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ABSTRACTS

# Abstracts of invited lectures, oral and poster presentations given at the Joint International Congress: 14th Congress of the Mediterranean Phytopathological Union and International Society of Mycotoxicology (Mediterranean Branch) meeting, Istanbul, Turkey, 25–29 August 2014

The joint 14th Congress of Mediterranean Phytopathological Union and International Society of Mycotoxicology (Mediterranean Branch) meeting was held in Istanbul, Turkey, on August 25–29 2014. The invited lectures, oral and poster presentations covered aspects of plant diseases caused by fungi, bacteria, viruses, protozoa and non-parasitic disorders, and with disease control. Particular attention was given to the Congress theme "Plant health management for ensuring food security, safety and quality in the Mediterranean area: Challenges and prospects". In addition, round-table discussions was held on "*Xylella fastidiosa*: a serious menace to the Mediterranean fruit industry" (chaired by Anna Maria D'Onghia, Italy), and "Epidemiology and control of new spreading virus diseases of economic importance in the Mediterranea basin" (chaired by Khaled Makkouk, Lebanon). Abstracts of the invited papers, and of the offered oral and posters presentations are given in this issue.

## Invited speakers

**Systematics and pathogen names in plant pathology: the Botryosphaeriaceae as a case study.** A.J.L. PHILLIPS. *Departament of Life Sciences, Faculty of Sciences and Technology, Universidade Nova de Lisboa, Monte de Caparica, Portugal. E-mail: alp@fct.unl.pt*

It has been estimated that nearly 70% of damaging forest insects and pathogens established in the US between 1860 and 2006 most likely entered on imported live plants, and it is likely that a similar figure applies to other hosts and countries. Considering that the trade in live plants around the world has become a major industry valued at more than € 350 billion annually the need for strict control on the potential import of pathogens is critical. Implementation of such control measures depends on correct and accurate identification of pathogens. Correct identification of a fungus is also fundamental for the correct diagnosis of a plant disease. Indeed, reliable identification is one of the main challenges facing plant pathologists and mycologists. Unfortunately, many fungal species names are based on morphological or other phenotypic characters, which has resulted in ambiguous definitions frequently encompassing several morphologically similar species, and duplication of names. The application of DNA sequence-based phylogenetic methods has dramatically influenced the taxonomy and systematics of several fungal genera resulting in unambiguous species definitions, resolution of cryptic species complexes and a clearer view of the relationships between pathogens and saprophytes. In turn, this has resulted in numerous name changes that have caused confusion amongst plant pathologists. The recent adoption of the one fungus one name policy has added a further layer of confusion leaving many pathologists wondering if all of this is really necessary. Using the Botryosphaeriaceae as an example I will explain why name changes have been necessary and how they will ultimately make our lives as pathologists so much easier.

**Potential threatening diseases of Mediterranean fruit crops and strategic innovative surveillance tools.** A.M. D'ONGHIA. *Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) / Mediterra-*

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The entrance and establishment of invasive pathogens of fruit tree crops across the Mediterranean region are serious threats to food security as well as menaces of public and environmental concern, since some of these crops, such as olive, citrus and grapevine, represent a considerable part of the Mediterranean heritage. Governments allocate extensive resources for monitoring and eradication, as well as for plant health services; they cooperate regionally and globally in prevention, early warning and control of invasive pathogens, most of which are strictly regulated in the EU and in other Mediterranean countries. Within this contest, the Mediterranean citrus, olive and grapevine industries seem particularly vulnerable due to the threats posed by the following destructive agents: (i) the severe forms of *Citrus tristeza virus* (CTV), which may soon spread in the whole area by *Toxoptera citricidus*, the most efficient vector, apparently present only in Portugal and Spain; (ii) Huanglongbing, the main destructive disease of the citrus industry worldwide, caused by different forms of *Ca* Liberibacter (asiaticus, africanus, americanus) which are not yet present in the Mediterranean region, although the psyllid vector *Tryoza eritreae* is already established in Madeira and Canary islands; and (iii) *Xylella fastidiosa,* a destructive bacterium mainly for citrus and grapevine species in South and North America, which is vectored by sharpshooter leafhoppers and froghoppers or spittlebugs. This pathogen has just entered the Mediterranean region and is associated with 'olive quick decline syndrome', which is causing the death of many ancient olive trees in Italy. These pathogens may soon invade the whole region and their introduction and dissemination can be contained by strengthening the national quarantine system and promoting the use of certified material. Unfortunately, there is a lack of efficient preventive control measures in most of the countries. Therefore it is necessary to adopt sound and sustainable pathogen surveillance and management, based on a thorough knowledge of the territory and of the time and space evolution of the infection since the first outbreak. To this aim, a successful surveillance model has been applied by Puglia Region in Italy for the control of CTV; this model integrates innovative tools of territorial analyses (forecasting maps, tree counting, space analysis, satellite images) with advanced sampling (MAIB-S) and diagnosis (DTBIA, real-time PCR) methods. This strategy enables the set up of an efficient and advanced surveillance system, also for other pathogens, providing valid support to institutional decision making.

**New frontiers in teaching plant pathology - the scope on crop protection.** A. Von TIEDEMANN, S. WEIGAND. *University of Göttingen, Faculty of Agricultural Sciences, Department of Crop Sciences, Göttingen, Germany. E-mail: atiedem@gwdg.de*

The ongoing global population growth, although expected to slow down in the next decades, will require a further substantial increase in agricultural production by about 70% until 2050. There is no doubt that this is only achievable by increasing yield levels and reducing losses due to pests and diseases. Such losses in the six major crops are estimated to exceed 65% with no crop protection and are still above 30% when applying the contemporary strategies and tools of crop health management (Oerke, 2006). Hence, crop protection, besides breeding for improved cultivars, is a key to meet the century challenge of feeding a growing world. Besides developing and introducing modern agro-technologies, building the human capital for implementimg such progress is crucial. Thus, education in key subjects of the agricultural sciences, particularly the crop sciences, is an essential prerequisite to master the future. While the awareness of the fundamental role of agriculture for the global well-being has strongly increased in the last decade and boosted the interest in agricultural education among the young generation in many countries like Germany, the related academic sector in universities has become an endangered one. This is mainly due to recent developments in which the basic sciences have obtained an increasing weight in many academic and research institutions. For crop protection, this mainly applies for the significant growth in significance of molecular biology. As a result, basic research in plant pathology or entomology have moved into the institutions of basic biosciences while being eroded in the agricultural faculties. In the competition for funding, basic biosciences outcompete applied research initiatives as they seemingly do better comply with the criteria of scientific excellence. As a result, the broader scope and systems approach typically represented by agricultural science and urgently needed to address the burning issues of agriculture now and in the future is being lost in academic research and education. In its Madeira declaration of 2004, EPPO has addressed this problem stating that the "whole scientifc basis of the phytosanitary field is eroding, … while the need for phytosanitary expertise, training and research is substantially increasing." Similar concerns have been raised by the American Phytopathological Society (APS) and by the German Society of Plant Protection and Plant Health (DPG). Similarly, the German Science Foundation (DFG) has stressed the unique role of agricultural research as a systems- and solution-oriented science in its memorandum of 2005 (Anonymous, 2005). The displacement of agricultural sciences as systems science by basic disciplines ignores the fact that both scientific levels are required to warrant further progress and meet the challenges in agriculture. While the biosciences generate novel

tools e.g. for detecting, differentiating and understanding pathogens and pests in an evidence-oriented approach, agricultural sciences are the next link in the knowledge chain to apply cutting-edge knowledge and methodologies in order to pave the way for solving problems in practice and to generate innovations. In this situation, study programmes focusing on key areas like crop health management are needed. As a response, in 2010, an international practice-oriented and science-based master programme on Crop Protection has been launched at the University of Göttingen (https://www.uni-goettingen.de/de/crop-protection-msc/ 148939.html). It is unique in its scope and design linking basic science and practice as well as academia and the industry sector. Key components of the four-semester programme are courses on all subjects relevant for crop health management, including plant diseases and pests, integrated control, pesticide chemistry, use and registration as well as lab courses on molecular techniques in plant pathology/entomology and biocontrol. A unique feature of the programme is an internship where students work on a project in the industry. Besides this, industrial partners give valuable input to courses on pesticide regulation and registration. In addition, they support follow-up PhD projects, for the best performing students. The interest in the programme from students all over the world sharply increases and allows for a competitive selection. Currently, attempts are made to link the programme to similar approaches in other universities in Europe and beyond, in order to build an international network of crop protection education, enabling us to generate the human capital which we will need in the future.

**Arguments and actions for the establishment of plant medicine as a new university science and as a new profession for global agriculture.** E. TJAMOS. *Emeritus Professor of Plant Pathology, Agricultural University of Athens, Greece, President of the Hellenic Society of Phytiatry. E-mail: ect@aua.gr*

The American Phytopathological Society (APS, world's top Phytopathological Society), initiated collaboration in its most recent congress activities in organizing joint meetings between the International Association for the Protection Sciences (2011), and the Entomological Society of America (2011). This strongly indicates that we, the plant health specialists, have to work together from education and research to extension for several important agro-economical, humanitarian and environmental reasons. This presentation highlights the major problems related to the current situation in plant medicine concerning education, research and application around the globe. It presents convincing arguments analysing why we have to move to the establishment of the University science of Plant Medicine (Phytiatry). It elaborates joint actions to be taken to develop the profession of Plant Doctor with more focused education for qualitative and profitable agriculture, securing food desperately needed. Because real specialists in Plant Medicine are almost lacking, allowing the amateurism to prevail. When the APS emphatically underlines the problem in its website and USA Universities have already established postgraduate degrees in plant medicine. When the British Society for Plant Pathology tries dynamically to attract new students to plant pathology, we the scientists of the Mediterranean basin could initiate the move for establishing Plant Medicine as a University science. Major problems with accurate plant disease diagnosis and effective management cannot be limited to diseases and considered separately from pests particularly when non-parasitic diseases create severe diagnostic difficulties with large financial consequences. There are also acute problems related to postharvest plant medicine, to the impact of mycotoxins and chemicals to food safety, integrated pest management and organic farming. I believe that joint efforts to establish a new unified science can secure the future of Plant Health Sciences in general and Plant Pathology in particular. That is why APS produced and provides excellent you tube videos to emphasize the emerging need for plant health specialists. Recent approaches in UK caused by the diminishing availability of plant pathologists are really alarming, while the establishment of plant medicine in Florida and Nebraska has already diffused and adopted by Universities in Japan, China and Taiwan. The Tempus project in plant medicine is a current initiative in several Mediterranean countries. Overwhelming amateurism is prevailing particularly in Mediterranean agriculture from non-specialized scientists in extension. When the Bologna process created problems in agricultural education in Spain and Italy, all speak about specialized scientists. When studies in animal husbandry, in food science, in biotechnology in agriculture, in agricultural engineering and economics are separated from classical agronomy why not establishing Plant Medicine. Time to dare and move to Plant Medicine as a separate University science.

**The future challenges for IPM in the Mediterranean.** I. PERTOT[1,2]. [1]*Fondazione Edmund Mach (FEM), Research and innovation Centre, S. Michele all'Adige (TN), Italy.* [2] *International Organization for Biological and Integrated Control. E-mail: ilaria.pertot@fmach.it*

Integrated Pest Management (IPM) is an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides. Although several definitions of IPM exists, for example

EPA in US defines it as "an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment", while in the EU directive, IPM means "careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. IPM emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms", a wide consensus exists on the advantages of applying IPM in agriculture. Thanks to IPM, the use of chemical pesticides is continuously decreasing on several crops in many countries. Although IPM is a well-established concept, it has to face several challenges in order to reach a wide application in the Mediterranean. The availability of non-chemical tools is still limited in many crops and many countries. A lot of research have been carried out in the last decades to identify alternatives to chemicals, however, extremely few new-concept control solutions have reached the market and/or end-users. At the same time, the reduction of registered chemical active ingredients and more strict regulations have left growers with few tools. Harmonization of regulations on pesticide use in the Mediterranean, access for growers to new technologies and commercial products, implementation of area-wide alternative solutions, development of alternatives to chemicals for specific Mediterranean crops, impact of climate change on pests and the increased spread of new invasive pests are just some of the major constrains that IPM in the Mediterranean has, and will have, to face. A clear definition of main goals and a strict assessment of feasibility and sustainability of the IPM solution is nevertheless the first step to be accomplish in order to be successful. The availability of guidelines for IPM of agricultural crops and standardises methods of testing effects of pesticides on beneficial species is also mandatory. Several studies and surveys indicate that a key feature determining the degree of success in implementing IPM is the extent to which stakeholders (researchers, advisors, growers and other key players as trader, food retailers, etc.) interacted and worked together. This is particularly true for the implementation of the most innovative and advanced IPM solutions. A strong collaboration between neighbouring countries is also crucial in case of some area-wide IPM solutions, especially when facing migratory pests. International collaboration and exchange of knowledge in the development and promotion of biological and integrated production systems is a key step to cope with future challenges in IPM in the Mediterranean.

**What future for biological control of plant diseases?** G. VANNACCI. *Department of Agriculture, Food and Environment, University of Pisa, President of the Italian Phytopathological Society. E-mail: giovanni.vannacci@unipi.it*

Biological control of plant diseases is still in its infancy, but pointers indicate that the interest for this crop protection strategy is widely increasing. Before discussing the future for biocontrol, we need to agree on its definition, as "Biological control" really represents a large array of ideas. In this talk we will delimit the concept of "Biological control" to the purposeful utilization of introduced organisms, other than host plants, to suppress the activities and populations of one or more plant pathogens, keeping in mind that biocontrol tools have to be considered only a facet of the Integrated Pest Management concept. As all infants, development of Biological Control will be driven by several factors, including scientific achievements, national and supranational regulations, availability of new pesticides, public perception, market structure among others. These drivers will be brought into question in order to foster a sustainable approach to crop protection.

**Tools and strategies to mitigate the effect of mycotoxins.** A. VENÂNCIO, L. ABRUNHOSA, C. SANTOS and N. LIMA. *CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal. E-mail: avenan@deb.uminho.pt*

In food safety management there are many hazards which have to be considered. Among them are mycotoxins, which are chemical agents produced in foods by filamentous fungi. Human exposure to mycotoxins can occur by a direct route (consumption of food contaminated by the fungus) or by an indirect route (consumption of animal products contaminated through animal feed). The need to properly control the presence of these toxic agents in foods presents challenges for their prevention and removal from food commodities. Many tools or strategies may be employed to reduce mycotoxin levels in food commodities. The most important are the preventive ones, since they avoid commodity losses in the first place. In some instances, the early detection of mycotoxins is needed, so that the correct preventive action may be applied. However, absence of mycotoxins has not yet been achieved, making the need of further actions for the reduction of contamination. These measures, which are technologically diverse, are usually classified into physical, chemical or biological. Physical

methods consist of segregation, sorting, cleaning, peeling and others that aim to remove the most contaminated fractions of the commodities. Chemical methods consist of the use of compounds to destroy toxins, as is the case for ozone (ozonation). Finally, biological methods use microorganisms to decompose, transform or adsorb toxins from contaminated products or to avoid the toxic effects when mycotoxins are ingested. Most of biological technologies are mediated by enzymes, making them more specific than most physical and chemical methods. As a preventive example, the direct detection of fungal pathogens causing internal infection in food tissues without laborious previous treatment is an urgent need, and the use of tools based on matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) analysis is a promissing solution. MALDI-TOF MS has demonstrated high potentiality on the identification of filamentous fungi at species level by analysing the intact fungal cells. However, the direct detection of fungal pathogens causing internal infection in agricultural commodities is still in its infancy. The use of lactic acid bacteria (LAB) is seen as solution to reduce the exposure to dietary mycotoxins because of the unique mycotoxin decontaminating characteristic of some LAB. Either by the adsorption of mycotoxins to the cell wall or by their degradation into less toxic compounds it is possible to reduce the exposure to these contaminants. In the case of ochratoxin A (OTA), to the best of our knowledge, no reports have clearly demonstrated its biodegradation by LAB. Previous studies have either concluded that OTA was adsorbed onto the LAB cell walls or did not allow elucidation of the mechanism of elimination of OTA. From a perspective of implementing a food safety model, some recent advances in the above mentioned strategies to mitigate mycotoxin contamination will be presented.

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**Almond witches' broom phytoplasma, an emerging lethal disease of stone fruits with potential threat to the Mediterranean area.** Y. ABOU-JAWDAH[1], M. JAWHARİ[1], E. CHOUEİRI[2], P. A. BIANCO[3], A. ALMA[4], M.MOLINO-LOVA[2]. [1]*American University of Beirut, Faculty of Agricultural and Food Sciences, Department of Agricultural Sciences, Beirut, Lebanon;* [2]*Lebanese Agricultural Research Institute, Department of Plant Protection, Tal Amara, Rayak, Lebanon;* [3]*Università degli Studi di Milano, Di.S.A.A. - Dipartimento di Scienze Agrarie e Ambientali, Milan, Italy;* [4]*1DISAFA, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: abujawyf@aub.edu.lb*

Almond witches' broom (AlmWB) is a fast-spreading lethal disease of stone fruits associated with '*Candidatus Phytoplasma phoenicium*' that belongs to 16S rRNA group IX. AlmWB was first observed on almond trees in the 1990s in North Lebanon; it is characterized by proliferation, small chlorotic leaves, development of witches' broom on the stems followed by dieback and death of almond trees. So far, AlmWB has been reported only in Lebanon and Iran. To monitor and study this disease, cooperation was established between the ministry of agriculture, the agriculture research institute, three Lebanese Universities, two Italian Universities and an NGO. Recent surveys showed that AlmWB had become widely spread in Lebanon, that epidemics had occurred also on peach and nectarine trees, and that the disease had killed or damaged about 200,000 trees. Therefore, the disease was considered of quarantine importance and containment measures were initiated in major stone fruit production areas. This presentation will shed the light on some aspects of the disease epidemiology. It will focus on the spread of the phytoplasma in Lebanon, development of specific, sensitive and reliable PCR and real-time PCR detection methods, and on preliminary data obtained on the identification of potential insect vectors and alternative hosts, and on integrated disease management. Finally, the importance of phytosanitary control measures, the adoption of a national strategy and regional cooperation in order to contain the further spread of the disease will be discussed.

**Virus diseases affecting the Mediterranean stone fruit industry.** K. ÇAĞLAYAN. *Mustafa Kemal Univ., Agriculture Facul, Plant Protection Depart, Hatay, Turkey. E-mail: kcaglayano@yahoo.com*

The Mediterranean region provided approx. 30% of the world supply of stone fruits, and this confirms the strategic role of the fruit sector and its potential on the world market. Over the last 10 years, an intensive and diversified evolution of stone fruit production has been observed within the Mediterranean countries among which Turkey is the major producer with 2,412,268 tons accounting for 24.5% of the total production of Mediterranean countries. Peach, apricot and plum are the most important crops contributing 47%, 19% and 14% of the total production, respectively. Although *Prunus* spp. can be affected by many viruses belonging to *Ilarvirus*, *Potyvirus*, *Trichovirus*, *Nepovirus*, *Foveavirus*, *Capillovirus* and *Ampelovirus*, the first three genera cause the most important diseases in Mediterranean countries. Sharka, caused by *Plum pox virus* (PPV), is the most devastating disease of stone fruits, mainly affecting apricot, peach and plum trees. The disease was described for the first time in 1917 on plums and in 1933 on apricot in Eastern Europe. Since then the virus has progressively spread to a large part of the European

continent, around the Mediterranean basin and in the Middle East. Sharka disease has serious agronomic and political consequences because it causes severe economic losses. Estimated costs associated with sharka management worldwide in the last 30 years exceed €10,000M. The introduction of infected plant propagation material is the most important means of long distance spread of PPV. The virus is also non-persistently transmitted by a number of aphid species. Three ilarviruses, *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV) are important in the Mediterranean stone fruit industry. Extended field surveys for ilarviruses of stone fruits, coordinated by the Mediterranean Agronomic Institute of Bari (Italy), were carried out in the Mediterranean region during 1992 to 2007. The results of enzyme-linked immunosorbent assay (ELISA) testing of approximately 24,000 trees (almond, apricot, plum, peach, and sweet and sour cherry) demonstrated high incidence (23.5%) of ilarvirus infection. Among ilarviruses that infect stone fruits, PNRSV, ApMV, and PDV occur worldwide, whereas *American plum line pattern virus* (APLPV) was limited to the United States and Canada. However, recently it has been isolated from trees of different *Prunus* species in several Mediterranean countries. Reports on nepoviruses are extremely rare from the Mediterranean countries because their vectors (*Xiphinema diversicaudatum*, *Longidorus elongatus*, *L. attenuatus* and *L. macrosoma*) like temperate regions with cool and humid conditions, but do not thrive well under the warmer and drier climate. Among the *Trichovirus* genus, *Apple chlorotic leaf spot virus* (ACLSV) is the most important due to its widespread occurence in many Mediterranean countries especially in apricot. Although latent infection is observed frequently, severe symptoms can be also reported depending on the viral isolate-plant cultivar combination. *Apricot latent virus* (ApLV), a *Foveavirus*, is mostly latent in apricot, which is the natural host for this virus. It was reported only from France, Greece and Turkey in Mediterranean region. *Plum bark necrosis-stem pitting associated virus* (PBNSPaV) is currently classified as a tentative species in the genus *Ampelovirus*, and occurs in Italy, Turkey, Marocco and Jordan among Mediterranean countries. Symptoms of PBNSPaV consist of bark necrosis, stem pitting, delayed budbreak and reddening of leaves on plum, apricot, almond, cherry and peaches. The general picture of the sanitary situation of the Mediterranean stone fruit industry was studied by many workers in different periods and spreading of important virus diseases can be a real risk for the virus-free production of propagation material. In the Mediterranean basin, there is still a high risk of further spread of virus diseases due to transporting of latently infected plant material that have escaped the quality controls and/or the quarantine procedures.

## Oral and poster presentations

**Study and definition of the syndromes of the esca complex of grapevine in Capitanata (Southern Italy).**
A. CARLUCCI, M.L. RAIMONDO, F. CIBELLI and F. LOPS. *Department of Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy. E-mail: antonia.carlucci@unifg.it*

Esca is a disease that has always affected the vineyards of Puglia, Italy, and those throughout the world. It is a disease complex, as several abiotic and biotic factors combine in its complexity. In the province of Foggia, viticulture is one of the most important sources of income. The present study was carried out as part of a research project funded by the *Fondazione Cassa di Risparmio di Puglia*, with the aim to define the most widespread symptoms on grapevines in the Foggia area, and to correlate the external symptoms with the internal (xylem tissue) symptoms, and the fungal species isolated from the symptomatic tissues. The external symptoms consisted of "tiger striped leaves" and "diffuse chlorosis of the leaves", which were often accompanied by leaf-margin necrosis, and sudden and unexpected death of whole plants. The following internal symptoms were associated with the first symptoms: brown spots with exudates; small circular brown patches; extended brown patches that covered large portions of the xylem system; and degradation of the wood (white rot). The following internal symptoms were associated with the external symptoms of leaf chlorosis and leaf-margin necrosis: superficial and deep subcortical browning, from a few mm to 1 cm, seen in longitudinal stem sections; and deep brown parts that penetrated into the deep medulla. Widespread necrosis of the foliage and the branches, and degradation of the vascular tissues were associated with the external apoplexy symptoms. The fungi isolated from the woody tissues were identified by morphological and molecular characterisation. The correlations observed between the internal and external symptoms and the fungi isolated were as follows. The tiger striped leaves were associated with: brown spots, which were in turn correlated to the presence of *Ph. chlamydospora*; brown circular areas, correlated to *Phaeoacremonium* spp.; and white rot, correlated to *F. mediterranea*. The leaf chlorosis with leaf-margin necrosis was associated with: brown subcortical surfaces, which correlated to *Pleurostomophora richardsiae*; brown subcortex and deeper areas, correlated to fungi belonging to the family of the Botryosphaeriaceae spp. The results of these investigations are consistent with the literature, although the presence of *Pl. richardsiae* associated with symptoms of esca disease is reported here for the first time in Italy (Foggia province). Therefore, in our opinion, we believe that *Pl. richardsiae* can be included among the causative agents of esca disease. In addition, the direct correlation between leaf chlorosis and leaf-margin necrosis and fungi of the Botryosphariaceae, suggests that this definition of the external symptoms

can be included among the signs associated with the complex syndromes of esca disease.

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**Pathogenic and genetic diversity of *Pyrenophora tritici-repentis* in Algeria and consequences of new virulence type identification in some basis of *Pyrenophora tritici-repentis* / wheat pathosystem.** H. BENSLIMANE[1], L. LAMARI[2], A. YAHYAOUI[3], F. OGBONNAYA[4,] Z. BOUZNAD[1], A. BENBELKACEM[5] and M. BAUM[4]. [1]*Ecole Nationale Supérieure d'Agronomie, Département de Botanique, El-Harrach, Algiers, Algeria;* [2]*University of Manitoba, Department of Plant Science, Canada;* [3]*International Center for Agricultural Research in Dry Areas, Aleppo, Syria;* [4]*International Maize and Wheat Improvement Center, Apdo. Postal 6-641, 06600, Mexico DF, Mexico;* [5]*Institut National de la Recherche Agronomique d'Algérie, Unité de Recherche de Constantine, Algeria. E-mail: h.benslimane@ensa.dz*

Tan spot, caused by *Pyrenophora tritici-repentis*, is a major wheat disease in Algeria and worldwide. An isolate population, originating from diverse wheat growing regions in Algeria, was studied to determine which races are present and to identify new races. A differential host set, including both bread and durum wheat were inoculated with the isolates. In order to study genetic diversity among this population, DNA isolates were analyzed using fluorescent Amplified Fragment Length Polymorphism (fAFLP). Initially, 78 primer combinations were tested, of which 12 were selected and applied to the 61 isolates. Races 1, 4, 5, 6, 7, and 8 were found (the first time races 1, 4, 7, and 8 had been identified in Algeria) and a new virulence pattern was discovered. Isolates with this pattern induced necrosis in durum wheat but failed to induce any disease in the common wheat genotypes in the differential set. Our results displayed the highly variable pathogenic nature of the pathogen in Algerian wheat fields, and that durum wheat seems be an additional factor of this variability. They also revealed high genetic diversity in this population, with 61 different haplotypes among the 61 isolates selected. Cluster analysis of molecular data showed that clustering of the isolates was independent of their race classification, geographic origin, or host plant. However, one isolates (Ptr24) that showed a new virulence pattern was clearly distinguished from the rest of the population studied. This isolate had not only new virulence but also different genetic makeup to other *P. tritici-repentis* isolates. These primary data indicated that the *P. tritici-repentis* / wheat pathosystem should be further investigated and revised; the presence of this putative new race involves a significant impact on some bases of this pathosystem. The current model establishing the relationship between different races is permissible only when the host/pathogen interaction is considered for bread wheat. The introduction of durum wheat in the host differential set makes the hypothesis invalid, and requires additional studies to completely decipher host-pathogen interactions for tan spot of wheat.

**Multiplex pcr assay for one-step detection and determination of mating type alleles in *Togninia minima*.** M. ARZANLOU, A. NARMANI and A. BABAI-AHARI. *University of Tabriz, Faculty of Agrciulture, Plant Protection Department, Tabriz, Iran. E-mail: arzanlou@tabrizu.ac.ir; arzanlou@hotmail.com*

Esca is an economically important disease on grapevine. *Togninia minima* is one of the main fungi involved in this disease, worldwide. It has a biallelic heterothallic mating system. A multiplex PCR test was developed for one-step detection of the species as well as the mating type. A primer set specific for *T. minima*, with expected amplicon size of 500 bp, was designed based on the alignment of sequence data for the β-tubulin gene from the known *Phaeoacremonium*. A Mat1-2 gene specific primer set for *T. minima*, with expected amplicon size of 230 bp, was designed based on the *Mat1-2* gene sequence of this species. The specificity of the new primer sets were verified on DNA extracted from a set of *Phaeoacremonium* and other fungal species frequently occurring on grapevine in separate reactions. Both primer sets were then successfully combined in a multiplex PCR for the one-step identification and determination of mating types of *T. minima*. A 500 bp amplicon was obtained only from *T. minima* isolates and none from the other *Phaeoacremonium* spp. Amplification of 230 bp band confirmed *T. minima* isolates that have the Mat1-2 allele. The β-tubulin primer set served as an internal control to confirm that the PCR reaction with the mating-type primer set had worked properly. The reliability of the multiplex PCR assay was further confirmed by crossing representatives from Mat1-1 and Mat1-2 isolates under laboratory conditions. Approximately 3–4 weeks after mating, the *Togninia* teleomorph appeared on the cane and agar surface between Mat1-1 and Mat1-2 isolates of *T. minima*. The control plates (self crossings) did not produce perithecia after 2 months of incubation.The new multiplex PCR assay can facilitate rapid screening of mating types in populations of *T. minima*.

**Postharvest fungal pathogens on pomegranate fruit (*Punica granatum* L. var. hicaz) in cold storage conditions**. P. KINAY TEKSÜR[1], F. ŞEN[2], F. YILDIZ[1,] N. ÖZALTACA[1], A.K. SELVI[1] and A. KALIN[3]. [1]*Ege Univer-*

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The purpose of this study was to determine the postharvest diseases on pomegranate fruits stored in cold storage conditions. In addition, the relationships between the postharvest treatments and diseases were determined. Thirty-three cold storage facilities in Antalya, Manisa and İzmir, Turkey, were visited and samples were taken from cold storage rooms. Relevant information relating to each cold storage room was collected. Fruit showing disease symptoms was brought to the laboratory, and isolations were made from theses. The average incidence of disease ranged from 30% to 50% in cold storage rooms. From these rooms, *Botrytis cinerea* was the most dominant pathogen with average proportion of 40% disease incidence. Incidence of other postharvest pathogens was: *Coniella granati*, 15%; *Penicillium* spp., 12%, *Alternaria alternata*, 12%; *Aspergillus niger*, 8%.

**Biodiversity of *Phytophthora* taxa in Mediterranean maquis ecosystems.** B. SCANU, B. T. LINALDEDDU, A. DEIDDA and A. FRANCESCHINI. *Università degli Studi di Sassari, Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Viale Italia 39, Sassari, Italy. E-mail: bscanu@uniss.it*

The Mediterranean basin is recognized as a global biodiversity centre accounting for more than 25,000 plant species that represent almost 10% of the world's vascular flora. In particular, the maquis vegetation on Mediterranean islands and islets constitutes an important resource of the Mediterranean plant diversity due to its high rate of endemism, accounting for 4.3% of all plant species worldwide. Since 2009, a severe and widespread dieback and mortality of *Quercus ilex* trees and several other plant species of the Mediterranean maquis has been observed in the National Park of La Maddalena archipelago (northeast Sardinia, Italy). Infected plants showed severe decline symptoms such as branch dieback, epicormic shoots, bleeding cankers on the stems, root losses and sudden death associated with a significant reduction of natural regeneration. First studies revealed the involvement of the highly invasive *Phytophthora cinnamomi* and several other fungal pathogens. Subsequent detailed research led to a better understanding of these epidemics, showing that the aetiology is more complex than initially assumed and that multiple other *Phytophthora* spp. are also involved, some of them undescribed. A total of 13 *Phytophthora* spp. were isolated from bark tissues, roots and soil samples collected from symptomatic trees and shrubs such as *Arbutus unedo*, *Asparagus albus*, *Juniperus phoenicea*, *Juniperus oxycedrus*, *Pistacia lentiscus*, *Quercus ilex* and *Quercus suber*. Based on morphological characters, growth–temperature relations and sequence analysis of the ITS and *Cox*1 gene regions, the isolates were identified as: *Phytophthora alticola*, *P. asparagi*, *P. bilorbang*, *P. cinnamomi*, *P. cryptogea*, *P. gonapodyides*, *P. melonis*, *P. nicotianae*, *P. parvispora*, *P. psychrophila*, *P. quercina*, *P. syringae* and two informally designated taxa, *P. aplerotica* prov. nom. and *P. ornamentata* prov. nom., both within ITS Clade 6. Studies are currently underway to formally describe the new species in conjunction with large scale pathogenicity trials to confirm Koch's postulates for the new host/*Phytophthora* associations. The implications of these findings for disease management and plant health policy are discussed.

**Role of *VdLaeA* in virulence and physiology of the vascular wilt pathogen *Verticillium dahliae*.** A.M. GIANNAKOPOULOU[1,2], A.A. GKATZOUNI[1] and D.I. TSITSIGIANNIS[1]. *[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology, Athens, Greece; [2]John Innes Centre, The Sainsbury Laboratory, Norwich, UK. E-mail: dimtsi@aua.gr*

Fungal secondary metabolites are compounds with high degrees of specialization, possessing various roles concerning toxin production, sporulation processes and biosynthesis of substances with special biotechnological and pharmaceutical interest. Previous studies have shown that the phytopathogenic fungus *V. dahliae* produces phytotoxins and other molecules that induce programmed cell death or other forms of host resistance. The exact nature of these compounds remains unknown. In *Aspergillus,* the global regulator of secondary metabolism *laeA* encodes a nuclear protein that is required for the expression of secondary metabolite genes, while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of the *V. dahliae* genome with the *laeA* gene of *A. nidulans* led to the discovery of a homologous gene that was named *VdLaeA*. This gene was deleted in *V. dahliae* in order to clarify whether products of secondary metabolism play any roles in virulence and physiology of this fungus. Greenhouse pathogenicity experiments revealed that the transformed ΔVdLaeA strains reduced the disease levels in eggplant, tomato and *Arabidopsis thaliana* hosts. ΔVdLaeA strains were also altered in the rate and morphology of conidium germination, mycelial development and microsclerotia formation. Additionally, the role of *VdLaeA* in programmed cell death, as well as gene expression data of the regulatory gene *VdLaeA in planta* will be presented. This research expands understanding of the role of secondary metabolites in *V. dahliae* virulence.

**Identification of putative defense-related genes in the genome of *Chondrus crispus*; insights from the genome consortium.** A. ZAMBOUNIS[1,2,3] and C.M.M. GACHON[2]. [1]*CERTH, Institute of Applied Biosciences, Thessaloniki, Greece;* [2]*SAMS, Scottish Marine Institute, Oban, United Kingdom;* [3]*INRA-AgroParisTech, UMR1290 BIOGER, Grignon, France. E-mail: antonios.zambounis@versailles.inra.fr*

The 105 Mbp genome of the *Chondrus crispus* (Irish Moss) is the first available genome of a red macroalga, which has recently become available. The genome depicts features typical of compact genomes, characterized by gene-dense regions surrounded by repeat-rich regions dominated by transposable elements. We report the outputs of manual annotations that we employed in the *C. crispus* genome, targeting genes/domains potentially involved in defense reactions and signal transduction pathways. We systematically curated a variety of putative defense-related genes. Some gene families usually highly expanded in other plant genomes are also fairly abundant in *C. crispus*. We identified 45 Sel1 repeats-containing genes (SLRs) which are presumably involved in interactions with specific ligands during pathogenicity responses. Additionally, 26 tetratricopeptide repeat (TPR)-containing genes were also annotated, two of them fused with a nucleotide-binding domain (NB-ARC domain) which is functionally involved in signalling pathways. In the *C. crispus* genome we also curated 85 genes which were comprised by WD40-repeats (also known as WD or beta-transducin repeats). WD40-repeats proteins are a large family found in all eukaryotes, typically involved in protein-protein interactions, and are implicated in pleiotropic functions ranging from signal transduction and transcription regulation to cell cycle control and apoptosis. This repeated domain may serve as a multi-domain folding scaffold for specific protein binding. We observed intensive fluctuation in number of WD40-repeats per gene and we addressed the hypothesis that different kinds of short range re-arrangements (deletions/duplications) may have occurred on them. Furthermore, in some cases WD40 genes were fused either with Toll/interleukin-1 receptor (TIR) domains or with NB-ARC domains. Only 11 leucine rich repeat (LRR) containing genes were found, while other plants genomes typically contain hundreds of these. No TIR-NBS-LRR homologues of plant resistance genes were identified. Orthologues of apoptosis-related genes were also identified and annotated, leading to an overall picture similar of that found in other plant species. This work was published as part of the *Chondrus crispus* genome consortium paper (Collen *et al.*, P.N.A.S, 2013)

**Investigation of genetic variability of '*Candidatus* phytoplasma prunorum' infecting stone fruit trees.** Ç. ULUBAŞ SERÇE[1], M. GAZEL[2] and K. ÇAĞLAYAN[2]. [1]*Niğde University, Faculty of Agricultural Sciences and Technologies, Niğde, TURKEY;* [2]*Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department, Hatay, Turkey. E-mail: culubas@gmail.com*

Particular gene regions of *Candidatus* Phytoplasma prunorum isolates (the cause of European stone fruit yellows, ESFY) from Turkey were examined to have new information on virulence and mechanisms responsible from their evaluation. Apricot, plum and peach tree samples and *Cacopsylla pruni* psyllid individuals collected from 11 provinces from Turkey (Isparta, Hatay, Yalova, Bursa, Mersin, Niğde, Konya, Muğla, Burdur, Malatya and Erzurum) were analyzed for the pathogen using PCR-RFLP. A total of 17 phytoplasma isolates were investigated for genetic variability. Immudominant membrane proteins (*imp*), which constitute a major portion of the total cellular membrane proteins and have considerable amino acid sequence variations in most phytoplasmas, were targeted for genetic variability studies. This gene region was amplified in three isolates from Yalova (plants), eight from Mersin (five from plants, three from insects), five from Bursa (insects) and one from Isparta (plant). DNA sequences of this gene were provided from PCR products, and multiple alignments of these sequences were performed with nucleotide sequences of the *imp* gene from Genbank. Investigated isolates included in the I3 genotype (two isolates from Mersin) and the I26 genotype (three isolates from Yalova, six from Mersin, one from Isparta) among the 30 genotypes (I1-I30) which constituted*imp* genes genotypes in previous studies (Danet *et al.*, 2011). The I26 genotype has been found only in Turkey and has not been previously identified in Europe, and 10/17 isolates was included in this group. The I3 genotype has been identified only in one isolate from Germany, 2/17 the investigated isolates were included in this group. The analyzed isolates were therefore different from isolates of Europe, and this situation has important implications for phytoplasma epidemiology.

**Toward the understanding of host specificity of *Orobanche crenata* populations to legumes.** M. ENNAMI[1], F. GABOUN[1], R. ABDELWAHD[1], L. BELQADI[2], L. GHAOUTI[2], F. ABBAD ANDALOUSSI[1] and R. MENTAG[1]. [1]*National Institute of Agricultural Research (INRA), Biotechnology Unit, Rabat, Morocco;* [2]*Institut Agronomique et* Vétérinaire Hassan *II, Plant biotechnology Department, Rabat, Morocco. E-mail: rachidmentag@yahoo.ca*

Legumes are are vitally important in Moroccan agriculture. They play strategic roles in improving soil fertility and structure, and they are commonly used in rotation with cereals. During the last decades, however, legume production had decreased due to several biotic and abiotic stresses. *Orobanche crenata* represents major biotic

constraint. This root holoparasitic angiosperm attacks a wide range of host species, including a number of commonly cultivated crops. In Morocco, this parasitic weed is particularly problematic in Faba bean and lentil fields. For the first time, natural seed populations of *O. cenata* produced on different hosts were quantified for their host specificity to *Vicia faba*, *Lens culinaris* and *Anacyclus radiatus*. Pot and rhizotron bioassays of parasite and host growth and development were performed. Evaluation under controlled conditions gave close monitoring of the infestation and rapid evaluation of the sensitivity of host plants to specific *O. crenata* populations. The three host species showed distinct patterns of host specificity. Thus, *Orobanche* populations developed on *A. radiatus* was more host-specific, while *O. crenata* seed populations developed on *V. faba* and *L. culinaris* were more specific to legumes species only. The impacts on host development of this parasitic weed was more pronounced on food legumes.

**Patulin is not involved in the development of blue mold decay on apples.** J. TANNOUS[1,2,3], S.P. SNINI[1,2], R. EL KHOURY[1,2,3], Y. LIPPI[1,2], A. EL KHOURY[3], A. ATOUI[4], R. LTEIF[3], I.P. OSWALD[1,2] and O. PUEL[1]. *[1]INRA, UMR 1331 Toxalim, Research Centre in Food Toxicology, 180 Chemin de Tournefeuille, F-31027 Toulouse, Cedex, FRANCE; [2]Université de Toulouse III, ENVT, INP, UMR 1331, Toxalim, F-31076, Toulouse, France; [3]Université Saint-Joseph, Centre d'Analyses et de Recherches (Faculté des Sciences), Campus des Sciences et Technologies, Mar Roukos, Mkallès, P.O Box 11- 514 Riad El Solh, 1107 2050 Beirut, Lebanon; [4]Laboratory of Microorganisms and Food Irradiation, Lebanese Atomic Energy Commission-CNRS, P.O. Box 11–8281, Riad El Solh, 1107 2260 Beirut, Lebanon. E-mail: opuel@toulouse.inra.fr*

Patulin is a polyketide-derived mycotoxin produced by numerous filamentous fungi. *Penicillium expansum* is by far the most worrisome species. This fungus causes blue mold decay of apples and produces significant amounts of patulin. The biosynthetic pathway of patulin is well-characterized, but its genetic bases remain largely unknown, with only few characterized genes in less economic relevant species. A putatif patulin cluster was described in a non-producing strain of *Aspergillus clavatus* NRRL1, and a nonfunctional "fossil" patulin cluster was also identified in non-producing species such as *P. chrysogenum* and *P. digitatum*. In *P. expansum*, the species of greatest concern, the cluster is not yet sequenced. The present study identified the patulin cluster in *P. expansum* strain NRRL35695 and the assessed the role of patulin in the apple infection process. Primers specific to conserved regions were designed on the basis of some orthologous gene sequence alignments, to amplify and sequence large gene fragments. The intergenic regions were then amplified in order to obtain genes islets. GeneWalking methodology was then used to obtain the remaining gene sequences and link islets together. The characterized patulin gene cluster of *P. expansum* consists of 15 genes (*PatA-PatO*). The gene expression study under patulin permissive and restrictive conditions showed a correlation between gene expression and patulin production. Putative binding sites for fungal transcription factors, such as NRFA, AbaA, StrE, SltA, PacC and BrlA, were shown in the 5' regions of 14/15 genes. A BLAST analysis revealed that the *PatL* gene encodes for a protein that presents a high homology with AFLR, the specific regulatory factor of aflatoxin biosynthesis. The disruption of *PatL* caused the inability to produce patulin with marked decrease of *Pat* gene expression. In the complemented ΔPatL:*PatL* strain, the ability to produce patulin was restored. Pathogenicity studies on apples indicated that the ΔPatL strain was still able to infect apples in the same way and with an equivalent rate compared to wild type strain. This demonstrates that patulin has no active role in the development of blue mold decay on apples.

***Diaporthe foeniculina* associated with severe shoot blight of lemon in Lebanon.** W. HABIB[1], E. GERGES[1], I. ANTELMI[2], F. BAROUDY[1,2], E. CHOUEIRI[3] and F. NIGRO[2]. *[1]Lebanese Agricultural Research Institute, Laboratory of Mycology, Department of Plant Protection, Fanar, P.O. Box 90-165 Jdeidet El Metn, Lebanon; [2]Università degli Studi di Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy; [3]Lebanese Agricultural Research Institute, Department of Plant Protection, Tal Amara, Lebanon. E-mail: whabib@lari.gov.lb*

*Diaporthe* (anamorph *Phomopsis*) comprises endophytes and plant pathogens causing blights, cankers and diebacks on several economically important crops. Some species have a wide host range and more than one species can occur on the same host. In July 2013, several woody samples were collected from lemon trees [*Citrus limon* (L.) Burm.f.] showing severe shoot blight symptoms, in commercial orchards at Sarafend (Saida) and Sammourié (Akkar), in Lebanon. Isolation on potato dextrose agar (PDA) amended with streptomycin sulphate (0.5g $L^{-1}$) from the xylem of infected shoots consistently revealed the occurrence of several *Diaporthe* species. Colonies were grouped according to their morphology on PDA, and the most frequent was selected for further studies. Morphological characteristics were assessed on PDA and water agar amended with sterilized citrus stems. Colonies were grown at 25°C with 12/12h alternative fluorescent light and darkness. Pycnidia were 550–950 μm ($\bar{x}$ = 811 μm) wide, 400–720 μm ($\bar{x}$ = 581 μm) long, multilocular, immersed, ostiolate, without necks, scattered or aggregated, dark brown to black; conidia were exuded in yellow and white creamy cirrhi. Conidiophores were cylindrical, hyaline, filiform, branched above the

septa, 1–3 septate, 20–32.5 × 2–3 μm ($\bar{x}$ = 25.3 × 2.2μm), with phialidic conidiogenous cells, tapering from root to apex. Alpha conidia were unicellular, fusoid with rounded apices and obtuse bases, hyaline, biguttulate, 6.3–10×2–3.8μm ($\bar{x}$ = 7.8×2.6μm). Beta conidia were unicellular, filiform, curved, with rounded ends, hyaline, eguttulate, 20–32.5×1–1.7 μm ($\bar{x}$ = 25.9×1.4 μm). Two-week-old cultures were entirely white on surface and reverse, cottony with slightly fringed margins. Growth rate at 25°C was 11.2 mm $d^{-1}$. Based on the recent taxonomy of *Diaporthe* species, isolates were assigned to *Diaporthe foeniculina* (Sacc.) Udayanga & Castl., synonym of *D. neotheicola* A.J.L. Phillips & J.M. Santos. Molecular analysis of nuclear rDNA internal transcribed spacer (ITS), and partial sequences of the β-tubulin gene confirmed morphological identification, giving 99% similarity with reference strains of *D. foeniculina*. The species has a wide host range including almond, fennel and persimmon. Preliminary results from pathogenicity tests indicate that *D. foeniculina* induces blight symptoms on lemon plantlets. Further tests are in progress to determine its pathogenicity on lemon twigs and fruits and to characterize the other *Diaporthe* spp. from Lebanon.

***Neofusicoccum australe* and *Phytophthora alticola*: the main pathogens associated with declining *Eucalyptus camaldulensis* trees in Sardinia (Italy).** A. DEIDDA, B.T. LINALDEDDU, B. SCANU and A. FRANCESCHINI. *Università degli Studi di Sassari, Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Viale Italia 39, Sassari, Italy. E-mail: adeidda@uniss.it*

*Eucalyptus* L'Hér. is a large genus in the family *Myrtaceae* which includes evergreen trees and shrubs, mostly native to Australia. Due to their rapid growth and adaptability to different environmental conditions *Eucalyptus* species have been widely introduced in both hemispheres for pulpwood production. In Sardinia (Italy), *Eucalyptus* plantations were established in the 20th Century primarily in areas reclaimed from marshland, and subsequently all over the island where they are currently cultivated as ornamental plants, windbreaks and for honey production. In recent years, a severe and unusual disease of unknown aetiology has been observed in several artificially established plantations of *Eucalyptus camaldulensis* throughout the island. The affected plants showed leaf chlorosis, crown thinning, shoot and branch dieback, cankers on branches and trunks, epicormic shoots, exudates, root losses and symptoms of sudden death. Since there is no information about this unusual disease and given the high ecological and economic relevance of these ecosystems, a survey was carried out during 2013 to establish the causal agents involved in the disease. A total of 391 botryosphaeriaceous isolates were obtained from 510 symptomatic branch samples, from which five species (*Neofusicoccum australe*, *N. luteum*, *N. mediterraneum*, *N. parvum* and *N. vitifusiforme*) were identified using morphological characters and DNA-based techniques. *Neofusicoccum australe* was the dominant species (86.7% of isolates), followed by *N. mediterraneum* (5.1%). In addition, 26 *Phytophthora* isolates were obtained from soil and root samples collected from symptomatic *Eucalyptus* trees at six of the 12 investigated sites. Based on morphological and cultural features and analysis of ITS sequences, all *Phytophthora* isolates were identified as *Phytophthora alticola*. All isolates produced papillate, persistent, ovoid, limoniform to distorted sporangia and oogonia with thick-walled oospores and paragynous antheridia. Our results provide the first evidence for combined involvement of *N. australe* and *P. alticola* as causes of the decline affecting *E. camaldulensis* trees in Italy. This is the first report of *P. alticola* in the northern hemisphere.

**Geographical distribution and virulence spectrum of *Phoma tracheiphila* causal agent of mal secco disease of citrus in the Mediterranean basin**. Z. SANA[1], C. SAMIR[1], I. ANTONIO [2] and M. AHMED[1]. *[1]Centre of Biotechnology of Borj Cedria, Laboratory of Plant Molecular Physiology, Tunisia: [2]University of Bari, Department of Agro Forestry and Environmental Biology and Chemistry, Italy. E-mail: sana.ziadi@cbbc.rnrt.tn*

This work aimed to determine the geographic distribution of Mal Secco Disease in citrus orchards of six Mediterranean countries, to determine patterns of virulence variability of the *Phoma tracheiphila* population of 51 isolates, and also to establish relationships between geographic distribution and pathotypic distance of *P. tr* population structure over our sampling spatial scale. Based on the unweighted pair-group method with arithmetic averaging clustering and mean disease rating scores, three distinct virulence groups were identified. The isolates were classified into 20 pathotypes, and extensive virulence variability was detected in the isolates causing Mal Secco Disease of citrus in the Mediterranean basin. A regression plot between geographical distance and pairwise virulence showed that virulence is independent of the geographical origin, indicating that isolates collected from a specific country have different degrees of virulence. The lack of significant correlation between geographic structure and virulence confirmed the absence of an isolation by distance pattern, suggesting non-regular and non-gradual dispersal of the pathogen over this spatial scale.

**Biodiversity of toxigenic fungi in the Mediterranean area.** A. MORETTI, G. MULÈ, G. PERRONE, S. SOMMA, A. SUSCA and A. F. LOGRIECO. *Research National*

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Study of the biodiversity of toxigenic fungi occurring on food crops provides important information for evaluation of the mycotoxicological risks related to their occurrence. The importance of surveys of toxigenic fungal biodiversity is also related to the changes of environmental conditions worldwide, which significantly influence their distribution and profile. Long-standing surveys in the Mediterranean Basin have provided a valuable source for increasing knowledge on the biodiversity of toxigenic fungi, through collection and the study of a wide number of fungal strains belonging to *Aspergillus*, *Fusarium*, and *Penicillium* genera, isolated from samples of cereals, mainly maize and wheat, grape and dried fruits from different countries. The biodiversity studies of strains were based both on a multi-locus phylogenetic approach by sequencing part of the β-tubulin (βt), calmodulin (caM), RNA polymerase II (RPB2) and elongation factor 1$\alpha$ (EF-1$\alpha$) genes, and analysis of their toxin production. The identification of strains from natural samples has led to a total of approx. 1400 strains belonging to *Aspergillus*, approx. 400 to *Penicillium*, and approx. 1200 to *Fusarium*. *Aspergillus* section *Nigri* isolates were predominant compared to *Aspergillus* section *Flavi* strains. In addition, representative strains for each species or population, phylogenetically determined, were analyzed for their mycotoxin production in order to define a typical mycotoxin profile for each group of fungal strains. Considerable diversity was detected in the toxigenic fungal species/strains at a global level from the different crops and for their multi-toxin production risk. This highlights that mycotoxin risk in food commodities is related to the co-occurrence of groups of toxigenic fungi that are both genetically closely or distantly related or distant. Deeper investigations on the potential additive and/or synergistic effects of the combinations of more mycotoxins on a single crop are needed. The extensive diversity of the fungal strains identified in the Mediterranean Basin is a unique biological source for investigations on new emerging toxigenic fungal species, and on new mycotoxin/commodity combinations.

**Occurrence of toxigenic and non-toxigenic *Fusarium* species in major cereal grains used in human consumption in Iran.** M. SHAMS-GHAHFAROKHI[1], A. MOHAMMADI[1] and M. RAZZAGHI-ABYANEH[2]. [1]*Tarbiat Modares University, Faculty of Medical Sciences, Department of Mycology, Tehran, Iran;* [2]*Pasteur Institute of Iran, Department of Mycology, Tehran, Iran. E-mail: shamsm@modares.ac.ir*

*Fusarium* spp. are important in the etiology of human diseases and for their ability to contaminate a wide variety of agricultural crops and subsequent production of carcinogenic mycotoxins (fumonisins). The aim of this study was to evaluate *Fusarium* spp. contamination of maize and wheat samples from ten provinces of Iran, with special attention to the ability of the isolates for production of fumonisin $B_1$ ($FB_1$), to improve public health risk evaluation. Thirty-two maize and 15 wheat samples collected from the major cultivations areas were surface disinfected with 1% sodium hypochlorite solution for 2 min. *Fusarium* species were isolated by the flotation method on Malachite green agar. Pure cultures on Potato Dextrose agar (PDA) were identified using a combination of macroscopic and microscopic morphological characteristics. The ability of the isolates to produce Fumonisin $B_1$ was evaluated by thin layer chromatography (TLC), and the amounts of fumonisin $B_1$ produced were assessed by high performance liquid chromatography. A total of 55 *Fusarium* isolates belonging to four species were isolated. Twenty seven of 32 maize samples (84.4%) and 11 of 15 wheat samples (73.3%) were contaminated with *Fusarium* species. These were *F. verticillioides* (20 isolates), *F. proliferatum* (20 isolates), *F. subglutinans* (ten isolates) and *F. nygamai* (five isolates) based on macroscopic and microscopic morphological criteria. Twenty-two of the isolates (40%) belonging to *F. verticillioides* (eight isolates), *F. proliferatum* (ten isolates), *F. subglutinans* (three isolates) and *F. nigamayi* (one isolate) produced $FB_1$, at a range from 230 to 9565 $\mu g\ mL^{-1}$. These results indicate a high degree of contamination of maize and wheat with *Fusarium* strains belonging to the *Gibberella fujikuroi* species complex, particularly *F. verticillioides* and *F. proliferatum*. This contamination can is a potential public health threat, as a meaningful number of isolates were reported to produce high levels of carcinogenic $FB_1$.

**Multi-mycotoxin risks and related toxigenic fungi in the Mediterranean area.** A. LOGRIECO, G. MULÈ, S. SOMMA, A. SUSCA and A. MORETTI. *Research National Council, Institute of Sciences of Food Production, Bari, Italy. E-mail: antonio.logrieco@ispa.cnr.it*

The occurrence of toxigenic fungi and related mycotoxins in plant products which are important in Mediterranean diets is one of the most important social and economic concerns in the Mediterranean Basin. Production of such toxins may start in the field, or occur after harvest, during storage or processing. The mild Mediterraenan climate and associated crop diversity present an environment that can be highly conducive to the development of mycotoxigenic plant pathogens. Increasing levels of contamination by mycotoxins in various Mediterranean crops (particularly, toxins produced by *Alternaria*, *Aspergillus*, and *Fusarium*) and their high toxicity, have obliged many gouvernmental authorities to impose or consider severe limitations on allowable my-

cotoxin occurrence in agricultural commodities. However, although the European Commission has set up the maximum levels of most important mycotoxins, there are no rules or recommendations for the co-occurrence of more than one toxin. The risk of multi-toxin contamination of food commodities is still poorly recognized even if it is well-known that each mycotoxin has a specific target and the complex of mycotoxins could have a wide range of effects on human and animal health. This presentation is an overview of the information on the occurrence of toxigenic fungi and mycotoxins in plants, food and feed, in Mediterranean countries. The expanding research on toxigenic fungi and related mycotoxins, particularly in the last decade, has produced a large body of information on this topic. Differences in environmental conditions and plant cultivation in the various Mediterranean countries, and climate changes, have significantly influenced the distribution of specific toxigenic fungi and related mycotoxicological problems. The contamination by the main mycotoxions of the most sensitive crops, such as maize, wheat, grape, dried fruits and nuts, will be descibed with special emphasis on: a) fumonisins, trichothecenes, and zearalenone, for *Fusarium* species on cereals; b) aflatoxins from *A. flavus* and related species on cereals, dried fruits and nuts; c) ochratoxin A and fumonisins from the black *Aspergilli* on grape; and d) *Alternaria* toxins from *Alternaria* species, on cereals. The risk that several mycotoxins from diverse fungi occur together on several crops in the whole Mediterranean Basin is realistic and needs further investigation.

**Prevention of aflatoxin and ochratoxin a production by the application of HACCP approach.** E. GÖKMEN and D. HEPERKAN. *Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Istanbul, Turkey. E-mail: ece.gokmen@gmail.com*

Consumers need food products in order to sustain their lives. Because of direct effects of food products on human health, food safety and food quality should be ensured. Therefore, a systematic approach, known as Hazard Analysis and Critical Control Points (HACCP), has been developed. This system is specialized on food safety during processes from raw materials to the consumption of food products. HACCP intends prevention of potential hazards from farm to table**.** Potential hazards can be eliminated or reduced to acceptable limits with this approach. Potential hazards associated with food products can be classified as physical, chemical or biological. One of the well known chemical hazards are mycotoxins. Approximately 400 compounds are known as mycotoxins. These are secondary metabolites of moulds. Most frequently produced mycotoxins are aflatoxins, ochratoxin A, fumonisins, patulin and tricothesenes. Mycotoxins are mainly synthesized by mycotoxigenic strains of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria.* In this presentation, prevention of aflatoxin and ochratoxin A by application of HACCP systems is emphasized. Four different food products (dry-cured meat products, pistachio, peanut butter and wine) have been studied. HACCP plans for each product are indicated. After HACCP plans are effectively applied to the factories, levels of food safety and food quality will be increased by completely eliminating the potential hazards, or reducing the potential hazards to acceptable limits. Additionally, frequency and efficiency of inspections will be increased. General information about aflatoxins and ochratoxin A is outlined, and information about HACCP systems and their application for the four food products are emphasized.

**Robust processing method to produce candidate certified reference material for aflatoxin in dried fig.** H. ÖZER[1], H.İ. OKTAY BASEGMEZ[1] and N. TOKMAN[2]. *[1]TÜBİTAK Marmara Research Center (MRC), Food Institute, Gebze, Kocaeli; [2]TÜBİTAK National Metrology Institute (UME), Chemistry Group, Reference Materials Laboratory, Gebze, Kocaeli. E-mail: hayrettin.ozer@tubitak.gov.tr*

Aflatoxins are structurally related compounds produced as secondary metabolites mainly by toxigenic strains of *Aspergillus flavus* and *A. parasiticus* in/on food and feeds. Among the mycotoxins, aflatoxins could pose threats to human and animal health because they are toxigenic, carcinogenic, mutagenic, and teratogenic. These compounds can cause liver cancer in humans. Aflatoxins are classified as human carcinogenic substances (Group I) by the International Agency for Research on Cancer (IARC). As a result of their potential threat for health, regulations on mycotoxins have been established in many countries in order to protect consumers from their harmful effects. Based on the regulatory limits, accurate and reliable determination of mycotoxins in foods becomes very important for food safety and international trade. The traceability of aflatoxin measurement results can be achieved through the use of a matrix of certified reference material (CRM). However, for the determination of aflatoxins in dried fig, such a matrix CRM is not yet available. The proposed study material is a candidate CRM for the determination of aflatoxin ($B_1$, $B_2$, $G_1$ and $G_2$) levels in dried fig. Production and certification of CRM includes steps of planning, feasibility, processing, homogeneity and stability tests, and characterization. This study presents statistical evaluation of the results obtained at the planning and feasibility steps. Processing of raw material, selection of the units for the homogeneity and stability tests and characterization for real scale production have been carried out, and this information will be distributed to laboratories in order to control the accuracy of

their results. The raw material to be used in this study was obtained from the province of Aydin and stored at -18 ºC until processing. Raw material was blended by a blade mixer, dehydrated using a freeze dryer, ground and sieved. The product (particle size between 125–500 μm) was collected and packed in dark-coloured polyethylene bottles. Samples were selected for homogeneity and stability tests. The validated international standard method (AOAC Official Method no. 999.07) was used for determination of aflatoxins in candidate CRM. Relative standard deviations achieved were less than 5%, both for homogeneity and stability tests.

**Next generation sequencing approaches to identify T-DNA tagged genes in *Colletotrichum higginsianum* pathogenicity mutants.** A. ZAMBOUNIS, M.-H. LEBRUN and R. O'CONELL. *INRA-AgroParisTech, UMR1290 BIOGER, Thiverval-Grignon, France. E-mail: antonios.zampounis@versailles.inra.fr*

*Colletotrichum higginsianum* causes anthracnose on many cruciferous crops, as well on *Arabidopsis thaliana*. This fungus uses a hemibiotrophic infection strategy, forming melanized appressoria for host penetration, bulbous biotrophic hyphae inside living host cells, followed by thin necrotrophic hyphae. The *Colletotrichum-Arabidopsis* pathosystem provides a model for analysing plant-fungal interactions because the pathogen can be stably transformed and extensive genetic tools and resources are available to dissect host responses. Annotated genomes are also available for both partners. Previously, random insertional mutagenesis of *C. higginsianum* using *Agrobacterium tumefaciens*-mediated transformation identified 40 mutants that were reduced in pathogenicity. These mutants were classified according to their infection phenotypes, such as melanin- or penetration-deficient, induction of plant defence responses or impaired in the switch to necrotrophy. In order to identify the T-DNA tagged genes in 23 of these mutants, we used whole-genome resequencing with IIlumina (100 bp paired-end reads) at relatively low coverage (26-fold). An efficient bioinformatics pipeline was developed to identify putative insertion sites by mapping the paired reads against the reference genome assembly of *C. higginsianum* and the library-specific T-DNA sequences. We particularly focused on identifying chimeric (fungal/T-DNA) reads spanning integration sites, thereby providing their exact location, and pairs in which one read mapped to the fungal genome and one mapped to the T-DNA. We identified a total of 39 integration sites among the mutant libraries, including remarkable T-DNA insertion patterns such as tandem or inverted arrays of T-DNA in head-to-tail orientation at a single site in the genome or closely-spaced independent insertions. We identified mostly 'precise' junctions, i.e. where T-DNA border and flanking genomic region were joined without a gap, associated with short deletions of genomic DNA at the integration sites. T-DNA border truncations at both ends of the T-DNA sequences were also frequently found. Furthermore, the genomic location of the T-DNA integrations was predominantly in exons. Some of these tagged genes encoded for fungal-specific transcriptional regulators or enzymes putatively involved in pathogenicity signalling pathways. In order to verify the predicted T-DNA integration sites, sequences spanning the insertion loci were recovered from mutant genomic DNA by PCR using specific primers.

**Hyperspectral discrimination of *Erwinia amylovora* infections in pear plants.** Y. ALNAASAN[1,2], F. SANTORO[2], Franco VALENTINI[2], A. FABI[1], A. CALZOLARI[3], A.M. D'ONGHIA[2] and L. VARVARO[1]. *[1]Tuscia University, DAFNE, Viterbo, Italy, [2]CIHEAM-IAMB Istituto Agronomico Mediterraneo di Bari, IPM, Valenzano, Italy; [3]Servizio Fitosanitario – Regione Emilia-Romagna, Italy. E-mail: y_nassan@hotmail.com*

Fire blight, caused by *Erwinia amylovora*, is the most serious bacterial disease of pear in Italy. Excluding antibiotics, which are prohibited in the EU, no chemicals can effectively control the infections. The eradication of heavily infected plants and the removal of the symptomatic branches are currently the common means for fireblight management. Large-scale monitoring is difficult due to the huge number of trees to be inspected. The present study aimed to use hyperspectral data (proximal sensing) to discriminate between healthy and fire blight infected, symptomatic or asymptomatic, pear trees, to apply the remote sensing technology as support for pathogen surveillance. Greenhouse and field trials were carried out in spring and summer 2013. In the greenhouse, a number of pear seedlings were inoculated by *E. amylovora* strains and sterilized water was used for the control. Leaf spectral reflectances were acquired three times after the artificial inoculation, at of 10 d intervals post-inoculation. Two field trials were also conducted in Emilia-Romagna. Based on fire blight symptoms, spectral reflectances were acquired on two levels (leaf and canopy) from symptomatic an asymptomatic pear trees. All collected spectral reflectances were preprocessed, analyzed (to calculate some Spectral Vegetation Indices) and compared using multivariate analysis (Principal Component Analysis, Stepwise Discriminant Analysis, and Canonical analysis). Hyperspectral data discriminated between non-infected and fire blight infected pear. Wavebands of 410, 510, 642, 682, 972, 1132, 1157, and 1245 nm, along with some vegetation indices (Greenness Index, Pigment Specific Normalized Index and Blue-Green Pigment Index) identified infected pear plants in the greenhouse trial. Although no significant

differentiation between symptomatic and asymptomatic trees was detected from analyzing reflectance data in field trials, a noticeable trend of disease discrimination was observed using some vegetation indices (calculated at the leaf and canopy levels) such as Yellowness Index, Water Band Index and Anthocyanin Reflectance Index.

**On the biology of four isolates of *Nattrassia mangiferae* Nattrass: the effect of media.** W. NORI[1] and H. EL ATTA[2]. [1]*University of Kordofan, Faculty of Natural resources and environmental studies, Department of Forestry and range, El Obeid, Sudan;* [2]*King Saud University, Faculty of Food Science and Agriculture, Department of Plant production, Riyadh, Kingdom of Saudi Arabia. E-mail: waffanori@gmail.com*
The aim of this study was to characterize four isolates of *Nattrassia mangiferae* previously isolated from infected *Ficus* spp. and to determine the effects of various media on the growth of these isolates. Different sources of carbon (at 20 g $L^{-1}$) and organic nitrogen (2 g $L^{-1}$) were used. The media used were: Potato dextrose agar (PDA), Potato sucrose agar (PSA), Dextrose asparagine agar (DAA), PDA + asparagine (PDAA), Dextrose leucine agar (DLA), PDA + leucine (PDAL) and Natural medium (Ficus sawdust agar). The choice of the carbon sources was made in such away to include a monosaccharide (dextrose), a disaccharide (sucrose) and polysaccharide (starch and cellulose in potato and ficus sawdust agar formations respectively). PDA leucine supported the most extensive growth of all *N. mangiferae* isolates. However, there were differences in time required by each isolate to attain the maximum colony radius of 45 mm. Growth was faster in Isolate types 3 and 4 (cylindrical conidia group) than in Isolate types 1 and 2 (spherical conidia group). Among all isolates, Isolate 3 was the fastest growing type, and grew almost equally on all the carbon and nitrogen sources assessed. Growth in PDA and PDA leucine confirmed the presence of four independent isolates. In the remaining media, Isolates 3 and 4 on one hand and isolate 1 and 2 on the other hand, behaved similarly. Although there are four isolates, nevertheless, they are interrelated in pairs.

**Innovative methods of biological indexing for the efficient and fast detection of citrus virus and virus-like diseases.** K. DJELOUAH, G. CAVALLO and A.M. D'ONGHIA. *CIHEAM-Mediterranean Agronomic Institute of Bari, Italy. E-mail: djelouahl@iamb.it*

Biological indexing onto citrus woody indicators still remains a compulsory tool for detecting most of the citrus graft-transmissible diseases, particularly Concave gums, Cristacortis, Impietratura, Gummy bark and unknown diseases, despite the disadvantages of being laborious, time-consuming and skill-demanding. For this reason, this technique is still included in the sanitary assays in the framework of programmes for the certification of citrus propagation material. In order to improve the traditional method of biological indexing, which is based on the graft-inoculation of 1-year-old indicator seedlings, comparative trials were performed using three indexing techniques based on cuttings, thus overcoming the numerous constraints based on seedlings production under greenhouse conditions. These techniques included: the indicator cutting (Indicator cutting - IC) method, (bud-inoculation and grafting onto a citrus rootstock); the cutting of the sample to be tested (Sample cutting - SC) (is bud-inoculation with the indicator and placing onto a citrus rootstock); and indicator cutting (after bud inoculation, cuttings are rooted (Indicator rooted cutting - IRC) with 2000 ppm IBA hormone. The sources of inoculum for the trials were from the main citrus virus and virus-like diseases. All tested indexing techniques allowed more rapid appearance of symptoms than in the traditional method; clear symptoms of the tested diseases were observed 2 weeks post inoculation on the new emerging leaves of the specific indicator cuttings (IC) and indicator rooted cuttings (IRC). In the sample cutting method (SC), however, symptoms were not observed earlier. About 4 weeks later symptoms could be shown in the traditional indexing method. Among the tested methods, IRC was the most promising being low cost, highly reliable, rapid and easy to apply. However, the rooting of some indicator species (i.e. Mexican lime, Dweet tangor) was a limiting factor. This problem was overcome by the other two methods for which the cutting is cleft grafted onto a rootstock and not rooted.

**Development of LAMP methodology for *Monilinia* spp. detection, and correlation with environmental parameters.** M. SI AMMOUR, T. YASEEN, F. SANTORO and A.M. D'ONGHIA. *CIHEAM-IAMB Istituto Agronomico Mediterraneo di Bari, IPM, Valenzano, Italy. E-mail: y.thaer@iamb.it*

Brown rot, an important disease of all stone fruit species in the Mediterranean region caused by *Monilinia* spp., is now posing serious threats due to the arrival of *M. fructicola*, which was considered a quarantine pathogen in Italy before 2010. A loop-mediated isothermal amplification (LAMP) assay was developed in combination with the application of a prototype for data acquisition (data logger, gravitational spore trap and solar shield), distributed throughout a cherry orchard, in order to rapidly detect and monitor *Monilinia* spp. in correlation with microclimatic conditions for pathogen development. The LAMP assay was developed with primers based on the intergenic spacer (IGS) of the ribosomal DNA sequences of *M. laxa*, *M. fructigena* and *M. fructicola.* The assay specifically and rapidly de-

tected and quantified the pathogen using both real-time PCR and a Smart DART device prototype (Diagenetix Inc). The pathogen quantity detected by the assay was correlated with the monitored meteorological data. A heterogeneous inoculum distribution in the field was observed. In addition, the greatest spore concentrations were recorded during the pink bud stage and beginning of flowering phases of host development, which matched the rainfall of 5 mm, a cumulative temperature of 10–14ºC and high cumulative relative humidity values of 70–90%. The combination of small sensors with gravitational spore traps allowed greater accuracy of monitoring microclimatic conditions, thus reporting the risky conditions for pathogen development and distribution in the grove. This innovation has very considerable potential for quantification and identification of any target DNA of any organism or contaminant. The results from this study provide a new framework for a Decision Support System (DSS) for *Monilinia* spp.

**Increased knowledge of *Spongospora* diseases of potato indicates severe challenges for reducing their yield- and quality-limiting effects.** R. FALLOON[1,2], R. LISTER[1], R. BUTLER[1], D. CURTIN[1], F. SHAH[1], S. SINTON[1], R. GENET[1], M. PAGET[1] and U. MERZ[3]. [1]*The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand;* [2]*Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand;* [3]*Plant Pathology, IBZ, Swiss Federal Institute of Technology, Zurich, Switzerland. E-mail: richard.falloon@plantandfood.co.nz*

*Spongospora subterranea* has long been recognised as an important pathogen of potato (*Solanum tuberosum*), causing powdery scab of tubers. This disease poses problems for seed tuber production, and in potatoes grown for processing or fresh consumption. Recent research has shown that *S. subterranea* has greater effects than previously recognised. The life cycle stages of the pathogen in host roots can reduce plant productivity, and reduce crop yields. Greenhouse experiments have shown that *S. subterranea* disrupts water and nutrient uptake by potato plants, resulting in reduced growth. These effects occur similarly in cultivars that are resistant to tuber powdery scab and those that are susceptible to this disease. Root malfunction is associated with the pathogen zoosporangium stage, including cycles of zoospore infection and multiplication in host roots during early plant growth. Later root infection stages manifest as prolific production of root galls (hyperplasia), from which sporosori containing highly resistant resting spores are produced. These are released into the soil, providing long-surviving inoculum to threaten future potato crops. Recent surveys in New Zealand and Australia have indicated that *Spongospora* root disease is widespread in potato crops, and, along with other soilborne diseases, causes significant yield reductions. Management of *Spongospora* diseases is difficult because the pathogen can survive for many years, either as resting spores or through infection of alternative hosts. The ability of *S. subterranea* to produce root galls both in tuber-resistant and tuber-susceptible cultivars means that even where tuber-resistant cultivars are grown, the pathogen can multiply to infest cropping soils. We are beginning to assess potato breeding lines for susceptibility to root infection, which may provide cultivars with effective resistance both to *Spongospora* root diseases and tuber powdery scab. In the short term, management of these diseases will rely on long (multi-year) rotation intervals between potato crops, avoiding infested soils, use of certified healthy seed tubers, seed tuber- and soil-applied pesticides, manipulation of soil nutrients, and planting of tuber-resistant cultivars. The *S. subterranea* diseases of potato (root malfunction, root hyperplasia and tuber powdery scab) continue to challenge the economic viability of intensively managed, high input potato crops.

**Control of plant virus diseases in cool season legume crops.** K.M. MAKKOUK[1], S.G. KUMARI[2], J.A.G. VAN LEUR[3] and R.A.C. JONES[4,5]. [1]*National Council for Scientific Research, Beirut, Lebanon;* [2] *International Centre for Agricultural Research in Dry Areas; (ICARDA), Tunis, Tunisia;* [3]*NSW Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia;* [4]*School of Plant Biology and Institute of Agriculture, University of Western Australia, Nedlands, WA 6009, Australia;* [5]*Department of Agriculture and Food, South Perth, WA, 6151, Australia. E-mail: khaled.makkouk@cnrs.edu.lb*

Cool-season food legume crops become infected with a wide range of viruses, many of which cause serious diseases and major yield losses. This review begins with discussion of which viruses are important in the principal cool season food legume crops in different parts of the world, the losses they cause and their economic impacts in relation to control. The main types of control measures available are then described: host resistance, phytosanitary measures, cultural measures, chemical control and biological control. Examples are provided of successful deployment of the different types of measures to control virus epidemics in cool season food legume crops. The need for integrated control approaches is emphasized, because single control measures used alone rarely reduce virus-induced yield losses adequately in these crops. Development of effective integrated virus disease management (IDM) strategies depends on an interdisciplinary team approach to: (i) understand the ecological and climatic factors which lead to damaging virus epidemics; and (ii) evaluate the effectiveness of individual control measures. In addition to using virus-resistant cultivars, other IDM components include: sowing virus-tested seed stocks,

selecting cultivars with low seed transmission rates, using diverse phytosanitary or cultural practices that minimize the virus source or reduce its spread, and use of selective pesticides in an environmentally responsible way. The implications of climate change in increasing problems associated with control, and the opportunities to control virus diseases more effectively through new technologies, are discussed.

**Aflatoxin biocontrol, an interesting experience for Europe and the Mediterranean basin.** A. MAURO[1], P.J COTTY[2] and P. BATTILANI[1]. [1]*Università Cattolica del Sacro Cuore, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Institute of Entomology and Plant Pathology, Piacenza, Italy;* [2] *The University of Arizona, School of Plant Sciences, U.S. Department of Agriculture-Agricultural Research Service, Tucson, AZ, USA. E-mail: paola.battilani@unicatt.it*

*Aspergillus flavus* is the main fungus responsible for aflatoxin production in maize at a global level. Aflatoxin contamination was recently a serious issue for the Mediterranean basin and Europe, in particular in 2012. The distribution of atoxigenic strains of *A. flavus*, able to compete with the toxin producer strains, have been demonstrated as the most powerful strategy to reduce aflatoxin contamination in several crops, included maize. The selection of atoxigenic endemic strains adapted to climatic and environmental conditions, and as good competitors, are crucial to optimise their efficacy. A population of 138 *A. flavus* isolates, collected from Italian maize kernels during the period 2003–2010, from five districts of northern Italy, was characterised with the aim of selecting a candidate biocontrol agent. Forty-six percent of the strains were atoxigenic and none of the aflatoxin producers synthesised both B and G aflatoxins. Forty-eight vegetative compatibility groups (VCGs) were obtained; 25 included only atoxigenic isolates and the remaining only toxigenic isolates. Aflatoxins and cyclopiazonic acid gene analyses showed different profiles, and some VCGs lacked both gene clusters. A candidate biocontrol agent was then selected after *in vitro* evaluation with competition tests. It was from the most common atoxigenic VCG (IT6) isolated in different years and locations. Field trials were carried out in 2012 and 2013, with aflatoxin $B_1$ reduction greater than 80% without increase in ear infection and the overall quantity of *A. flavus* on the crop. Good recovery of the distributed strain was confirmed on ears at harvest. The strain is now ready to move from the large study scale as a valuable new support for maize growers.

**Implementation of biological control strategies in the farming industry: a seed to field approach.** D. ANGELOPOULOU, E. NASKA, V. DIMITRAKAS, S. TJAMOS and E. PAPLOMATAS. *Agricultural University of Athens, Laboratory of Plant Pathology, Athens, Greece. E-mail: epaplom@aua.gr*

The vascular wilt pathogens *Verticillium dahliae* and *Fusarium oxysporum* cause severe yield reductions in a variety of important annual crops worldwide. Control of vascular wilts has relied on soil fumigation. However, the use of the main soil fumigant, methyl bromide, has been banned in the European Union since 2010, creating a demand for novel crop protectants. As such, the use of biocontrol agents (BCAs) is an appealing management strategy. In the present study, two BCAs (*Paenibacillus alvei* K165 or the nonpathogenic *Fusarium oxysporum* F2) and two release strategies (seed coating or amendment of transplant soil plugs) were assessed against, respectively, Verticillium wilt of aubergine and Fusarium wilt of tomato.. Mixing the transplant soil plug with K165 or F2, at a rate of 10 and 20% (v/v), respectively, reduced Verticillium wilt symptom development; while Fusarium wilt severity was reduced by K165. Statistical analysis also showed that disease severity was negatively correlated to the size of the rhizosphere BCA population. In addition, qPCR analysis showed that both BCAs induced the expression of the pathogenesis-related (PR) proteins PR1 and PR4 in the stems of aubergines before and after inoculation with *V. dahliae*. This project was co-funded by the Greek Ministry of Education and Religious affairs and the European Union in the framework of the operational programme "Competitiveness and entrepreneurship" OPCII, NSRF 2007–2013.

**Perspectives on control of grapevine trunk diseases associated with fungal pathogens.** L. MUGNAI[1], S. DI MARCO[2] and G. SURICO[1]. [1]*Università degli Studi di Firenze, Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione Patologia vegetale e Entomologia, P.le delle Cascine 28, 50144 Firenze, Italy;* [2]*CNR, Istituto di Biometeorologia, Via Gobetti 101, 40129 Bologna, Italy. E-mail: laura.mugnai@unifi.it*

Trunk diseases of grapevine are major causes of damage to quality and quantity of production. They can also severely reduce vineyard longevity. The harmful effects occur in wine and table grape vineyards, often beginning in the first years of new plantations. Three types of wood symptoms and consequent diseases occur on grapevines: wood decay, caused in Europe and the Mediterranean area by the same agents responsible for wood decay in other crops (e.g. citrus, olive); cankers, causing the death of the cordons, again associated with pathogens of several other tree crops in addition to grapevine, and vascular disease, causing malfunctioning of leaves bringing progressive weakening and finally death of affected vines. These diseases, namely,

white decay of the wood caused by *Fomitiporia mediterranea*, Eutypa and Botryosphaeriaceae cankers and dieback, as well as other diseases or syndromes in the Esca complex (mainly grapevine leaf stripe disease, GLSD), are influenced by a range of cultural, climatic, environmental factors, and are impossible to counteract with one single disease management solution. Within this general picture three different approaches were set up and are bringing initial promising results for the control of GLSD and, indirectly, of the other grapevine trunk disease syndromes. Firstly, protection of pruning wounds is important as these are infection sites for all wood pathogens. New infections begin from winter pruning every year, increasing the complexity of the microbial community able to enter and colonize grape wood tissues, the interaction among all these organsisms and between these organisms and the plant itself. Pruning wound protection is a common approach for the control of all these diseases, and considerable effort has been given to identifying products and protocols suitable for organic viticulture and/or for integrated pest management. Secondly, application of wood penetrating chemical formulations has been assessed. Thirdly, foliar treatments with innovative products have been examined, which stimulate plant defence reactions. These last two approaches are particularly relevant for diseases in which wood infections cause malfunctioning of the leaves. Positive or promising results obtained in field trials in Italian vineyards are reported. The possibilities for future application of these approaches as an integrated strategy for the control of grapevine trunk diseases will be discussed. A COST Action FA1303 "Sustainable control of GTDs" (which commenced in November 2013) supports collaborations and exchanges of information on grapevine trunk diseases at the European level.

**Characterization of cotton-infecting begomoviruses in alternative hosts from cotton growing regions of Pakistan.** M.I. FAREED, M. TAHIR and A.G. KAZI. *National University of Sciences & Technology, Plant Biotechnology, Atta-Ur-Rahman School of Applied Biosciences, H-12 Sector, Islamabad, 44000, Pakistan. E-mail: irfan_fareed_bhatti@yahoo.com*

Begomovirus (genus *Begomovirus*; family *Geminiviridae*) causes severe yield losses in tropical and sub-tropical regions of the World. More than nine species of cotton-infecting Begomoviruses have been reported. Weeds and ornamental plants are thought to act as reservoirs for different begomoviruses, and maximum virus recombination can take place in alternative hosts. Suspected infected leaf samples of castor bean, cotton, tobacco, tomato, hibiscus, okra, ageratum, *Digera arvensis* and Papaya plants with leaf curl, mosaic and vein thickening symptoms were collected from areas in Pakistan in 2010–11. PCR amplification using diagnostic primers showed the presence of a begomovirus, and the whole genome of the virus was cloned. All the plants, except castor bean, were known hosts. The complete nucleotide sequence was determined to be 2,759 nt (accession No. HE985227). Alignments showed the greatest levels of nucleotide sequence identity (98.8%) with *Cotton leaf curl Burewala virus* (CLCuBuV; accession No. JF416947). The virus in castor beans lacked an intact C2 gene, as is typical of CLCuBuV in cotton. An amplification product of ca. 1.4 kb was obtained in PCR with primers for betasatellites, and the complete nucleotide sequence of a clone was determined to be 1373 nt (HE985228). The sequence showed 96.3% nucleotide sequence identity to the recombinant *Cotton leaf curl Multan betasatellite* (CLCuMB; JF502389). This was the first report of CLCuBuV and its betasatellite infecting castor bean, demonstrating that this plant is an alternative host of the virus.

**Silencing of *Erwinia amylovora* sy69 AHL-quorum sensing by a *Bacillus simplex* AHL-inducible *aiiA* gene encoding a zinc-dependent *n*-acyl-homoserine lactonase.** A. HANANO, M. HARBA, M. AL-ALI and H. AMMOUNEH. *Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), B.P. Box 6091, Damascus, Syria. E-mail: samy_hanano@yahoo.fr*

Quorum sensing in Gram-negative bacteria is regulated by the diffusible signal molecules *N*-acyl-L-homoserine lactones (AHLs). These are degraded by lactonases. In this study, six *Bacillus simplex* isolates were characterized and identified as a new quorum-quenching species of *Bacillus*. An *aiiA* gene encoding an AHL-lactonase was identified, based on evidence that: (i) it showed high homology with other *aiiA* genes of *Bacillus* sp.; (ii) the deduced amino acid sequence contained two conserved regions, $^{104}$SHLHFDH$^{111}$ and $^{165}$TPGHTPGH$^{173}$, characteristic of the metallo-β-lactamase superfamily; and (iii) the protein had zinc-dependent AHL-degrading activity. Additionally, the expression of the *aiiA* gene was significantly up-regulated by 3-oxo-AHL. The AHL-lactonase inhibited multiplication of the 3-oxo-C6-AHL-producing plant pathogen *Erwinia amylovora* sy69 both *in vitro* and *in planta*. These results provide support for the use of the quorum-quenching functionality of *B. simplex* in the integrated control of the devastating fire blight pathogen.

**Early blight of potato and tomato in Algeria: importance of disease and phenotypic diversity of pathogenic species.** D. AYAD, A. MANSOURI, Y. MOULAY, A. KEDAD and Z. BOUZNAD. *Ecole Nationale Supérieure d'Agronomie, El Harrach Algiers, Algéria. E-mail: aydji@yahoo.fr*

In Algeria, potato and tomato occupy an important place in human diets. After late blight, potato production is limited by early blight, a severe disease caused by the genus *Alternaria* which can cause considerable yield losses (Kumar *et al*., 2008). Classification of *Alternaria* species is complex, making their identification difficult; phylogenetic studies encompass *Alternaria* spp. in several groups (Hong *et al*., 2005). The detection and identification of *Alternaria* spp. is based on specific media culture and on a morphological, physiological (toxins production) and molecular characterization (Simmons, 2007). Early blight of potato and tomato is most often attributed to two species responsible: *A.solani* and A.*alternata*. A recent survey in Algeria showed that symptoms occur on leaves as two types of necrotic spots: small, numerous, dispersed, brown to black spots and large necrotic spots with concentric rings. Fungal isolates showed different types of culturural characteristics, varying from light to dark green and light to dark brown, with intensity of sporulation varying from no conidia to abundant sporulation. Microscopic observations showed two types of conidia: small conidia typical of the group *A. alternata* with abundant sporulation and three types of conidiophores: (1) conidiophore with a single long chain of conidia; (2) branched conidiophore with short chains of conidia, each having a small beak; and (3) branched short conidiophore bearing clusters conidia. These observations indicate that other species, such as *A. arborescens* or *A. infectoria*, are present in Algeria. Group *A. solani* has large conidia and less abundant sporulation which is sometimes scarce or absent. This class of isolates showed yellow to orange pigmentation in culture. The large conidia have a diameter more or less wide and usually have elongated beaks. Among isolates of *A. solani* obtained from tomato there are probably *A. tomatophila* or *A. grandis.* Molecular characterization will better define the phenotypic diversity of *Alternaria* spp. to allow improved disease management.

**Abscisic acid regulates important elements in the sirna machinery against *Bamboo Mosaic Virus*.** M. ALAZEM and N.-S. LIN. *Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan. E-mail: m.alazem@gmail.com / mazen@gate.sinica.edu.tw*

In the absence of specific *R*-resistance genes, the RNAi machinery is considered to be the main defense against viruses. Abscisic acid (ABA) also regulates several components of the RNAi machinery. Taking into account this regulation, we investigated whether ABA may regulate members of the *AGO* family, the main slicers of viral genetic material in the RNAi system. By assessing the effects of exogenous and endogenous ABA on the expressions of *AGOs*, we found that ABA positively regulates several members in this family. In complementation experiments, ABA mutants *aba2-1* and *aao3* reduced the transcript levels of those positively regulated *AGOs*. Infection with *Bamboo Mosaic Virus* (BaMV) showed similar effects to that of ABA on *AGO* genes. The combination of BaMV infection with exogenous or endogenous ABA strongly induced the same set of the ABA-regulated *AGOs*. *AGO1* and its regulator *miR168a* were found irrelative to ABA-mediated resistance to BaMV. However, other *AGOs* showed anti-BaMV activity by reducing BaMV levels in plants over-expressed with either of these *AGOs*. The data presented here show tight connections between ABA and *AGO* transcripts, suggesting an ABA-transcriptional regulation for these *AGOs*, and adding another defense layer that ABA controls in plants.

**Protection of durum wheat against *Fusarium culmorum* attack by seed biopriming with rhizosphere bacteria.** N. MNASRI[1], S. ELKAHOUI[2], S. CHENNAOUI[1], K. HESSINI[1], M. MRABET[3], S. GARGOURI[4] and N. DJÉBALI[1]. *[1]Laboratory of Molecular Physiology of Plants, Centre of Biotechnology of Borj Cedria, BP 901 Hammam-Lif 2050, Tunisia; [2]Laboratory of Bioactive Substances, Centre of Biotechnology of Borj Cedria, BP 901 Hammam-Lif 2050, Tunisia; [3]Laboratory of Legumes, Centre of Biotechnology of Borj Cedria, BP 901 Hammam-Lif 2050, Tunisia; [4]Laboratory of plant protection, National Institute of Agronomic Research of Tunis, rue hédi karray 2049, Tunisia. E-mail: dnaceur@yahoo.fr*

Fusarium foot and root rot (caused mainly by *Fusarium culmorum* in Tunisia) leads to considerable yield losses in durum wheat (*Triticum durum L.*) crops. We evaluated the potential of 60 rhizosphere bacterial strains for biocontrol activity against *F. culmorum*. The study of the bacterial effect on the growth of two isolates of *F. culmorum* (Fc2 and Fc3) *in vitro* showed that 33 strains inhibited growth of Fc2, and 51 strains inhibited Fc3. The antagonism was due to inhibition by contact or at distance. None of these bacterial strains produced chitinase.. Some of the bacteria produced diffusable and / or volatile compounds that inhibited growth, sporulation and macroconidium germination of *F. culmorum*. Morphological and molecular identification of these antagonistic bacteria showed that they mainly belonged to the genus *Bacillus*. Dual inoculation of durum wheat (cv. Karim) seeds by the *Bacillus* sp. strains and *F. culmorum* macroconidia suspensions showed that the bacteria generally improved germination and seedling vigour and reduced the percentage of infected seeds *in vitro*. In greenhouse trials, antagonistic bacteria, especially strain B16E, increased coleoptile length, root fresh weight, seedling vigour index and photosynthesis parameters of plants. The B16E strain induced the greatest decrease in *F. culmorum* severity in comparison to control plants.

**Stimulation of plant immune systems as a holistic approach to sustainable and durable crop protection.** S. MOLINARI and P. LEONETTI. *National Research Council of Italy (CNR), Institute for Plant Protection, Bari, ITALY. E-mail: s.molinari@ba.ipp.cnr.it*

Plants have developed sophisticated molecular mechanisms to detect pathogens and pests and to activate immune responses. Immune responses are regulated by phytohormones, that are low molecular weight molecules which interact in a complex network to also regulate many aspects of plant growth, photosynthesis, flowering, reproduction, seed production and response to environmental abiotic challenges. Plant immune systems should be considered as within plant growth processes; thus, expression of constitutive defense systems occurs only at the cost of plant growth, and risks allocating resources to defense in the absence of natural pathogens and pests. An effective alternative is to fine-tune immune responses by modulating the "immunological memory" of plants, as it occurs in animals. An aspect of this modulation may be represented by the so-called "priming" by which previously attacked plants respond more quickly or more strongly to a subsequent attack than non-attacked plants. Such priming is termed Systemic Acquired Resistance (SAR), which typically induced following effector-triggered immunity (ETI) or, in other words, *R* gene-mediated resistance. This is effective against a wide range of biotrophic pathogens. Priming is a relatively low-cost mechanism of advancing plant defense, as resources are not used until the threat returns. There is an epigenetic regulation of priming, as there is a lack of significant transcriptional changes in primed plants unless they are exposed to the priming agent/hormone. Defense responses in plants primed with a low, non-effective concentration of a defensive hormone are also faster and stronger than those in non-primed plants. Moreover, priming can be transferred to offspring. Hormone network interactions are as important for plant immune system expression that pathogens and pests have developed sophisticated molecular mechanisms to deregulate the biosynthesis of hormones and/or to interfere with hormonal signaling pathways thus impairing plant defense response. A natural stimulation of plant immune systems should be pursued by enriching the soil of fields, particularly those intensively cropped, with beneficial organisms such as arbuscular mycorrhiza forming fungi (AMF) and plant growth promoting rhizobacteria (PGPR). This research strategy aims to develop a holistic approach to durable crop protection that considers the very complex relationships occurring between plants and changing natural environments, which may be characterized by a large array of biotic attackers, also in view of new concerns as global warming.

**Fusarium head blight of wheat in algeria: level of grain contamination and evaluation of wheat cultivars for resistance to *Fusarium culmorum* and *F. graminearum*.** S. TOUATI-HATTAB[1], S. HADJOUT[2]; L. MEKLICHE, A. KEDAD[2], Z. BOUZNAD[2] and C. BARREAU[3]. *[1]Amar Telidji University, Laghouat, Algeria; [2]National School of Agronomy, ENSA, Algiers, Algeria; [3]National Institute of Agronomic Research, INRA, MycSA, Bordeaux, France. E-mail: touatisihem@yahoo.fr*

Despite the large areas of wheat crops in Algeria, production remains low, very irregular and insufficient to cover the country needs. This situation places the country as the greatest importer of durum wheat in the world market. When environmental conditions and susceptibility of the varieties are met, important losses reducing both the amount and the quality of yield are caused by pathogens. Recent surveys carried out in different wheat production areas indicate the importance of Fusarium head blight. The causative fungi probably also affect the quality of grain through mycotoxin production. During crop years 2009, 2010 and 2011, analyses of wheat seeds (250 lots of soft and durum wheat harvested in different Algerian areas at different bioclimatic stages) have shown wide species diversity in which the most dominant are *F. graminearum* and *F. culmorum*. Experiments were conducted *in vitro* and *in vivo* (artificial inoculation in the field) with the two most damaging species. During 2011 and 2012 variability was detected in the behaviour of the genotypes for these pathogens. Different evaluation criteria of disease development using a scale of notation, percentage of grains lost, thousand grain weight, and AUDPC, revealed that the common cultivars and lines derived from crosses have different susceptibilities to fungal isolates, with slightly higher aggressiveness in the case of *F. culmorum*; some wheat lines showed greater resistance than their parents. Furthermore, durum wheat cultivars showed greater susceptibility than the soft wheat cultivars. These preliminary studies show the possibilities of yield improvement from wheat cultivation and better sanitary quality of the grains harvested through the use of resistant or tolerant cultivars with regard to infection by *F. graminearum* and *F. culmorum*.

**Epidemiological estimation of foliar rust diseases of wheat and assessment of different rust pathogens isolated from rainfed areas of Punjab, Pakistan.** S.Q. BATOOL, S. IFTIKHAR and S.S. AHMAD. *Department of Environmental Sciences, Fatima Jinnah Women University, The Mall Rawalpindi. E-mail: batool.syedaqamar@gmail.com*

This study was conducted to check pre- and post-harvest conditions of soil and existing spores of fungal pathogens related to foliar disease. A survey was conducted

to assess foliar rust disease severity in rainfed areas of Pothohar region of the Punjab. Four districts (Jhelum, Rawalpindi, Attock, and Chakwal) were selected for collection of disease severity data. Twenty-one 21 locations were surveyed and total of 63 soil samples were collected from different fields. A questionnaire-based survey was conducted to gather information from local farmers about their crop management practices. Physiochemical analysis of soil samples was carried out to check soil conditions before wheat sowing. Soil fertility was measured, including organic matter and NPK values. Heavy metals (Pb, Cd, Cr, Cu, Ni and Zn) were also extracted from soil samples for nutrient deficiency assessments. Fungi were isolated from samples to verify preliminary soil conditions for disease development. Fungi isolated from soil samples were mainly *Fusarium, Aspergillus, Mucor,* and *Penicillium*. Results of soil parameters showed profiles of healthy soils and indicated that foliar rust disease is caused by biotic factors. These included pathogens which spread via wind and caused crop yield reductions. The results also showed that different cropping patterns contribute slightly to disease development and low crop yields result from differences in soil nutrients. Inoculum of the pathogens causing yellow rust, brown rust and powdery mildew was collected to identify the evolving races, and to study virulence behaviour of these pathogens on different host germplasm. Diseases of wheat related to elemental deficiencies show similar symptoms, so there is need to accurately identify sources of disease, so that effective controls are implemented to ensure high crop yields. Study of the behaviour of fungal pathogens will help to manage foliar diseases of wheat in controlled and environmentally friendly ways.

**Assessment of mycoflora, mycotoxins and nutritional profile in ensiled and fresh corn fodder.** S. IRAM and N. SULTANA. *Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan. E-mail: iram.shazia@gmail.com*

This study evaluated mycoflora, mycotoxin and nutritional profiles in fresh and ensiled corn fodder. Ensiling is the preservation of chopped green plant material by process fermentation under anaerobic conditions. Silage is considered to be least susceptible for fungal attack due to the acidic environment that develops. During ensiling processes, improper compression, condensation and high humidity can cause aerobic spoilage, leading to undesirable growth of fungi that would subsequently lead to mycotoxin production. Mycotoxin contamination not only deteriorates quality of silage but could negatively impact human health. The aim of the study was to analyze corn fodder samples before and after ensiling by general screening of mycoflora, detecting commonly occurring mycotoxins (aflatoxin) and assessing nutritional parameters. Mycoflora was determined using the serial dilution method and oxytetracycline media. Aflatoxins were quantified by high performance liquid chromatography techniques coupled with an immunoaffinity column. The method was optimized by standard curve calibration. Nutritional parameters were analyzed by methods described by AOAC. Among the commonly occurring fungi like *Aspergillus*, *Fusarium* and *Penicillum*, only *Aspergillus* was observed/detected in all samples. Five species of *Aspergillus* were observed, including *Aspergillus flavus, A. ochraceous, A. fumigatus*, and these were prevalent in fresh and ensiled corn fodder. Among all, *A. niger* (87.5%, 70.85%), *A. flavus* (79.15, 75%) and *A. fumigatus* (66.66%, 95%) were most dominant (respectively) in fresh and ensiled corn fodder. *Aspergillus ochraceous and A. terrus* were least commonly detected. Total fungal colonies were ranged from $1 \times 10^3 - 6 \times 10^3$ cfu $mL^{-1}$ for all species in both fresh and ensiled corn fodder. *Aspergillus fumigatus* and *A. flavus* showed greatest total fungal counts of $6 \times 10^3$ cfu $mL^{-1}$. Total observed fungal density was less than the safety limit of $1 \times 10^4$cfu $mL^{-1}$. The aflatoxin standard curve analysis shows good linearity, with detection limits of 0.1 μg $mL^{-1}$. Aflatoxin contamination was detected in samples of fresh (37.5%) and ensiled (41.66%) fodder, with an average, respectively, of 8.36 and 9.49 ppb. The average detected value was below the permissible levels of Europeon Commission (20 ppb). Nutritional profile results showed that pH of fresh fodder declined from 5–6 to 3.6–4.6. Ideal silage has pH of 4.2. Dry matter loss was observed after ensiling that indicated good fermentation. Overall, other nutritional parameters remained stable after ensiling. Corn silage has balanced nutrional value, but fungal and aflatoxin contamination may reduce quality.

**Effects of toxic metals on filamentous fungi isolated from the contaminated soil in Pakistan.** A. RASOOL and S. IFTIKHAR. *Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan. E-mail: anam.rasool32@gmail.com*

This study investigated the metal tolerance potential of indigenous filamentous fungi, because of the importance of the organisms for bioremediation of wastewater and contaminated soils, Certain metals are important for biological activity. However all metals, whether essential or inessential will show toxicity at certain levels. A total 17 fungi were isolated and preserved from contaminated peri-urban agricultural areas of Multan and Gujranwala in Pakistan, for further detailed investigation of heavy metal tolerance. *Aspergillus niger, A. fumigatus* and *A. flavus* were isolated both from soil and water samples from Multan, while *A. terreus* and *Penicillium.* were only isolated from soil samples. *Aspergillus versicolor, A. flavus, A. niger* and *Fusarium ox-*

*ysporum* were isolated from contaminated soils and water samples from Gujranwala, and *Penicillium* sp. was isolated only from water samples. These fungal isolates were assessed for tolerance to metal compounds $Cu(SO_4)_2.5H_2O$, $Cd(NO_3)_2$, $Cr(NO_3)_2$ and $Pb(NO_3)_2$. The tolerant strains were selected with metals concentrations of 100 ppm and compared to control nil added metals in the medium. The degree of tolerance was measured by radial growth (cm) in the presence of various heavy metals and compared to the controlsIsolates *Penicillium* sp. and *A. flavus* from Gujranwala soil showed maximum tolerance index 2.1 at 100 ppm toward Cr and 4.8 at 100 ppm toward Cd respectively. *Aspergillus versicolor* (isolated from waste water) exhibited the greatest tolerance index toward Cu and Pb, while showing sensitivity against other metals. From all the collected samples the Gujranwala soil and water contained fungi with more tolerance toward the heavy metals as compared to Multan area. In future similar strains will be tested with other heavy metals for the confirmation of tolerance, and tolerant strains will be used for bioremediation of heavy metal contamination.

***Ceratocystis manginecans*, the causal agent of a destructive mangoe disease in Pakistan.** A. RASHID[1], S. IRAM[2], I. AHMAD[1], F.S. FATEH[1] and G. BAHAR. *[1]PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Center, Islamabad, Pakistan. [2]Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi. E-mail: asma_friend@yahoo.com*

Mango trees (*Mangifera indica* L.) are affected by a serious wilt disease, recognized as mango sudden death (MSD), was first reported in Muzafargargh Punjab, Pakistan (1995). Mango sudden death is an emerging problem in Pakistan. The disease has been observed in almost all mango growing areas, and severity varied from 2–5% in Punjab and 5–10% in Sindh. Symptoms on affected trees include bark splitting, discoloration of the vascular tissues, wilting, gummosis and at the end rapid death of affected plants. Although many researchers are working on this disease, some reviews are still contradictory and perplexed. If pathogens are not identified, and trees are not rescued before large scale damage to the fruit, this will ultimately lead to the lower fruit production. A more detailed investigation is required to understand the association of *Ceratocystis* with MSD and the species involved. This study focused on geographical distribution, pathological and genetic characterization of fungal pathogens (*Ceratocystis* sp.) of mango. A total of 45 isolates were obtained from geographically different regions of Punjab and Sindh. Pathogenicity of these isolates was tested through artificial inoculation on different hosts (potato tubers, detached mango leaves, detached mango twigs and mango plants) under controlled conditions, and all were proved pathogenic with varying degrees of aggressiveness in reference to uninoculated controls. This proved that out of these four methods, potato tuber inoculation was most ideal as this fixed the inoculum on the target site. Increased fungal growth and spore numbers may be due to the soft tissues of potato tubers. Lesion area on potato tubers was in the range of 7.09–0.14 $cm^2$, followed by detached mango twigs which were ranged from 0.48–0.09 $cm^2$. All pathological results were proved highly significant ($P<0.05$), but isolate to isolate variability was non-significant, although this affected lesion area. Re-isolation of respective fungi was achieved with 100 percent success, veritying Koch's postulates. Calculation of the Pearson correlation coefficient (r), indicated positive correlations between the lesion length *vs* area and width *vs* area. DNA of fungal pathogens was successfully extracted using the phenol chloroform method. Amplification was done through ITS, b-tubulin gene, and Transcription Elongation Factor (*EF1-α*) gene primers and the amplified amplicons were sequenced and compared from NCBI which showed 99–100% similarity with *Ceratocystis manginecans*. *Ceratocystis manginecans* formed one strongly supported sub-clade through the resulting phylogenetic tree. These results obtained support establishment of relation of isolates with their region, and will give information about pathogenicity levels of isolates that willbe useful for developing management policies to reduce the effects of MSD in mango orchards.

**Exogenous Sirna-mediated protection of plants from *African cassava mosaic virus* infection**

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RNA silencing is an adaptive antiviral defence mechanism in plants. The unifying feature of RNA silencing is the production of small interfering (si) RNAs of 21-25 nucleotides that are generated from the viral genomes. Profiles of viral (v) siRNAs also indicate that certain regions, namely hotspots, of the viral genome are more prone to RNA silencing-mediated degradation than others. We hypothesised that direct targeting of viral genome by synthetic hotspot siRNAs could confer protection of plants from virus infection. To test this idea, we obtained a high resolution profile of ACMV vsiRNAs using northern blotting and high-throughput deep sequencing. One hotspot and one coldspot on each DNA component of ACMV, a ssDNA virus causing severe destruction to cassava crop production, a staple food for more than 700 million people all over the world, were selected and *in vitro* transcribed to pro-

duce viral sense and anti-sense small RNAs. To test the turnover of these synthetic siRNA on ACMV infection, co-inoculation of double-stranded (ds) hotspot siRNA onto *Nicotiana benthamiana* prevented ACMV infection of plants, in which viral DNA replication was almost undetectable. The plants remained healthy through the course of the experiments. On the other hand, the sense, anti-sense sRNA of hotspot vsiRNA or cold spot vsiRNA had less or no impact on ACMV infection or disease severity. Furthermore, we further demonstrated that the systemically acquired resistance by applying exogenous hotspot siRNA has a threshold effect and requires a functional *RDR6*. These data show that hotspot vsiRNAs bear a functional significance on antiviral RNAi, suggesting that they may have the potential as an exogenous biological agent for controlling destructive viral diseases such as cassava mosaic disease.

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**Comparison of aflatoxin $b_1$ levels in Iranian and Indian spices using ELISA detection.** A.S.M. NEJAD[1] and V. RABBANI[2]. [1]*Member of Young Researchers and Elite Club, Sari Branch, Islamic Azad University, Sari, Iran;* [2]*Department of Food Engineering, Istanbul Aydin University, Istanbul, Turkey. E-mail: asmozafarinejad@yahoo.in*

This study was carried out to detect the presence of aflatoxin $B_1$ ($AFB_1$) in 36 samples of spices from Iran and India that were inclued of chilli powder (12 samples), black pepper powder (12) and whole black pepper (12). Enzyme-linked immunosorbent assay (ELISA) was applied to analyse $AFB_1$ in the samples. All the analyses were done twice. Aflatoxin $B_1$ was found in all the spice samples, the concentration of $AFB_1$ in Iranian samples ranged from 63 to 6276 ng/kg, and in Indian samples ranged from 31 to 246 ng $kg^{-1}$. The mean of $AFB_1$ concentration in the chilli powder was significantly greater ($P$<0.05) than the whole and powdered black pepper. However, none of the samples exceeded the maximum prescribed limit i.e. 5000 ng $kg^{-1}$ (5 μg $kg^{-1}$) of European Union regulations for aflatoxin $B_1$. Although the present study was not wide, it provides valuable information on aflatoxin $B_1$ levels in Iranian and Indian spices.

**Plant growth promoting rhizobacteria as biocontrol agents against *Xanthomonas campestris* pv. *oryzae* (bacterial leaf blight in rice).** M.A. ZAHID[1], S. YASMIN[2], S.M. SHAHZAD[3], Y. IFTIKHAR[1] and M. HUSSAIN[1]. [1]*University of Sargodha, University College of Agriculture, Department of Plant Pathology, Sargodha, Pakistan;* [2]*Biocontrol Lab Microbial Phytotechnology Group Soil and Environmental Biotechnology Division National Institute for Biotechnology and genetic Engineering (NIBGE) SEBD, Faisalabad, Pakistan;* [3]*University of Sargodha, University College of Agriculture, Department of Soil and Environmental Sciences, Sargodha, Pakistan. E-mail: awais.3@hotmail.com*

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a serious problem in rice crops and causes substantial economic losses to farmers throughout the world. PSA medium was used to isolate Xoo from diseased leaves, and pathogen identity was confirmed through PCR by using specific primers TXT4R. The virulence of Xoo strains was also confirmed by *in vivo* pathogenicity tests. Plant growth promoting rhizobacteria (PGPR) were isolated from the rhizospheres of rice plants grown in different regions of Punjab, Pakistan. Three hundred bacterial isolates were screened *in vitro* for their antagonistic activity against Xoo using the hole plate diffusion method. Isolates SA33, Tk229, Tk179, SP12 and SA37 showed positive antagonism against Xoo. In greenhouse experiments, isolates SP12, Tk229 and SA37 were effective in reducing BLB incidence, with disease suppression up to 79%, 72% and 68%, respectively. These three isolates were further studied for growth promotion mechanism. Strain TK229 showed significant indole acetic acid activity and phosphorous sloubulization. Rice plants sprayed with fresh suspension of isolate SP12 showed 75% protection from Xoo, followed by TK229 and SA37 with 66% and 62% protection, respectively. Kinetics of these bacterial strains was also studied in order to assess them for efficient fresh spray application. These strains can also be used along with other strategies to achieve increased crop protection, and to sustain rice yields.

**Biological control of whitefly *Bemisia tabaci* by *Encarsia sophia* on selected host plants.** A.U. NISA[1] and E. UL HAQ[2]. [1]*PARC Institute of Advanced Studies in Agriculture, Department of Plant and Environmental Protection, Islamabad, Pakistan;* [2]*Insectary-Biological Control Labs., IPMP, National Agricultural Research Centre, Park Road, Islamabad, Pakistan 45500 Benazir Bhutto Women Hostel NARC, Park Road, Islamabad, Pakistan. E-mail: asmat277@gmail.com; asmat1201@yahoo.com*

Whitefly *Bemisia tabaci* Gennadius (Homoptera, Aleyrodidae) is a serious insect pest of cotton crops in Pakistan. It causes economic losses by sucking plant cell sap and by transmitting *Cotton leaf curl virus*(CLCV). Application of insecticides give only limited control of whitefly which necessitates the adoption of integrated pest management by incorporating biological control as an integral component. *Encarsia sophia* Girault & Dodd (Hymenoptera: Aphelinidae) is a parasitoid of nymphal whitefly that can potentially suppress population of the pest. Mass rearing of parasitoids is a main limitation to launch inundative releases in the field. For rearing of *E. sophia* the culture of whitefly was maintained at In-

sectary-Biological Control Labs, National Agricultural Research Centre (NARC), Islamabad. Whitefly rearing was studied by comparing some biological parameters of the insect on three host plant species, i.e. cotton, brinjal, and tomato at 27±1°C and 50–55% R.H. The biology of *E. sophia* was studied at 25 ± 1°C and 50–55% R.H. The parameters studied were incubation period, larval, pupal, and adult developmental duration, and sex ratio. Maintenance of whitefly cultures was more efficient on brinjal plants than on tomato or cotton. The life cycle of whitefly was completed in shorter time 19.8±0.19 d on brinjal as compared to 20.53 ± 0.28 d on tomato and 23.05 ± 0.23 d on cotton plants. The observed male to female sex ratio was 1:1.6 on brinjal and 1:1.5 on cotton and 1:1.5 on tomato. Average incubation period of *E. sophia* in whitefly nymphs was 2.10 d, larval duration 6.6 d, and pupal duration 5.5 d. Total duration for adult emergence was 14.2 d. These studies lead to the successful rearing of this important parasitoid *E. sophia* under laboratory conditions and provided a foundation for its mass production and release in the field.

**Identification and biological control of basal stem and root rot of snapdragon.** N. RAMADAN. *University of Mosul, Collage of Science Department of Biology, Iraq. E-mail: nadeem.ramadan53@yahho.com*

For the first time wilt symptoms have been detected on the snapdragon (*Antirrhinum majus* L) plants cultivated in the parks of Mosul University. Isolation of fungi from wilted plants onto PDA medium revealed the presence of seven genera including; *Alternaria, Aspergillus, Cladosporium, Fusarium, Macrophomina, Penicillium* and *Stemphyllium. Fusarium oxysporum* was the most frequently isolated fungus, approaching $3.6 \times 10^2$ Colony Forming Units/g soil. *Fusarium oxysporum* significantly reduced the chlorophyll content of infected plants, and inhibition of chlorophyll content increased up to 82% in severe infections. The infection caused significance stunting of plants, with plant height inhibited by 58% in dead plants. Antagonism ability of *Trichoderma* species including *T. harzianum, T. viride*, and *T. reesei* bio-resistance against *F. oxysporum* showed control efficiency of 1.67, 2.33, and 2.67 respectively.

**Use of nutritional supplements for the management of rootknot nematode (*Meloidogyne incognita*) infecting potato.** A.S. GONDAL[1], N. JAVED[1], S.A. KHAN[1] and M. SHAHID[2]. [1]*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan;* [2]*Plant Pathology Research Institute, Faisalabad, Pakistan. E-mail: amjadshahzasd@live.com*

A range of synthetic chemicals has been widely used as the major control measure against plant pathogens. Environmental pollution, degradation, pesticide resistance development and other agronomic concerns have prompted scientists to seek alternative disease management strategies. *In vitro* studies were conducted to evaluate the efficacy of nutritional supplements, including micro-power, humic acid and plant protectors containing benzoic acid, against rootknot nematode (*Meloidogyne incognita*) infection on a susceptible potato cultivar. Each treatment applied as single applications or in combined treatments significantly reduced the numbers of galls and egg masses, and promoted overall plant growth, compared to un-amended controls. Application of aqueous solutions of 4% plant protector + 4% micro-power + 2% humic acid increased the number of leaves, root and shoot development, and tuber weight, and decreased the root weight with minimum number of females, root galls and egg masses. Greatest nematode fecundity was recorded in the control treatment resulting in poor plant growth and development of greater numbers of galls and egg masses. The significantly lower number of galls and egg masses and enhanced plant growth from the combined application of plant protector 4%, micro power 4% and humic acid 2% indicated this treatment to be superior.

**Sensitive detection of *Potato virus Y* in potato crops of Pakistan.** M.F. ABBAS and CA. RAUF. *Pir Mhr Ali Shah Arid Agriculture University, Faculty of Crop and Food Sciencs, Department of Plant Pathology, Rawalpinid, Pakistan. E-mail: fahimuaar@yahoo.co.uk*

A large number of potato germplasm lines were screened in Pakistan against PVY through ELISA, which is inadequate to detect the virus at initial stages of infection. The increasing incidence of PVY is getting to severe proportions in the main potato growing areas of Pakistan. PVY was confirmed serologically through DAS-ELISA and was on susceptible indicator host plants (*Nicotiana rustica, N. tabacum* and *Chenopodium album*). Ninety-six treatments were adapted for optimizing of RT-PCR assays by applying six concentrations of total nucleic acid, four of Taq DNA polymerase and sense and antisense primers, respectively. The optimum reaction for PVY detection was found to be 10–20 pM of primer and 2 units of enzyme. Designed CP gene specific sense and antisense primer successfully amplified approx. 800 bp coat protein gene fragments through PCR assay. ELISA negative samples were further certified with optimized RT-PCR, and few samples produced the appropriate band in PCR, while no band was observed in negative controls. The application of the molecular technique in plant diagnostic laboratories of Pakistan will reduce the risk of PVY dispersal, and this applied research will greatly assist in the reduction of PVY losses in the potato crops of Pakistan.

**Exploration of antagonistic potential of selected biocontrol agents against *Macrophomina phaseolina*.** S. HYDER, S.T. SAHI, A. HANNAN and A.S. GONDAL. *Department of Plant Pathology University of Agriculture Faisalabad, Pakistan. E-mail: sajjad1614@yahoo.com*

*Macrophomina phaseolina* (Tassi) Goid is an important soil-borne fungal pathogen, having wide host range and causing significant yield losses to agricultural crops. Being cost-effective and eco-friendly, biocontrol is preferred to health hazardous and potentially noxious chemical controls. Selected agents including *Trichoderma harzianum*, *Penicillium digitatum*, *Aspergillus niger* and *A. flavus* were tested for their antagonistic potential against *M. phaseolina*in dual culture assays under laboratory conditions. Percent zone inhibition of pathogenic fungi was recorded when compared against untreated controls. *Penicillium digitatum gave* significant percentage zone reduction (72%) followed by *A. flavus* (52%), *T. harzianum* (48%) and *A. niger* (35%). Application of environmentally friendly antagonistic microbes is best alternative to health hazardous chemical formulations. Moreover, identification and extraction of mycotoxins from the most efficient fungal biocontrol agents can lead to development of various antibiotics and effective drugs against human ailments as alternatives to synthetic chemical formulations.

**Histopathological changes in mango (*Mangiferae indica*) seedlings inoculated with *Ceratocystis manginecans*, the cause of mango sudden decline in Pakistan.** A. REHMAN[1], I.A. KHAN[2], G. NAVEED[1], A.S. KHAN[2], I. AHMAD[3], A. RAZA[4] and A.A. KHAN[5]. *[1]Department of Plant Pathology, University of Agriculture Faisalabad 38040- Pakistan.; [2]Institute of Horticultural Sciences, University of Agriculture Faisalabad38040-Pakistan; [3]Department of Horticulture Bahau Din Zakriya University Multan- Pakistan; [4]College of Agriculture, Sub Campus Burewala- University of Agriculture Faisalabad Pakistan; [5]Center of Agriculture Biology and Biotechnology, University of Agriculture Faisalabad. E-mail: arb041@gmail.com*

Mango production is hindered by the attack of number of diseases such as malformation, anthracnose, leaf spots and die back. However, the recently occurring mango sudden decline caused by *Ceratocystis manginecans* is considered as the most serious threat to mango industry of Pakistan. This diseasesappears as white and black streaks in vascular bundles under the bark, gum exudation and ultimately sudden death of affected tress within few weeks.. The present study aimed to find sources of resistance to the disease. One-year-old mango seedlings of indigenous germplasm with good morphological and physiochemical properties were artificially inoculated with *C. manginecans*. Assessment of upwards and downwards movement of *C. manginecans* in the vascular tissues by fungal mycelium was examined in the cross sections of artificially inoculated seedlings. Upward movement of fungus was greater than downward movement. Maximum lesion length in both upward and downward directions was found in seedlings of host line KHW-515 followed by KHW-506 and KHW-490, while least lesion length was recorded in seedlings of KHW-481. Re-isolation of the pathogen confirmed that *C. manginecans* is responsible for wilting and death of infected mango seedlings. Histopathological studies indicated that infection of the xylem vessels results in the blockage of vascular system by fungus colonization and tylosis formation. Mycelium movement in vascular systems and tissue discolouration is the mechanism responsible for wilt and death of infected mango seedlings.

**Evidence of *Strawberry latent ringspot virus* infections in fig (*Ficus carica*).** T. ELBEAINO[1], F. TURGUT[2], M. GUMUS[2] and M. DIGIARO[1]. *[1]Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy; [2]University of Izmir, Faculty of Agriculture, Department of Pant Protection, Izmir, Turkey. E-mail: elbeaino@iamb.it*

*Strawberry latent ringspot virus* (SLRSV) is a cosmopolitan virus that infects several plant species. Its presence in fig (*Ficus carica*) was investigated in 140 samples collected from the main fig-growing areas of two Mediterranean countries (Lebanon and Turkey). Leaf samples were collected from symptomatic (mosaic, chlorotic blotching, leaf deformation, ringspots) and asymptomatic fig trees, and used for the total nucleic acids (TNA) extraction, reverse-transcription and RT-PCR assays using two couple of primers specific to SLRSV. Eighteen percent (nine of 50 tested) of Lebanese samples, and 24.4% (22 of 90 tested) of Turkish samples were infected. Ten SLRSV-PCR amplicons (292 bp), four from Lebanon and four from Turkey, were sequenced. Blast analyses of the nucleotide sequence portions shared 93–98% identity with SLRSV isolates present in the database, in particular with the isolate X75165 that was the most similar to those analyzed in this study. The phylogenetic tree constructed with the nucleotide sequences of SLRSV from both countries gathered all isolates in a single cluster, regardless their geographic origin. In the attempt to identify the possible etiological role of SLRSV in the fig symptoms observed, the presence of other fig-infecting viruses, i.e. *Fig leaf mottle associated virus 1* (FLMaV1), *Fig leaf mottle-associated virus 2* (FLMaV2), *Fig mild mottle-associated virus* (FMMaV), *Fig latent virus 1* (FLV1), *Fig mosaic virus* (FMV), *Fig cryptic viru*s (FCV), *Fig fleck-associated virus* (FFkaV), and *Fig Badnavirus 1* (FBV1) was also investigated in all collected samples. Results of RT-PCR showed that FMV and FBV1 were the prevailing viruses and that almost

all samples were infected by more than one virus, thus hindering the evaluation of the exact role of SLRSV in inducing symptoms in fig trees. To our knowledge, this is the first report on SLRSV infection on fig.

**Comparative study of antagonistic activity of some *Trichoderma* spp. isolates against *Fusarium* spp. and *Microdochium nivale* involved in fusarium head blight and root rot of wheat**. H. BOUREGHDA, N. ABDALLAH and Y. DANE. *Ecole Nationale Supérieure Agronomique (ENSA), Département de Botanique, El Harrach, Algiers, Algeria. E-mail: hou.boureghda@gmail.com.*

Fusarium root rot and Fusarium head blight of wheat are considered as serious worldwide diseases, which may affect yields and also grain contamination by mycotoxins. Yield losses may be as high as 50%. Both of diseases are caused by a complex of *Fusarium* spp. and *Microdochium nivale*, which can be associated with attack on roots, collars and spikes of wheat plants. In Algeria as in the Mediterranean area, in recent years, the most predominant species is *F. culmorum* which can be associated with root and collar rot and head scab. We have compared the antagonistic activity of three *Trichoderma* spp.: *T. atroviride* (Ta.13), *T. harzianum* (Th.6) and *T. longibrachiatum* (TL.9) against six *Fusarium* species (*F. culmorum*, *F. graminearum, F. avenaceum, F. lateritium, F. solani, F. verticilliodes*) and *M. nivale*, obtained from wheat collars and spikes, based of *in vitro* bioassays. These were carried out using direct and indirect confrontations. In the case of direct confrontation, a net reduction of pathogen growth was observed with variability in the sensitivity of *Fusarium* spp. and *M. nivale* towards *Trichoderma* species. Percentages of colony growth reduction ranged from 12.3% to 100%. The greatest growth reduction of all *Fusarium* species and *M. nivale* was obtained with isolate Ta.13 (*T. atroviride*) where 100% reduction occurred towards *F. culmorum*, *F. solani* and *M. nivale.* In direct confrontations, pathogen colonies were invaded by *Trichoderma*, with variability of this behaviour from total recovery, partial or no recovery by the antagonist. In the case of *Fusarium* spp., total or partial overlap occurred with *T. atroviride* and *T. longibrachiatum*, and no overlap occurred with *T. harzianum*. Nevertheless, full recovery of the pathogen colony was observed for *M. nivale*, with the three species *Trichoderma* isolates. Furthermore, all *T. atroviride* isolates overgrew the pathogen colonies and sporulated above, showing the greatest parasitic capacity (mycoparasitism) of this species towards the pathogen. In indirect confrontation (no direct contact) between the pathogen and the antagonist, where inhibition occurs as a result of volatile antifungal substances produced by the antagonist, significant reductions of pathogen growth occurred, compared to the controls. Percentage reduction varied between 5 and 75%, and the greates reduction was obtained with *T. atroviride* (Ta.13). This isolate produces a coconut odour in PDA culture. Aaccording to Samuels *et al.* (2002) and Dodd *et al.* (2003), this odour is characteristic of the volatile antibiotic 6-pentyl-$\alpha$-pyron (6PP) described only from *T. atroviride* and *T. viride* of the section *Trichoderma.* The filtered culture broth (2 L) of strain Ta.13 was extracted with ethyl acetate and was dried ($Na_2SO_4$) and evaporated under reduced pressure. The residue recovered was subjected to flash column chromatography (Sigel; 50 g) with gradient elution, which resulted in six fractions. Thin-layer chromatography using 6PP as control showed that the extract 2 was pure 6PP. By *in vivo* bioassay, isolate Ta.13 (*T. atroviride*), shown to be most effective by *in vitro* tests, was assessed against the species *F. culmorum* by seed treatment before sowing wheat in soil infested with *F. culmorum.* Percentage disease severity index reduction of 70% was obtained, showing the potential effectiveness of this isolate in wheat protection against root rot and crown rot..

**Population genetic diversity of *Mycosphaerella pinodes* in Western Algeria, indicated using AFLP fingerprinting.** B. MOHAMED[1], S. BENALI[2], D.E. HENNI[3] and C. NEEMA[4]. *[1]Institut des Sciences de la Nature et de la Vie, Université de Khemis Meliana, Khemis Meliana, Ain Defla, 44000. Algérie; [2]Institut des Sciences Agronomiques, Université de Chlef, BP151, 02000, Algérie; [3]Institut des Sciences, Université d'Es Senia, 31000 Oran, Algeria; [4]UMR de Pathologie Végétale, INRA/INA-PG/Université Paris VI, 16 rue Claude Bernard, 75231 Paris, France. E-mail: benseti@yahoo.fr*

The genetic diversity and population structure of *Mycosphaerella pinodes* (*Dydimella pinodes*) in Algeria were investigated using AFLP markers. A total of 75 isolates from different collection sites corresponding to four geographic regions were analyzed. Data from AFLP loci were used to estimate gene diversity, genetic distances, and to make indirect measures of gene flow between population groups. Extensive diversity was detected in the *M. pinodes* population, regardless of the population group. The percentage of polymorphic loci varied from 41% in the semi-arid superior region to 50% in the subhumid region. Nei's gene diversity across loci was 0.471 and the Shannon's index across loci was 0.663. A high level of differentiation (Gst = 0.308) and low gene flow (Nm = 1.118) were found among population. Based on the analysis of molecular variance, 58% of the genetic variation of *M. pinodes* was within populations, 31% of the variation was between geographical regions and 11% among populations within a region. Cluster analyses using individual isolates failed to group them according to population. This is the first report on genetic diversity and population structure of *M. pinodes* on pea in Algeria.

**Occurrence of toxigenic fungi, aflatoxins and ochratoxin a in wheat, rice, dried fruits, and spices commercialized in Algeria.** A. RIBA[1,2], C. VERHEECKE[3], S. ZEBIRI[2], K. BOUTI[2], N. AZZOUNE[1,2], N. MAHDI[2] and N. SABAOU[2]. [1]*Université M'hamed Bougara, Faculté des Sciences, département de Biologie, Boumerdès. Algeria;* [2]*Ecole Normale Supérieure de Kouba. Laboratoire de Biologie des Systèmes Microbiens (LBSM), Kouba, Alger, Algeria;* [3]*Université de Toulouse, INPT-ENSAT, Laboratoire de Génie Chimique, UMR 5503 (CNRS/INPT/UPS), 1 Avenue de l'Agrobiopole BP 32607 Auzeville Tolosane 31326 Castanet-Tolosan. France. E-mail: riba_amar@yahoo.fr*

This study aimed to determine the occurrence of toxigenic fungi, aflatoxins (AFs) and ochratoxin A (OTA) in wheat, dried fruits, spices and rice consumed in Algeria. A total of 380 samples (210 wheat and derivates products, 44 spices, 96 dried fruits, and 30 rice) were analyzed. Wheat samples were collected during the following phases: pre-harvest, storage in silos and after processing. Spices, dried fruits and rice samples were collected randomly from locally popular markets. Dilution plating and direct plating were respectively used for isolation of fungi for ground and grain samples. Aflatoxins and OTA contamination levels were determined by detection using high-performance liquid chromatography coupled with fluorescence. Mould analysis showed that the commonly isolated fungi were species of *Aspergillus*, *Penicillium*, *Fusarium, Mucor* and *Eurotium*. Species belonging to the section *Nigri* and to the section *Flavi* were predominantly isolated in all samples. Among isolates of *Aspergillus* section *Flavi* examined, 45% produced high levels of aflatoxins. The most frequent chemotypes correspond to isolates able to produce both aflatoxin B and cyclopiazonic acid, followed by the producers of only aflatoxin B. The ability to produce OTA by isolates belonging to species of *Aspergillus* and *Penicillium* spp., revealed that 40% of isolates were OTA-producers. All isolated strains of *A. ochraceus*, *A. alliaceus* and *A. carbonarius* produced high levels of OTA. Aflatoxin B1 was detected in 56.6% of the wheat samples with contamination levels ranging from 0.13 to 37.42 μg $kg^{-1}$. This mycotoxin was detected in 90% of the dried fruits with contamination levels ranging from 0.16 to 25.82 μg $kg^{-1}$. Twenty-three of the 36 spices (63.9%) were found to be positive for aflatoxin B1 with quantities ranging from 0.10 to 26.50 μg $kg^{-1}$. Ochratoxin A was detected in 40% of the wheat samples at levels ranging from 0.20 to 41.55 μg $kg^{-1}$. The study has revealed the widespread occurrence of aflatoxigenic and ochratoxigenic strains in Algerian food, and highlight the importance of the post-harvest care of grains. Also, it is necessary for Algerian authorities to establish the maximum limits for ochratoxin A.

**Effects of seed dressing pesticides on the spread of *Faba bean necrotic yellows virus* on faba bean and *Barley yellow dwarf virus-pav* on barley and oat.** S.G. KUMARI[1], A. EKZAYEZ[1] and A. NAJAR[2]. [1]*International Center for Agricultural Research in the Dry Areas (ICARDA), Tunis, Tunisia;* [2]*Institut National de Recherches Agronomiques, (INRAT), Tunisia. E-mail: s.kumari@cgiar.org*

The effects of two seed-dressing pesticides in reducing the spread of aphid-transmitted *Faba bean necrotic yellows virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*) and *Barley yellow dwarf virus-PAV* (BYDV-PAV, genus *Luteovirus*, Family *Luteoviridae*) wer investigated under field conditions at Mornag Research Station, Tunisia, for two growing seasons (2012/2013 and 2013/2014), using artificial virus inoculation. Four faba bean (Najah, Bachar, Badii and Baj-90), two barley (Rihan and Manal) and two oat (Bizantha and Meliane) Tunisian varieties were used for the experiments. Seeds were treated before sowing with Celest top (25 g $L^{-1}$ difenoconazole + 25 g $L^{-1}$ fludioxonil + 262.5 g $L^{-1}$ thiamethoxam) at three rates (0.75, 1.5, 3.0 cc $kg^{-1}$ of seeds) and with Apron Star 45 WS (200 g $kg^{-1}$ thiamethoxam, 200 g $kg^{-1}$ mefenoxam, 20 g $kg^{-1}$ difenoconazole) at three rates (1.25, 2.5, 5 g $kg^{-1}$ of seeds), and untreated seeds were used as experimental controls. The experiments were carried out in a randomized complete block design with two replicates for each treatment. Four weeks after sowing, all faba bean plants were artificially inoculated with FBNYV using the *Acyrthosiphon pisum* as a vector, and barley and oat plants were inoculated with BYDV-PAV using *Rhopalosiphum padi* as a vector. Aphid populations were also observed for 48 h after inoculation to investigate the effect of seed treatment on the viruliferous aphids. Virus infection was recorded visually 4–5 weeks after inoculation, based characteristic symptoms of the two viruses. Spread of both viruses and yield losses were significantly decreased in treated plots compared with untreated plots. For example, incidence of BYDV in barley and oat, and FBNYV in faba bean was reduced from 100% (cvs. Bachar, Najah, Rihan, Bizantha) in untreated plots to 0, 4, 3 and 12% in plots treated with Celest top (3 cc $kg^{-1}$ of seeds), and 3, 5, 45 and 3% in plots treated with Apron star (5 g $kg^{-1}$ of seeds). Based on these results, seed treatment with Celest top and Apron Star can effectively reduce the incidence of two persistently transmitted aphid-borne viruses, BYDV and FBNYV, affecting cereal and legume crops, respectively. Detailed information on the inoculation methodology and the differences among treatments and growing seasons will be presented.

**Spreading of *Alfalfa mosaic virus* in lavandin in Croatia.** K. VRANDEČIĆ[1], J. ĆOSIĆ[1], I. STANKOVIĆ[2], K: MILOJEVIĆ[2], A. BULAJIĆ[2] and B. KRSTIĆ[2]. [1]*University of J.J. Strossmayer, Faculty of Agriculture, Kralja Petra Svačića 1d, 31000 Osijek, Croatia;* [2]*Institute of Phytomedicine, Department of Phytopathology, University of Belgrade-*

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In 2012 and 2013, a survey was conducted to detect the occurrence and distribution of *Alfalfa mosaic virus* (AMV) in lavandin crops growing in continental parts of Croatia. A total of 73 lavandin samples from six crops in different localities were collected and analyzed for the presence of AMV and *Cucumber mosaic virus* (CMV) using commercial double-antibody sandwich (DAS)-ELISA kits. AMV was detected in 62 samples collected at three different localities, and none of the samples tested positive for CMV. For further analyses, six selected samples of naturally infected lavandin plants originating from different localities were successfully mechanically transmitted to test plants *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana benthamiana*, and *Ocimum basilicum*, confirming the infectious nature of the viruses. Molecular detection was performed by amplification of a fragment of 751 bp in all tested samples, using the specific primers CP AMV1/CP AMV2 that amplify the part of coat protein (CP) gene. The RT-PCR products derived from isolates 371-13 and 373-13 were sequenced (KJ504107 and KJ504108, respectively) and compared with the AMV sequences available in GenBank. CP sequence analysis, conducted using MEGA5 software, revealed that isolate 371-13 showed the highest nucleotide of 99.5% (100% amino acid identity) with an isolate from Argentina originating from *Medicago sativa* (KC881010), and the sequence of isolate 373-13 had the highest identity with Italian AMV isolate from *Lavandula stoechas* (FN667967) of 98.6% (99% amino acid identity). Phylogenetic analysis showed clustering of selected isolates into four molecular groups and lavandin AMV isolates from Croatia grouped into two distinct groups. Isolates 70-12 and 373-13 clustered in group IV, together with the majority of isolates selected for phylogenetic analysis, while isolate 371-13 grouped in molecular group III. Determination of variability within the population of AMV in lavandin crops, but also establishing relationships with the isolates originating from other host plants in Croatia, will contribute to better understanding of the epidemiology of this pathogen, especially related to virus reservoirs in nature and the way the virus is introduced into crops.

**Morphological and virulence variation among isolates of *Mycosphaerella pinodes* the causal agent of pea leaf blight.** S. BENALI[2], B. MOHAMED[1], D.E. HENNI[3] and C. NEEMA[4]. *[1]Institut des Sciences de la Nature et de la Vie, Université de Khemis Meliana, Khemis Meliana, Ain Defla, 44000. Algérie; [2]Institut des Sciences Agronomiques, Université de Chlef, BP151, 02000 Algérie; [3]Institut des Sciences, Université d'Es Senia, 31000 Oran, Algeria; [4]UMR de Pathologie Végétale, INRA/INA-PG/Université Paris VI, 16 rue Claude Bernard, 75231 Paris, France. E-mail: setiben@yahoo.fr*

Mycosphaerella blight, caused by *Mycosphaerella pinodes* (Berk. et Blox.) Vestergr., is an important disease, causing severe damage in peas. Variability of 20 Algerian isolates of *M. pinodes* representative of four agro-climatic regions were investigated on the basis of cultural, morphological and pathogenicity characteristics. Variations were detected in colony colour, radial growth pattern and production of pycnidia and pycnidiospores. Significant differences ($P<0.05$) were observed both in pycnidia and pycnidiospore size among isolates. Pycnidia varied from 145 × 143 µm to 280 × 265 µm and pycnidiospores from 11.5 × 2.3 µm to 22.5 × 6.3 µm. Using the factor analysis, the first principal component (pc) was shown to be more related to the growth and sporulation aspect, hence, the colony growth and both the pycnidia and pycnidiospore density were more related to the first pc, while the second pc contributed for the pycnidiospores size. The isolates were also evaluated for their pathogenicity on seven cultivars in controlled conditions. Cluster analysis based on disease rating on a scale of 1 to 5, indicated high similarity. In addition, using the Euclidian distances method, the clusters were subdivided at 70% of similarity into seven pathotype groups. The two first pathotypes included most of the isolates (70%), representing isolates from the four agro climatic regions. However, members of same group were different in their cultural and morphological characteristics. A detailed study to investigate molecular and genetic basis of diversity is suggested.

**Importance of the *Agrobacterium tumefaciens* from almond nurseries in Chlef region in Western Algeria.** H. ABDELKADER[1], S. BENALI[1] and B. MOHAMED[2]. *[1]Institut des Sciences Agronomiques, Université de Chlef, BP151, 02000 Algérie; [2]Institut des Sciences de la Nature et de la Vie, Université de Khemis Meliana, Khemis Meliana, Ain Defla, 44000. Algérie. E-mail: setiben@yahoo.fr*

Crown gall is a destructive disease that occurs worldwide. It is considered to be great economic importance in almond and other stone fruit tree nurseries, due to the extensive losses. Based on their morphological characteristics on MacConkey medium and YMA medium, ten isolates of *Agrobacterium tumifaciens* were selected. Colonies of these isolates after 48 h at 28°C were circular, convex, smooth and translucent, and were easily suspended in water. The bacterial cells were rod shaped with rounded ends and were either single or in pairs. The isolates were Gram negative, and optimum growth temperature was between 25 and 27°C. All strains are positive for motility, catalase, and oxydase. These isolates all oxidized lactose to 3-ketolactose. All the strains

oxidized sucrose, D-mannitol, D-sorbitol, indol, inositol, melibioze, D-galactose, L-arabinose, rhamnose, amygdalin, lactose and glucose. Furthermore, the isolates also transformed arginin, lysin, ornithin, gelatin and starch. The pathogenic nature of isolates was confirmed by a bioassay on carrot disks. Additionally, Koch's postulates for all isolates were fulfilled.

**Apple scab on commercial cultivars: an emerging disease of economic importance in Maragheh apple orchards in Northwest Iran.** S. DAMADI. *University of Maragheh, Agriculture Faculty, plant protection Department, Maragheh, Iran. E-mail: smdamadi@yahoo.com*

The Maragheh area in northwest of Iran is one of the major apple production areas in the country. In commercial orchards mainly two cultivars of apple, Red and Golden, are cultivated, and some native cultivars are cultivated in conventional gardens. Occurrence of apple scab, caused by *Venturia inaequalis* (Cooke) Wint., on resistant cultivars is an important problem. Until recent years this disease was endemic in the area, on one of the native cultivars named Gara yaprag (black leaf), but did not affect commercial cultivars, Red and Golden. The infection rate was severe and symptoms appeared as large lesions on leaves and fruits, resulting in extensive damage. As the fruit of this cultivar is only used by the owners as fresh fruit or to prepare apple jam, garden owners do not apply any chemicals or use any control measures. In the past two years extensive field surveys showed that the disease has shifted to commercial cultivar Red. Symptoms were observed as small spots on fruits and leaves. Infection rates were so low that they did not get the attention of growers, but pose a threat to apple yields in the future. Furthermore, there is also the possibility of infection of the cultivar Golden. The problem has threrfore been investigated, and the cycle of disease was studied. Apple scab is now considered a threat to apple production and incidence and severity is under close watch. The disease cycle is characterized by two phases. The asexual phase occurs during the vegetative period of apple growth in the late spring and summer, and causes economic losses characteristic of the disease. The sexual phase occurs during the autumn and winter when the pathogen overwinters as pseudothecia in dead plant material. More research is underway to understand the nature of transferring the disease from the native cultivar to commercial cultivars. Control measures have been recommended to growers, who are applying chemical to control the disease and prevent spread of the pathogen.

**Mycoflora of feed for rainbow trout (*Oncorhincus mykiss*) from major farms in Iran.** S. ALINEZHAD[1], S.-R. SABERI[1], M. RAZZAGHI-ABYANEH[2] and M. SHAMS-GHAHFAROKHI[3]. *[1]Department of Veterinary Medicine, Higher Education Institute of Applied Science and Technology of Jahad Agriculture, Tehran, Iran; [2]Pasteur Institute of Iran, Department of Mycology, Tehran, Iran; [3]Tarbiat Modares University, Faculty of Medical Sciences, Department of Mycology, Tehran, Iran. E-mail: isopod47@yahoo.com*

Mycoflora of trout feed was determined in major farms of Iran. Sampling was done on pellets and their ingredients including wheat, wheat flour, soya, fish powder, gluten and starch, in the factory and in the farm, according to the instruction of Institute of Standard and Industrial Research of Iran. Samples were transferred to the laboratory in nylon bags and cultured for fungi on specific media, i.e. Aspergillus flavus and parasiticus agar (AFPA) and Dichloran rosebengal chloramphenicol agar (DRCA). Fungal colonies were purified from primary cultures by sub-culturing on Sabouraud dextrose agar plates and identified using macro- and microscopic morphology. A total of 133 fungal isolates were isolated, belonging to 11 genera. Most contamination occurred in wheat flour (17.3%) and gluten (15.8%). *Aspergillus* was the most prominent fungus isolated (54.9%) followed by the *Penicillium* (10.5%) and *Absidia* (9.8%). *Aspergillus flavus* was the most frequently isolated fungus (36.8%). A total of 109 fungal species from 11 genera were isolated from pellets and feed ingredients in the factory. The most contamination was in pellets (22.0%), followed by wheat flour (21.1%) and gluten (19.3%). *Aspergillus* species were present in all the samples, while *Pseudallscheria* and *Ulocladium* were isolated only from the pellets. *Aspergillus* was the most prominent genus (56.0%), followed by *the genera Penicillium* (12.8%), *Absidia* (11.0%) and *Pseudallscheria* (10.1%). From the farm samples, a total of 24 fungal species were isolated, of which 50% were *Aspergillus flavus*. These results show the importance of fungal contamination of rainbow trout feed not only for loss of quality but also for potential production of mycotoxins as serious public health hazards.

**Health micro propagation of potato (*Solanum tuberosum* L.).** F. LARBI - BOUGHRAROU[1], F. HASSINI[1], K. TALEB[1] and A. AISSAT[2]. *[1]Agronomic National High School, Algiers, Algeria; [2]National High Institute of Agriculture, Blida, Algeria. E-mail: faziaboughrarou@yahoo.com*

Every year, seed potatoes certified free of viruses have revealed the presence of virus after their culture. We tested plant material consisting of four varieties of seeds of class A considered free of viruses, according to regulations, with a lower infection rate of 6%. However, we found that of 57 tubers, 42 are affected at a rate of 27%. Application of Enzyme immunoassay ELISA, made for a rapid diagnosis of viruses of potato revealed: from PVM from the mother tuber varieties and Elvira Folv, PVS from mother tuber varieties and

Elvira Diamand, PLRV from potato varieties Diamand mother, and Elvira Folva. Juvenile plants are obtained by the technique of *in vitro* micro propagation using a medium without growth hormones. A serological test is applied to *in vitro* plants and this revealed the presence of: PVS in Diamand and Elvira varieties, and PRLV in the Elvira variety. We note that the rate of virus in explants decreases gradually as one approaches the meristem of micropropagated plants. The *in vitro* plants free of viruses are selected. Combined with the ELISA test, micro propagation is a sanitary filter. The *in vitro* plants infected with virses can be eliminated.

**Morphological and molecular characterization of *Penicillium* species occurring on grape and raisin in the northwestern region of Iran.** A. KHODAEI[1], A. BABAY-AHARI[1], M. ARZANLOU[1] and J. HOUBRAKEN[2]. [1]*University of Tabriz, Faculty of Agriculture, Department of Plant Protection, Tabriz, Iran;* [2]*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. E-mail: ababaiahari@yahoo.com*

*Penicillium* is a cosmopolitan mould genus with agricultural, industrial and medical significance. Members of this genus contaminate agricultural products during pre- and post-harvest conditions, resulting in reduced crop yields and quality. The Iranian grapevine industry currently covers a total area of 2,275 $km^2$ and makes a significant contribution in the country's economy. However, there is a paucity of knowledge on the biodiversity of fungal species occurring on grapes and raisins in Iran. This study aimed to characterize *Penicillium* species occurring on grapes and raisins in vineyards of northwestern region of Iran, using morphological and molecular data. A total of 66 *Penicillium* isolates were recovered from symptomatic grape berry samples collected from vineyards in East, West Azerbaijan and Qazvin provinces during the 2011–2013 growing seasons, and raisin samples were prepared from grape processing factories. A combination of morphological and *β-tubulin* gene sequence data revealed a rich diversity of *Penicillium* species in sampled areas. Twelve species of *Penicillium* were present on grape, with *Penicillium expansum* being the dominant species with the isolation frequency of 33.3%. *Penicillium expansum* is a known producer of the mycotoxin patulin, and an effective management strategy is recommended to prevent mycotoxin formation in grapevines products. Only one isolate of *Penicillium* was isolated from raisin samples. The other species included *P. brevicompactum, P. crocicola, P. crustosum, P. glabrum, P. olsonii, P. sumatrense, Talaromyces. atroroseus* and *T. minioluteous*. The latter two species were until 2011 classified in *Penicillium*.

**Influence of essential oils on *Passalora fulva* (syn. *Cladosporium fulvum*) in *in vitro* conditions.** A. NOVAK[1], D. IVIĆ[1], J. ĆOSIĆ[2], K. VRANDEČIĆ [2] and D. JURKOVIĆ[2]. [1]*Croatian Centre for Agriculture, Food and Rural affairs; Institute for Plant Protection, Rim 98, Zagreb, Croatia;* [2]*Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Kralja Petra Svačića 1d, Osijek, Croatia. E-mail: adrijana.novak@hcphs.hr*

Tomato leaf mould, caused by *Passalora fulva* (Cooke U. Braun & Crous) (synonym: *Fulvia fulva* (Cooke) Ciferri), is an important disease in tomato greenhouse production in Croatia. Several fungicides are registered in Croatia for the control of *P. fulva*, including tetraconazole, azoxystrobin or difenconazole, but increased interest in biological control in tomato greenhouses is evident among producers. As a preliminary screening, the effects of essential oils for the possible control of *P. fulva* was tested. Six essential oils were tested for *in vitro* antifungal activity on two *P. fulva* isolates, representing two races from different locations. Sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), aniseed (*Pimpinella anisum*), common thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomum verum*) essential oils were used. Seven to 10-day-old conidia were used for conidial germination tests at 10, 3.3, 1 and 0.33 μl $mL^{-1}$ concentrations of essential oils. Results were recorded after 2, 7 or 10 d. Number of germinated conidia, as well as the length of germtubes were recorded, according to the method by Banihashemi and Abivardi (2011). Statistical analysis of germtube lengths was carried out using analysis of variance. Relative inhibition of conidium germination on substrate with essential oils was calculated. In addition, measurement of germtube lengths was conducted and used to determine EC 50 values for all essential oils tested. The most promising results were obtained using clove essential oil (main components, eugenol and eugenyl acetate). When used in 1% concentration, only 9% of conidia germinated after 2 d, and 21% germinated after 10 d. Less than 50% of germinated conidia, compared to control, was recorded on rosemary and aniseed essential oils. Cinnamon essential oil showed the least germination inhibition. When used in 0.3 μL $mL^{-1}$ concentration, this oil had no inhibitory effects on conidium germination, whereas use in 3.3 μL $mL^{-1}$ and 1 μL $mL^{-1}$ led to conidia germination of over 60%. $EC_{50}$ values were 1,24 μL $L^{-1}$ for clove oil, 1,857 μL $L^{-1}$for rosemary oil and 2,5118 μL $L^{-1}$ for aniseed oil. Overall, laboratory test showed the potential of clove, rosemary and aniseed essential oils to be used as biological agents in tomato leaf mold control.

**Induction of PR proteins by the biocontrol agents *Trichoderma harzianum* and the non-toxigenic *Aspergillus flavus*, in onion seedlings infected with *Aspergillus niger*.** N. ÖZER[1], D. ATEŞ[2], M.P. ALEANDRI[3], P. MAGRO[3] and G. CHILOSI[3]. [1]*Namik Kemal University, Faculty of Agriculture, Department of Plant Protec-*

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Isolates of *Trichoderma harzianum* (TRIC7 and TRIC8) and a non-toxigenic *Aspergillus flavus* (AS3), obtained from onion growing soils, have been found to be effective by seed treatments against black mould of onion caused by *Aspergillus niger* (AN), and to induce the accumulation of antifungal compounds in sets, under pot and field conditions. Induced systemic resistance by these antagonistic fungi appears to be one of the mechanisms involved in onion protection. The aim of the present study was to explore the capability of such antagonists to also induce a set of pathogenesis related (PR) proteins (β1,3-glucanase, chitinase and peroxidase) as a part of the defence responses built up by seedlings following antagonist and AN-treatment of seeds, separately and in the combination of pathogen and antagonist, during 3–9 d post infection. In the case of pathogen-antagonist treatment, sterilized seeds were treated with a mix of spore suspension of antagonist and pathogen ($1 \times 10^7$ spores $mL^{-1}$ each) (simultaneous inoculation). Protein crude extracts were obtained from treated and control seedlings. β1,3-glucanase activity was measured spectrophotometrically in a reaction mixture containing sodium acetate buffer (pH 5·2) and laminarin 0.1% w/v. Chitinase activity was determined by measuring the amount of reducing sugars in a reaction mixture containing colloidal chitin in 0.05M citrate-phosphate buffer. Peroxidase activity was assayed by measuring the absorbance increase at 470 nm in a reaction containing guaiacol and $H_2O_2$ in sodium phosphate buffer (pH 5.4). Isoenzyme separation by isoelectric focusing (IEF) was performed horizontally with a mini IEF cell apparatus using 0.4 mm thick polyacrylamide gels containing ampholyte with a pH range of 3.5–10. **β1**,3-glucanase activity was induced in response of *Trichoderma* isolates and pathogen inoculation. Chitinase was induced by TRIC8 and AS3 treatment, and in the system TRIC8-pathogen-onion. The three antagonists separately increased peroxidase activity. Among them, TRIC8 and AS3 induced peroxidase activity on seedlings concomitantly infected with pathogen. In addition both AS3-treated alone and AS3-pathogen-treated seedlings gave differential induction of peroxidase isoforms. These results indicate that AS3 and TRIC8 can prevent the onset of black mould of onion by the induction of a complex of defence compounds including PR proteins.

**Antifungal activity of plant extracts and *Trichoderma* spp. against *Alternaria solani* and *A. alternata*.** Z. BOUZNAD[1], A.KEDAD[1], AYAD D.[1] and K. PASTIRCAKOVA[2]. *[1]Ecole Nationale Supérieure d'Agronomie El Harrach, Algiers, Algeria; [2]Slovak Academy of Sciences, Institute of Forest Ecology, Branch for Woody Plant Biology, Akademicka 2, SK-94901 Nitra, Slovakia. E-mail: z.bouznad@ensa.dz*

Potato (*Solanum tuberosum* L) occupies an important place in agricultural production in Algeria, and the crop remains threatened by abiotic and biotic stresses. Surveys carried out in different areas of potato crops since 2011, show that early blight caused by *Alternaria solani* and *A. Alternata*, proved is very harmful, would cuase considerable yield losses, especially in warm regions where climatic conditions are favourable to the development of the pathogens. The traditional control method is based on the use of fungicide products. However the harmful effects of this practice on humans, animal health and the environment are well known. Through this work, we have sought to find other more environmentally-friendly means of disease control, including the use of biological products (plant extracts and the biological agents *Trichoderma atroviride* and *T. longibrachiatum* with effectiveness against other pathogenic fungi. Several tests have been conducted *in vitro* by direct and indirect confrontation of the isolates of *Alternaria* spp. with isolates of *Trichoderma* spp., and confrontation of isolates of *Alternaria* spp. using extracts from three species of plant: *Thymus* spp., *Inula viscosa* and *Allium sativum*. The first results obtained *in vitro* showed high inhibition of mycelial growth of four isolates of *Alternaria solani* and four isolates of *A. alternata*, by the *Thymus* sp. extracts) and by some isolates of *Trichoderma* spp. This research has provided interesting data on the possibility of using alternative methods to control the early blight, which cause economic and significant losses on potato and tomato, and opens interesting prospects for further *in planta* studies.

**Leaf resistance to *Erysiphe necator* in some Turkish grapevine genotypes: preliminary studies on the role of leaf wax layers.** N. ÖZER[1], C. ÖZER[2], E. SOLAK[2], K. GINDRO[3] and S. SCHNEE[3]. *[1]Namik Kemal University, Faculty of Agriculture, Department of Plant Protection, Tekirdağ, Turkey; [2]Viticultural Research Station, Tekirdağ, Turkey; [3]Agroscope in Changins, Nyon, Switzerland. E-mail: nurayozer@hotmail.com*

Powdery mildew of grape (*Erysiphe necator* Schwein) is caused by an obligate biotrophic fungus growing on leaf surfaces. The epicuticular leaf wax layer is a physical barrier preventing pathogen attack. We investigated the development of *E. necator* on different grapevine genotypes showing different resistance levels towards this fungus and analysed the biocide effects of leaf wax materials. Different genotypes of *Vitis vinifera* **(Özer Karasi**, Italia × Mercan-174 [bred by Research Station of

Viticulture, Tekirdağ/Turkey, registered and candidate genotypes respectively], Gürcü, Isabella, Mercan [local varieties]), showing different resistance levels to powdery mildew based on symptomatological observation, were used in the experiments. Sensitive cultivars, Cabernet Sauvignon and Italia, were chosen as controls. Young leaves from rooted cuttings grown in a glasshouse (16 h photoperiod, 60% RH) were detached and disinfected in a solution of 40g/l calcium hypochlorite for 10 min. Leaf discs (0.8 mm) were placed in Petri dishes containing water agar (20g/l) supplemented with benzimidazole (30mg/l) and their adaxial surfaces were inoculated by slight brushing conidia from sporulating zones of infected leaves of cv. Kozak Siyahi. Leaf discs were observed 1, 3 and 7 d post inoculation (dpi) using an epifluorescence microscope. Healthy leaf surface waxes were extracted by dipping intact leaves in chloroform. The extracts were separated by thin layer chromatography (TLC) on silica gel plates (TLC plates 60 F254), using hexane:diethyl ether:acetic acid (78:20:4 v:v:v). The antigerminative effect of each spot was evaluated. Those showing inhibition rates over 75% were analysed by gas chromatography (GC/MS). Whereas the complete infection cycle was accomplished in 7 dpi on susceptible cultivars, the pathogen was stopped at the primary hyphae stage on Italia × Mercan-174, Gürcü, Mercan and Özer Karasi, and at the secondary hyphae stage on Isabella. Some fractions of cuticular waxes obtained by TLC exhibited high rates of inhibition of conidium germination. Current chemical investigations have identified several compounds known for their antifungal activity, such as β-Santalol, 1,25-dihyroxcholecalciferol, 20-hydroxyecdysone, thiophene and bithienyl. This study highlights the likely contribution of foliar cuticular layers in the control of powdery mildew using resistant cultivars.

**Genetic variability and population structure of *Ascochyta rabiei* in Morocco using microsatellite markers.** S. KRIMI BENCHEQROUN[1], S. AHMED[2], A. HAMWIEH[3], M. IMTIAZ[4], R. MENTAG[5] and S.M. UDUPA[2]. *[1]National Institute of Agricultural Research (INRA), P.O. Box 589, Settat, Morocco; [2]International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box. 6299, Rabat, Morocco; [3]International Center for Agricultural Research in the Dry Areas (ICARDA), Cairo, Egypt; [4]International Maize and Wheat Improvement Center (CIMMYT-Pakistan), Islamabad, Pakistan; [5]National Institute of Agricultural Research (INRA), P.O. Box 6809, Rabat, Morocco. E-mail: krimisanae@gmail.com*

Ascochyta blight, caused by *Ascochyta rabiei* Lab. (teleomorph: *Didymella rabiei*) is an economically important fungal disease on chickpea in Morocco, and other parts of the world. The genetic diversity and population structure of *A. rabiei* in Morocco (41 isolates from four geographical regions) and 55 isolates from Syria and Turkey were investigated using nine polymorphic microsatellite markers. Genetic analysis based on 47 amplified alleles revealed a high level of genetic diversity among Moroccan *A. rabiei* isolates (*Ht* = 0.59) with the majority attributed to diversity within subpopulations (*Hs* = 0.51). Small genetic differentiation (*Gst* = 0.16) and a significant gene flow (*Nm* = 2.53) were detected among pathogen populations originated from Morocco, Turkey and Syria, suggesting limited geographic delimitation and a high pathogen migration probably due to seed exchange. However, high genetic similarity was observed between isolates from Syria and Turkey (86% similarity index) and between the Morroccan regions Chaouia and Abda/doukkala (83%), indicating that these isolates may be clonally related. These results will be useful in breeding for Ascochyta blight-resistant chickpea cultivars and development of efficient control strategies in Morocco.

**Determination of resistance in some wheat cultivars against *Fusarium* spp. isolates in Trakya region of Turkey.** N.D. KÖYCÜ and N. ÖZER. *Namik Kemal University, Agricultural Faculty, Plant Protection Department, 59030 Tekirdağ, Turkey. E-mail: dkoycu@nku.edu.tr*

*Fusarium* diseases of wheat (seedling blight, foot rot, scab and *Fusarium* head blight - FHB) caused by a number of species, mostly by *F. graminearum, F. culmorum, F. avenaceum, F. poae and F.tricinctum*, are common in the Trakya Region. The aim of this study was to determine the pathogenicity of *Fusarium* species isolates from the diseased roots and stem bases of wheat plants, and the reactions of common bread wheat cultivars to these pathogens. As the causal agent of these diseases, *Fusarium* spp. were collected in the years of 2009 and 2010. Pathogenicity seed tests of 40 *Fusarium* isolates (monoconidium cultures) were implemented on the bread wheat cultivars Pehlivan and Flamura 85. Seeds of the cultivars were surface-sterilised by immersing for 3 min in 1% sodium hypochlorite solution. Sterilized seeds were placed on wet filter paper in five Petri dishes, each representing one replicate, with ten seeds per dish. An agar plug (0.5 cm diam.) with mycelium only was cut from 12-d-old cultures, then placed to the embryo base of each seed. The seeds were incubated at 22°C. The hypocotyls and roots of seedlings were monitored for disease severity (%) after 5 d. The most pathogenic isolates were used for resistance tests of seven bread wheat cultivars. Healthy wheat seeds were sown in pots filled with sterile soil inoculated with *Fusarium* species. Pots were kept at 20°C, and 12h light and 12h dark each day. In the seed test, disease severity was 40–100% in both cultivars. *F. culmorum*, *F. tricinctum* and *F. acuminatum* isolates were the most pathogenic. The severity and incidence (%) of the disease was assessed in seedling roots 35 d after inoculation.Disease severity by these isolates

was 64–87% for the seven wheat cultivars. *Fusarium culmorum* was the most virulent on the wheat cultivars, while *F. tritinctum* and *F. acuminatum* isolates were less virulent on all tested cultivars. The cv. Golia was most sensitive to the disease among the tested cultivars.

**Effectiveness of cyprodinil + fludioxonil mixture against *Botrytis cinerea*, and their residues in grapes and wine.** N.D. KÖYCÜ[1], N. ÖZER [1] and N. DELEN[2]. [1]*Namik Kema University, Agricultural Faculty, Plant Protection Department, Turkey;* [2]*Ege University, Agricultural Faculty, Plant Protection Department, Turkey (Retired). E-mail: dkoycu@nku.edu.tr*

'Gray mould' in grapes, caused by *Botrytis cinerea* Pers. Fr., is a destructive disease responsible for severe economic damage in vineyards, both to grape production and also to wine quality. The pathogen is present at pre- and post-harvest periods in vineyards. Gray mould is also a common vineyard disease in Turkey. Spores of *B. cinerea* are required to be free of moisture on surfaces of grape berries in order to germinate and infect. In this study the effectiveness of cyprodinil + fludioxonil (Switch 62.5, Syngenta) on *B. cinerea* was evaluated in two experimental vineyards in which the wine cultivars Emir (white) and Zinfandel (rose), both sensitive to disease, were cultivated. In addition, residues of the mixture components were determined in grapes and wine. The trials were carried out as two experimental programmes. Programme I included spraying at the stages of post-bloom, berries pea-size, veraison and 21 d before harvest. Programme II was conducted at the stages of veraison and 21 d before harvest. Disease severity was determined at the harvest using a standard scale. Residue analysis was carried out using Gas-chromatography. The weather conditions contributed to severe grey mould. Experiment average temperature, relative humidity and rainfall were, respectively, 20.16ºC, 78.84% and 13.48 mm, respectively. In Programme I the best disease control was achieved, and disease severity was significantly reduced on both cultivars. In programme I the mixture was more than 70% effective in both cultivars due to decreased of the flower infections although the Programme II showed very low effect to control the disease. The concentrations of cyprodinil + fludioxonil in grapes and wine harvested from both spraying programs were well below the European Maximum Residue limit. The greatest concentrations of cyprodinil and fludioxonil in grapes were, respectively, 0.62 mg $kg^{-1}$ and 0.45 mg $kg^{-1}$.

**New Tunisian barley variety "imen" selected for resistance to *Barley yellow dwarf virus -PAV* and adapted to semi-arid conditions.** A. NAJAR[1], S. G. KUMARI[2,4], H.B. GHANEM[1], H SAIED[3], S. GRANDO[2,5] and M. BAUM[2,6]. [1]*National Agricultural Research Institute of Tunisia, 2049 Ariana, Tunisia;* [2]*International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria;* [3]*University of Aleppo, Syria;* [4]*present address: ICARDA, Tunis, Tunisia;* [5]*present address: ICRISAT, India;* [6]*present address: ICARDA, Amman, Jordan. E-mail: asmanajara@yahoo.fr*

Barley is the second most widely cultivated cereal crop in Tunisia where it actually covers an average area of 500,000-600,000 ha, providing an estimated mean yield of 1.5 tons/ha. Barley straw and grain is a major resource for animal feeding especially in the north and the central regions of Tunisia. Recent studies have shown that virus diseases may cause severe damage and yield loss in barley crops. Surveys conducted in Tunisia showed that *Barley yellow dwarf virus-PAV* (BYDV-PAV) is the most important virus, that is widely spread by its most efficient aphid vector *Rhopalosiphum padi.* Genetic resistance is the method of choice for controlling virus infections. A screening programme for resistance to BYDV-PAV was initiated during the 2002/2003 cropping season, with ten F2 segregating barley populations provided by ICARDA, as starting material. Crosses were made with resistant cultivars carrying the *Yd2* resistance gene, used worldwide in barley breeding programmes. Approx. 200 resistant lines were selected based, on serological tests (ELISA, TBIA) to monitor virus multiplication, severity of symptoms and a molecular markers for the detection of the*Yd2* gene. From this genetic pool, 23 resistant lines were then evaluated for agronomic performance in a semi-arid growing area (Kef) during seven cropping seasons (2006/2007 to 2012/2013). "Manel" and "Rihane", the most commonly grown barley varieties were used as checks. This programme led to the selection of an interesting line that showed constant superiority to the check cultivars, and this was given the name "Imen". This new cultivar gave 11 and 19% more biomass than the two checks, respectively, and 13% increase in grain yield. Based on these data, "Imen" has been officially registered in 2014 in the Tunisian catalogue of plant varieties for commercial use by farmers.

**Comparative effects of oregano essential oil and vermicompost on bioagressors of *Olea europea*.** L. BRAHIMI, H. HALLADJ and Z.-E. DJAZOULI. *SAAD DAHLAB University, Faculty of Agro-Veterinary Sciences and Biological Department of Agricultural Sciences, BP270, road Soumaa, Blida, Algeria. E-mail: bellabed.brahimi_lati@yahoo.fr; zahroor2002@yahoo.fr*

Integrated production in sustainable agriculture seeks to improve the efficiency of organic inputs across formulations, thus prolonging persistence at field by incorporating synergists products themselves being non-toxic at the doses used in order to increase protective

action and maintain the culture viability. The present study evaluated effectiveness of a biofertilizer (vermicompost) and a biopesticide (formulated essential oil of oregano) compared to a synthetic pesticide (Methomyl), on pests of the olive tree (*Olea europaea*). The study was carried out in the olive groves. From elementary plots used for our investigations, weekly monitoring of the abundance of pests was carried out before the application of the treatments, and continued until 10 d after treatment. Period adopted corresponds to period of activity of Methomyl. A disparity in the degree of toxicity of the different treatments was detected, the three treatments which have a repressive effect diverge depending on the physiology of each pest. For example, with *Aceria olea* presents comparable levels from the three treatments, unlike *Euphyllura olivina* where essential oil of oregano had greater efficiency. The results show a moderate biocenotic resumption in species having received biological treatments. However, as a result of Methomyl, there is a pronounced disturbance of the abundance of different groups of pests. We conclude that the alternatives to chemicals, including biological control through the use of biopesticides and biofertilizers, has potential for integrated production and protection of the environment and preservation of natural resources.

**Occurrence and molecular characterization of *Apple chlorotic leaf spot virus* on apple and pear in Egypt.** M. HASSAN[1,2]. [1]*Department of Agricultural Botany, Faculty of Agriculture, Fayoum University, Egypt;* [2]*Department of Plant Pathology, University of Arkansas, Fayetteville, 72701, USA. E-mail: maa22@fayoum.edu.eg*

Samples of apple and pear were collected from different orchards in Egypt. Presence of *Apple chlorotic leaf spot virus* (ACLSV) was investigated using RT-PCR. ACLSV was found to be widespread among apple and pear samples collected from either traditional areas of apple and pear culture in Qalyubia Governorate or new reclamation land in Menoufia Governorate. The population structure of the virus was studied using the collected isolates. Sequences of a part of the CP encoding gene of isolates from different hosts and locations were compared with those available at NCBI GenBank, showing similar divergence levels. Further phylogenetic analysis using the sequenced region indicated that ACLSV-Egyptian isolates clustered with no correlation found based on host or geography.

**Distribution and characterization of species of Botryosphaeriaceae associated with stem end rot disease of mango fruit, determined using molecular, morphological and pathological data.** S. IRAM and S. ABRARI. *Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan. E-mail: iram.shazia@gmail.com*

This study was based on molecular, morphological and pathological characterization of postharvest fungal pathogens associated with stem end rot of mango fruits collected from 12 locations of Pakistan. The postharvest mango disease stem end rot arises from quiescent infections. During 2013, surveys were conducted in farmer and demonstration blocks of six districts, of Punjab (Multan, Muzaffar Garh, Rahim Yar Khan) and Sindh (Matiyari, Mirpur Khas, Tando AllahYar). Fresh mango fruit samples were assembled and kept in the laboratory for ripening at room temperature. Disease assessments were made by recording disease symptoms, colour and ripeness of fruit each day for 2 weeks. Disease severity on each sample was determined based on affected area of fruit by using 0–5 point grading scale (0% no disease, 1–5% trace, 6–25% mild, 26–50% moderate, 51–75% severe, and 76–100% very severe disease). The same disease symptoms were observed on samples of each location but incidence and severity were variable. Stem end rot was found 100% prevalent in all the locations. Incidence of disease was determined to be high in farmer blocks. The causal pathogens were isolated from 47 infected samples (22 from Punjab and 25 from Sindh), by the tissue segment method on potato dextrose agar and specific malt extract agar. Two species of Botryosphaeriaceae (*Lasiodiplodia theobromae* and *Botryosphaeria dothidea*) were detected by molecular sequencing analysis. Total genomic DNA was isolated from all the fungal isolates using phenol DNA extraction method. A pair of ITS primer was used to amplify the obtained DNA. The amplified PCR products were resolved using 2% agarose gel, stained with 0.5 ug/ml ethidium bromide. Isolate band size was in the range of 580–600bp. The amplified products from ITS-5.8S-rDNA were sequenced and compared with previously identified sequences in Genbank, which showed 96–100% similarity with *Lasiodiplodia theobromae* and *Botryosphaeria dothidea*. Sequences obtained in this study were aligned and a neighbour-joining tree was rooted to determine the phylogenetic relation among the isolates of the two detected species. Isolated pathogens were morphologically identified by determining cultural and conidial characteristics. Average conidial length, width and growth rates of both species were determined after 1 week of incubation. Pathogencity tests were performed by artificial inoculations on detached mango twigs, detached mango leaves and potato tubers, to determine the aggressiveness of post harvest fungal pathogens. All isolates produced lesions on twigs and potato tubers, but detached leaves were not good hosts. Lesion area on detached twigs was in the range of 2.6–12.5 cm² and on potato tubers 5.8–19.6 cm². Results obtained from pathological studies were analyzed statistically and proved non significant at $P>0.05$ through analysis of variance.

Standard error (SE) was calculated to determine the differences of length, width and area between inoculated pathogens. Isolates showed more aggressiveness on potato tubers as compared to detached mango twigs. Isolates from Punjab were more aggressive compared to isolates of Sindh. Fungal isolates were revitalized with 100% same results from artificially inoculated hosts, and Koch's postulates were verified. This study has shown relationships of isolates with region, and has given information about pathogencity level of isolates. This will be useful for developing effective management policies to reduce the afflictions caused by mango stem end rot.

**Effects of some plant activators on salad lettuce inoculated with *Botrytis cinerea* in pot conditions. Ü.** ESER[1] and A. COŞKUNTUNA[2]. *[1]Black Sea Agricultural Research Institute, Samsun, Turkey; [2]Namik Kemal University, Agricultural Faculty, Plant Protection Department, Tekirdağ, Turkey. E-mail: atuna@hotmail.com*

Gray mould, caused by *Botrytis cinerea*, has been considered most destructive fungal disease in greenhouse-grown lettuce in Turkey. The primary symptoms of the disease include plant wilting accompanied by a fuzzy gray growth at the base of plants, which contains masses of air borne spores. Effective control of the disease has been achieved with range of fungicides, but their widespread use has led to the development of pathogen resistance. Recommended treatments involving the alternate use of fungicides with different modes of action, are not always effective. Furthermore, public concern over fungicide residues on vegetables has increased markedly. As a result, there is a need for satisfactory alternative methods for managing the disease, including biocontrol and cultural practices. Harpin, a protein produced by *Erwinia amylovora* that induces a hypersensitive response in certain hosts, marketed as Messenger, is a systemic acquired-resistance inducer that also promotes plant growth. Crop-Set is a product based on plant extracts and *Lactobacillus acidophilus* fermentation production which optimizes plant physiological processes. It aids in stressful situations by providing nutrients essential to growth. In this study, effects of application of two plant activators: harpin protein (Messenger TM, 0,1 g $mL^{-1}$ water) and *Lactobacillus acidophilus* fermentation production + plant extract + mineral substance (Crop-set, 0,5 mL/100 mL water) and one test fungicide fenhexamide (Teldor 1 mL/1000 mL water) on gray mould disease were investigated in pot conditions. Plant activator applications were realized at interval of 14 d with five replicates using hand sprays. After first applications, *B. cinerea* spore solution of $1 \times 10^5$ cfu $mL^{-1}$ was inoculated onto the plants. Harpin protein application reduced disease on cultivars Chianti and Yedikule at the rates of 30% and 69% respectively. The effects of application of activators with active ingredient of *L. acidhophilus* were 30% and 58% reductions, and with fenhexamid were 90% and 93% reductions respectively. Promising results were achieved on preventing disease at the end of the study. Combinations of two plant activators, or plant activator + fungicide have been shown to be more efficient and environmentally acceptable for gray mould control.

**Distribution and population structure of *Monilinia* spp. on peach, nectarine and plum in Croatia.** T. FAZINIĆ[1], D. IVIĆ[1], A. NOVAK[1] and T. MILIČEVIĆ[2]. *[1]Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Plant Protection, Zagreb, Croatia; [2]University of Zagreb, Faculty of Agriculture, Department of Plant Pathology, Zagreb, Croatia. E-mail: tina.fazinic@hcphs.hr*

During 2012 and 2013, a national survey was conducted in 13 Croatian counties with intensive stone fruit production, to identify the possible presence of *Monilinia fructicola* (G. Winter) Honey in commercial plantations of peach, nectarine and plum. Additionally, population structure of *Monilinia* spp. was analyzed on peach, nectarine and plum fruits, as well as the distribution of *Monilinia* species throughout Croatian counties. Isolates were identified using a synoptic key for described by Lane (2002) and in EPPO Standard PM 7/18. Identification was confirmed for isolates that were identified as *M. fructicola*, following the Côté *et al*. (2004) PCR protocol with species-specific primer pairs MO368-5/MO368-10R and ITS1-Mfc1/ITS4-Mfc1, as described by Ioos and Frey (2000). PCR reaction using the primer pair ITS1-Mfc1/ITS4-Mfc1 was more reliable for molecular diagnosis of *M. fructicola*. In 2012, *M. fructicola* was found only in one peach orchard near Vrgorac in Split-Dalmatia County, in 19 of 20 collected samples. This was the first record of this quarantine fungus in Croatia (Ivić *et al*., 2014). In 2013, *M. fructicola* was determined again in Split-Dalmatia County, but also in Međimurje, Zagreb, Dubrovnik-Neretva and Vukovar-Srijem County. In Istria County, *M. fructicola* was identified on plum and this was the first record of *M. fructicola* on plum in Croatia. Among 353 *Monilinia* spp. isolates collected from peach, nectarine and plum during the two-year survey, 130 (36.8%) were *Monilinia laxa* (Aderhold & Ruhland), 123 (34.9%) were *Monilinia fructigena* (Aderhold & Ruhland), while 100 (28.3%) were confirmed to be *M. fructicola*. Now it is known that *M. fructicola* is present both in continental and Mediterranean part of Croatia, even though the results of the first year indicated that *M. fructicola* might be established only in a relatively isolated area near Vrgorac. Moreover, *M. fructicola* is a dominant *Monilinia* sp. in this fruit-growing area. Out of 78 samples collected near Vrgorac, *M. fructicola* was present in 74 (95%).

**First report on *Grapevine leafroll associated virus 1* infecting pomegranate trees (*Punica granatum* L.) in Turkey.** K. ÇAĞLAYAN, M. GAZEL, E. ELÇİ and C.T. ÇINAR. *Mustafa Kemal University, Agriculture Faculty, Plant Protection Department, Hatay, Turkey. E-mail: kcaglayano@yahoo.com*

Pomegranate (*Punica granatum* L.) has been cultivated since ancient times through the Mediterranean region, Asia, Africa and some parts of Europe with the main production countries being mainly Mediterranean and Asian countries such as India, Iran, China and Turkey. Turkey is one of the leading producers and exporters, and total pomegranate production reached 315,000 tons in 2012, corresponding to one of the largest pomegranate economies in the world. Hatay, located in Eastern Mediterranean region, is one of the most important centers of diversity in pomegranate in Turkey. Symptoms resembling virus infection in local pomegranate cultivars were observed recently in late summer-autumn periods in Hatay province. Pomegranate leaves showing yellowing, chlorotic spots, vein clearing and oak-leaf symptoms were sampled to verify possible virus association with these symptoms. DAS-ELISA and RT-PCR analysis were used for the detection of suspicious viruses infecting fruit trees, grapevines and potatoes such as *Plum pox virus, Prunus necrotic spot virus, Apple mosaic virus, Apple chlorotic ring spot virus, Apple stem grooving virus, Arabis mosaic virus, Grapevine leafroll associated virus* 1, 2, 3, 4-9, 6, 7, *Grapevine virus A, Cucumber mosaic virus, Pepino mosaic virus, Potato virus X, Potato virus Y* (PVYN), *Potato virus M* and *Potato leafroll virus*. Plant sap extraction for ELISA and Nucleic acid extraction for PCR analysis were performed from leaves and shoots of three strong symptomatic and twenty suspicious plants. Due to high content of metabolic compounds such as polyphenols and anthocyanins in pomegranate tissues, nucleic acid extractions did not result in high quality RNA in most of the conventional procedures used in this study, except for a modified CTAB based method. One set of primers F5′-GTTACGGCCCTTTGTTTATTATGG-3′ and R5′-CGACCCCTTTATTGTTTGAGTATG-3′ was used to amplify the coat protein gene (397 bp) of GLRaV-1. According to ELISA and RT-PCR analysis only three symptomatic plant samples yielded positive results for *Grapevine leafroll associated virus-1* (GLRaV-1) among tested viruses, whereas all other samples were negative for all tested viruses. To datw, there are only a few reports on *Cucumber mosaic virus* and Hop stunt viroid in pomegranates. To the best of our knowledge, this is the first report to show GLRaV-1 in pomegranate trees.

**Effect of gaseous ozone and 1-methylcyclopropene treatments on the development of *Penicillium expansum* and patulin production in apple fruit.** V. PAPADOPOULOS[1], I. MINAS[2], C. MYRESIOTIS[3], A. MOLASSIOTIS[2] and G. KARAOGLANIDIS[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P.O.Box 269, 54124 Thessaloniki, Greece; [2]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Pomology, 54124 Thessaloniki, Greece; [3]Aristotle University of Thessaloniki, Faculty of Agriculture, Pesticide Science Laboratory, P.O.Box 1678, 54124 Thessaloniki, Greece. E-mail: gkarao@agro.auth.gr*

Blue mould, caused by *Penicillium expansum,* is the most important postharvest rot of apple fruit, while *P. expansum* is a common producer of patulin, one of the most important mycotoxins. Control of the disease is achieved by fungicide treatments. However, development of fungicide resistance and social concerns regarding pesticide residues necessitate research for alternative control methods. The effect of gaseous ozone ($O_3$) exposure (0.3 μL $L^{-1}$) and/or 1- methylcyclopropene (1-MCP – 0.5 μL·$L^{-1}$, 24 h, 0°C) treatment on the development of blue mould and patulin production on apple fruit (cvs 'Granny Smith' and 'Fuji') was investigated. Exposure of fungal cultures at gaseous $O_3$ for 0, 2, 8, 24 and 72 h resulted in significant reductions of mycelial growth during exposure, but growth resumed after cultures were removed from ozone enriched atmosphere. Similarly, exposure of fungal cpores to $O_3$ for 8 h or more resulted in a significant reduction of spore viability. 1-MCP-treated and untreated fruit were artificially inoculated with *P. expansum* and subjected for 3 months to cold storage (0 °C, RH 95%) either in an $O_3$ enriched atmosphere or in a conventional cold room. Measurements of disease incidence and severity showed that 1-MCP treatments of Granny Smith fruit provided a slight reduction (10%) in disease incidence, while $O_3$ treatments did not reduce this further. However, in 1-MCP-treated Granny Smith fruit disease severity was reduced by 30% ($P<0.05$) compared to untreated control fruit. In contrast, neither MCP nor $O_3$ treatments had any effects both on disease incidence and severity on Fuji fruit. $O_3$ treatment of both 1-MCP-treated and untreated fruit resulted in a complete inhibition of spore production. Measurements of patulin production in rotted fruit showed that on Granny Smith fruit 1-MCP treatment resulted in a 4-fold increase of patulin accumulation, while ozone treatments had no effect on patulin content. Ripening features, including ethylene production, fruit firmness and soluble solids content, were remarkably depressed by 1-MCP application, particularly evidenced in Granny Smith. These results indicate that ozone treatments do not contribute to the reduction of blue mould on apple fruits, while MCP treatments may reducet disease severity. However in Granny Smith fruit 1-MCP may increase the risk for higher patulin accumulation.

**Geographic distribution of *Aspergillus* spp. in fig orchards of the Southern Peloponnese, Greece, de-**

**termined with a GPS-map system.** V. DEMOPOULOS[1], D.F. ANTONOPOULOS[1], T.J. MICHAILIDES[2], T. AMORGIANNIOTIS[3] and E. GEORGOPOULOS[4]. [1]*Technological Educational Institute of Peloponnese, Department of Agricultural Technology, Laboratory of Plant Protection, Kalamata, Greece;* [2]*University of California Davis, Department of Plant Pathology, Kearney Agricultural Research and Extension Center, Parlier, USA;* [3]*University of Patras, Department of Computer Engineering and Informatics Computer Science and Engineering, Pattern Recognition Laboratory, Rio, Greece;* [4]*Technological Educational Institute of Peloponnese, Department of Agricultural Technology, Laboratory of Computer Science, Kalamata, Greece. E-mail: vdimo@teikal.gr*

Aflatoxins are naturally occurring mycotoxins produced mainly by two species of *Aspergillus*, *A. flavus* and *A. parasiticus*, and are considered as the most notoriously known carcinogenic substances. Although aflatoxin contamination of dried figs can occur at all cultivation and postharvest stages, if figs are properly stored, aflatoxins do not develop during storage and the problem becomes exclusively a preharvest one. In order to estimate the endemic population of *Aspergillus* spp. in the southern Peloponnese, the largest fig tree cultivation area in Greece, soil samples from 46 commercial fig orchards (six replicates per orchard) were collected during July to August 2013. The distribution of the sampling sites was determined and performed based on the production volumes in 2010, 2011, and 2012 of the fig orchards and the geographical coordinates of each sampling site were recorded using GPS. Soil samples of 0.2 g were spread on Petri plates containing modified rose bengal chloramphenicol agar selective medium supplemented with dichloran and streptomycin. The plates were incubated at 30°C for 3 d and the grown colonies of *Aspergillus* spp. were counted. Approximately 10% of the colonies were collected and single spore strains were isolated for further analysis. The population data of the fungi were plotted on a GPS map system, and the whole target region was interpolated using the Barnes spatial interpolation method. The map showed that certain locations within the studied area display much higher population densities of *Aspergillus* spp. than others. The addition of more data on the proposed map, such as the evaluation of adaptability, pathogenicity, and aflatoxigenicity, as well as possible fungicide efficacy on growth and aflatoxigenicity of the isolated strains will help develop this map into a comprehensive risk assessment predictive tool for aflatoxin contamination of dried figs in the area. The present study constitutes the first stage of an Archimedes III research project, cofinanced by the European Union and the Greek Ministry of Education, aiming to develop a suitable strategy to control aflatoxin contamination in dried figs in the southern Peloponnese following an integrated plant protection management approach.

**Occurrence of *Lasiodiplodia citricola* in vineyards showing decline in southern Italy.** M.L. RAIMONDO, F. CIBELLI, A. CARLUCCI and F. LOPS. *Department of Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy. E-mail: antonia.carlucci@unifg.it*

During surveys carried out in the summer of 2012 and 2013 in the province of Foggia (Apulia, southern Italy), in vineyards with grapevines affected by Esca disease, symptoms of decline were often observed on the grapevine trunks, cordons, canes and shoots. These external symptoms included leaf chlorosis and marginal necrosis, and were associated with internal symptoms of subcortical longitudinal brown streaking and brown xylematic sections. The grapevine plants were sampled and subjected to mycological analysis. One hundred and six isolates showing morphology similar to Botryosphaeriaceae were grown for investigations using molecular tools, including microsatellite-primed polymerase chain reaction (MSP-PCR). Thirty representative isolates from seven MSP-PCR groups were used for phylogenetic analysis, based on amplification of internal transcribed spacer (ITS) regions and part of the translation elongation factor 1-alpha (EF1-$\alpha$) gene. The nucleotide sequences compared with those retrieved from GenBank (http://www.ncbi.nlm.nih.gov) showed 99% to 100% similarity with Botryosphaeriaceae spp. Multigenic analysis that combined ITS and EF1-$\alpha$ sequences was performed with PAUP version 4.0b10 (Swofford 2003) using maximum parsimony command blocks. This analysis allowed the identification of the following species: *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata, Diplodia corticola, Dothiorella iberica*, *Dothiorella sarmentorum Lasiodiplodia theobromae, Lasiodiplodia citricola* and *Neofusicoccum parvum*. The fungal identification using molecular tools was confirmed using microscopic analysis. In July 2013, green shoots of young grapevine plants of cvs. Lambrusco and Sangiovese were grown in pots in a greenhouse, and inoculated with representative isolates of these Botryosphaeriaceae spp., including uninoculated controls. Thirty days after inoculation, the extent of the vascular discoloration caused by the fungi was very variable. In particular, *L. citricola*, *L. theobromae* and *N. parvum* were more virulent than the other *Botryosphaeriaceae* spp. To our knowledge, this is the first report of *L. citricola* as a pathogen on grapevine, because it has only been reported as a pathogen on *Citrus* (Abdollahzadeh *et al.*, 2010) and *Juglans regia* (Chen *et al.*, 2013).

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**Appearance and management of hop stunt disease in Slovenia.** S. RADIŠEK[1], T. GUČEK[1], J. JAKŠE[2],

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In August 2007, a severe outbreak of stunted hop plants of variety Celeia was observed in a hop garden located in the Savinja valley. Affected plants showed symptoms of severe stunting, shortening of the internodes of the main bine and lateral branches, leaf curling, small cone formation and dry root rot. Based on extensive diagnostic analysis of symptomatic plants "hop stunt viroid (HSVd)" and a citrus viroid were identified as casual agents. Since 2007, the disease was found in ten hop farms located in the vicinity of the first outbreak. The majority of the affected hop gardens were planted with the variety Celeia, and the rest with the varieties Bobek, Savinjski golding and Aurora. The disease incidence varied among hop gardens from 1–30% and increased rapidly (up to 10%) each subsequent year, predominantly along plant rows. HSVd has previously been reported as a causal agent of hop stunt disease in Japan, South Korea, USA and China. With the aim of total eradication and preventing further spreading, the Slovenian Institute of Hop Research and Brewing and the Phytosanitary Inspectorate, co-ordinated by the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection, carried out a monitoring survey of hop gardens, which included visual inspections of hop gardens, sampling, laboratory analysis and expert support. In addition, strict phytosanitary measures have been taken and included in Slovenian legislation to prevent further spread and to eradicate HSVd infections. Results are presented of monitoring surveys in the period 2007-2013, new diagnostics approaches, experiences with the eradication process and first epidemiological analysis of hop stunt disease in Slovenia.

**The complexity of fusarium head blight may lead to constant wheat mycotoxin contamination: a key study in Italy.** L. COVARELLI[1], G. BECCARI[1], A. PRODI[2], S. GENEROTTI[1,3], F. ETRUSCHI[1,3], G. MECA[3], C. JUAN[3], E. FERRER[3] and J. MAÑES[3]. *[1]University of Perugia, Department of Agricultural, Food and Environmental Sciences, Borgo XX Giugno 74, 06121, Perugia, Italy; [2]Alma Mater Studiorum Bologna University, Department of Agricultural Sciences, Viale Fanin 46, 40127, Bologna, Italy; [3]University of Valencia, Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, Av. Vicent Andrès Estellès s/n, 46100, Burjassot, València, Spain. E-mail: lorenzo.covarelli@unipg.it*

Fusarium head blight (FHB) is an important disease of wheat, causing yield losses and grain contamination by several mycotoxins including trichothecenes (DON, NIV, T2, HT2, DAS) and "emerging" mycotoxins such as beauvericin (BEA) and enniatins (ENs). We identified and characterized mycotoxin producing *Fusarium* species isolated from 162 durum and soft wheat samples from an area of central Italy in 2009 and 2010. Furthermore, the presence of mycotoxins in the grain was determined by LC-MS/MS and strains of *F. graminearum s. str.* (FG), *F. culmorum* (FC), *F. avenaceum* (FA), *F. poae* (FP), *F. equiseti* and *F. sporotrichioides* were tested *in vitro* for their ability to synthesize trichothecenes, BEA and ENs. In general, FG was the most frequently isolated species. In 2009, the occurrence of FA and FP was greater than in 2010. Among FG strains, the 15-ADON chemotype was the most frequent, followed by NIV and 3-ADON chemotypes, while all FC isolates belonged to the 3-ADON chemotype. All FP strains were NIV chemotypes. *In vitro* trichothecene production confirmed molecular characterization. All FA strains showed the ability to biosynthesize ENs *in vitro* but not BEA. All FP strains resulted to be NIV and BEA producers and some of them co-biosynthesized ENs. Regarding grain contamination, NIV was always detected at appreciable levels while T2+HT2 toxins were mostly found in durum wheat samples in 2009, with 6% of samples exceeding maximum levels (ML) recommended by the EU. Presence of ENs and co-contamination by BEA and ENs were also found. DON levels were always below ML. In general, a high presence of "secondary" FHB causal agents and their mycotoxins, which have not yet been legally regulated in the EU, were detected in the grain. Climatic conditions (rainfall, temperature) were the predominant factors influencing mycotoxigenic species composition and mycotoxin contamination. NIV contamination occurred in all climatic conditions, suggesting that it may often represent an underestimated risk.

**Survey of ochratoxin a-producing fungi and ochratoxin a in grapes grown under organic and integrated systems in Umbria, central Italy.** L. TOSI, G. BECCARI, A. SENSI, F. GRADASSI, M. ORFEI, M.V. CONSALVI and L. COVARELLI. *University of Perugia, Department of Agricultural, Food and Environmental Sciences, Borgo XX Giugno, 74, 06121, Perugia, Italy. E-mail: laura.tosi@unipg.it*

A survey was conducted in 2013 five Umbrian organic and integrated vineyards, to determine the occurrence of mycotoxigenic fungi in grapes, to identify the isolated mycotoxigenic species and to assess their ochratoxin

A (OTA) production *in vitro*. Four integrated vineyards were located at Magione (plain) and Castel Rigone (hill) (Perugia, central Italy) and included white grape (Chardonnay) and red (Cabernet Sauvignon) varieties, while the organic vineyard, located at Cannara (Perugia, central Italy), included three red varieties (Cabernet Sauvignon, Sangiovese, Sagrantino) and one white variety (Grechetto). Grapes were collected from mid-June to September at four development stages: fruit set, pea-size berries, veraison and harvest. Small pieces from each bunch were transferred into moist chambers while eight berries were plated on PDA and MEA nutritive media. Vineyard phytosanitary conditions were also monitored throughout the growing season by the use of moist chambers. This revealed that vines were affected by downy and powdery mildews due to incorrect and inadequate use of control measures. *Alternaria*, *Penicillium*, *Cladosporium*, *Aspergillus* were the most frequently isolated genera from grapes from setting to harvest. In particular, *Alternaria* spp., *Penicillium* spp. and *Cladosporium* sp. were abundant over the entire season while *Aspergillus* spp. populations increased from veraison to harvest. *Aspergillus* colonization was prevalent on the secondary branches and berries of the bunches. The incidence of *Aspergillus* spp. increased with berry ripening on all wine cultivars. *Botrytis cinerea* colonization was low during the grape growing season but severe attacks were noticed at harvest on Cabernet Sauvignon in an integrated vineyard located in the plain. Fifty-three *Aspergillus* spp. were isolated from berries collected in the integrated (26) and organic (27) vineyards. Based on molecular identification, most of *Aspergillus* isolates were identified as *A. niger sensu stricto*, *A. tubingensis* and *A. japonicus*. Other black aspergilli were identified as belonging to *A. niger* aggregate. *Aspergillus carbonarius*, reported as the main OTA producer, was never isolated. According to ELISA assays, all *Aspergillus* isolates produced OTA *in vitro* with concentrations varying from 2.1 to 77.5 $\mu g\ L^{-1}$.

**Development of a multiplex one-step real-time RT-PCR assay for the detection and quantification of *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV).** I.S. FOTIOU, P.G. PAPPI, K.E. EFTHIMIOU, V.I. MALIOGKA and N.I. KATIS. *Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Lab of Plant Pathology, 54124 Thessaloniki, Greece. E-mail: katis@agro.auth.gr*

*Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV), both members of the genus *Ilarvirus*, are among the most important viruses of the stone fruit trees, causing high economic and agronomic losses. These viruses are mainly transmitted through infected propagating material, so the application of sensitive assays is crucial for reliable indexing of plants and prevention of virus spread. A multiplex one-tube quantitative real time RT-PCR was developed to simultaneously detect both viruses and quantify RNA targets. Appropriate primers and probes were designed, based on the nucleotide sequences of PNRSV and PDV isolates recovered from databases. After alignment of the conserved regions of the polymerase and movement protein genes, primers were designed to amplify 111 and 121 bp DNA fragments from PNRSV and PDV respectively, and TaqMan probes were designed to anneal within these sequences. For determining the quantification limits of the assays, PNRSV and PDV *in vitro* synthesized RNA transcripts were used in ten-fold serial dilutions. The linear range of quantification was from $10^2$ to $10^8$ and 40 to $4 \acute{} 10^8$ RNA transcripts for PNRSV and PDV, while the amplification efficiencies were 99.8% and 93%, respectively, for the two viruses. Multiplex RT-PCR showed no significant differences in the detection limits compared to the single virus assays. Additionally, no competitiveness of primers was observed. The real time RT-PCR was tested with a collection of isolates of different geographical origins. Overall, the multiplex assay exhibits high sensitivity and could be used for the reliable detection of PDV and PNRSV in routine testing of plant material, as well as in certification schemes for stone fruit trees.

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**Incidence of aphid-borne viruses in cucurbit crops in Greece.** C.K. XANTHIS[1], V.I. MALIOGKA[1], H. LECOQ[2] and N.I. KATIS[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Lab of Plant Pathology, 54124 Thessaloniki, Greece; [2]INRA, Unite Pathol Vegetale, UR407, F-84140 Montfavet, France. E-mail: katis@agro.auth.gr*

Cucurbit viral diseases have been associated with high economic losses worldwide, but only limited information on their incidence is currently available in Greece. During 2012 and 2013, surveys were carried out in open field cucurbit crops of 19 prefectures in Greece. Eight hundred and eighty samples were collected showing typical virus-like symptoms such as mosaic, mottling, leaf yellowing, deformations and blistering, fruit discolorarion and deformation. Samples were analyzed by double-antibody sandwich (DAS)-ELISA and reverse-transcription polymerase chain reaction (RT-PCR) against *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV-W), *Moroccan watermelon mosaic virus* (MWMV) transmitted non-persistently and *Cucurbit aphid borne-borne yellows virus* (CABYV)

transmitted in a persistent, circulative non-propagative manner. In zucchini, watermelon and melon samples WMV was the most common virus, present in, respectively, 64%, 61% and 82% of the samples from these plants. In zucchini crops during both growing seasons the incidences of WMV, MWMV, CABYV, CMV, PRSV and ZYMV were, respectively, 64%, 41%, 38%, 19%, 4% and 0.4% respectively. MWMV was only detected in South-Western Greece in zucchini and watermelon crops. Percentages for the more frequently detected viruses in zucchini crops in the two prefectures (South-Western Greece) where MWMV was first reported, were, respectively, 68%, 43%, 55%, 12%, 7% and 0.4% for MWMV, WMV, CABYV, CMV, PRSV and ZYMV. In watermelon plants the percentage of MWMV was less than in zucchini (12%), and the virus was not detected in melon and cucumber plants. Multiple infections were also observed for zucchini, with the most frequent combination being MWMV + CABYV (27%) followed by MWMV + WMV (13%) and MWMV + PRSV (2%). Also, 128 weed samples, belonging to nine families, were collected from within MWMV-affected zucchini fields, and these were tested by RT-PCR for the presence of MWMV. Only the *Amaranthus retroflexus, Chenopodium album, Conyza bonariensis* and *Malva sylvestris* were found to be infected.

**Cucurbit chlorotic yellows virus: a new serious pathogen of cucurbit crops in Greece.** C.G. ORFANIDOU, V.I. MALIOGKA and N.I. KATIS. *Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Lab of Plant pathology, 54124 Thessaloniki, Greece. E-mail: vmaliogk@agro.auth.gr*

In 2011, an outbreak of a yellowing disease was observed in greenhouse cucumber (*Cucumis sativus*) and melon (*C. melo*) crops, and in 2012 in open field watermelon (*Citrullus lanatus*) crops, in the island of Rhodes. Similar symptoms were observed in November 2013 in a greenhouse cucumber crop in Crete (Tympaki). Disease incidence ranged from 10-40% in 2011, while in the next two years incidence ranged from 90–100%. Symptoms were similar to those caused by the whitefly transmitted criniviruses (family Closteroviridae) *Cucurbit yellow stunting disorder virus* (CYSDV), *Beet pseudo-yellows virus* (BPYV) and *Cucurbit chlorotic yellows virus* (CCYV), and by the aphid transmitted polerovirus (family Luteoviridae) *Cucurbit aphid-borne yellows virus* (CABYV). In all affected crops, dense populations of whiteflies were observed, whereas aphid colonies were very rare. Leaf samples from cucumber (from Rhodes and Tympaki), melon and watermelon were collected and tested for the presence of the above viruses using RT-PCR methods. CCYV was detected in all the samples tested including cucumber, melon and watermelon plants, while CYSDV was detected in 59% of the samples, with most infections occuring in cucumber. CABYV was detected in low incidence in watermelon in mixed infections with CYSDV and CCYV, whereas BPYV was not detected in any sample. To verify CCYV presence, the partial HSP70h and RdRp regions of a cucumber isolate from Tympaki and a watermelon isolate from Rhodes were directly sequenced, revealing 99–100% identity with CCYV isolates from Lebanon. CCYV was successfully transmitted by *Bemisia tabaci* biotype Q in six out of eight inoculated cucumbers, and infected plants started showing interveinal yellowing 2 weeks post inoculation. Although CYSDV, BPYV and CABYV were previously known to induce yellowing symptoms in cucurbits in Greece, our findings highlight CCYV as a new serious threat of these crops in the country.

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**Investigations on the epidemiology and control of olive anthracnose in Apulia, Southern Italy.** F. NIGRO[1], I. ANTELMI[1], I. PENTIMONE[1], M. FERRARA[2] and A. IPPOLITO[1]. *[1]Università degli Studi di Bari - Aldo Moro, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy; [2]Consiglio Nazionale delle Ricerche (CNR), Istituto di Scienze delle Produzioni Alimentari (ISPA), Bari, Italy. E-mail: franco.nigro@uniba.it*

Anthracnose has long been recognized as one of the most important and severe olive diseases in the Mediterranean basin, mainly in Portugal, Italy, Spain, and Greece. The causal agents are *Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc. and *C. acutatum* species complex, although previous studies had identified *C. gloeosporioides* (originally known as *Gloeosporium olivarum*) as the primary incitant. More recent investigations have shown that groups in the *C. acutatum* complex can be erected as distinct species, and prevail in areas where anthracnose epidemics occur. The severe outbreaks of olive anthracnose observed in Apulia during recent years, caused by the occurrence of climatic conditions conducive to the disease, the reduction of cultural interventions, and new features about the aetiology and the disease cycle, have been investigated, focusing on some epidemiological and control aspects. Data on the epidemiology of *Colletotrichum* spp. on drupes showed that the incidence of latent fruit infections can reach 25-30% in September. Field trials on the efficacy of QoI-based fungicides applied at different timing (new shoot growth, pre-blooming, fruit set, veraison) indicated good efficacy of the mixture trifloxystrobin + tebuconazole in reducing the disease. This fungicide mixture significantly reduced the inoculum density of *Colletotrichum* spp. on fruit surfaces and the incidence of latent

infections, as compared to the traditional copper applications. The new information on the etiology, disease cycle, and latency of anthracnose infections raises substantial questions about the traditional chemical control practices, with reference to both the active ingredients and the timing of fungicide applications. Protectant fungicides can be ineffective against pathogens that are able to survive within fruits or as mycelia under the epidermis. Application of systemic fungicides has proved effective in field trials, and spring sprays contribute to reducing the inoculum density for autumn infection.

**The prevalence of leaf rust in the wheat cultivated area in Sakarya province of Turkey, the reactions of some lines, and relationships with climate.** V. URİN[1] and N. ÖZER[2]. [1]*Maize Research Station, Department of Plant Protection, Sakarya, Turkey;* [2]*Namik Kemal University, Faculty of Agriculture, Department of Plant Protection, Tekirdağ, Turkey. E-mail: vesurin@hotmail.com*

Wheat is one of the most important grains cultivated in Turkey. Leaf rust, caused by *Puccinia recondita tritici*, restricts wheat yields, and causes important losses especially along coastline when climate conditions are suitable for disease development. The disease is very common every tear in Sakarya province every year because the climate favours the disease development. The most preferred method for controlling the disease is to improve resistant genotypes. The purpose of this research was to determine the distribution of the leaf rust of wheat, to detect the reactions of some cultivars and lines to the disease, and to determine relationships between the disease and climate parameters in Sakarya province for two years. A survey was carried out in at least 0.5% of wheat cultivated area in Sakarya. Nine cultivars and forty lines in a bread wheat improvement programme, administered by Maize Research Station in Sakarya, were scored using the Cobb scale for the leaf rust reaction type, and a 2-1000 scale for the disease severity under natural infection conditions. In addition, four cultivars and six lines were observed under natural conditions, and climate parameters were measured at weekly intervals, to determine the correlations between disease severity and climate. In survey carried out in 2010 and 2011, it was determined that the prevalence of leaf rust was 39% and the severity of the disease was 12%. The cultivars Karatopak, Tahirova-2000, and lines 7 and 10 at the wheat were resistant to the disease. The sensitive cultivars Beşköprü, Hanli and Line 9 were highly infected by the pathogen at the beginning of the disease development. Correlations between climate and disease severity using the mean of weekly temperature and humidity values were evaluated and regression models were developed for Sakarya province. Leaf rust severity was positively correlated with temperature, but was negatively correlated with humidity. The correlation between minimum temperature and disease severity for some cultivars and lines were statistically significant.

**Occurrence and spread of aphid-transmitted viruses in honeycomb breeding experimentation in lentil (*Lens culinaris* L.).** E.K. CHATZIVASSILIOU[1], E. NINOU[2], V. DIMITRAKAS[1], C. PANKOU[2], A. LITHOURGIDIS[3] and I. TOKATLIDIS[2]. [1]*Agricultural University of Athens, Department of Plant Science, Plant Pathology Laboratory, Athens, Greece;* [2]*Democritus University of Thrace, Department of Agricultural Development, Orestiada, Greece;* [3]*Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Greece. E-mail: echatz@aua.gr*

"Honeycomb breeding" is an innovative method which evaluates individually grown plants under ultra-low densities. Increased plant spacing favours aphid landings in the field. The viruses they transmit represent major pathogens for lentil (*Lens culinaris* L.). The aim of this study was to record and analyze the presence and spread of the aphid-transmitted viruses among evaluated and selected lentil plants in a honeycomb experimental design. In a replicated trial established in the farm of the Aristotle University of Thessaloniki (Greece), the lentil landrace "Elassona" originating from central Greece (Thessaly province) was evaluated for three successive years (2012–2014). Ultra-spaced individual plants (80 × 80 cm apart) have been surveyed biweekly from March to April for symptoms suggestive of virus infection. Randomly selected diseased plants among were analyzed in ELISA tests for the presence of the viruses known to occur in the country: the persistently transmitted *Bean leafroll virus* (BLRV, *Luteovirus, Luteoviridae*) and *Pea enation mosaic virus-1* (PEMV-1, *Enamovirus, Luteoviridae*), as well as the seed and non-persistenlty transmitted *Alfalfa mosaic virus* (AMV, *Alfamovirus, Bromoviridae*), *Bean yellow mosaic virus* (BYMV, *Luteovirus, Luteoviridae*), *Cucumber mosaic virus* (CMV, *Cucumovirus, Bromoviridae*) and *Pea seed-borne mosaic virus* (PSbMV, *Potyvirus, Potyviridae*). In 2012, 63.5% out of 2,395 plants showed disease symptoms, among which 24% were tested positive for BLRV, 12.3% for CMV, 0.8% for PEMV and 0.3% for AMV. In 2013, disease symptoms were recorded on 77.8% of 2,170 plants; 22.7% of them were infected by PEMV, 19% by BLRV, 16.9% by CMV and 7.1% by AMV. In 26.1% of the plants in 2012, and 15.7% in 2013, none of these viruses were. A major increase in the number of diseased plants occurred in May of both years, which was associated with rising temperatures. Experimentation in progress during 2014 shows a similar trend. In both 2012 and 2013 no virus was detected in the plants selected for high yield. The spatial spread of the diseased plants appeared to be uniform within the field and the breeding material. The implications of our results for breeding efficiency are discussed.

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**Virus diseases of grapevine plants grown in the east and southeast regions of Turkey.** O. ÇİFTÇİ[1], Ç. ULUBAŞ SERÇE[2] and B. GÜLER[1]. [1]*Plant Protection Research Station, Diyarbakir, Turkey;* [2]*Niğde University, Agricultural Sciences and Technologies Faculty, Niğde, Turkey. E-mail: culubas@gmail.com*

Turkey is one of the important countries for viticulture as the center of the grapevine gene pool covering Middle Asia-Mediterranean zone. Grape production in Turkey was 6.4% of world production in 2012, and 22% of this production was from The East and Southeast Regions of Turkey. Field surveys were carried out in grapevine growing areas of these two regions, to assess the phytosanitary status of commercial orchards during spring and autumn of 2013. Total of 222 samples (45 from Adiyaman, 25 from Diyarbakir, 52 from Malatya, 100 from Mardin provinces) in were collected in spring from grapevines showing virus symptoms. Another survey was performed in autumn, and total of 146 samples (two from Batman, 55 from Diyarbakir, 69 from Elaziğ, 15 from Mardin, five from Şanliurfa provinces) were collected. Most of the samples were from local varieties such as Ağbesni, Islahiye Karasi, Kabarcik, Boğazkere, Şire, Ağin, Öküzgözü, Köhnü, Mazruni, Tahannebi, Trakya and Ilkeren. All samples were tested by ELISA for the presence of *Grapevine leafroll associated virus-1* (GLRaV-1), *Grapevine leafroll associated virus-2* (GLRaV-2), *Grapevine leafroll associated virus-3* (GLRaV-3), *Grapevine leafroll associated virus-4* (GLRaV-4), *Grapevine leafroll associated virus-5* (GLRaV-5), *Grapevine leafroll associated virus-6* (GLRaV-6), *Grapevine leafroll associated virus-7* (GLRaV-7), *Grapevine leafroll associated virus-9* (GLRaV-9), *Grapevine fanleaf nepovirus* (GFLV), *Grapevine fleck virus* (GFkV), *Grapevine virus A* (GVA), *Raspberry ringspot nepovirus* (RpRSV), *Strawberry latent ringspot nepovirus* (SLRSV), *Tomato black ring nepovirus* (TBRV), *Arabis mosaic virus* (ArMV) and also by PCR for *Grapevine red blotch associated virus* (GRBaV). The overall infection level in all samples was 18.02%. The most commonly detected virus was GFLV (10.81%), followed by GFkV (5.85%) and the mixed infection of GFLV + GFkV (2.70%) in the spring samples. GLRaV 4-9 (9.59%), GLRaV 1 and 3 (2.75%) and mixed infections with GLRaV 1 + 3 and GLRaV 4-9 (0.68%) were detected among the samples collected in autumn. GVA, RpRSV, SLRSV, TBRV, ArMV, GLRaV-2 and GRBaV were not detected. Our results revealed deteriorated phytosanitary status for the local grapevine varieties.

**Survey of bacterial diseases on stone fruits in Lebanon.** P. MOUBARAK[1,3], C. SEBAALY[2], F. VALENTINI[1], A.M. D'ONGHIA[1] and L. VARVARO[3]. [1]*Istituto Agronomico Mediterraneo di Bari (CIHEAM), Dipartimento di Protezione Integrata delle Colture Orto-Frutticole Mediterranee, Via Ceglie 9, 70010 Valenzano (BA), Italy;* [2]*Lebanese Agricultural Research Institute (LARI), Fanar, P.O. Box 90-165, Jdeidet-El-Metn, Lebanon;* [3]*Università degli Studi della Tuscia, Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia (DAFNE), 01100 Viterbo (VT), Italy. E-mail: moubarak.p@gmail.com*

Many bacterial diseases, caused mainly by *Pseudomonas* spp., *Xanthomonas* spp. and *Agrobacterium* spp., attack stone fruits throughout the world. The high economic losses and the lack of efficient chemical controls for these diseases create a need for alternative control methods, including approaches based on prevention, biological control and plant resistance. These approaches require accurate knowledge of the genetics, ecology and pathogenicity of the causal agents. In Lebanon, stone fruits cover 10% of the total cultivated land, and are considered of high economic importance. Since research on stone fruit bacterial diseases were limited in this country, a survey was conducted between April and June 2013, as a start for further studies. A total of 303 samples from peach, plum, apricot, cherry, almond and nectarine were collected from the most important agricultural areas, and many bacterial isolates were obtained. These were subjected to morphological, physiological and biochemical tests; the bacteria were first cultured on several semi-selective media, then submitted to gram tests, LOPAT and GATTa tests for identification and characterization. Results were confirmed by molecular tools, using specific primers and rep-PCR. *Pseudomonas syringae* pv. *syringae* (*Pss*) and *P. syringae* pv. *morsprunorum* race 1 (*Psm* 1) causing bacterial canker, were the prevalent bacteria, that potentially constitute threats to stone fruit cultivation in Lebanon. We were able to identify and conserve 101 *Pss*, 31 *Psm* 1 and six unclassified *Pseudomonas* isolates. None of the other bacterial species pathogenic to stone fruits were isolated during this survey, a result which rebuts previous reports.

**Multiyear monitoring of *Botrytis cinerea* in treated vineyards.** P. CAMPIA, S.L. TOFFOLATTI, G. VENTURINI and A. VERCESI. *University of Milan, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, via Celoria 2, 20133 Milano, Italy. E-mail: paola.campia@unimi.it*

Grey mould, caused by *Botrytis cinerea* Pers., is a serious disease on several grapevine cultivars characterized by tight grapevine clusters. Fungicides are usually applied to control *B. cinerea*, but their repeated use in vineyards

can select resistant strains of the pathogen. İn northern Italy one/two treatments per year are carried out on the most susceptible varieties, particularly using anilinopyrimidine and SDHI fungicides. No data are currently available about the status of resistance of this pathogen Lombardy. The aim of this syudy was to monitor the sensitivity distribution of the pathogen strains to the botryticides most commonly used in vineyard over 3 years, and to determine possible correlations between the sensitivity levels and both the mating type and the presence of transposons *Boty* and *Flipper*. For three consecutive years (2011, 2012 and 2013), 20 monoconidial strains were isolated at harvest from symptomatic samples collected in three vineyards regularly treated against *B. cinerea*. The vineyards were located in the main viticultural provinces in Lombardy: Brescia, Pavia and Sondrio. The 180 isolated strains in were tested for sensitivity to boscalid and cyprodinil, and their mating types and the presence/absence of transposons were determined. The Resistance Factor, RF ($EC_{50}$ of the selected strain/average $EC_{50}$ of the untreated vineyard), was calculated for the strains showing high $EC_{50}$s. RFs > 10 indicate reduced sensitivity. The presence of transposable elements (*Boty* and *Flipper*) and *MAT1-1* and *MAT1-2* were detected using PCR specific primer pairs on all the strains after DNA extraction. The average $EC_{50}$ values observed for boscalid were similar to those calculated for *B. cinerea* in Europe, while higher values of this parameter were obtained for cyprodinil. A reduction in sensitivity has been assessed only in the vineyard treated for several years with anilinopyrimidines. The great majority of the strains were characterized by the presence of both transposons, while *MAT 1-1* and *MAT 1-2* were usually equally represented in the populations associated with each vineyard.

**A new method for determination of *Alternaria* mycotoxins alternariol, alternariol monomethyl ether and tentoxin in pomegranate fruits and juices, using a quechers-based extraction procedure and HPLC-DAD.** C. MYRESIOTIS[1], S. TESTEMPASIS[2], G. KARAOGLANIDIS[2] and E. PAPADOPOULOU-MOURKIDOU[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Pesticide Science Laboratory, P.O.Box 1678, 54124 Thessaloniki, Greece; [2]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, P.O.Box 269, 54124 Thessaloniki, Greece. E-mail: gkarao@agro.auth.gr*

A simple and accurate analytical method for the simultaneous detection of three *Alternaria* mycotoxins (alternariol, alternariol monomethyl ether, and tentoxin) in pomegranate fruits and juices was developed and validated. Mycotoxins were extracted from samples by acidified acetonitrile and water using a QuEChERS-based extraction method. High-performance liquid chromatography coupled with diode array detector was used for the analysis of the extracts. Extraction, chromatographic and detection parameters were optimized for increasing sensitivity and accuracy of the method. Chromatographic separation was achieved on a Hypersil BDS-*C18* analytical column with a gradient programme consisting of a mixture of water and acetonitrile containing 50 μl $L^{-1}$ trifluoroacetic acid at a constant flow rate of 1 mL $min^{-1}$. This method provided satisfactory validation parameters in terms of linearity, accuracy, precision, selectivity, limits of detection (LODs) and quantification (LOQs), requiring only 10 mL of organic solvent per sample. The linearity range was sufficient within the tested concentration range, with correlation coefficients higher than 0.9937 in all cases. Recovery studies were performed at five fortification levels (0.05, 0.1, 0.25, 0.5 and 1.0 μg $g^{-1}$ or μg $mL^{-1}$) in pomegranate fruits and juices in four replicates. The mean recoveries in pomegranate fruits ranged from 82.0% to 107.5% and between 96.3% and 109.4% in pomegranate juices. The uncertainty associated to the analytical method, expressed as RSD, was lower than 10.9% for all compounds. The limits of detection LODs were from 0.015 μg $g^{-1}$ (alternariol, alternariol monomethyl ether) to 0.02 μg $g^{-1}$ (tentoxin), while the LOQs were 0.05 μg $g^{-1}$ for alternariol and alternariol monomethyl ether, and 0.066 μg $g^{-1}$ for tentoxin. The optimized and validated method was applied to measure the presence of the target mycotoxins in real samples (ten fruits and six juices) from different lots commercialized in Northern Greece, but mycotoxins were not found in any tested samples. Also, artificially inoculated pomegranates with six different *Alternaria alternata/tenuissima* species group and *A. arborescens* isolates, obtained from pomegranate fruit, and known to produce the target mycotoxins in pure cultures, were analyzed. Alternariol concentrations determined were from 0.3 to 50.5 μg $g^{-1}$, alternariol monomethyl ether from 0.5 to 32.3 μg $g^{-1}$, while *tentoxin* was not detected. The proposed analytical method provides a basis for monitoring major *Alternaria* mycotoxins in pomegrtanates by analytical laboratories.

**Influence of root inoculations with vesicular arbuscular mycorrhizae and rhizomax for the control of dry and wet root rots of chickpea.** S. SHAKOOR, M. INAM-UL-HAQ and R. AHMED. *Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. E-mail: raees.agri@gmail.com*

Chickpea is one of the most important crops grown worldwide, including Pakistan. However, root diseases are one of the most important limiting factors in chickpea production. In Pakistan, chickpea crops are susceptible to various root pathogenic fungi including *Macrophomina phaseolina* (causing dry root rot) and *Rhizoctonia solani* (causing wet root rot). Considerable

evidence has accumulated in recent years to support and identify the benefits associated with the use of VA mycorrhizae in crop protection. In this study when different treatments of Rhizomyx and VAM were applied, it was observed that Rhizomyx and VAM produced significant control of the root pathogenic fungi, by minimizing the percent infection to minimum levels. *Glomus etunicatum*, *Glomus mosseae* and Rhizomax inoculation alone and in combinations significantly increased shoot length, shoot fresh weight, shoot dry weight, and root fresh weight in plants inoculated with *M. phaseolina* and *R. solani* over that in the uninoculated control plants. Application of most of the VAM species and Rhizomyx in different concentrations showed positive impacts on chickpea plant growth, by improving plant height, plant fresh weight and plant dry weight. Endophytes colonize the roots of plants similar to that of root pathogenic fungi, and biological control with endophytes offers an effective strategy for the management of chickpea root pathogenic fungi.

**Analysis of diversity in acid lime and Candidatus phytoplasma aurantifolia, the cause of acid lime witches broom.** A.M. AL-SADI, R.A. AL-YAHYAİ, S. AL-ABADİ, H. AL-MOQBALİ and I.H. AL-MAHMOOLİ. *Department of Crop Sciences, Sultan Qaboos University, Sultanate of Oman. E-mail: alsadi@squ.edu.om*

Acid lime (*Citrus aurantifolia*) is among the top four fruit crops in terms of production and area of cultivation in Oman. Witches' broom, which was first observed in Oman in the 1970s, is considered the most serious disease of acid lime. The disease spread to other areas of the country, and was reported in the UAE, Iran, India and Saudi Arabia. Area cultivated with lime trees in Oman is currently 50% of that in 1990 and the disease killed over half a million lime trees. The symptoms are characterized by clustering of leaves, which become small and light green to yellow, affected trees are usually killed within 5–8 years of symptom appearance. Genetic analysis provided evidence that the low level of genetic diversity of acid lime in Oman and frequent movement of planting material across districts are the main factors which contributed to the spread and high susceptibility of acid limes to the disease. Phylogenetic analysis of Candidatus Phytoplasma aurantifolia, based on 16S-23S rDNA, imp and secA genes, showed that phytoplasma isolates from Oman, Iran and the UAE clustered in different groups. At least five phytoplasma isolates from Oman, the UAE and Iran were identical to each other based on this analysis. This study provides evidence that phytoplasma in the three countries could have the same origin. However, phytoplasma populations in the three countries differ in their level of genetic diversity. The implications of these findings on the management of witches' broom of lime are discussed.

**Assessment of antifungal activity of saponins from *Medicago sativa* against *Fusarium verticillioides*.** C. LANZANOVA[1], A. TORRI[1], A. TAVA[2], D. CARMINATI[2], S. LOCATELLI[1], E. LUPOTTO[3], and C. BALCONI[1]. *[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di Ricerca per la Maiscoltura (CRA-MAC), Bergamo, Italy; [2]Consiglio per la Ricerca e la sperimentazione in Agricoltura, Centro di Ricerca per le produzioni Foraggere e Lattiero Casearie (CRA-FLC), Lodi, Italy; [3]Consiglio per la Ricerca e la sperimentazione in Agricoltura – Dipartimento di Biologia e Produzioni Vegetali, CRA-DPV, Roma, Italy. E-mail: chiara.lanzanova@entecra.it*

Among the secondary metabolites associated with plant defenses, saponins play a significant role. These compounds are triterpene glycosides present in several plant species such as *Leguminosae*, in which they are reported to be involved in plant defence mechanisms due to their antimicrobial, insecticide, nematocide and allelopathic activities. The *Leguminosae* have been extensively investigated for their saponin content and within this family of plants, the *Medicago* genus represents a particularly rich source of bioactive saponins. The antifungal activity of the saponins was investigated on a large spectrum of human and plant pathogenic fungi Saponin biological properties make these natural substances interesting compounds as antimicrobial and bio-control agents against crop pathogens and pests. Fungi of the genus *Fusarium* are widely distributed pathogens of maize, causing diseases on seedlings, roots, stalks, ears and kernels. In addition, *Fusarium* spp. can also affect grain quality, producing mycotoxins, including fumonisins produced by *F. verticillioides*. Mycotoxin contamination in maize grain is a global threat to safety of human and animal food. In this research, purified saponin mixtures from alfalfa leaves and roots have been tested against *F. verticillioides* in *in vitro* bioassays. Different amounts (2 mg, 1 mg, 500 μg and 250 μg) of saponins (leaf and root), were uniformly distributed on the surface of 5 mL potato dextrose agar) in Petri dishes, and inoculated with 100 spores of strain #294 *F. verticillioides*; colony diameters were evaluated from 2 to 7 d after inoculation. As controls, *Fusarium* (CRTL) and 50% ethanol (saponins dissolving solution) treated plates were used. After 7 d, fumonisins were extracted from the mycelium and quantified by ELISA test. The percentage of inhibition was calculated with respect to 50% ethanol. The experiment showed how saponins extracted from different *Medicago* plant tissues inhibited the growth of *F. verticillioides* and fumonisin production. Preliminary results suggest that saponins could be valuable new tools in the control of mycotoxigenic fungal pathogens of maize.

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***Weissella confusa* and *Lactococcus lactis* show high antifungal activity against fumonisin producing *Aspergillus niger* isolated from dried fig.** C. DASKAYA-DİKMEN and D. HEPERKAN. *Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Istanbul, Turkey. E-mail: daskayac@itu.edu.tr*

*Weissella confusa* and *Lactococcus lactis* are commonly found in fermented foods and beverages. *Weissella confusa* produces a bacteriocin-like substance and shows antifungal activity. *Lactobacillus lactis* is a highly efficient probiotic organism. Moreover, *L. lactis* produces most known bacteriocins, such as nisin, lacticin, and lactococcin, and also shows antifungal activity. This study investigated the antifungal effect of *W. confusa* and *Lc. lactis* isolated from boza against fumonisin producing *Aspergillus niger* isolated from dried fig. The antifungal activity of lactic acid bacteria (LAB) cultures was determined by agar overlay assay. Depending on the diameter of inhibition zones of mould growth, the LAB cultures were assessed as antifungal cultures. In order to determine the antifungal activity of the LAB metabolites, supernatants were screened using the mould agar spot and microdilution broth technique. The antifungal activity of supernatants was determined by measuring the diameter of the mould growth in mould the agar spot tests and measuring optical density in the microdilution broth assays. Both LAB cultures gave large inhibition zones (12–15 mm) for the *A. niger* isolate. The metabolites of both LAB cultures caused complete inhibition of mould growth. The supernatants showed highly inhibitory effects (89.9%–90.4%) against *A. niger.* As a consequence, *W. confusa* and *L. lactis* isolates can be used as possible antifungal agents against *A. Niger.*

**The canadair project: maize/*Fusarium* interaction and ear rot resistance.** C. BALCONI[1], C. LANZANOVA[1], A. TORRI[1], H. HARTINGS[1], S. LOCATELLI[1] and E. LUPOTTO[2]. [1]*Consiglio per la Ricerca e la sperimentazione in Agricoltura, Unità di Ricerca per la Maiscoltura (CRA-MAC), Bergamo, Italy;* [2]*Consiglio per la Ricerca e la sperimentazione in Agricoltura – Dipartimento di Biologia e Produzioni Vegetali, CRA-DPV, Roma, Italy. E-mail: carlotta.balconi@entecra.it*

CRA-MAC recently focused research activity on the identification of genetic and molecular bases of maize resistance to *Fusarium verticillioides* through i) artificial inoculation screening of germplasm of local varieties, inbred lines from local breeding programmes or commercial hybrids and ii) implementation of microarray experiments. Four inbred lines, showing differential patterns of susceptibility/tolerance, have been used in transcriptome analyses. The Agri-Food Canada, Eastern Cereal and Oilseed Research Centre (ECORC-Ottawa) research Group of Linda Harris is involved in the investigation of transcriptional changes taking place during the maize/ *Fusarium graminearum* interaction. The Canadian group carried out transcriptional analyses of resistant and susceptible maize genotypes, analysing transcriptional changes in kernel tissues. In addition, the ECORC group has developed a recombinant inbred line (RIL) population (F6) of >400 lines derived from CO441 (resistant) × B73 (susceptible), segregating for resistance to *F. graminearum*. Within the framework of the CANADAIR project, a bilateral collaboration between CRA-MAC and ECORC, which will allow complementation and integration of transcriptome data, and the genes that are regulated during maize response both to *F. graminearum* and *F. verticillioides* will be identified. Commonly regulated genes could act as functional markers of resistance in both diseases. The tests performed on maize lines will allow the identification of genetic materials with affordable resistance to both pathogens. This collaboration will see the exchange of data and materials. Microarray results will be shared in order to highlight the most repeatable and therefore affordable data. During 2012 and 2013 seasons, the most *F. verticillioides*-resistant and susceptible lines identified by CRA-MAC were tested for *F. graminearum* resistance by the ECORC group; similarly, the most resistant and susceptible RILs identified by ECORC were provided to CRA-MAC and tested through field artificial inoculation for resistance to *F. verticillioides.* Preliminary results of the extension of the mycelium on different (resistant and susceptible) ears and the accumulation of mycotoxins (fumonisin) produced by the pathogen will be presented.

This research is developed in the frame of CANADAIR Project, Ministero delle Politiche Agricole Alimentari e Forestali (MiPAAF) coordinate by Dr. E. Lupotto (CRA-DPV).

**A study of antifungal activity of some plant extracts against the barley fungal pathogen *Pyrenophora teres*.** K. TAIBI[1], F. BENTATA[2], M. LABHILILI [2], F.E. EL ALAOUI FARIS[1], J. IBIJBIJEN[3] and A. EL AISSAMI[1]. [1]*Laboratory of Botany, Mycology and Environment,, Faculty of Sciences, Mohammed V-Agdal, University, B.P. 1014. Avenue Ibn Battouta B, Rabat, Morocco;* [2]*Biotechnology and Breeding, Conservation and Valorisation of Phytogenetic. Resources Unit, Laboratory of Phytopathology, CRRAR, INRA, B.P 6356, Avenue Mohamed Belarbi Alaoui Rabat, Morocco;* [3]*Laboratory of environment and soil microbiology, Faculty of Sciences, Méknès. E-mail: bentataiav@yahoo.fr*

Net blotch, caused by *Pyrenophora teres* f. sp. *teres*, is one of the main diseases of barley (*Hordeum vulgare L.*). This cereal is agronomically and socio-economically important in Morocco. The most used control strategy against the disease is chemical treatments. Increasing evidence of fungicide-resistant pathogenic fungal species is obvi-

ous. Looking for new possibilities of antifungal treatments or sources of antifungal substances is a activity. Some medicinal plants exert strong antifungal properties and could be conveniently used as alternative sources of antifungal treatments in many areas. In this study, antifungal activities of *Oxalis pes-caprae, Allium roseum*, *Allium sativum* and *Daphne gnidium* were investigated. Potato dextrose agar dishes amended with 20 g $L^{-1}$ of plant aqueous extracts, were tested against two Moroccan isolates of *P. teres*. There was no antifungal activity from the *O.pes-caprae* and the *A. roseum* extracts against the *P. teres* isolates. In contrast, the *A. sativum* and *D. gnidium* extracts showed significant antifungal activity, with inhibition rates greater than 70%. These results provide promising base line information for the potential use of medicinal plants with antifungal activity for the treatment of net blotch of barley. Promising plant species have been identified for further investigation.

**Determination of resistance of some sunflower genotypes to downy mildew, utilizing artifical inoculation.** T.H. ÇİFTÇİGİL[1], G. EVCİ[1], V. PEKCAN[1], M. İbrahim YILMAZ[1] and Y. KAYA[2]. *[1]Trakya Agricultural Research Institute, Edirne, Turkey; [2]Trakya University Plant Breeding Research Center, Balkan Campus, 22100, Edirne, Turkey E-mail: hilalciftcigil@gmail.com*

Downy mildew of sunflower is caused by *Plasmopara halstedii* (Farl.) Berlese et de Toni, an oomycete with high virulence. The fungus is soilborne, airborne and seedborne. Soilborne and seedborne spores cause systemic infections, airborne spores causes secondary infections. Systemic infections can occur when plants are infected at seedling stages, and secondary infections can occur when zoospores are blown by wind from infected plants to the leaves of healthy plants. The pathogen is aggressive and has great potential to develop new races. To date, 14 races of *P. halsedii* have been identified in Europe, and more than 35race identified worldwide. When environmental conditions, primarily humidity and temperature, are suitable for infection, epidemics of downy mildew occur. During the springs of 2007, 2008, and 2011 serious outbreaks of downy mildew occurred on sunflower plants grown in commercial fields in Trakya, a part of Marmara region of Turkey. Disease contol measures can be divided into three types, agrotechnical, chemical and breeding for resistance. Due to adverse effects of pesticides on the environment and humans, using new and appropriate plant protection control methods in the field has increased the importance of nature and planting downy mildew-resistant hybrids. Although genetically resistant hybrid cultivars have been growing in Europe for many years, they have just started to be grown in Turkey. In this study, leaf samples were collected from mildew infested plants in different areas representing the whole Trakya region. A mixture of all different races was constituted and these leaf samples were dried for 24–48 h, and were then stored at -80 ºC. Spore solutions were prepared from these stored plant samples. As an experimental control in the experiment, host lines 6973-R, 2517-A, 6626-A, 9661-A were used as a sensitive varieties. F4, F5, and F6 generations of restorer were tested in this research, and 22 genotypes were observed for reaction to the disease. These resistant genotypes of restorer breeding programme of TTAE will be ultilized to develop new resistant hybrid cultivars.

***Phytophthora cinnamomi* associated with ink disease of european chestnut in Greece.** G.T. TZIROS and S. DIAMANDIS. *Hellenic Agricultural Organization, Forest Research Institute, 57006, Vassilika, Thessaloniki, Greece. E-mail: gtziros@yahoo.gr*

Ink disease of European chestnut (*Castanea sativa* Mill.) represents one of the major threats for chestnut orchards and coppice forests in Greece, especially since introduced hypovirulence of *Cryphonectria parasitica* on a nationwide scale has been successful in limiting the chestnut blight. In Greece, ink disease has previously been associated with three *Phytophthora* species, *P. cambivora*, which is the prevailing species in chestnut orchards and natural stands, as well as two satellite species, *P. citricola* and *P. cryptogea*. In 2012, approximately 20% of 8- to 10-year-old chestnut trees in three orchards in Northern Greece showed disease symptoms, including decline of the crowns, dead leaves and burrs which remained attached to the trees over the winter and necrosis with flame-shaped margins of the inner bark below the collars. In some cases there was wilting which was followed by progressive or rapid death of the diseased trees. A *Phytophthora* sp. was consistently isolated from root and soil samples and identified as *P. cinnamomi* on the basis of morphology and ITS sequencing. All the isolates examined produced ovoid to ellipsoid, persistent, nonpapillate sporangia, were heterothallic, of mating type A2 and produced plerotic oospores and amphigynous antheridia. Pathogenicity of *P. cinnamomi* isolates was tested by a soil infestation method using 2-year-old seedlings. Five weeks after inoculation, all inoculated seedlings, but none of the controls, showed wilting, collar and root rot. *Phytophthora cinnamomi* was re-isolated, fulfilling Koch's postulates. This is the first time that *P. cinnamomi* has been found as the causal agent of ink disease in Greece. We believe that *P. cinnamomi* was introduced into the chestnut orchards via transport of infected nursery stock, confirming the importance of the nursery pathway in the spread of aggressive, invasive plant pathogens. This pathogen constitutes a potential major threat to *C. sativa* in Greece,

and as it has a wide range of hosts, could be a potential threat to horticultural, ornamental and forestry plant species that are of economic and aesthetic importance.

***Plum pox virus* strain M is prevalent in stone fruit trees in Greece.** A. DIMITRIADOU, V.I. MALIOGKA and N.I. KATIS. *Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Lab of Plant Pathology, 54124 Thessaloniki, Greece. E-mail: vmaliogk@agro.auth.gr*

*Plum pox virus* (PPV) is the causal agent of sharka, one of the most devastating diseases of *Prunus* sp. PPV shows considerable genetic diversity, as to date nine virus strains have been characterised: M, D, Rec, EA, C, SoC, T, An and CR. In Greece, the virus was first recorded in 1967, and subsequently it has been established in *Prunus* orchards throughout the country. Though its presence has been known for more than 40 years, there have been no studies on virus diversity in Greece. During the spring and summer of 2013, a total of 460 samples were surveyed from diverse geographical areas of Greece and from different *Prunus* species, namely peach, apricot, plum, cherry, almond and *Prunus maliformis*. Samples originated from trees showing typical PPV infection symptoms and from asymptomatic cherry and almond trees. Initially, the virus was detected with a one tube RT-PCR reaction using primers targeting the CP region of PPV. All positive samples were further tested with two step RT PCR protocols using strain specific primers corresponding to the M, D, Rec and T strains. Overall, 238 samples were found positive for PPV. None of the cherry (0/100) or almond (0/122) trees were infected. The majority of the positive samples (235/238) belong to the PPV-M strain, whereas three isolates were typed as PPV-D and were geographically confined, as they originated from the same plant collection located in a public research institute in northern Greece (Pomology Institute, Naoussa, Greece). This survey demonstrate the prevalence of the PPV-M strain in samples collected from orchards and single trees across the country. Further research is currently underway to enhance our knowledge of the virus diversity in Greece. A. Dimitriadou was financed by the Act "Scholarships programme SSF (State Scholarships Foundation/IKY)" from resources of the Operational Programme "Education and Lifelong Learning", of the European Social Fund (ESF) of the NSRF, 2007–2013.

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***Trichoderma* seed treatment can induce resistance to *Fusarium verticillioides* in maize.** S. GALLETTI[1], R. PARIS[1], S. CIANCHETTA[1], P.L. BURZI[1] and E. LUPOTTO[2]. *[1]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per le Colture Industriali, Bologna, Italy; [2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Dipartimento di Biologia e Produzione Vegetale, Roma, Italy. E-mail: stefania.galletti@entecra.it*

The possibility to biologically control toxigenic pathogens is currently under investigation in the frame of the CANADAIR project, funded during 2012–2014 by the Italian Ministry for Agricultural and Forestry Politics to sustain the exchanges between Italian and Canadian researchers. A wide collection of wild-type fungal isolates belonging to *Trichoderma* has been screened for antagonism against *Fusarium verticillioides*. This fungus can endophytically infect maize without visible symptoms, but can also cause seedling blight and root, stalk, kernel or ear rot. At any stage, this fungus can synthesize fumonisins, a family of polyketide-derived micotoxins, considered carcinogenic for both animals and humans. Eleven isolates, out of 238 screened, were capable both to inhibit by 60% the colony growth and the spore production of the pathogen *in vitro*, so they were also tested *in vivo* for the ability to colonize maize roots and antagonize the pathogen in the maize rhizosphere. Three isolates, out of 11, effectively protected maize roots, reducing the infection by 70%, in comparison with the untreated controls. These three isolates were also assayed for ability to induce systemic resistance in maize against *F. verticillioides*. The stems of young maize plants obtained from *Trichoderma* treated seeds was inoculated with the pathogen, then the leaves were sampled for expression analysis of genes involved in induced resistance mechanisms. One of the isolates clearly induced JA/ET-dependent responses, enhancing the expression of genes like *hpl*, while another isolate enhanced the expression of *pr1* and *pr5* genes, involved in SA-dependent responses. The effect of induced resistance was also verified by the quantification of pathogen DNA in the stem portion above the inoculation sites. Amount of pathogen DNA was less in the plants obtained from treated seeds than in the untreated plants. Species identification of these *Trichoderma* isolates is in progress in collaboration with researchers of Agriculture and Agri-Food Canada, Ottawa. These isolates can thus be considered promising candidates to be used as seed treatments in order both to reduce root infection and to induce systemic resistance in maize against *F. verticillioides*.

**Multiplex RT-PCR detection of three sweet potato potyviruses in portugal, and analysis of coat protein gene variability among isolates.** C.M.R. VARANDA, S. SANTOS, M.D.M. OLIVEIRA, M.I.E. CLARA and M.R. FÉLIX. *Laboratório de Virologia Vegetal, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade*

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High incidence (ca. 30%) of sweet potato plants exhibiting virus-like symptoms such as leaf distortion, mosaic, chlorosis and purple borders was observed in a field in southwest Portugal. Symptomatic plants were collected and tested for the presence of the four potyviruses *Sweet potato virus C* (SPVC), *Sweet potato virus 2* (SPV2), *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus G* (SPVG). DsRNA fractions were extracted from leaves and used as templates in multiplex Reverse transcriptase – Polymerase Chain Reaction (RT-PCR) assays, utilizing specific primers for each of those viruses. The final amplified reaction products were of the expected size for SPVC (836 bp), SPV2 (589 bp) and SPFMV (369 bp), and the assays revealed triple infections due to SPVC, SPFMV and SPV2 in 25% of the tested plants. No single or double infections were observed and no amplicon expected from SPVG infections was found. Direct sequencing of PCR products revealed they corresponded to the coat protein gene (CP), and further confirmed virus identity showing, respectively, 98%, 99% and 99% homology to SPVC, SPV2 and SPFMV. Comparison of the CP genomic and amino acid sequences of the viral isolates here recovered with that of ten other retrieved from the GenBank from isolates obtained in different countries, showed very low variability. Maximum nucleotide distance values were of 0.006 for SPV2, 0.036 for SPFMV and 0.039 for SPVC. The application of this multiplex RT-PCR assay revealed for the first time the presence of SPVC and SPFMV affecting the sweet potato in Portugal, as well as the common occurrence of triple virus infections of sweet potato under field conditions.

**Studies on vector transmission of Mediterranean *Citrus tristeza virus* isolates by *Aphis gossypii, A. spiraecola*, and *Toxoptera aurantii*, and molecular analysis of the coat protein gene generated by aphid passage.**
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*Citrus Tristeza Closterovirus* (CTV) is a globally distributed citrus pathogen that has caused losses of more than 100 million trees worldwide. This virus is naturally adapted to replicate in *Rutaceae* host cells causing three prominent syndromes, including quick-decline (QD), stem pitting (SP) and seedling yellows (SY). High dispersal of CTV populations through aphid species was responsible for several outbreaks within the Mediterranean area. Six CTV populations from Italy, Palestine, Croatia, Egypt and Albania, and associated with different biological activities, were subjected to experimental transmission trials through the insects *Aphis gossypii*, *A. spiraecola* and *Toxoptera aurantii*. Aiming of the studied isolates after aphid passage, The virus sources and their cognate sub-isolates were serologically tested by the specific monoclonal antibodies (Mab) MCA13, to highlight their pathogenic behavior. Molecular screening of gene variations were also based on Single Strand Conformation Polymorphism analysis, multiple molecular markers genotyping of the 5'ORF and coat protein CPg sequencing. One mild (MAIB_Q1294) and one severe (SG29) isolate from Italy showed T30 and SY genotypes, respectively, and were easily vector transmissible (>50%) by *A. gossypii* compared to the other isolates (7.5 to 18.5%). The Albanian MAIB_Q3 isolate from the T36 QD-inducing genotype was not transmitted by any of the vector species. The mild MAIB_Q1294 isolate generated only discernible stunting and vein clearing symptoms on CTV-infected Mexican lime sub-isolates, and did not recognize the MCA13 Mab after transmission. Cognates from the remaining isolates reacted positively with the MCA13, and showed moderate to severe stunting and pronounced leaf deformations. SSCP patterns yielded minor gene mutations on *p*18 compared to *p*20, *p*23 and *p*25 genomic regions. Unrandom Single Nucleotide Polymorphisms (SNP) exhibited on the CPg were followed with slight changes in the deduced amino-acid sequences when translated into proteins. Since the CPg is strongly involved in symptom expression and acts as a powerful gene silencing suppressor, minor alterations generated by aphid transmission may induce divergence on the pathogenecity of the parental CTV source.

**Incidence of Verticillium wilt of olive in Lebanon.**
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Verticillium wilt of olive (VWO) is a severe vascular disease caused by the soil-borne fungus *Verticillium dahliae* Kleb., which has been reported in almost all olive growing areas around the world. In Lebanon, olive is one of the most important tree crops, spread within the regions on 57,000 ha. In recent years, olive production has greatly increased, accompanied with intensification of the cropping management and illegitimate import

of low-quality olive cuttings. This has led to deterioration of the sanitary status of the crop and increased reports of tree death and VWO symptoms. To evaluate the spread of VWO in Lebanon, 132 olive orchards distributed on 19 districts over four regions, were visited in 2012, and samples of twigs and/or branches were collected from 303 trees. Isolation on PDA amended with streptomycin sulphate (0.5 g $L^{-1}$) revealed that the pathogen occurred in 38.6% of the visited orchards and the frequency of *V. dahliae*-infected trees was 25.7%. The highest disease incidence was in Bekaa Valley (35.3%), followed by South Lebanon (27.3%), North Lebanon (25.7%) and Mount Lebanon (5.0%). Furthermore, the pathogen was detected in 13.1% of the sampled young trees (less than 5 years old) and 25.0% of the centenarian trees. The most reliable diagnostic symptoms were the combination of defoliation, leaf roll and/or internal wood browning, where 54.0% of infected trees exhibited these symptoms. Simple lateral defoliation, the most frequently observed symptom, was mainly associated with insect attacks (24.6%), high incidence of peacock's eye disease (22.2%) and, to a lesser extent (18.1%), with the occurrence of *V. dahliae*. In addition, the pathogen was recovered from 7.7% of dead olive trees, and interestingly from 3.8% of apparently healthy trees. Both syndromes described in literature for VWO, slow decline and apoplexy, were also reported. The widespread occurrence of the pathogen in all olive growing areas of Lebanon, causing in some cases severe infections, makes VWO the main current threat for the Lebanese olive industry. Results obtained from this survey indicate that appropriate prophylaxis, reliable diagnostic tests, and procedures for the certification of *V. dahliae*-free planting material are crucial to limit the spread of the pathogen.

**Immergence and importance of septoria blight on durum wheat in Morocco.** F. BENTATA[1], M. LABHILILI [2], I. MAAFA[2], A. EL JAOUADI[1], F.E. EL ALAOUI FARIS[1], J. IBIJBIJEN [3], A. EL AISSAMI[1] and M. NACHIT [4]. [1]*Biotechnology and Breeding, Conservation and Valorisation of Phytogenetic. Resources Unit, Laboratory of Phytopathology, CRRAR, INRA, B.P 6356, Avenue Mohamed Belarbi Alaoui Rabat, Morocco;* [2]*Laboratory of Botany, Mycology and Environment, Faculty of Sciences, Mohammed V-Agdal University, B.P. 1014. Avenue Ibn Battouta B, Rabat, Morocco;* [3]*Laboratory of environment and soil microbiology, Faculty of Sciences, Méknès;* [4]*ICARDA: International Center of Agricultural Research in Dry Area, Morocco. E-mail: bentataiav@yahoo.fr*

In Morocco, the main fungal diseases that attack the cereals are Septoria leaf spot, tan spot, leaf rust, yellow rust and head blight. *Septoria tritici* previously attacked only bread wheat, until 2008–2009, which was favourable for development of diseases) also attacked durum wheat. The pathogen was present in 100% of the visited fields. The majority of the cultivated varieties are susceptible to the disease. Severity ranged from traces to 100%, and the prevalence was 84%, followed by yellow rust and leaf rust. Since then, study and screening of Septoria susceptibility has been carried out in the genetic improvement of durum wheat. Genetic diversity of the pathogen isolates collected on durum wheat has also been studied. In 2014, surveys in different agroclimatic areas and in the principal areas of production of cereals in Morocco, have shown that in 57 fields visited, Septoria has present, with severity ranging from traces to 80%. *Septoria* has become a regular problem in Morocco.

**The competence of seven aphid species (Hemiptera: aphididae) to transmit *Moroccan watermelon mosaic virus* (MWMV).** E.K. CHATZIVASSILIOU[1], P.D. MPENARDIS[1] and D.Ch. PERDIKIS[2]. [1]*Agricultural University of Athens, Department of Plant Science, Plant Pathology Laboratory, Athens, Greece;* [2]*Agricultural University of Athens, Department of Plant Science, Agricultural Zoology and Entomology Laboratory, Athens, Greece. E-mail: echatz@aua.gr*

*Moroccan watermelon mosaic virus* (MWMV; genus *Potyvirus,* family *Potyviridae*) is an emerging pathogen for cucurbits. In Greece (Peloponnese), this virus was identified in 2012 as the causal agent of severe decline of zucchini crops. Only a few insect species are reported to transmit MWMV, so we aimed to record the vector competence of seven species of Greek aphid fauna. Non-persistent transmission experiments and arena tests were performed using zucchini (*Cucurbita pepo* L.) cv. Amerigo F1 plants. In the non-persistent experiments, aphids were starved for 2 h and subsequently were given 3 min acquisition access on WMMV infected plants. Aphids were then deposited in groups of five or ten on each of ten test plants, for a 24 h inoculation period. The test was repeated five times. In the arena tests, the same number of aphids was deposited on an infected plant in between eight healthy receptor plants, inside an insect proof cage, for 24 h; this test was repeated four times. Myzus persicae (Sulzer) (one aphid/plant) was used as a control. Plants were tested for virus presence 3 weeks later using ELISA. To compare the transmission rates of the species tested, the frequency of transmission by a single aphid was calculated. In the non-persistent tests, six out of the seven tested species transmitted the virus at rates ranging from 6% to 94%. *Aphis spiraecola* Patch was the most efficient vector species, followed by *M. persicae* and A. gossypii Glover. *Macrosiphum rosae* (Linnaeus), *A. fabae* Scopoli and *Uroleucon sonchi* (Linnaeus) were recorded as new vectors of MWMV, but with lower transmission efficiency. No transmission was obtained by *Brevicoryne brassicae* (Linnaeus). In arena tests,

lower transmission rates were recorded (up to 50%). In these tests, M. persicae spread MWMV more efficiently than *A. spiraecola*, A. gossypii or *M. rosae*. The information obtained has improved understanding of MWMV epidemiology.

**Climatic variables and crop management practices influencing chocolate spot (*Botrytis fabae* and/or *Botrytis cinerea*) in Morocco faba bean fields.** I.E. BAZI[1,3], S. KRIMI BENCHEQROUN[2], F. GABOUN[1], R. ABDELWAHD[1], Z.E.A. TRIQUI[3], F. ABBAD ANDALOUSSI[1] and R. MENTAG[1]. *[1]National Institute of Agricultural Research (INRA), Biotechnology Unit, Rabat, Morocco; [2]National Institute of Agricultural Research (INRA), Plant pathology Unit, Settat, Morocco; [3]Mohamed V University, Faculty of Sciences, biology Department, Rabat, Morocco. E-mail: rachidmentag@yahoo.ca*

Faba bean (*Vicia faba*) is a major legume crop grown in Morocco. It is a multi-purpose crop that plays an important socio-economic role, and is also important for improving soil fertility and structure. Faba bean is commonly used in rotation with cereals. However, during recent decades, faba bean production has decreased due to several biotic stresses. Chocolate spot, caused by *Botrytis fabae* and/or *Botrytis cinerea* is one of the most yield-limiting constraints of faba bean. Different production practices and environmental conditions can influence disease occurrence, epidemic development and damage to crops. Field surveys were conducted during 2011 and 2013 cropping seasons to assess the incidence and severity of this disease in the most Moroccan faba bean productive regions, and to investigate the relationships of disease intensity with climatic variables and crop management practices. Disease incidence and severity varied among districts and cropping seasons. Mean disease incidence varied from 52 to 100% in most fields, while disease severity index ranged from 12 to 23%. High precipitation, high weed density, and high weed coverage made significant contributions ($P$<0.001) to incidence of the disease in farmer fields. Disease severity was more influenced by high weed density, and high weed coverage. Our results indicate that effective weed control is a major practice that can reduce the impact of chocolate spot in Morocco.

**Preliminary screening of some cucurbits cultivars for tolerance to the bacterial fruit blotch pathogen *Acidovorax citrulli* in Turkey.** N. USTUN and N. ARSLAN. *Plant Protection Research Station, Bornova, Izmir, Turkey. E-mail: nursen_ustun@yahoo.com*

Bacterial fruit blotch of cucurbits, caused by *Acidovorax citrulli*, is a devastating disease threatening the watermelon and melon industries all over the world. Use of resistant cultivars can be one of the most reliable approaches for the disease control. No complete resistance was found in commercial cucurbit cultivars up to now, but differences in the disease response and relative tolerance was reported in some melon cultivars or lines. The susceptibility of various commercial cultivars of cucurbits commonly grown in Turkey was evaluated using seedling-inoculation assays. Tested cultivars were Suha and Eyfel for squash, Aswan and Crimson Tide for watermelon, Golden West for melon, and Divan and Beith Alfa for cucumber. Seeds of the cultivars were sown in pots (two seeds per pot) containing a soil mixture of equal parts of perlite and peat. Emerging seedlings were grown in a controlled climate room (25–28ºC, 60–70% RH, 16 h daylight). Two-week-old seedlings were artificially inoculated by spraying with bacterial suspension of two melon strains of *A. citrulli* ($10^6$ CFU $L^{-1}$. Ten plants per cultivar were inoculated with each strain. Control plants from each cultivar were sprayed with sterile water. Following inoculation, plants were covered by plastic chambers to maintain 90–100% RH for 48 h. The chambers were then removed and plants were incubated at the 25–28°C and 70–80% RH for the subsequent 12 d after inoculation. Severity of the disease for the strains was scored using 0–5 scale (0 = no symptoms, 1 = necrotic lesions on ≈25% of leaves, 5 = necrotic lesions on ≈50% of leaves, 7 = necrotic lesions on ≈75% of leaves, and 9 = necrotic lesions on most leaves). Statistical analyses were performed using the Tukey–HSD test. All of the cultivars were susceptible to the disease. However, some differences in the severity of the disease symptoms were observed. The melon cultivar Golden West and squash cultivars Suha and Eyfel were significantly ($P$ = 0,05) more affected than the watermelon (Crimson Tide and Aswan) and cucumber (Beith Alfa and Divan) cultivars tested.

**Preliminary study on the emerging disease on Volkamer lemon (*Citrus volkameriana* Tan. & Pasq.) in Egypt.** K. DJELOUAH[1], M. KHATER[1,2]., A.M. ABDEL–SALAM[3], H. FAHMY[4], R. ABOU SERIE[5] and A.M. D'ONGHIA[1]. *[1]CIHEAM-Mediterranenean Agronomic Institute of Bari, Italy; [2]Agricultural Research Center, Central Laboratory for Agricultural Climate, Giza, Egypt; [3]University of Cairo, Faculty of Agriculture Department of Plant Virology, Giza, Egypt; [4]Ministry of Agriculture, Certification center of Bahteem, Cairo, Egypt; [5]Agriculture Research Center, Hortculture Research Instiute, Giza, Egypt. E-mail: djelouah@iamb.it*

Citrus, the main fruit crop in Egypt, is mainly grafted onto Volkamer lemon rootstock, which is tolerant to *Citrus tristeza virus* (CTV), and is suitable under desert conditions. During recent years, this rootstock has been affected by a new disease, displaying gumming in the bark and pitting in the wood of affected trees,

and causing serious economic consequences for growers and nurserymen. Symptoms of Volkamer lemon disease resemble those of Citrus cachexia on mandarin and mandarin-like species, the agent of which is the *Hop stunt viroid* (HSVd). A preliminary survey by visual observations was carried out in seven citrus plantings in four Egyptian Governorates, and this showed more than 25% of symptomatic trees in the investigated groves, but the main disease outbreaks were found in sweet orange/volkamer lemon plantings located in the desert cultivated area. A study to identify the putative causal agent of the emerging volkamer lemon disease was carried out using molecular assays for the detection primarily of HSVd and of other pathogens which may induce similar symptoms in citrus, including *Citrus exocortis viroid* (CEVd), *Citrus viroid III* (CVd III), *Citrus bent leaf viroid* (CBLVd), and *Hibiscus green spot virus* (HGSV). Results of laboratory assays indicated high HSVd infection (79%), followed by CEVd (33%), CBLVd (27%) and CVd-III (23%). All the detected viroids were also found in some asymptomatic trees. Although further investigations on the putative agent/s are necessary, the results of this work provide evidence of an alarming situation for citrus production in Egypt, considering that the use of Volkamer lemon, as a tristeza tolerant rootstock, may be compromised by the emerging and rapidly-spreading of Volkamer disease.

**Elimination of *Spiroplasma citri* from washington navel orange [*Citrus sinensis* (L.) Osb.] using somatic embryogenesis from undeveloped ovules of aborted seeds.** R. MOUJAHED[1], D. FRASHERI[1], K. DJELOUAH[1], A. CARRA[2], F. CARIMI [2] and A.M. D'ONGHIA[1]. [1]*Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) - Mediterranean Agronomic Institute, Via Ceglie 9, 70010 Valenzano (BA), Italy;* [2]*Institute of Plant Genetics/CNR, Research Division of Palermo, Corso Calatafimi 414, 90129 Palermo, Italy. E-mail: frasheri@iamb.it*

*Spiroplasma citri*, a phloem-restricted prokaryote, is the agent of the Citrus stubborn disease. It is a graft-transmissible pathogen which is also naturally transmitted by leafhopper vectors. Somatic embryogenesis from undeveloped ovules, which proved to be effective in the elimination of several infectious agents of citrus, has been applied in the elimination of this pathogen. Immature fruits were collected 6–8 weeks after anthesis from one infected source of Washington navel orange. After surface sterilization of the fruits, undeveloped ovules were excised without integuments and individually placed in Petri dishes containing MS semi-solid medium supplemented with BAP and sucrose. Explants were *in vitro* cultured in a growth chamber at 25 ± 1°C with 16h light photoperiod and subculturing was carried out every 3–4 weeks. Somatic embryos were derived directly from the undeveloped ovules without a callus induction phase, 1 to 3 months after culture initiation. Individual somatic embryos were germinated in hormone-free MS solid medium supplemented with sucrose. From all germinated embryos, 83.5% were able to develop high embryogenic lines percentage (100%). After 2–3 months the plantlets derived from different embryogenic events were *in vivo* acclimatized by minigrafting onto sour orange seedlings and maintained in greenhouse at 32°C. Results of PCR applied on 8- and 24-month-old regenerated plants showed no infection by *S. citri*.

**Effects of spray programmes in vineyards and different sun-drying systems on formation of ochratoxin A in raisins.** N. ÖZALTACA[1], P. KINAY TEKSÜR[1] and K. HİZALER[2]. [1]*Ege University, Faculty of Agriculture, Department of Plant Protection, 35100 Bornova, Izmir, Turkey;* [2]*Bereketli Tarim, Sarigöl, Manisa, Turkey. E-mail: pervin.kinay@ege.edu.tr*

Turkey is the biggest raisin producer and exporter country in the world. The major problem on raisins is not only residue on but also ochratoxin A (OTA) produced by fungi. OTA is a common and well-known mycotoxin in raisins produced by two main genera of fungi, *Aspergillus* and *Penicillium*. In this study, effects of preharvest spray programmes in vineyards and different types of sun-drying systems on formation of OTA were studied on raisins in Manisa, Sarigöl. The spraying programme, farmer's programme and control were compared in three separate parcels. The spraying programme was started after the last fungicide applications for powdery mildew in the vineyard. After each fungicide application, microbial load were tested on fresh field-treated grapes. All grapes were dried on concrete or soil under the open sun after harvesting. In a second group, the grapes were harvested and stored as fresh with $SO_2$ and without $SO_2$ in cold storage conditions for 2 months. Disease assessments and quality parameters were also analyzed in bunches in the vineyards. Raisins were stored in normal storage conditions and in a cold storage room in sacks or polyethylene bags for 8 months. After analyses, amounts of OTA were below the legal limits on raisins at the beginning and the second month of the study. Generally, preharvest fungicide applications reduced the growth of *Aspergillus* spp. *Aspergillus* spp. populations were found to be very high on raisins dried on soil.

**Studies on effectiveness of potassium and sodium organic salts against grape moulds on sultani seedless grapes.** K. HIZALER [1] and P. KINAY TEKSÜR[2]. [1]*Bereketli Tarim, Sarigöl, Manisa, Turkey;* [2]*Ege University, Faculty*

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Many significant problems of fungicide residues and resistance occur with chemicals used against bunch rots of grapes. This study investigated the effect of pre-harvest applications of potassium (PBC) and sodium bicarbonates (SBC) organic salts on bunch rots of grape as an alternative to fungicide chemicals. In the pre-harvest period, PBC (1%) and SBC (2%) were applied twice as either individually or in combination. During the commercial harvest time, bunch decays were evaluated, and after harvest the plots were covered with polypreplyene, and one month later the decay was examined. Development of bunch rots in treated bunches was greater than in control plots. Phytotoxic effects were observed on leaves and bunches on treated plots. The decay development on bunches at PBC application under cover was less than 10% compared to control. The application inhibited decay by 34%. In addition, SBC and the mixture of SBC + PBC had 19% and 11% efficacy, respectively. Decay development in cold storage conditions was very high, but the lowest decay was found from the PBC application. For *in vitro* tests, PBC, SBC, and their mixture were effective at low doses to reduce mycelial growth of *B. cinerea* and *Cladosporium* sp., and at much higher doses for *A.niger* and *Alternaria* sp.

**Surveillance and morphological characterization of *Fusarium* isolates associated with lentil wilt.** R. ALTAF, Ch. Abdul RAUF and F. NAZ. *Fungal Plant Pathology Lab. Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi. E-mail: aridpmas@gmail.com*

Lentil *(Lens culinaris* Medikus) is an important dietry legume as a source of protein in many parts of the world, especially South Asia including Pakistan. The crop is vulnerable to wilt, a serious soil-borne threat incited by the fungus *Fusarium oxysporum* f. sp. *lentis*. In view of the potential threat Fusarium wilt can pose to lentils, this project was initiated for disease assessment, morphological characterization of recovered isolates of the pathogen and determination of their pathogenicity. Nine districts including 28 locations were surveyed during the crop season of 2012–13, of which 21 showed 100% disease prevalence. In total, 15 isolates of *F. oxysporum* f. sp. *lentis* were recovered. The mean length and width of microconidia of these isolates ranged from 4.4 to 6.7 μm and 2.3 to 3.2 μm, respectively. The micoconidia were oval for all the isolates except for isolate FOL-6 (two-celled oval) and FOL-10 (oval pyriform). The mean lengths and widths of macroconidia ranged from 9.9 to 29.7 μm and 3.0 to 5.0 μm, respectively. The macroconidia were straight for all the isolates except FOL-6 and FOL-12, which had slightly curved macroconidia. The mean diameter of chlamydospores ranged from 7.0 to 15.8 μm. During pathogenicity testing of 15 isolates on cv. Masoor-93, gave mean disease severity indices ranging from 0 (FOL-1, FOL-8 and FOL-11) to 0.72% (FOL-3), and on line ILL 4605 ranged from 0 (FOL-1, FOL-3, FOL-5, FOL-8, FOL-10 and FOL-13) to 0.66% (FOL-2). This line proved to be more resistant than Masoor-93.

**Biocontrol of plant pathogens, scope and limitations.** A.S. GONDAL. *Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi, Pakistan. E-mail: amjadshahzad@live.com*

Plant diseases need to be controlled to maintain the quality and supply of food. Different approaches may be used to prevent, mitigate or control plant diseases. Together with agronomic and cultural practices, farmers rely on the use of noxious chemical and fertilizers to get better yield of the produce. Environmental degradation, Insecticide resistance, resource losses, and agronomic concerns have prompted growing interest in alternative disease management techniques. Biocontrol of plant pathogens being ecofriendly and cost-effective, can contribute significantly to the improvements in crop productivity. Using Insecticide biocontrol agents have almost no harmful effects on humans and the environment and lead to the inability of pests to develop resistance. However, biocontrol often does not result better pest management in field conditions. Limitations involving research necessary in seeking a biological control solutions to an agricultural problem is often demanding in scientific and technical terms. To adequately practice biocontrol of plant diseases, firm understanding of the host population, pests along with their natural enemies, and their behavioural ecology is necessary, as the pest population will continue to exist at a level determined by the host properties, natural enemies and the habitats they occupy. The effectiveness of biocontrol agents must always be considered relative to man's economic threshold.

**Study of the influence of wheat varieties in the protection by *Trichoderma atroviride* P. Karsten against some isolates of *Fusarium* spp. causing root rot and head blight of wheat.** I. LARABA, D. KOUDRI, H. BOUREGHDA. *Ecole Nationale Supérieure Agronomique (ENSA), Département de Botanique, El Harrach- Algiers-ALGERIA. E-mail: laraba.imene@yahoo.fr*

Fusarium head blight (FHB) and root rot are serious diseases of wheat caused by several species of *Fusarium* and *Microdochium nivale*. In addition to the severe yield losses, the contamination of the grains with mycotox-

ins is a serious problem for human and animal health. The evaluation of efficiency of the antagonist species *Trichoderma atroviride* (Ta.13) against the an isolate of *F. culmorum* (FC 03-12) and of *F. graminearum* (FG 01-12) was carried out using *in vitro* and *in vivo* bioassays. The isolates were obtained from wheat collars and spikes exhibiting typical symptoms of disease. The ability of *T. atroviride* to reduce the growth of *F. culmorum* and *F. graminearum in vitro* was measured by two techniques: the direct confrontation of culture medium and indirect confrontation. Direct confrontation of the colonies of *T. atroviride* with those of the pathogen resulted in inhibition and growth arrest compared to the control. In the indirect confrontation assay, reductions in colony diameter were observed, which indicates the ability of *Trichoderma* to produce volatile antifungal substances. The highest percentage of mycelial growth reduction (80%) was observed with isolate FC 03-12 in direct confrontation, and with isolate FG 01-12 isolate (29%) in indirect confrontation. Four wheat varieties (Vitron, Waha, Hiddab and Ain Abid) were used in the *in vivo* test for the evaluation of the effectiveness of the Ta.13 isolate in protecting wheat seedlings against isolates of *F. culmorum* and *F. graminearum*. Seed treatment with Ta.13 before sowing in a soil infested by the pathogen led to a significant decrease of disease severity compared to the untreated control. The greatest percentage of disease reduction (77%) was obtained with Hiddab variety treated by Ta.13 and inoculated by *F. culmorum*. In addition, the varieties Hiddab and Vitron which gave the greatest highest disease indices for the positive controls showed the highest percentages of reduction in disease index. These results indicate that there is a difference in the protection against the disease depending on the wheat variety used.

**Elucidation of the interactions between the mycotoxigenic fungi *Fusarium proliferatum - Fusarium verticillioides* and maize germplasm.** M.K. ILIADI[1], A.A. GKATZOUNI[1], P. TERZOPOULOS[2], E.J. PAPLOMATAS[1] and D.I. TSITSIGIANNIS[1]. [1]*Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology, Athens, Greece;* [2]*SPIROY Company, House of Agriculture, Athens, Greece. E-mail: dimtsi@aua.gr*

*Fusarium verticillioides* and *Fusarium proliferatum* cause ear and grain rots, and produce the carcinogenic mycotoxins fumonisins in maize that are very harmful to human and animal health. *Fusarium* infections and fumonisin contamination occur as maize kernels reach physiological maturity, and increase during the season up to the harvest date. A major strategy to control these pathogens and to reduce the detrimental effects of fumonisins is breeding of less susceptible plant genotypes. The objectives of this study were to characterize different *Fusarium* species isolated from maize plants in Greece, and to evaluate a number of hybrid lines after infection with a mixture of four different *F. verticillioides* and *F. proliferatum* strains. The goal was to collect data on disease incidence and severity and fumonisin production, and to determine if there was positive association between visible symptoms caused by *Fusarium* infection and mycotoxin concentration. In *in vitro* experiments of corn kernel infections, a number of hybrids showed significant reductions in symptom development, and in conidium and fumonisin production by the mixture of the four different *Fusarium* strains. The hybrids that showed significant resistance are currently being evaluated in field trials and at the molecular level to investigate if genes involved in plant defences are activated. A better understanding of the resistance mechanisms would facilitate the implementation of strategic agriculture to breed resistant germplasm, and contribute to reduction of ear rot and production of fumonisins in maize.

**Elimination of *Citrus variegation virus* by seed cryotherapy.** G. LOMBARDO[1], D. FRASHERI[2], M. SIRAGUSA[3], R. SCHICCHI[4], K. DJELOUAH[2], F. DE PASQUALE[3] and A.M. D'ONGHIA[2]. [1] *University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) Botany and Plant Ecology Division, Palermo, Italy;* [2] *CIHEAM - Mediterranean Agronomic Institute of Bari (MAIB), Valenzano, Italy;* [3] *Consiglio Nazionale delle Ricerche (CNR), Istituto di Genetica Vegetale, Palermo, Italy;* [4] *University of Palermo, Department of Agricultural and Forest Sciences, Palermo, Italy. E-mail: donghia@iamb.it*

*Citrus variegation ilarvirus* (CVV) is one of the first viruses reported in *Citrus*, which produces crinkling, puckering and variegation of leaves in field trees and, occasionally, variegated and deformed fruits. This virus is primarily transmitted by grafting. However, no consistent data are available concerning CVV seed transmission, which is apparently low. The elimination of CVV in citrus genotypes is carried out by shoottip grafting. In the case of seed sources, virus elimination directly in the seeds becomes essential. In cryotherapy, plant pathogens such as viruses, phytoplasmas and bacteria are eradicated by exposing explants to liquid nitrogen. This method allows treatment of a high number of samples and results in a high frequency of pathogen-free regenerants. Three polyembryonic Citrus genotypes (sour orange, mandarin and lemon), which were found to be infected by CVV, were used for trials of virus seed transmission and CVV elimination by dehydration/cryopreservation of the seeds. The dehydrated seeds were placed in cryovials which were then plunged into liquid nitrogen at -196°C. ISSR markers were used to discriminate between zygotic and nucellar plants after treatment. TAS-ELISA and RT-PCR were applied for

CVV detection in the seed components before cryopreservation and in the produced seedlings after treatment. CVV seed transmission was detected for the first time in sour orange and lemon genotypes, by serological and molecular means. All tested *Citrus* genotypes showed CVV infection in both components of the seed plunged in liquid nitrogen. However, all plantlets developed from cryopreserved seeds were CVV-free 2 years after transplanting.

**Comparaison of the effectiveness of biological and chemical control against *Botrytis cinerea* Pers. the cause of grey mould on grapevine.** K. DERNANE and H. TRAIKIA. *Ecole Nationale Supérieure Agronomique (ENSA), Département de Botanique, El Harrach- Algiers-Algeria. E-mail: kawtherdernane@yahoo.com*

*Botrytis cinerea* is a fungal pathogen responsible of grey mould on grapes, causing serious damage on vineyards throughout the word. Two means of grey mould control were investigated using *in vitro* methods: biological control by the study of the antagonistic effects of two species of *Trichoderma, T. longibrachiatum* (T4) and *T. atroviride* (Ta13), and chemical control by studying the efficacy of fungicide (Switch) for reducing mycelial growth of *B. cinerea*. The study was on nine strains of the pathogen, which were isolated from grapes and branches with typical symptoms of grey mould. Biological control gave more efficient results regarding the application of the antagonist agent. The Ta13 isolate gave the best results, with 95% reduction of mycelial growth for direct confrontation and 57% for distant confrontation. The percentage reduction in growth in direct confrontation was 99%, and was also recorded for Ta13 against isolate BCV16. Less reduction ((79%) was recorded when confronting isolate I3 with T4. For the remote confrontation test, lower growth (40%) was recorded when confronting isolate I3 with T4. The highest proportion (79% was recorded for isolate I6 with isolate Ta13. The results in the Switch efficacy essay of mycelial growth showed weak resistance with a highest inhibition rate of 100% on the two isolates BCV 16 and BCV 19. The lowest inhibition rate was 81% recorded for the isolate I3.

**Characterization of the microbial quality of lettuce from different crop systems in Cyprus.** E. SAVVA, G. BOTSARIS and D. TSALTAS. *Cyprus University of Technology, Faculty of Geotechnical Sciences and Environmental Management, Department of Agricultural Sciences, Biotechnology and Food Science, Limassol, Cyprus. E-mail: dimitris.tsaltas@cut.ac.cy*

Vegetables and fruits, and in particular leafy greens which are usually consumed raw, are increasingly recognized as important vehicles for transmission of foodborne pathogens. Contamination with pathogenic and sometimes antibiotic resistant bacteria can occur in different steps of the production and distribution chains, making food safety of vegetables an important priority. Lettuce is the most commonly consumed vegetable worldwide, growing in close proximity to soil and soil amendments, while rain and irrigation water are facilitators of microbial movement and contamination. In addition most parts of the vegetable are eaten raw after variable ways of rinsing, which are not always sufficient to assure microbial safety. It is of scientific and public interest to explore how different production systems affect the presence of foodborne pathogens, since organic amendments are of preference to inorganic fertilizers, and hydroponic production is increasing. The predominant microflora were caharactrized in lettuce produced with different production systems, in order to highlight possible risks from leafy vegetable microbial quality. Samples were taken from all Districts of the Republic of Cyprus (Nicosia, Limassol, Pafos, Larnaca and Ammochostos) from conventional, organic, hydroponic and aquaponic farms, and open field and greenhouse growth were also compared. Whole lettuce plants, surface soil samples (20 m radius around plants) or swabs from hydroponic/aquaponic and irrigation water from all crop systems were sampled. All samples were analysed for: total microbial count, coliforms, *E. coli* and yeast and moulds using microbiological plate counts on PCA, VRBLA, and DRBC media. Our results describe qualitative and quantitative impacts of different production systems on the microbial quality of lettuce.

**Highly dynamic exon shuffling in *Ectocarpus siliculosus* putative pathogen receptors.** A. ZAMBOUNIS[1,2], M. ELIAS[3], L. STERCK[4,5], F. MAUMUS[6] and C. GACHON[7]. *[1]University of Thessaly, School of Agricultural Sciences, Volos, GREECE; [2]current address: INRA-AgroParisTech, UMR1290 BIOGER, Grignon, France; [3]University of Ostrava, Department of Biology and Ecology, Ostrava, Czech Republic; [4]VIB, Department of Plant Systems Biology, Ghent, Belgium; [5]Ghent University, Department of Plant Biotechnology and Bioinformatics; Ghent, Belgium; [6]INRA Centre de Versailles-Grignon, Unité de Recherche en Génomique-Info-UR 1164, Versailles, France; [7]SAMS, Scottish Marine Institute, Oban, United Kingdom. E-mail: antonios.zampounis@versailles.inra.fr*

Pathogen recognition is the first step of immune reactions. In plants, direct or indirect pathogen recognition is often mediated by numerous fast evolving receptors, many of which contain ligand-binding and signal transduction domains. In order to identify candidates potentially involved in plant defense, we mined the genome of the brown alga *Ectocarpus siliculosus* for homo-

logues of defense genes mainly searching for for putative pathogen receptors. Whilst homologues of plant resistance genes were absent from the genome, we identified two families of candidate pathogen receptors that apparently evolved new ligand-binding specificities by a highly original, controlled and dynamic exon shuffling mechanism. These presumably fast-evolving receptors, which contain ligand-binding and signal transduction domains, were members of the leucine rich repeat (LRR)-containing GTPases of the ROCO family (LRR-ROCO proteins) and of a group of 24 tetratricopeptide repeats (TPR)-containing genes fused with signal transduction domains harbouring a central nucleotide-binding domain (NB-ARC domain). Among the 251 proteins which were predicted to contain LRRs in *E. siliculosus*, we found 37 LRR GTPases of the ROCO family (associated to 20 related pseudogenes) and another 15 LRR-kinases, the structure and the genome organization of which seemed compatible with a putative defense function. Both gene families exhibited high birth and death rates, while a diversifying selection is acting on their LRR and TPR domains, presumably affecting their ligand binding specificities. We observed that each LRR repeat is encoded by only one exon, and that the intense exon shuffling underpinned the variability of LRR domains. Moreover, hypervariable solvent-exposed aminoacid residues were subjected to positive selection, an unusual feature reflecting strong evolutionary pressures, such as the ones imposed by a host-pathogen arms race. The genomic organization, structural and evolutionary features of these candidate pathogen receptors were very similar to other plant pathogen recognition systems. We hypothesize that brown algae might generate their immune repertoire *via* controlled somatic recombination.

**Evaluation of two selective agar media for detection of *Phytophthora* and *Pythium* species.** Ş. TÜRKÖLMEZ[1], S. DERVIŞ[2], O. ÇIFTÇI[1] and Ç. ULUBAŞ SERÇE[3]. *[1]Diyarbakir Plant Protection Research Station, 21110 Yenişehir, Diyarbakir, Turkey; [2]Department of Plant Protection, Agriculture Faculty, Mustafa Kemal University, 31034 Antakya, Hatay, Turkey; [3]Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde University, 51240 Niğde, Turkey. E-mail: s_turkolmez@hotmail.com*

Two new growth media, grated apple corn meal agar (GACMA) and grated carrot corn meal agar (GCCMA), were evaluated for the selective isolation and presumptive identification of *Phytophthora* and *Pythium* spp. Of a total of 196 crown and root samples from fruit trees showing root and crown rot symptoms, 84 yielded no growth and 112 yielded growth on one or both media. Overall, 98 *Phytophthora* spp. and 14 *Pythium* spp. isolates were attained from crown or root samples. The apple-based medium, GACMA, was for the first time prepared and used for isolation and culturing *Phytophthora* and *Pythium* species. GACMA had advantage for long incubation in Petri plates without drying as well as profound sporangia formation without aerial mycelium. Since GCA and CMA were routinely used for *Phytophthora* and *Pythium* isolations, both media were combined in one mixture adding CMA to GC instead of standard agar, and therefore GCCMA was formed. *Phytophthora* spp. cultures formed abundant aerial mycelium and very fast radial growth on GCCMA. In addition to standard antibiotics and fungicides (pimaricin, ampicillin, rifampicin and PCNB), the use of fungicide Tocata® TR and insecticide Lambda-cyhalothrin in both media yielded excellent suppression of bacteria, *Fusarium* spp. and mite contamination. We conclude that both media are sensitive and specific for the isolation of *Phytophthora* and *Pythium* from root and crown samples. The main advantages of these media reside in their sensitivity and selectivity, which enabled the recovery and presumptive identification of these oomycetes within 5 d and reduced unnecessary fungal growth.

**Post-harvest control of bacterial soft rot pathogens of onion bulbs in storage.** M. ABDALLA and S. HAMZA. *Mansoura University, Faculty of Agriculture, Plant Pathology Dept., Mansoura, Egypt. E-mail: abdalla.elaidey@yahoo.com*

General characteristics of bacterial pathogens based on the biochemical and physiological tests indicated that 41 of 88 bacterial isolates collected from rotted onion bulbs were identified as; *Erwinia cacticida* (two), *Erwinia carotovora* subsp. *atroseptica* (five), *Erwinia carotovora* subsp. *betavasculorum* (six), *Erwinia carotovora* subsp. *carotovora* (16), *Pantoea* spp. (five) and *Burkholderia cepacia* (seven). A storage trial for fresh harvest and cured onion bulbs collected from field plots that were treated either with irrigaion, withholding irrigation 4 months before harvest or four different levels of fertilizers (N, K). Assessments of bacterial bulb rot incidence (DI%) were carried out every 2 months at regular intervals during 10 months of storage for onion bulb samples after external treatments with propionic acid spray (0,025%), streptomycin sulfate spray (100 ppm) and actinomycetes (*Streptomyces coelicolor*) dust formulation. The highest DI% (21%) occurred in the check treatment after 10 months of storage. Compared with this control treatment; significant reduction in DI% was found in most treatments after all periods of storage. The highly significant reduction in DI% attributed to spray with propionic acid were 2.1%, 1.5%, 1.3%, 0.7% and 0.0%; followed by dust treatment with actinomycetes were 4.3%, 3.7%, 3.2%, 1.9% and 1.5%, respectively, after 2, 4, 6, 8 and 10 months. However; low significant differences or reductions in DI% were found between irri-

gation and withholding irrigation treatments. Among fertilizers treatments; N1K1 treatment (150 kg N + 24 kg $K_2O$/fed) gave significant reduction in DI%, reaching 4.4%, 5.5%, 6.3% and 7.2%, respectively, after 2, 4, 6 and 8 months of storage, but after 10 months the reduction in DI% was not statistically significant.

**Fungi precursors of woody decay (white rot) of olive plants in southern Italy.** S. FRISULLO[1], M. CONTURSI[1], L. PRUDENTE[1] and A. MORETTI[2]. [1]*Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi di Foggia, Via Napoli, 25,71121 Foggia, Italy;* [2]*Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Via Amendola 122/O, 70126, Bari, Italy. E-mail: antonio.moretti@ispa.cnr.it*

Olive plants of Ogliarola and Cellina di Nardò cultivars grown in the Lecce area showing discoloured wood tissue symptoms were associated with ten different fungal species in 2001 and 2006 crop seasons. Pathogenicity tests were performed in order to establish the main agents of these symptoms. Strains belonging to a *Phaeoacremonium rubrigenum, Phaeoacremonium aleophilum, Phialophora richardsiae, Phaeoacremonium mortoniae, Phaeoacremonium angustius, Phaeoacremonium inflatipes* and *Phaemoniella clamydospora*, proved to be the main species inducing woody decay-like symptoms in olive trees. More recently, a 3 year survey (2011–2014 crop years) carried out in Lecce Province evaluated growing woody decay symptoms on olive branches, showing the occurrence of several fungal species that were isolated from withered branches of the two varieties. Five fungal species, *Verticillium dahliae*, *Phaeoacremonium aleophilum*, *Phaemoniella clamydospora*, *Fusicoccum aesculi* and *Fomitiporia mediterranea*, have been selected to test their pathogenicity by performing single and dual assays, through artificial inoculation on 4-year-old plants of both varieties. When inoculated alone, *Verticillium dahliae* was the only species causing severe branch blight symptoms after 3 years in the olive plants, while *Phaeoacremonium aleophilum* caused severely discoloured wood tissue symptoms; *Phaeomoniella clamydospora* and *Fusicoccum aesculi* showed the same symptoms of *Phaeoacremonium aleophilum*, but at a significantly lesser extent. *Fomitiporia mediterranea*, inoculated alone, did not show any disease symptom. When plants were inoculated with the other four species in dual pathogenicity assays, *Fomitiporia mediterranea* caused discoloured wood tissue symptoms on branches and early woody decay symptoms on all inoculated olive trees.

**Determination of mycotoxin $Fb_2$ in Anatolian raisins.** Z. ASLANOĞLU and D. HEPERKAN. *Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Istanbul, Turkey. E-mail: zaslanoglu@itu.edu.tr*

Thirty-eight samples of five different types of raisins (Antep, Besni, Maraş, Urumu and Dimişki) from regions of Southeastern and Eastern Anatolia (Antep, Adiyaman, Kahramanmaraş anf Bingöl) were analyzed for the presence of the mycotoxin $FB_2$. 47.3%, 28.9%, 15.7%, 7.9% of the samples were, respectively, from Gaziantep, Bingöl, Adiyaman and Kahramanmaraş. An analytical method, based on immunoaffinity column clean-up and high performance liquid-chromatography (HPLC) with fluorimetric detection, was used to determine the occurrence of $FB_2$ in the raisin samples. To ensure homogeneity before analysis, laboratory samples are normally slurried with water in the ratio of one part fruit to four parts water, and test materials in this form were used in the study. The test portions were extracted with relevant solvent. The extracts were filtered, diluted with phosphate-buffered saline, and applied to an affinity column. The analysis of $FB_2$ was performed using HPLC. After the seperation of $FB_2$, contents were detected by reversed phase column assays performed by detectors. The limit of detection (LOD) and limit of quantification (LOQ) for $FB_2$ were, respectively, 8 $\mu g\ kg^{-1}$ and 8,63 $\mu g\ kg^{-1}$, and the recovery value was 75.5%. Of all the 39 raisin samples analysed in the survey 2.6% contained $FB_2$ at 8.8 $\mu g\ kg^{-1}$. The sample contaminated with $FB_2$ was Antep type raisin, which is black and seeded originated from Bingöl. 5.9% of sample of Antep raisins were contaminated with $FB_2$. No $FB_2$ was detected for the samples from Gaziantep, Adiyaman and Kahramanmaraş. In order to prevent the formation of $FB_2$ in raisins, during and after harvest, Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) are very important. Preventing $FB_2$ formation in raisins is important both for protection of public health and avoidance of economic losses. During production, every step should be meticulously controlled, and storage conditions should be appropriate. Regular mycotoxin analysis should be carried out to prevent contamination.

**Relationships between the endemic populatıon of *Aspergillus* spp. and soil properties and altitude for fig orchards of southern peloponnese.** V. DEMOPOULOS[1], D.F. ANTONOPOULOS[1], E. TSIAVTARI[2], A. KOSTRIVA[1] and A. KOTSIRAS[3]. [1]*Technological Educational Institute of Peloponnese, Department of Agricultural Technology, Laboratory of Plant Protection, Kalamata, Greece;* [2]*Agricultural Institute of Kalamata, Kalamata, Greece;* [3]*Technological Educational Institute of Peloponnese, Department of Agricultural Technology, Laboratory of Horticulture, Kalamata, Greece. E-mail: vdimo@teikal.gr*

The effects of soil properties and altitude were studied to explore which factors influence the distribution

of the endemic population of *Aspergillus* spp. İn the fig orchards of southern Peloponnese. Six soil subsamples were collected as replications from each of the 46 fig orchards, and the samples were mixed and analyzed as one sample. Saturation percentage (SP), pH, electrical conductivity (EC), composition (sand, clay, silt), and the concentration of the nutrient elements P, K, Ca, Mg, N$\alpha$, B, Fe, Mn, Zn, Cu, as well as of organic matter, were measured for each combined sample. The altitude of each fig orchard was determined using GPS. No statistically significant correlations were found between the population of *Aspergillus* spp., and soil properties or altitude. Consequently, the explanation of the disjunct distribution of the *Aspergillus* spp. populations may be found in other soil properties, which could be affecting the epidemiology of the fungus, such as soil formation and relief, microclimate and history of cultivation practices of each individual fig orchard.

**Environmental *Aspergillus flavus* isolates: is there any association between phylogeny and aflatoxin biosynthesis?**

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With world-wide distribution, aflatoxigenic fungi are very important. *Aspergillus flavus* is particularly significant, composed of phenotypically and genotypically diverse groups in crops and in the environment, with the ability to contaminate crops and agricultural commodities with carcinogenic aflatoxins, and capability to produce over ten other mycotoxins, including cyclopiazonic acid, aflaterm and aspergillic acid. Toxicological data suggest that *A. flavus* populations largely behave as clones with certain groups scattered in either aflatoxigenic or non-aflatoxigenic strains. Distribution among toxigenic or non-toxigenic strains is completely dependent on biotic and abiotic environmental conditions which affect ecosystems. Although there are large amounts of data about different aspects of aflatoxigenic fungi, from epidemiology to genetics and molecular biology, little has been documented about genetic diversity of these fungi in relation of aflatoxin biosynthesis. This study focused on an association between the ability of producing aflatoxin by environmental strains of *A. flavus* isolated from soil and air as major natural reservoirs, and the genotypes provided by phylogenetic dendrograms.

**The role of the global regulator of secondary metabolism *AclaeA* in *Aspergillus carbonarius* physiology, virulence and ochratoxin A production.**

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*Aspergillus carbonarius* is considered one of the main fungi responsible for the sour rot in grapes and for the production of the carcinogenic mycotoxin ochratoxin A (OTA). The regulatory mechanisms of OTA production by *A. carbonarius* remain largely unknown. In *A. Nidulans*, the global regulator of secondary metabolism *laeA* encodes a nuclear methyltransferase protein that is required for the expression of secondary metabolite genes, while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of the genome of *A. carbonarius* with the *laeA* gene of *A. nidulans* resulted in the presence of an orthologous gene named *AclaeA*. The goal of this study was to investigate the role of the regulatory gene *AclaeA* in physiology, virulence and OTAproduction, by deleting this gene from the genome of the wild type *A. carbonarius* strain 5010. *AclaeA* was deleted by targeted gene replacement using *Agrobacterium tumefaciens* mediated transformation. Data of morphological characteristics, virulence in red and white grape varieties and ochratoxin analysis of ΔAclaeA mutants showed that these strains were defective in growth and in OTA production and were less virulent producing, 40–50% less conidia in three different cultivars of grape berries. Current studies are focused on the global regulatory role of *AclaeA* in secondary metabolism of *A. carbonarius* at transcriptional and metabolomics levels. The study of the regulatory gene *AclaeA* can contribute to broader understanding of the role of secondary metabolites during *A. carbonarius* – grape interactions.