine cultivar, i.e. *trans*-resveratrol was more abundant in calli of Italia than in those of Matilde. This compound consistently decreased when calli were grown in the presence of each of the three fungi, all of which were capable of converting it. When grown on media amended with resveratrol, *Pcb*, *Pal* and *Fme* showed inhibited growth, but were able to live in media containing resveratrol as the sole carbon source. In addition, *Pcb* and *Pal* produced scytalone and isosclerone, two phytotoxins recently isolated from liquid cultures of *Pcb* and *Pal*, as well as from calli infected by both mitosporic fungi. Stilbenes, flavonols and flavonol-*O*-glycosides are the prevailing compounds of xylem sap; their concentration increased or decreased in relation to the esca-associated fungus present in the grapevine trunk. Metabolic changes associated with the infectious process may be basically modified by polyphenols to fulfil vital requirements of plant defence.

BIOCHEMISTRY OF NC-S AND NC-R WHITE CLOVER CLONES EXPOSED TO OZONE. A. Francini, V. Picchi, C. Nali, G. Lorenzini. Dipartimento di Coltivazione e Difesa delle Specie Legnose "Giovanni Scaramuzzi", Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. Fax: +39.050.960622; e-mail: afrancini@agr.unipi.it.

Campaigns with white clover clones (*Trifolium repens* L. cv Regal, NC-S, ozone-sensitive, and NC-R, ozone-resistant), performed within the frame of the European network, confirmed how good this system is at displaying measurable differences in growth response to ozone. In this work, the response of these clones to a simulated photochemical episode (200 ppb of ozone for 5 h) was investigated under controlled conditions. As expected, the clones showed a different phenomenological response: 24 h after fumigation, only NC-S developed chlorosis and bifacial necrosis on mature, fully expanded leaves. Decrease of chlorophyll content was evident in NC-S (-9% and -25%, at 5 and 24 h by comparison with the controls). Five h after treatment, the photosynthetic efficiency significantly diminished in both clones (-56 and -27%, compared to the control, in sensitive and resistant clones, respectively) and a similar trend was found for the optimum quantum yield ratio (-3 and -4%). At the same time, CO₂ concentration in the substomatal chamber increased (26% and 12%), indicating a mesophyll limitation of the photosynthetic process. However, the day after, the photosynthetic performance of NC-R was similar to controls, indicating complete recovery. The activity of phenylalanine ammonia-lyase (the key enzyme of phenylpropanoid metabolism) was stimulated in both clones, in particular in NC-S, which showed a 11-fold increase compared to the control. A similar behaviour was observed in total phenol and carotenoids contents (+70 and +45%, respectively).

PHYTOTOXIC AND BIOCIDAL METABOLITES PRODUCED IN CULTURE BY PSEUDOMONAS AVELLANAE. M. Greco¹, M. Scortichini². ¹Dipartimento di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto Sperimentale per la Frutticoltura, Via di Fioranello 52, 00134 Roma, Italy. Fax: +39.067.9340158; e-mail: mscortichini@yahoo.it.

Pseudomonas avellanae is the causal agent of bacterial canker

and decline of hazelnut (*Corylus avellana* L.), which is characterized by an extensive withering of the trees during the vegetative season, which invariably leads to a rapid wilting (i.e. in one or two weeks). Two strains of the bacterium, from northern Greece and central Italy (province of Viterbo), were used to assess their *in vitro* capability to produce bioactive metabolites. After 11 days of growth at 16-25°C into still flasks containing the IMM medium with basic L-histidine instead of acid L-histidine, culture filtrates of both strains showed phytotoxic activity towards tobacco leaves (leaf necrosis and chlorosis), lemon fruits (necrosis and water-soaking) and bean pods (necrosis). Biocidal activity against *Rhodotorula pilimanae*, 600 units ml⁻¹, and *Bacillus megaterium*, 800 units ml⁻¹, was also observed. The metabolites contained in culture filtrates retained their activity after lyophilization, freezing at -80°C for three days, and heating at 50 and 80°C for 30 min. Shaken cultures had no phytotoxic activity. Likewise, no phytotoxic or biocidal activity was observed when cultures were grown in IMM medium with acid L-histidine, Woolley medium, potato-dextrose-casamino acid broth, and Gross minimal medium. The tested strains did not possess the *syrB* gene coding for the production of cyclic lipodepsinopeptides such as the syringomycin toxin.

MULTIPLICATION AND MOVEMENT OF CHRYSANTHEMUM YELLOWS PHYTOPLASMA IN THE HOST PLANT CHRYSANTHEMUM CARINATUM. C. Marzachi¹, P. Saracco¹, D. Bosco². ¹Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, 10135 Torino. ²Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Entomologia, Università degli Studi, Torino, Italy. Fax: +39.011.343809; e-mail: c.marzachi@ivv.cnr.it.

A 16SrI-B phytoplasma is associated with chrysanthemum yellows (CY), a disease transmitted by *Euscelidius variegatus* (Kirschbaum), *Euscelis incisus* (Kirschbaum) and *Macrosteles quadripunctulatus* (Kirschbaum). A model system was recently developed to study the relationships among the CY phytoplasma, the host plant (*Chrysanthemum carinatum*) and the insect vector (*M. quadripunctulatus*). In this work, CY- and Chrysanthemum-specific primers and TaqMan probes were developed and used for monitoring phytoplasma multiplication and movement in the host plant following controlled inoculation. CY-infected *M. quadripunctulatus* were transferred for three day inoculation on: (i) apical leaves, (ii) basal leaves, and (iii) on the whole daisy plant. The relative titer of phytoplasma was expressed as fg of CY DNA ng⁻¹ of daisy DNA following quantification by Real Time PCR in apical and basal leaves and roots at 3, 6, 10 and 13 days from the end of the three different inoculations. Preliminary results have shown that, following apical and basal inoculation, CY titer in apical leaves increases from 3 to 13 days after the end of inoculation, while it remains constant in the corresponding basal leaves. At the end of the experiment, CY in apical leaves is 6 times less concentrated following basal inoculation than in the corresponding leaves following apical inoculation. Following inoculation on the whole plant, CY titer in roots also increases from 3 to 13 days and at the end of the experiment CY is about 5 times more concentrated in roots than in apical leaves.

PHYSIOLOGICAL AND ULTRASTRUCTURAL STUDY OF LEAF TISSUES OF APRICOTS RECOVERED FROM EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA INFECTION. R. Musetti¹, L. Sanità di Toppi², F. Ferrini¹, A. Loschi¹, M.A. Favali², R. Osler¹. ¹Dipartimento di Biologia Applicata alla Difesa delle Piante, Via delle Scienze 208, 33100 Udine, Italy. ²Dipartimento di Biologia Evolutiva e Funzionale, Parco Area delle Scienze 11/A, 43100 Parma, Italy. Fax: +39.0432.558503; e-mail: Rita.Musetti@pldef.uniud.it.

Recovery is a spontaneous remission of symptoms of diseased

plants, which may or may not involve the elimination of the causal agent from the host. Recovery has been observed in phytoplasma infected apples, grapevines, and apricots, but the reasons underlying its onset are still incompletely known. In this paper we report on the localization of hydrogen peroxide (H_2O_2) and the role of peroxidases, malondialdehyde, and reduced glutathione in the leaves of apricot plants in three conditions: symptomless, showing symptoms of European stone fruits yellows phytoplasma (ESFYP) infection, or in a recovery phase. PCR analysis showed that ESFYP was present in all trees, regardless of their condition, thus confirming previous observations indicating that recovery of apricot does not seem to be related with the disappearance of phytoplasmas from the plants. H_2O_2 was detected cytochemically by its reaction with cerium chloride. It occurred in the plasmalemma of the phloem of recovered plant leaves, but not in symptomless or symptomatic samples. Furthermore, no difference in the localization of peroxidases was observed in leaf tissues labelled by DAB. Protein content of symptomless, symptomatic, and recovered leaves was not significantly different. By contrast, the guaiacol peroxidase activity had the following trend: symptomatic > recovered > symptomless. Reduced glutathione content showed the opposite trend: symptomless > recovered > symptomatic. Finally, no difference was observed in malondialdehyde concentration between symptomless, symptomatic and recovered leaves. The overall results suggest that H_2O_2 and related metabolites and enzymes appear to be involved in lessening both pathogen virulence and symptom expression.

EXPRESSION OF TOMBUSVIRUS OPEN READING FRAMES 1 AND 2 IS SUFFICIENT FOR THE REPLICATION OF DEFECTIVE INTERFERING BUT NOT OF SATELLITE RNA. L. Rubino, V. Pantaleo, B. Navarro, M. Russo. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; e-mail: l.rubino@area.ba.cnr.it.*

Carnation Italian ringspot virus (CIRV) and *Cymbidium ringspot virus* (CymRSV) are definitive members of the genus *Tombusvirus*, family *Tombusviridae*. Defective interfering (DI) or satellite (sat) RNAs often accompany tombusvirus infections. Neither of these RNAs codes for any protein but depends entirely on a helper virus genome for its replication (*trans*-replication). DI RNAs are shortened forms of genomic RNA, whereas satRNAs share limited sequence homology with the viral genome, except for a region of *ca* 50 nt in common with both viral genomes and DI RNAs. Yeast cells co-expressing CIRV replication proteins p36 and p95 support the RNA-dependent replication of several DI RNAs derived from the genome either of CIRV or CymRSV, but not the replication of a satRNA, originally associated with CymRSV. DI, but not satRNA was replicated in yeast cells co-expressing both DI and satRNA. By using transgenic *Nicotiana benthamiana* plants expressing constitutively CymRSV replicase proteins (p33 and p92) or, transiently, either these proteins or CIRV p36 and p95, it was shown that expression of replicase proteins alone was not sufficient for the replication of satRNA also in plant cells. However, it was also shown that replicating genomic RNA deletion mutants of CIRV that encoded only replicase proteins could sustain replication of satRNA in plant cells. These results suggest that satRNA has a replication strategy differing from that of genomic and DI RNAs, for it requires the presence of a *cis*-replicating genome acting as a *trans*-replication enhancer.

THE TRICHODERMA – PLANT INTERACTION IS MEDIATED BY AVIRULENCE PROTEINS PRODUCED BY THE FUNGUS. M. Ruocco¹, S. Lanzuise², S. Woo², P. Ambrosino², R. Marra², D. Turrà², S. Gigante², E. Formisano², F. Scala², D.J. Kip³, P.J.G.M. de Wit³, M. Lorito². ¹*Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy.* ²*Istituto di Protezione delle Piante del CNR, Sezione di Portici, Via Università 133, 80055 Portici (NA), Italy.* ³*University of Wageningen, The Netherland. Fax: +39.081.7755114; e-mail: lorito@umina.it.*

The molecular basis of *Trichoderma*-plant interaction is very complex and still incompletely understood. Colonization of the root system by rhizosphere competent strains of *Trichoderma* results in increased development of root/aerial systems, improved yields, and disease control. To understand the mechanisms involved we are using different approaches, including the production of transformants expressing genes that encode compounds able to affect plant response to pathogens. *Trichoderma* transformants carrying the avirulence gene *Avr4* from *Cladosporium fulvum* under the control of constitutive and inducible promoters were obtained and tested on tomato plants having the *Cf4* resistance gene. Necrosis and suberification zones, similar to the symptoms appearing during *Cladosporium*-tomato interaction, were found when the roots of the *Cf4* plants were treated with *Avr4-Trichoderma*. This demonstrates that selected *Trichoderma* strains are able to transfer to the plant molecules that can deeply affect metabolism, disease resistance, etc. Therefore, these beneficial fungi can be regarded as biotechnological tools to provide a variety of crops with useful compounds. Moreover, in *in vitro* competition assays the transformants were found to be more effective than the wild type as antagonists of *Alternaria alternata*. *Trichoderma* sends a variety of biochemical signals to the plants including avirulence molecules; therefore the presence of *avr*-like proteins in the fungus proteome was investigated. Proteome analysis has allowed isolation and sequencing of many proteins potentially having this function. From the extracellular protein extracts, a protein with structural characteristics similar to *Avr4* of *C. fulvum* purified and sequenced. This protein, (Hytra1) was found to be a hydrophobin with chitin binding activity, the typical eight cysteine residues, and four disulfide bridges. Infiltrations of the extracellular protein fractions containing Hytra1 resulted in a strong HR reaction on tobacco and tomato leaves.

CYTOLOGICAL AND HISTOLOGICAL INVESTIGATIONS IN THE INCOMPATIBLE INTERACTION BETWEEN TOMATO PLANTS BEARING THE *OL-2* GENE AND *OIDIUM NEOLYCOPERSICI*. V. Siciliano, M. Marte. *Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. Fax: +39.075.5856482; e-mail: sicilianovaleria@tiscali.it.*

Oidium neolycopersici is a widespread powdery mildew fungus, which can cause severe yield losses especially to greenhouse-grown tomato plants. The use of tomato cultivars resistant to the fungus represents an environment-friendly strategy for controlling the disease, alternative to the use of fungicides. Resistance to *O. neolycopersici*, supported by the single recessive *ol-2* gene, has been found in different species of the genus *Lycopersicon*, such as *Lycopersicon esculentum* var. *cerasiforme* (Ciccarese *et al.*, 1998; Plant Pathology 47: 417-419). Cytological and histological investigations, carried out to assess how and in which developmental stages *O. neolycopersici* growth is arrested by the expression of the *ol-2* gene, showed that the resistance was not associated with

the hypersensitive reaction and that appressoria of the fungus failed to produce haustoria in epidermal plant cells. A massive apposition was detected of wall material beneath the fungal appressorium, which lead to the formation of large papillae with a high level of autofluorescent compounds, probably antimicrobial phenolic derivatives. As a consequence, a slow growth and sporadic sporulation of the pathogen were observed. Like in the resistance of barley to *Blumeria graminis* mediated by the recessive alleles at the *mlo* locus, the particular nature and composition of the papillae could be responsible for resistance of ol-2 tomato plants to fungal penetration.

GOSSYPOL, A SUBSTANCE INVOLVED IN THE COTTON/*FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM* INTERACTION. E. Turco¹, C. Brubaker², J. Scown², G. Mergeai³, B. Mori⁴, C. Vizzuso⁵, A. Ragazzi¹. ¹Dipartimento di Biotecnologie Agrarie, Sezione di Patologia vegetale, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. ²Centre for Plant Biodiversity Research, CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia. ³Unité de Phytotechnie tropicale et d'Horticulture, Faculté Universitaire des Sciences Agronomiques, 2 Passage des Déportés, B-5030 Gembloux, Belgium. ⁴Dipartimento di Biologia Vegetale, Università degli Studi Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. ⁵Freelance. Fax: +39.055.3288273; e-mail: elena.turco@unifi.it.

The genus *Gossypium*, in which cotton, the most important fiber crop belongs, is characterized by the presence of terpenoid aldehydes ("gossypol"), in pigment glands of seeds and the aerial part of the plant. Gossypol is a known phytoalexin and additional terpenoid aldehydes are produced following infection by *Fusarium oxysporum* f.sp. *vasinfectum* (FOV). The use of cotton seeds by-products (oil, cakes, and flour) is strongly limited because gossypol is toxic to non-ruminant animals and causes male sterility in humans. For this reason, new cotton hexaploid "low gossypol" or "glandless" hybrids were developed. Experiments were carried out to test *in vitro* the effect of various levels of gossypol on some morphological parameters of FOV. No statistically significant difference was observed on colony growth, whereas a negative and significant correlation was evident between the gossypol concentration (mg l⁻¹) and conidial germination rate. For *in vivo* trials under greenhouse conditions, cotton hexaploid seedlings were infected with different FOV isolates collected from Australia and from other cotton-growing areas. High disease incidence was observed in FOV-infected hybrids as compared with control genotypes. Fourteen to 21 days after inoculation, hybrid seedlings showed a stronger and rapid development of symptoms, such as chlorosis, wilting, and leaf necrosis. In conclusion, the use of gossypol as an alternative method for the control of cotton wilt disease is suggested and information is provided on the possible sources of genetic resistance to FOV in the different cotton diploid genomes.

LATENT PHASE OF *BISCOGNIAUXIA MEDITERRANEA* QUANTIFICATION IN *QUERCUS* SPP. WITH REAL-TIME PCR BY TAQMAN™ CHEMISTRY. N. Luchi¹, P. Capretti¹, M. Pazzagli², P. Pinzani², C. Orlando². ¹Dipartimento di Biotecnologie Agrarie, Sezione Patologia vegetale, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. ²Dipartimento di Fisiopatologia Clinica, Sezione Biochimica clinica, Università degli Studi, Viale Pieraccini 6, 50139 Firenze. Fax: +39.055.3288273; e-mail: paolo.capretti@unifi.it.

Biscogniauxia mediterranea is a widespread fungal parasite of

Quercus spp. of the Mediterranean region, which normally causes stem cankers and wood decay. *B. mediterranea* is able to kill trees under water stress conditions mostly in the southern part of the Italian peninsula. The organism is often reported as an endophyte and is characterized by long latent periods of colonization. For predicting the fungal distribution under conditions of climatic change, from southernmost areas to northern regions, a study was setup by using Real-Time PCR (TaqMan™ chemistry). The occurrence and the extent of *B. mediterranea* colonization in *Q. cerris* and *Q. pubescens* were estimated by species-specific primers and a TaqMan probe labelled with different fluorescent dyes. The method was successfully used with axenic cultures and ascospores and also with infected and symptomless twigs and acorns. *B. mediterranea* was detected in 96% of asymptomatic *Quercus* spp. shoots collected from an infected forest.

ROLE OF POLYGALACTURONASE-INHIBITING PROTEINS IN *ARABIDOPSIS THALIANA*-*FUSARIUM GRAMINEARUM* INTERACTION. S. Ferrari¹, L. Sella¹, G. De Lorenzo², F. Favaron¹. ¹Dipartimento Territorio e Sistemi Agroforestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. ²Dipartimento di Biologia Vegetale, Università "La Sapienza", Piazzale A. Moro 5, 00185 Roma, Italy. Fax: +39.049.8272890; e-mail: simone.ferrari@unipd.it.

Fusarium graminearum causes ear blight disease on cereal crops worldwide, lowering grain quality and safety. The molecular basis of resistance to ear blight in cereal species is poorly understood. We used a recently developed experimental system, based on floral infection of *Arabidopsis thaliana*, to investigate the role of fungal endo-polygalacturonases (PGs) and plant polygalacturonase-inhibiting proteins (PGIPs) during *F. graminearum* infection. Both *Arabidopsis* PGIPs, AtPGIP1 and AtPGIP2, are effective inhibitors of *F. graminearum* PG. Plants expressing the beta-glucuronidase (*GUS*) reporter gene under the control of the promoter sequences of AtPGIP1 or AtPGIP2 showed that *Arabidopsis* PGIPs are expressed in flowers, but only in the stigma and in the abscission zones of floral organs. In contrast, *F. graminearum* penetration was observed in correspondence of petals and anthers. Plants transformed with AtPGIP1 or AtPGIP2 cDNAs under the control of the constitutive promoter CaMV 35S showed a significant reduction of disease symptoms upon fungal infection, suggesting that PG is a major virulence factor of *F. graminearum*. Overexpression of PGIPs may therefore be a successful strategy to reduce head blight incidence in cereal crops.

PHYLOGENETIC RELATIONSHIPS OF ITALIAN *FUSARIUM SOLANI* ISOLATES BASED ON SEQUENCES FROM THE NUCLEAR ITS-5.8 S r-DNA REGION. S. Vitale, M. Zaccardelli, M. Merighi, L. Corazza. Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Fax: +39.06.82070370; e-mail: mal.crit@ispave.it.

The genus *Fusarium* comprises a wide and heterogeneous group of fungi important for the food and drug industry, medicine, and agriculture. *Fusarium solani* (teleomorph = *Nectria haematococca*), a phytopathogenic fungus classified in the *Martiiella* section, is responsible for many economically important plant diseases such as root and fruit rot of *Cucurbita* spp., root and stem rot of pea, sudden death of soybean, foot rot of bean, and dry rot of potato tubers during storage. *F. solani* is subdivided into ten *formae specialis* (f.sp.) based on host range tests. Recently, molecular studies have identified about 50 different species on

the basis of DNA sequences. In this work, we have investigated the phylogenetic relationships of 34 *F. solani* isolates versus 12 known *Fusarium* spp. using sequence data from the nuclear ribosomal internal transcribed spacer (ITS)-5.8 S r-DNA region. Phylogenetic trees were constructed using the Neighbour-Joining method applied to the whole set of manually aligned sequences. The confidence of the branching was estimated by bootstrap analysis. *Fusarium cerealis* and *F. oxysporum* were used as outgroups for rooting the phylogenetic tree. Preliminary data show the clustering of seven isolates in a well-supported clade containing *F. solani* f.sp. *pisi*. Twenty-two isolates cluster in less supported clades including *F. solani* f.sp. *radicicola*, f.sp. *batatas* and f.sp. *xanthoxylis*. None of the isolate shows significant similarity to *F. solani* f.sp. *phaseoli*, *robinia*, *mori*, *piperis*, *eumartii* and the *Fusarium* species used as outgroups.

PHYTOPLASMA CLASSIFICATION. G. Firrao¹, C. Marcone², A. Bertaccini³. ¹Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi, Via Scienze 208, 33100 Udine, Italy. ²Dipartimento di Biologia, Difesa e Biotecnologie Agroforestali, Università della Basilicata, Campus Macchia Romana, 85100 Potenza, Italy. ³Dipartimento di Tecnologie e Scienze Agroambientali, Sezione di Patologia vegetale, Alma Mater Studiorum, Università di Bologna, Via G. Fanin 46, 40127 Bologna, Italy. Fax: +39.0432.558503; email: firrao@uniud.it.

Although phytoplasmas have not yet been cultivated *in vitro*, phylogenetic analyses based on various conserved genes showed that they represent a distinct monophyletic clade within the class *Mollicutes* and it was proposed to accommodate them within the genus 'Candidatus Phytoplasma' (International Journal of Systematic and Evolutionary Microbiology 54: 1257-69, 2004). The new genus comprises the 26 species listed below, although 7 of them have been named but still need to be validly described (in square brackets below).

'Candidatus Phytoplasma' species	Phylogenetic group according to Lee <i>et al.</i> , Int. J. Syst. Bacteriol., 4: 1153-69, 1998	Associated disease
'Ca. Phytoplasma asteris'	Aster Yellows group (16SrI)	Aster yellows
'Ca. Phytoplasma japonicum'	Aster Yellows group (16SrI)	Japanese Hydrangea phylloxy
'Ca. Phytoplasma aurantifolia'	Peanut Witches' Broom group (16SrII)	Acid lime witches' broom
['Ca. Phytoplasma pruni']	X-disease group (16SrIII)	X-disease
'Ca. Phytoplasma palmae'	Coconut Lethal Yellowing group (16SrIV)	Coconut lethal yellowing (Mexico)
['Ca. Phytoplasma cocostanzaniae']	Coconut Lethal Yellowing group (16SrIV)	Coconut lethal yellowing (Tanzania)
['Ca. Phytoplasma cocosnigeriae']	Coconut Lethal Yellowing group (16SrIV)	Coconut lethal yellowing (Nigeria)
'Ca. Phytoplasma castanaceae'	Coconut Lethal Yellowing group (16SrIV)	Korean chestnut witches' broom
'Ca. Phytoplasma ziziphi'	Elm Yellows group (16SrV)	Ziziphus jujube witches'-broom
['Ca. Phytoplasma vitis']	Elm Yellows group (16SrV)	Flavescence dorée
'Ca. Phytoplasma ulmi'	Elm Yellows group (16SrV)	Elm yellows
'Ca. Phytoplasma trifolii'	Clover Proliferation group (16SrVI)	Clover proliferation
'Ca. Phytoplasma fraxini'	Ash Yellows group (16SrVII)	Ash yellows
['Ca. Phytoplasma luffae']	Loofah Witches' Broom group (16SrVIII)	Loofah witches'-broom
'Ca. Phytoplasma phoenicium'	Pigeon Pea Witches' Broom group (16SrIX)	Almond lethal disease
'Ca. Phytoplasma mali'	Apple Proliferation group (16SrX)	Apple proliferation
'Ca. Phytoplasma pyri'	Apple Proliferation group (16SrX)	Pear decline
'Ca. Phytoplasma prunorum'	Apple Proliferation group (16SrX)	European stone fruit yellows
'Ca. Phytoplasma spartii'	Apple Proliferation group (16SrX)	Spartium witches' broom
'Ca. Phytoplasma rhamnii'	Apple Proliferation group (16SrX)	Buckthorn witches' broom
'Ca. Phytoplasma allocasuarinae'	Apple Proliferation group (16SrX)	Allocasuarina yellows
'Ca. Phytoplasma oryzae'	Rice Yellow Dwarf group (16SrXI)	Rice yellow dwarf
'Ca. Phytoplasma australiense'	Stolbur group (16SrXII)	Australian grapevine yellows
['Ca. Phytoplasma solani']	Stolbur group (16SrXII)	Stolbur and Bois Noir
'Ca. Phytoplasma cynodontis'	BGWL group (16SrXIV)	Bermuda grass white leaf
'Ca. Phytoplasma brasiliense'	'Ca. P. brasiliense' group (16SrXV)	Brazilian hibiscus witches'-broom

CHARACTERIZATION OF PSEUDOMONAS SYRINGAE PV. TOMATO ISOLATES FOR EFFECTOR GENES AND FIRST ANALYSES OF THEIR VIRULENCE ON SUSCEPTIBLE AND RESISTANT TOMATO GENOTYPES. M. Zaccardelli¹, B.A. Vinatzer^{2,3}, J.T. Greenberg². ¹Istituto Sperimentale per le Colture Industriali, Mi.P.A.F., Battipaglia (SA), Italy. ²Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, USA. ³Current address: Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, USA. Fax: +39.082.8340169; e-mail: m.zaccardelli@isci.it.

Pseudomonas syringae pv. *tomato* (*Pst*) is the causal agent of bacterial speck of tomato. As many other pathogenic Gram negative bacteria *Pst* is equipped with genes that encode effector proteins secreted into the host cell by a Type III secretion system. The function of the individual *Pst* effectors is largely unknown, but as a whole they manipulate plant cells to create an environment optimal for bacterial growth. Thirteen *Pst* isolates, eight from Southern Italy, two from Northern Italy and three from France, were analysed for the presence of 97 effector genes of the sequenced *Pst* strain DC3000, the sequenced *P. syringae* pv. *syringae* (*Pss*) strain B728a and of *P. syringae* pv. *maculicola* (*Psm*) strain ES4326. The *Pst* strains from Southern Italy were very similar to each other and very similar to an isolate from Northern Italy and two isolates from France. They were also similar, although to a lesser degree, to *Pst* DC3000. Conversely, one isolate from Northern Italy and one from France were very similar to *Pss* strain B728a and other three *Pss* isolates from melon cultivated in France. Probably, these two *Pst* strain were wrongly classified as *Pst*. In fact, they induced no symptoms when inoculated to different tomato genotypes. By contrast, the other *Pst* isolates were very virulent to susceptible tomato genotypes, whereas virulence of DC3000 was very low. Using Arabidopsis as host plant, DC3000 gave severe symptoms whereas the other *Pst* isolates did not give any symptoms on all Arabidopsis ecotypes tested.

ELECTRONIC NOSE ENABLES DISCRIMINATION OF FB1 PRODUCER AND NON-PRODUCER STRAINS OF FUSARIUM VERTICILLIOIDES. E. Gobbi¹, M. Falasconi², G. Sberveglieri². ¹Biodiversity s.r.l., Via Corfù 71, 25124 Brescia and Dipartimento di Biologia applicata alla Difesa delle Piante, Università di Udine, Via Scienze 208, 33100 Udine, Italy. ²SENSOR Laboratory, Department of Chemistry and Physics for Engineering, University of Brescia and INFN, Via Valotti 9, 25123, Brescia, Italy. Fax: +39.030.2450064; e-mail: micol@uniud.it.

Maize crops are subjected to fungal attacks resulting in contamination by mycotoxins that can pose a hazard to health deriving from consumption of maize and derivatives as animal feeds and/or human food. In Italy, the most common maize mycotoxin is fumonisin B1 (FB1), which is mainly produced by *Fusarium verticillioides* and is associated with the development of human oesophageal cancer. Early detection of mycotoxin contamination is crucial to prevent risks deriving to humans and animals from the entry of mycotoxins into the food chain. The aim of this work was to verify if the pattern of fungal volatile compounds (headspace) could be used as a marker for mycotoxin production and if the electronic nose could discriminate *Fusarium* strains in relation to their different ability to produce fumonisins. The electronic nose consists of an array of chemical sensors and a pattern-recognition algorithm. The sensor array "sniffs" the headspace from a sample providing a set of measurements, and then the pattern-recogniser compares the pattern of measurements with known existing patterns. The electronic nose could therefore represent a valid method for the detection of contamination because of its rapidity, simplicity and low cost. The headspaces from four isolates of

Fusarium verticillioides, (two FB1 producers and two non-FB1 producers) grown on synthetic medium, were analysed with an electronic nose. The effectiveness was investigated of the electronic nose EOS835-EN, equipped with an array of thin-film metal oxide sensors, in discriminating between mycotoxigenic and non mycotoxigenic strains of *Fusarium verticillioides*

FINGERPRINTING ANALYSIS OF PATHOGENIC AND NON-PATHOGENIC POSTHARVEST *PENICILLIUM* STRAINS. C. Oliveri¹, A. Campisano², G. Cirvilleri¹, A. Catara^{1,2}. ¹Dipartimento di Scienze e Biotechnologie Fitosanitarie, Università degli Studi, Via S. Sofia, 100, 95123 Catania. ²Parco Scientifico e Tecnologico della Sicilia S.C.p.A., Blocco Palma I – Strada V. Lancia, 95131 Catania. Fax: +39.095.7147278; e-mail: cataraan@unict.it.

Penicillium is a large genus with at least 150 species that often share morphological traits, which makes identification difficult at the species level. At present the most common identification method relies on the observation of morphological features and microscopical structures. To some extent, these characteristics are related to the physiological status of the cells and can therefore lead to misjudgement and errors in identification. A collection of *Penicillium* spp. was collected from fruits in storage and on the market, and from warehouse air. Terverticillate subspecies isolates were identified using the key developed by Pitt, both by colony and cellular morphology. Agronomically relevant features were screened, such as fungicide tolerance, pathogenicity to different species, virulence (determined as lesion extension on fruits after wound inoculation) and growth rate. Most (87.5%) air contaminating *Penicillium* strains from citrus packaging operations were not pathogenic to citrus species and other susceptible hosts. All isolates were then genetically characterised by ITS1, ITS2 and 58S rDNA gene amplification and amplicon restriction by endonucleases. MUCL collection isolates were used as control. AFLP fingerprinting was performed to assess its ability to discriminate species and to analyse correlation of alleles with morphological and agronomical features. ITS amplification and restriction allowed differentiation of only a few species, confirming that this technique is efficient at the genus level, but lacks specificity. The polymorphism detected within the assayed isolates by AFLP analysis confirmed the reliability of the technique both for discriminating fungal strains at the species level, and to isolate genes that are related to pathogenicity and agronomical important traits.

FUNGAL PATHOGENS ON SEEDS OF ITALIAN CULTIVARS OF *ORYZA SATIVA*. A. M. Picco, M. Rodolfi, E. Lorenzi, D. Rodino. Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Sezione di Micologia, Università degli Studi, Via S. Epifanio 14, 27100 Pavia, Italy. Fax: +39.038.234240; e-mail: apicco@et.unipv.it.

In the framework of a project addressing the genetic improvement and the study of biodiversity of rice grown in Italy, the presence of fungal pathogens on the seeds of *Oryza sativa* was evaluated. For each Italian cultivar listed in the Registro Nazionale Varietale we analyzed: one hundred each of unpolished and hulled seeds, according to the International Rules for Seed Testing Association; one hundred hulled seeds, both entire and shattered, according to a protocol for fungal endophytes detection in rice. From 91 cultivars, 84 and 66 epiphytic fungal taxa were isolated from unpolished and hulled seeds, respectively. The detected genera could be divided into three groups: (i) saprotrophic fungi, which do not appear to affect rice; (ii) storage fungi which can spoil the grains, represented by both *Aspergillus* and *Penicillium* spp. and mainly observed on the glumes; (iii) field fungi, includ-

ing members of genera known to be parasitic and semiparasitic on rice, able to lower seed quality, induce grain discoloration and infect plants. Among the isolated pathogens, the presence of *Alternaria* spp., *Epicoccum nigrum*, *Nigrospora oryzae* was significant on unpolished seeds, whereas *Fusarium verticillioides* and *Bipolaris* spp. prevailed on hulled seeds. As to the endophytic colonization of seeds, a total of 40 species were isolated among which *Alternaria alternata*, *Epicoccum nigrum* and *Fusarium* spp. prevailed. The endophytic presence of the two main seed-borne pathogens of rice, *Pyricularia grisea* and *Bipolaris* spp. was minor.

THE DATABASE OF PCR PRIMERS FOR PHYTOPATHOGENIC FUNGI. S. Ghignone¹, Q. Migheli². ¹Department of Plant Biology and IPP-CNR – University of Turin, Viale P.A. Mattioli 25, 10125 Torino, Italy. ²Department of Plant Protection - Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.011.6705962; e-mail: ste.ghi@libero.it.

Among the modern nucleic acid-based techniques, those involving polymerase chain reaction are most suited for early detection of phytopathogenic fungi, due to their high sensitivity and the automating potential. Primer sets can be designed for specificity at the genus, species, or physiological race level, so as to distinguish a given pathogen even from closely related entities. So far, very few are the examples of specific primer set collections reported in the literature, while no on-line repository is available. Here we present the first on-line searchable database of primer sets useful for the detection of plant pathogenic fungi. This web resource is totally implemented with open source software (PHP, MySQL). Primer set details can be retrieved by the organism name, primer name, nucleotide sequence comparison, target DNA, PCR technique, authors' name, journal and year of publication. Each record is directly linked to other reference databases to allow easy access to the correct nomenclature, taxonomic position and anamorph/teleomorph connections of the pathogen, GenBank deposited source sequences of primer sets and reference contents. The database is open to addition of data submitted by users, following system administrator reviewing of submitted data.

DIAGNOSTIC TOOLS FOR THE IDENTIFICATION OF *BRENNERIA NIGRIFLUENS*, THE CAUSAL AGENT OF PERSIAN WALNUT BARK CANCER. C. Moretti¹, F.M. Silvestri¹, E. Rossini², G. Natalini³, R. Buonaurio¹. ¹Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. ²Servizio Fitosanitario Regionale ASSAM, Via Alpi 21, 60131 Ancona, Italy. ³Servizio Fitosanitario Regionale ARUSIA, Via Fontivegge 51, 06124 Perugia, Italy. Fax: +39.075.5856482; e-mail: buonaurio@unipg.it.

After the first report from northern Italy (Veneto and Piemonte) in 1998, bark canker of Persian walnut (*Juglans regia* L.), caused by *Brenneria nigrifluens*, was recorded again from other Italian regions (Lazio and Campania). In 2002, we observed the disease in walnut plantations for timber production in 13 farms of central Italy (Umbria and Marche). The totality of 44 bacterial isolates from symptomatic plants were Gram-negative and had oxidative and fermentative metabolism. Twenty of them were submitted to API 20E system (bioMérieux) for identification because they were oxidase negative and therefore belonged to the Enterobacteriaceae. Four isolates (one from Umbria and three from Marche) gave the 7-digit code 0005773, which is identical to that reported for the type strain LMG 2694^T and the two reference strains LMG 5107 and LMG 5953 of *B. nigrifluens*. When trunks of young walnut plants were inoculated with these four isolates, typical symp-

toms of the disease developed three months after inoculation. A number of isolates associated with bark cankers, which were not pathogenic to walnut, were identified as *Erwinia rhapontici* using the API 20E system. Rep-PCR using primers REP showed that pathogenic isolates had high similarity (89%) with the type and reference strains of *B. nigrifluens*. The development is in progress of a molecular assay exploiting a 905 bp fragment amplified during REP-PCR, which seems to be characteristic for *B. nigrifluens*.

SELECTIVE DETECTION OF PSEUDOMONAS SYRINGAE PV. TOMATO OVER OTHER PSEUDOMONAS SYRINGAE PATHOVARS. V. Fanelli, C. Cariddi, M. Finetti-Sialer, D. Gallitelli. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; e-mail: vilmafanelli@yahoo.it.

Pseudomonas syringae pv. *tomato* is an ubiquitous seedborne pathogen that causes bacterial speck of tomato. We already reported that a 250-bp fragment, homologous to a region flanking a heat shock protein gene of *P. syringae* pv. *tomato* (Acc. No AE016871) could be used for the detection of this pathogen. However, this fragment was not highly specific since it cross-hybridised with pvs *morsprunorum* and *avellanae*, in dot blot assays. The sequence of another region flanking an heat shock protein gene of *P. syringae* pv. *tomato* was determined and used to design two new primers (Rtime-for and RTR-rev) and to synthesize a digoxigenin-labeled 138-bp DNA-probe. In dot blot, the 138-bp probe hybridised only to *P. syringae* pv. *tomato* with a detection limit for pure cultures and for bacteria released from soaked tomato seeds of about 20 CFU ml⁻¹. The primers Rtime-for and RTR-rev and a molecular beacon designed to hybridize with a 160 bp amplicon were used for the specific detection and quantification of *P. syringae* pv. *tomato* by Real-Time-PCR. The limit of detection, with a 10-fold dilution series of a DNA preparation was 1 pg ml⁻¹, with a highly significant correlation ($r^2=0,99$) between the amount of target DNA and cycle threshold (Ct). Using a fast protocol (CTAB method) for DNA extraction from pure bacterial cultures and contaminated seeds, the detection threshold was about 10² CFU/ml. The diagnostic tools developed proved highly specific for *P. syringae* pv. *tomato* and simple to use. Thus, they can be used for large-scale testing of seeds for the production of disease-free plants in nurseries.

IS GRAPEVINE VEIN NECROSIS A REACTION TO GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS? H. Bouyahia, D. Boscia, V. Savino, P. La Notte, C. Pirolo, M.A. Castellano, A. Minafra, G.P. Martelli. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911. e-mail: csvvdb08@area.ba.cnr.it.

Vein necrosis (VN), a virus-like disease of the grapevine first identified in France by Legin and Vuittenez (1973), induces necrosis of the veinlets on the underside of the leaf blade of its specific indicator *Vitis rupestris* x *Vitis berlandieri* 110 R. The disease is latent in all European grapevine cultivars and in most American rootstock species and hybrids, has a worldwide distribution, and has no known agent. VN is very common in southern Italy, so that over 50% (87 of 173) of the grapevine accessions selected in the course of sanitary improvement programmes in the last few years indexed positive on 110R. As assessed by ELISA, the same vines had a very low rate of infection (<3%) by detrimental viruses (GFLV, GVA, GVB, GLRaV-1, GLRaV-2, GLRaV-3, GFkV) commonly tested for in the course of selection. When the VN-positive

110R indicators were checked by PCR and Western blot for the presence of *Grapevine rupestris stem pitting-associated virus* (GRSPaV) a strikingly high association (over 95%) was observed between this virus and VN symptoms. Likewise, the totality of 72 mother plants of *Vitis rupestris* used as indicators in indexing trials and recently discovered to be infected by GRSPaV, induced VN reactions following grafting onto 110 R, whilst no VN reactions developed in 110R vines top-grafted on a single GRSPaV-free *V. rupestris*. Moreover, symptomatic bottom leaves from VN-infected 110R vines were consistently positive for GRSPaV whereas the symptomless top leaves from the same shoots were not. The very close association between GRSPaV and VN symptoms strongly suggests that this virus may be implicated in the aetiology of VN.

SYNERGISM OF ABC TRANSPORTER MODULATORS AND AGRICULTURAL FUNGICIDES IN CONTROL OF BOTRYTIS CINEREA. G. Carrideo¹, V. Lanzotti², E. Barile², G. Del Sorbo¹. ¹Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale vegetale, Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. ²Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via F. De Sanctis, 86100 Campobasso, Italy. Fax: +39.081.7755320; e-mail: giuseppecarrideo@vodafone.it.

ABC transporters (=ATP binding cassette transporters) are membrane-bound proteins that utilise ATP to transport a wide spectrum of compounds through biological membranes. Simultaneous or multi drug resistance (MDR) to several classes of agricultural fungicides depends on their decreased accumulation at the level of target site, as a consequence of energy-dependent efflux operated by ABC transporters. To reduce application rates of agricultural fungicides, we tested the effectiveness of two classes of non-toxic compounds, which inhibit ABC transporters: jatrophone diterpenes from *Euphorbia* sp. and the plant metabolite prenylchrysin. These compounds show strong inhibitory activity on the main human ABC transporter, P-glycoprotein, and the multidrug transporter Pdr5p of *Saccharomyces cerevisiae*, and show ability to reverse the MDR phenotype. These ABC transporter modulators were used in combination to test their synergism with a number of agricultural fungicides (tebuconazole, imazalil, fludioxonil, fenpiclonil, tolylfluanid and fenhexamid) in germ tube elongation inhibition tests on *Botrytis cinerea*. Results of *in vitro* experiments showed a clear synergism of jatrophanes with fludioxonil and tebuconazole and of prenyl-chrysin with tolylfluanid, whereas an antagonistic effect was observed between prenyl-chrysin and fludioxonil. Tests on tomato and bean leaves artificially inoculated with *B. cinerea* conidia confirmed the synergism of jatrophanes with tebuconazole and tolylfluanid with prenyl-chrysin.

EXTREME RESISTANCE TO LOCAL AND SYSTEMIC INFECTION FROM PLUM POX VIRUS STRAINS M AND D BY TRANSGENE-MEDIATED RNA SILENCING. E. Di Nicola Negri, A. Brunetti, V. Ilardi. Istituto Sperimentale per la Patologia vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. Fax: +39.06.8270372; e-mail: v.ilardi@ispave.it.

Plum pox virus (PPV), a single-stranded RNA virus, is the causal agent of Sharka, the most serious disease of stone fruits. PPV D and PPV M are the two major and important strains in terms of economic incidence. No useful natural resistance has been found yet. Expression of pathogen-derived sequences encoding self-complementary "hairpin" RNA that induces an efficient and predictable post-transcriptional silencing (Smith *et al.*, 2000. Nature, 407: 319-320) is a new and agriculturally sustainable biotechnological strategy to obtain virus-resistant plants

avoiding the production of transgenic viral proteins. To obtain a broad resistance against PPV strains four viral genomic regions, highly homologous between D and M, were selected as potential silencing targets. One of the four sequences starts at the 5' end of PPV genome and includes part of the P1 gene; the other three sequences partially cover the HC-Pro gene encoding a multifunctional protein involved, among other functions, in the suppression of RNA silencing. Each region was amplified by RT-PCR from a PPV M Italian isolate and arranged to form an intron-spliced RNA hairpin structure. The four sequences were introduced by *Agrobacterium* transformation into *Nicotiana benthamiana* plants under the transcriptional control of the CaMV 35S promoter. Resistance analysis was carried out challenging R1 transgenic plants with PPV M and PPV D. The results indicate that all four constructs confer an extreme level of resistance to local and systemic infection by both PPV M and D.

EVALUATION OF *POT-1* GENE IN *LYCOPERSICON HIRSUTUM* PI 247087 AGAINST ITALIAN *POTATO VIRUS Y* ISOLATES FROM NON POTATO HOSTS. A. Fanigliulo¹, G. Parrella², S. Comes¹, R. Pacella¹, A. Crescenzi¹. ¹Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Campus Macchia Romana 3A310, 85100 Potenza, Italy. ²Istituto per la Protezione delle Piante del CNR, Sezione di Portici, Via Università 133, 80055 Portici (NA), Italy. Fax: +39.0971.205703; e-mail: crescenzi@unibas.it.

Potato virus Y (PVY) is a widespread potyvirus and a serious pathogen of tomato in Europe. Recently, severe outbreaks of PVY isolates endowed with a high biological, serological, and molecular variability have been observed in tomato crops in Italy. The use of genetic resistance is the best strategy to control losses caused by this virus. The gene *pot-1* confers what appeared to be a durable resistance to PVY in the tomato wild relative *Lycopersicon hirsutum* PI247087, which nonetheless, was recently overcome by a PVY strain carrying a single aminoacidic change in the VPg. To determine whether other PVY strains able to overcome this resistance exist, *L. hirsutum* PI247087 seedlings were mechanically inoculated with 20 different virus isolates, available at the University of Basilicata that had been recovered from tomato, pepper and black nightshade in different Italian regions. Inoculated plants were monitored for symptoms development and analysed by TAS-ELISA 14 and 28 days post inoculation. None of the PVY isolates used in the present study was able to infect *L. hirsutum* PI247087. This can be taken as an indication that virulent PVY isolates able to overcome the *pot-1* mediated resistance may not occur in the main tomato growing areas of Italy. Based on this assumption, the production and release of tomato varieties carrying the *pot-1* gene could represent an attractive strategy to control PVY in open field tomato crops.

ISOLATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS AFTER TREATMENT OF *PLATANUS ACERIFOLIA* LEAVES WITH CERATO-PLATANIN, A MULTI-FUNCTIONAL PROTEIN FROM *CERATOCYSTIS FIMBRIATA* F.SP. *PLATANUS*. F. Fontana¹, R. Bernardi¹, M. Salvini², A. Scala³, S. Tegli³, L. Carresi³, L. Pazzagli⁴, M. Durante¹. ¹Department of Agricultural Plant Biology, Genetics Section, University of Pisa, Via Matteotti 1/B, 56124 Pisa, Italy. ²Scuola Normale Superiore, Piazza dei Cavalieri 56100 Pisa, Italy. ³Department of Agricultural Biotechnology, Plant Pathology Section, University of Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. ⁴Department of Biochemical Sciences, University of Firenze, Viale Morgagni 50, 50142 Firenze, Italy. Fax: +39.050.576750; e-mail: rbernard@agr.unipi.it.

Cerato-platanin (CP) is a 12.4 kDa protein produced by the

Ascomycete *Ceratocystis fimbriata* (Ell. and Halst.) Davidson f.sp. *platani* Walter, the causal agent of the canker stain of plane trees. CP has been shown by our research group to have a high content of β -sheets and a high percentage (40%) of hydrophobic residues, and to contain 4 cysteines forming two S-S bridges at Cys20-57 and Cys60-115. CP is the prototype of the cerato-platanin family, and its N-terminal region has a high similarity with that of cerato-ulmin, an *Ophiostoma* II class hydrophobin involved in Dutch elm disease. CP is able to self-assemble to give large, thioflavin T-positive molecular aggregates, showing some similarities with those observed in the moderately hydrophobic hydrophobins. CP is located in the fungal cell walls, is secreted early in culture filtrates, and interacts with the host leaves by eliciting phytoalexin synthesis, extended cell plasmolysis and crushing, and abundant starch accumulation in the chloroplasts. The CP gene was cloned in *Pichia pastoris*. The recombinant protein was properly folded and maintained its biological activity. In the present paper, the study of the expression of the host genes following treatment with CP was approached by using the suppression subtractive hybridisation method (SSH). We constructed forward and reverse subtracted cDNA libraries for the isolation of differentially expressed transcripts, and identified some genes that are switched on/off after the treatment. The main feature of this technique is the peculiar effectiveness in isolating rarely expressed transcripts.

MOLECULAR CHARACTERIZATION OF RESISTANCE-BREAKING STRAINS OF TOMATO SPOTTED WILT VIRUS INFECTING RESISTANT PEPPER CARRYING THE *TSW* GENE. P. Margaria, D. Pacifico, M. Ciuffo, M. Turina. Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. Fax: +39.011.343809; e-mail: m.turina@ivv.cnr.it.

Tomato spotted wilt virus (TSWV) (*Tospovirus*, *Bunyaviridae*) is the causal agent of an important disease of pepper (*Capsicum* sp.) occurring worldwide. The most effective and environmentally sound control strategy for this virus relies on the availability of resistant cultivars. Unfortunately all such cultivars seem to rely on a single dominant resistance gene called *Tsw*. This gene induces a hypersensitive-like reaction (HR) characterized by necrotic local lesions limiting the spread of the virus to the point of entry. Naturally occurring resistance-breaking TSWV strains (RB-TSWV) are a recent cause of major concern. A collection of RB strains was obtained during breeding work carried out to introgress the *Tsw* gene in some local *Capsicum* varieties. We are currently working with a collection of RB-TSWV strains in order to identify the specific genetic determinant that allows the virus to overcome the *Tsw* gene in *Capsicum*. A reverse genetic approach is so far unfeasible for minus strand genome plant virus such as TSWV. For this reason, we have set up a series of reassortment experiments between the wild-type strain Br01 and RB-TSWV strains. Our results confirm that S RNA, which encodes both the nucleocapsid N and a non-structural NSs protein, carries the genetic determinant responsible for the breakdown of *Tsw* resistance. Sequence comparisons between wild type and various full-length S RNA segments of RB strains allows us to speculate about the specific genetic determinant of the "avirulence" factor. Moreover, we are investigating the heterologous expression of N and NSs proteins from both wild type and RB TSWV strains with various vectors and leaf agroinoculation with the aim of eliciting the HR-like response characteristic of the TSWV-resistant *Capsicum* interaction.

POST-HARVEST TREATMENTS BY *EPICOCOCCUM NIGRUM* TO CONTROL BROWN ROT OF STONE FRUITS. M. Mari¹, L. Casalini¹, P. Melgarejo², A. De Cal², I. Larena², M. Liñán², J-F. Mandrin³, J. Mazollier³, A. Bellini⁴, P. Domenichini⁴, J. Usall⁵, R. Torres⁵. ¹CRIOF – DIPROVAL, Alma Mater Studiorum – Università di Bologna, Via Gandolfi 19, 40057 Cadriano (BO), Italy. ²INLA, Caretera De la Coruña km 7, 28040 Madrid, Spain. ³CTIFL – Centre de Balandran, BP32, 30127 Bellegarde, France. ⁴SIPCAM S.p.a., Via Sempione 195, 20016 Pero (Milano), Italy. ⁵Postharest Unit, CeRTA, Centre UdL-IRTA, Av. Rovira Roure 19, 25198 Leida, Catalonia, Spain. Fax: +39.051.76504; e-mail: mari@agrsci.unibo.it.

Brown rot caused by *Monilia laxa* and *Monilia fructigena* is a serious disease found in all commercially grown *Prunus* species in European Mediterranean areas. Losses of economic importance result from infection to flowers and from fruit rot at harvest and in the postharvest phase. Pathogens are controlled by fungicide applications during fruit development. Postharvest treatments are not made in France, Italy and Spain. In the last 15 years, interest in disease management practices alternative to chemical pesticide applications has increased due to the need to eliminate chemical residues from the fruits. The potential of *Epicoccum nigrum* for the biological control of *M. laxa* in the orchards has been demonstrated elsewhere (Melgarejo *et al.*, 1986; Madrigal *et al.*, 1994). Attempts to develop an effective and practical method for controlling brown rot disease by pre- and post-harvest *Epicoccum* treatments are described. Postharvest treatments were carried out in Spain, Italy and France. Naturally or artificially infected fruits were treated with *E. nigrum* fresh conidia or a formulate based on conidial paste. Concentrations of 10⁶, 10⁷, and 10⁸ conidia ml⁻¹ were tested for three years. After treatment, fruits were kept at 20°C and high humidity for 7 days. In postharvest trials carried out in Italy, a treatment with *E. nigrum* at the rate of 10⁸ conidia ml⁻¹ reduced significantly natural and artificial infections on nectarines with respect to untreated controls. Lower concentrations of fresh cells and formulate were less effective. No disease control was obtained in Spain and France (in these trials only 10⁶ conidia ml⁻¹ were tested). The effect of integration of pre-harvest *E. nigrum* treatments with physicochemical postharvest treatments was also evaluated on naturally infected fruits.

VALIDATION OF A-SCAB, A DYNAMIC SIMULATION MODEL FOR APPLE SCAB. V. Rossi¹, S. Giosuè¹, R. Bugiani², F. Spanna³, A. Galliano⁴. ¹Istituto di Entomologia e Patologia vegetale, Università Cattolica del S. Cuore, Piacenza, Italy. ²Servizio Fitosanitario Regionale, Regione Emilia-Romagna, Bologna, Italy. ³Settore Fitosanitario, Sezione di Agrometeorologia, Regione Piemonte, Torino, Italy. ⁴Creso S.c.a.r.l., Cuneo, Italy. Fax: +39.0523.599256; e-mail: vittorio.rossi@unicatt.it.

Scab, caused by *Venturia inaequalis*, is the main fungal disease of apples worldwide. The control of primary infections, caused by ascospores, is a key point in disease control. Optimal timing for fungicide sprays against these infections is usually defined using spore samplers and Mills tables together, the former providing information about inoculum density, the latter estimating infection severity based on temperature and leaf wetness. Unfortunately, spore samplers are expensive and time consuming, while the Mills tables frequently give unjustified alarms. To improve information about scab infection periods, A-Scab, a dynamic simulation model, was previously elaborated. It simulates maturation, ejection, deposition and infection probability of ascospores during the season based on hourly data of the air temperature, rainfall, relative humidity, and leaf wetness, and produces a risk index for each infection period and an estimate of disease outbreak.

The model has now been validated under different epidemiological conditions in apple orchards of Piedmont and Emilia-Romagna, between 2002 and 2004. The model was operated using meteorological data from the regional agrometeorological networks, and its outputs were compared with daily spore counts, disease onset, and its severity on leaves and fruits. The model produced accurate and robust simulations. There was a good agreement between estimated periods of scab appearance and disease onset in the orchard. The risk index was also able to estimate infection severity well, severe infections in the field being always associated with high values of the risk index. A-Scab can then be used for warning for apple scab control.

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FIRST RESULTS ON THE USE OF MOLECULAR MARKERS TO TRACK *METSCHNIKOWIA PULCHERRIMA* STRAINS AGAINST POSTHARVEST ROTS ON GOLDEN DELICIOUS APPLE. D. Spadaro, A. Garibaldi, M.L. Gullino. Centro di Competenza per l'Innovazione in Campo Agro-ambientale (AGROINNOVA) e Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia Vegetale, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Fax: +39.011.6708541; e-mail: davide.spadaro@unito.it.

Some strains of the yeast *Metschnikowia pulcherrima* isolated from the carposphere of apples showed high efficacy against *Alternaria* sp., *Botrytis cinerea*, *Monilia* sp. and *Penicillium expansum*. One of the major requirements for an antagonist to be used in biological control is its precise identification and traceability, to allow following its environmental fate in space and time, after release. The RAPD-PCR technique was used to study the competitive action of the strain GS88 of *M. pulcherrima* on the epiphytic microflora of apples cv Golden delicious. The OPB17 primer was chosen for the identification of this strain that was applied at three concentrations (10⁷, 10⁶ and 10⁵ cells ml⁻¹) for different times on the carposphere of the fruits. Eucariotic microorganisms (fungi and yeasts) and bacteria were isolated on selective media. A rapid extraction of the yeast genomic DNA was carried out. After the shortest dips, the number of fungi and isolates of other yeasts decreased, while the number of bacteria was higher, with superior concentrations of GS88. Two different ways of application were studied for another strain of *M. pulcherrima*. The strain GS37 was applied on apples of cv Golden delicious from an organic orchard by spraying and by immersion at three different concentrations (10⁸, 10⁷ and 10⁶ cells ml⁻¹). Immersion proved more effective than spraying at the same concentration of inoculum. A stability or increase in the number of bacterial cells was observed by increasing the initial concentration of the antagonist. To study the competition with other yeast cells a couple of STS (sequence tag site) primers specific for this strain, designed on the sequence of a specific AFLP band, were used.

CHARACTERISATION OF BINUCLEATE AND POLYNUCLEATE ANTAGONISTIC *RHIZOCTONIA* STRAINS. L. Ferraris, D. Valentino, F. Cardinale, G. Tamietti. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia vegetale, Università di Torino, Via L. da Vinci 44, 10195 Grugliasco (TO), Italy. Fax: +39.011.6708541; e-mail: giacomo.tamietti@unito.it.

Two isolates of *Rhizoctonia*, R2 binucleate and R3 polynucleate, effective against *R. solani* on radish were investigated for

their mode of action. Tomato seedlings were grown in soil colonised with R2, R3, a non-pathogenic suppressive *Fusarium oxysporum* strain, as standard, or in steamed soil. Plant leaves were challenge inoculated with *Botrytis cinerea*. Laminarinase and chitinase activity was determined in leaves before and after inoculation with *B. cinerea*. Microscopic observations of root/fungus interaction were also made. Root colonisation by the antagonists caused a significant increase of the tested enzymes in the leaves, which was higher in those inoculated with *B. cinerea*, showing induced systemic resistance. A reduction of *B. cinerea* lesions was sometime observed. R3 promoted tomato growth. For molecular classification, a taxonomically relevant ITS trait was amplified from R2 and R3. Amplicons were subjected to RFLP analysis in parallel with the pathogenic *R. solani* FAG and a whole collection of tester strains covering all known *R. solani* anastomosis groups. ITS from R2, R3 and FAG were entirely sequenced. BLAST analysis revealed 100% identity of R3 with *R. solani* AG4; FAG also showed the highest similarity, although less than R3, to AG4 isolates worldwide. R2 confirmed to be most similar to other binucleate *Rhizoctonia* spp. Diagnostic primers were designed based on ITS sequence alignment between R2, R3 and FAG. They are being tested for their efficiency and specificity as a tool for further analyses on R2 and R3 antagonistic mechanism on plant roots challenged by FAG.

THE INTERGENIC SPACER OF rDNA IN *TRICHODERMA* SPP.: SEQUENCE CHARACTERISATION. E. Mercatelli, S. Pecchia, S. Ciliegi, G. Vannacci. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. Fax: +39.050.543564; e-mail: mercatell@agr.unipi.it.*

The entire nuclear rDNA intergenic spacer (IGS) of 16 *Trichoderma* isolates belonging to the *Pachybasium* and *Trichoderma* sections was amplified using the primer pair 28STD and CNS1. PCR products of ca 1900 to 2400 bp were recovered from agarose gels, cloned, and sequenced. Comparing all sequences, two distinct regions were identified: one more variable (region A) containing the IGS 5' end, and one more conserved (region B) that included the IGS 3' end. The analysis of sequence substitution frequencies of the two regions showed that in region A 732 identical pairs were found out of 1704 total nucleotides (41.4%), with 59.4% in GC content. In region B identical pairs made up 723 out of 832 total nucleotides (86.9%), with 61.3% in GC content. Following multiple alignment, a phylogenetic relationships between isolates was built. Isolates belonging to section *Trichoderma* clustered separately from isolates belonging to section *Pachybasium*. Sequence analysis showed that the organisation of rDNA IGS is typical of rDNA spacers, consisting of a central repetitive region flanking unique sequences on either side. Imperfect inverted repeats of aligned 5'ETS sequences suggested the presence of secondary structures in *Trichoderma* species whose comparison between the two sections was made. The putative ends of the 28S and 18S rDNA were also identified.

OZONE AS SOIL FUMIGANT FOR CONTROL OF TOMATO CORKY ROOT. F. Ciccarese, O. Longo, D. Schiavone. *Dipartimento di Biologia e Patologia vegetale, Università degli Studi, Via G. Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442906; e-mail: fciccare@agr.uniba.it.*

The investigations carried out up to now on development of alternatives to methyl bromide for soil fumigation have re-proposed old chemical compounds or new formulations of neglected fumigants. All alternatives do not take into proper consideration the need of minimizing their environmental impact. The objective of this study was to determine and demonstrate the efficacy of soil treatment with ozone for controlling corky root of tomato caused by *Pyrenochaeta lycopersici*. Trials were made on greenhouse tomato grown in soil naturally infected by *P. lycopersici*. Before transplanting, ozone gas, manufactured on site with a generator, was injected into soil through drip tubing of 16 mm PVC tubes with 300 mm emitter spacing. The drip tubing was buried just next the transplanting rows, at a depth of 200 mm in the soil. Injection tubes were left in place throughout the duration of the trial and were used for subsequent irrigation and fertirrigation. The ozone was applied either alone as gas into moistened soil or was dissolved in irrigation water. The effectiveness of ozone fumigation was compared with untreated control and with dazomet fumigation. The results of these trials generally demonstrated the broad effectiveness of ozone treatment of soil in reducing the gravity of tomato corky root. Advantages of this method of soil fumigation from a point of view of environmental impact are discussed.

OCHRATOXIN A AND PRODUCING FUNGI IN SARDINIAN VINEYARDS. S. Serra, L. Maddau, A. Franceschini. *Dipartimento di Protezione delle Piante Sezione di Patologia Vegetale, Università degli Studi, Via De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; e-mail: salvase@uniss.it.*

New EC regulations will limit the Ochratoxin A (OTA) content in wine. Consequently several Sardinian vineyards were monitored to detect both OTA (by means of HPLC with fluorescent detector) and OTA producing species of *Aspergillus* and *Penicillium*. In 2001 and 2002 grape samples from different cultivars were collected in 14 vineyards of north and south Sardinia, at set times during ripening. Grapes were pressed and subjected to mycological and chemical analysis. Wine samples from some of these vineyards were subjected to chemical tests. All *Aspergillus* and *Penicillium* species isolated were tested for OTA production *in vitro* by TLC. *Aspergillus* populations varied from 0 to $3.9 \cdot 10^5$ CFU g⁻¹ and *Penicillium* populations from 0 to $5 \cdot 10^5$ CFU g⁻¹. Most isolates belonged to *A. niger* and *P. brevicompactum*. Among OTA producing species, *A. carbonarius* and *A. ochraceus* were sporadically isolated, but only the latter was positive to TLC test. OTA was found in 2 out of 45 grape samples tested (0.77 and 0.14 mg kg⁻¹) and in 2 out of 11 wine samples (< 0.05 mg l⁻¹). This survey indicates that the situation for Sardinian wines looks good, as the OTA limit is likely to be established at 2 mg l⁻¹. However, more extensive investigations are necessary to confirm the present data.