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"13th Congress of the Mediterranean Phytopathological Union" (MPU)

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Edited by
Marina Barba, Emma Motta,
Laura Tomassoli, Luca Riccioni

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Proceedings

13th Congress of the Mediterranean Phytopathological Union

Rome, 20-25 June 2010

Edited by Marina Barba, Emma Motta, Laura Tomassoli, Luca Riccioni

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Giardini di Ninfa

Introduction

The Mediterranean Phytopathological Union and the congress organizing committee welcome all of the participants, comprising over 200 scientists from Mediterranean and non-Mediterranean countries, who are attending the 13th Mediterranean Phytopathological Congress being held in the Plant Pathology Research Centre in Rome, Italy on 20-25 June 2010.

The study of the causes and effects of plant diseases plays an important role in limiting the losses in yield and quality of the crops and plant products that form the major component of human food. All efforts aimed at reducing the risk of epidemics and improving plant health management are very important both strategically and economically.

The danger of a plant disease epidemic arises as a consequence of interactions between different factors such as environmental conditions, plant variety, pathogen type, and human practices (such as agronomic and land use management techniques, and trade patterns). Moreover, increased human movement, the globalization of trade, and new propagation material and seed supply systems can influence the introduction and proliferation of diseases across the world.

The official responsibility for safeguarding plants against invasive pathogens is held by national plant protection agencies under the guidance of the International (ICPP) and European Plant Protection Organizations (EPPO), which play a crucial role in developing recommendations to avoid the introduction of harmful diseases into European and Mediterranean countries.

All of these issues are of central concern in the Mediterranean area, where agriculture makes a vital contribution the socio-territorial balance between the various member countries.

The 13th Congress of the Mediterranean Phytopathological Union, which coincides with the 50th anniversary of its journal, *Phytopathologia Mediterranea*, will provide the scientific community with the opportunity to meet and share information on new techniques and on the main phytopathological problems afflicting this agricultural region. The meeting will cover the areas of plant disease epidemiology, the diagnosis of plant pathogens, new or unusual disease reports, the variability of plant pathogens, plant-pathogen interactions, mycotoxins, and control strategies based on traditional and alternative methods.

Marina Barba

13th Congress of the Mediterranean Phytopathological Union

Rome, 20-25 June 2010

Programme

Sunday, June 20

17.30-19.00	Early Registration – Poster setup
19.00 -20.00	Welcome drink

Monday, June 21

08.30- 09.15	Registration – Poster setup
09.15-09.45	Welcome address – Opening Session
09.45-10.00	*Maria Ludovica Gullino* ISPP and Food Security
10.00-10.30	**Plenary lecture** ***Eleftherios C. Tjamos*** Phytiatry: Priorities and challenges in the Mediterranean basin and worldwide at the 21st century
Session I	**Disease epidemiology**
10.30-11.00	**Invited lecture** ***Richard Falloon*** The plant pathology contribution: a world perspective
11.00-11.15	***M. Mnari-Hattab****, S. Zammouri, F. Pellegrin and N. Gauthier* Molecular identification of new natural *Begomovirus* recombinants associated with tomato yellow leaf curl disease co-existing with parental viruses in legume crops and weeds in Tunisia
11.15 – 11.45	Coffee break
11.45-12.00	***S. Boukef,*** *B.A. McDonald, A. Yahyaoui, S. Rezgui and P.C. Brunner* Global distribution of *mtRFLP4* haplotype of *Mycosphaerella graminicola*

12.00-12.15 ***D.I. Tsitsigiannis,*** *M. Georgiadou, S. Agoritsis, G. Zakynthinos, T.H. Varzakas, S. Tjamos, P. Antoniou, M. Dimakopoulou, G. Karnavas, E. Paplomatas, S. Yanniotis, E.C. Tjamos*
Ecology, epidemiology and control of *Aspergillus* spp. in pistachio orchards in Greece

12.15-12.30 ***D. Giovanardi,*** *D. Dallai, E. Stefani*
Population features of *Xanthomonas arboricola* pv. *juglandis* and epidemiology of walnut blight in Romagna (Italy)

12.30-12.45 ***V. Sergeeva,***
Grapevine and olive diseases in Australia

12.45-13.00 ***S.M. Damadi****, J.A. Smith, M. Abbasi*
Additions to the rust mycobiota of maragheh area, NW Iran

13.00 – 14.30 Lunch

14.30-15.30 **Poster sessions I, II and III**

Session II: **Diagnosis of plant pathogens**

15.30-16.00 **Invited lecture**
Yusuf Abou-Jawdah
Diagnosis and molecular characterization of whitefly-transmitted viruses which affect solanaceous and cucurbit crops in the Mediterranean region

16.00-16.15 ***A. Tiberini,*** *L. Tomassoli , M. Barba*
Multiplex diagnosis of viral agents of tomato by DNA microarray technology

16.15-16.30 ***U. Čepin****, I. Gutiérrez-Aguirre, L. Balažic, M. Pompe-Novak, K. Gruden, M. Ravnikar*
One-step RT-qPCR assay for the detection and quantification of *Grapevine fanleaf virus*

16.30-16.45 ***G.L. Bianchi****, N. Bertazzon, F. De Amicis, M. Borgo, E. Angelini*
Multiplex real time RT-PCR for the detection of the most important grapevine viruses

16.45-17.00 ***P. Teymuri****, S.V. Alavi, H.R. Zamanizadeh*
A rapid and accurate method for detection of citrus viroids in Northern Iran

17.00 – 17.30 Coffee break

17.30 -18.00	**Invited lecture** ***Françoise Petter*** EPPO's diagnostic activities
18.00-18.15	***E. AL-Turaihi*** Diagnosis of date palm diseases caused by *Thielaviopsis paradoxa* (De Synes) Höhn
18.15-18.30	***A. Novak****, J. Cosic, D. Jurkovic, K. Vrandecic* Influence of different nutrient media on growth of *Passalora fulva in vitro*
18.30 -18.45	***S. K. Mukhtar,*** *A. A. Abnaouf, M. E. Abdelmohsin* Identification of root-knot nematode species from the North Kordofan area, Sudan, by morphology, esterase phenotypes and RAPDS

Tuesday, June 22

Session III	**New or unusual disease reports**
09.00-09.30	**Invited lecture** ***Anne-Sophie Roy*** Emerging plant diseases: the EPPO perspective
09.30-09.45	***S. Arous,*** *A. Marais, C. Faure, M. Le Romancer, T. Candresse* Detection of a new dsRNA virus in Kerguelen Islands native Apiaceae *Azorella selago*
09.45-10.00	*V. Ferraro, Lo Piccolo, G. Conigliaro, V. Mondello,* ***L. Torta****, S. Burruano* Frequent alterations in Sicilian olive-yeards: first pathogenicity tests
10.00-10.15	***A. Lehtijärvi,*** . H. *T. Doğmuş-Lehtijärvi, F. Oskay, A. G. Aday* Snow molds and scleroderris canker on *Pinus nigra* subsp. *pallasiana* on the Dedegül mountain in Turkey
10.15-10.30	***S.V. Alavi,*** *P. Teimouri, H.R. Zamanizadeh* First report on the new causal agent of concave gum disease on Thomson Navel orange in Northern Iran

10.30 - 11.15 Coffee break

Session IV	**Variability of plant pathogens**
11.15-11.45	**Invited lecture** ***Alan Phillips*** The importance of being correct: why the right fungus name matters
11.45-12.00	***N. Luchi,*** *D. Paffetti, K. Korhonen, J. Hantula, P. Capretti* Genetic variation of *Heterobasidion abietinum* populations: diversification across the South Europe and Mediterranean basin
12.00-12.15	***J.M. Santos,*** *V.G. Correia, A.J.L. Phillips* Towards meaningful species definitions in *Diaporthe* and *Phomopsis*
12.15-12.30	***S.A. Khodaparast,*** *S. Takamatsu* Identification of two genotypes of *Leveillula* powdery mildews on sunflower (*Helianthus annuus*) based on its sequences

12.30-12.45	*S. Vitale, A. Santori, E. Wajnberg, P. Castagnone-Sereno, L. Luongo,* ***A. Belisario*** Characterization of *Fusarium lateritium* isolates in nut grey necrosis disease of hazelnut
12.45-13.00	***A. Hussien***, *C. Saccone, S. Vicario, A. M. D'Onghia, T. Yaseen* Investigating the phylogenetic signal in pathogenicity phenotypes of *Fusarium* spp. on citrus seedlings
13.00-13.15	***R. Falloon,*** *U. Merz, R. A. Lister, A. R. Wallace* Morphology enumerates resting spores in collections of *Spongospora subterranea* sporosori

13.15 – 14.45 Lunch

Session IV	**Variability of plant pathogens** (continue)
14.45-5.00	***H. Hajjeh,*** *M. Miazzi, F. Faretra* Points mutation in *Erysiphe necator cyp51* gene
15.00-15.15	***R. Shamsi****, A. El-Ahme,d M. Nachit, A. Yahyaoui* Molecular characterization of *Phyrenophora tritici-repentis* races in Syria using AFLP technique
15.15-15.30	***N.A. Elamri****, D. Arnold, A. Vivian* Plasmid profiles of *Pseudomonas syringae* pv. *maculicola* and closely related pathovares
15.30-15.45	**S.** ***Nabhan****, T. Debener , M. Linde , K. Wydra* Fingerprinting methods (AFLP, MLST) for identification and characterization of pectolytic, soft rot causing bacterial strains from Syria in comparison to strains from worldwide origin
15.45-16.00	***L. Ferretti****, M. Saponari, R. Sciarroni, A. Fontana, R. Schimio, G. Albanese* Molecular investigation on genetic variability of *Citrus tristeza virus* (CTV) isolates recovered in Calabria (Southern Italy)
16.00-16.15	***A.M. Al Sadi****, S.A. Al-Hilali, R.A. Al-Yahyai, F.A. Al-Said* Occurrence, distribution and characterization of *Citrus tristeza virus* (CTV) in Oman

16.15-6.30 ***M. Luigi**, A. Roschetti, G. Albanese, M. Barba, F. Faggioli*
Molecular characterization of olive leaf yellowing associated virus isolates

16.30 – 17.00 Coffee break

17.00-17.15 ***T. Rana**, V. Chandel, Y. Kumar, R. Ram, V. Hallan and A. A. Zaidi*
Analyses of molecular variability of the capsid protein of *Apple chlorotic leaf spot virus*

17.15-17.30 ***I.N. Manoussopoulos***
Exploitation of genetic variability in the potyvirus helper component proteinase for constructing and analyzing a complex network of highly statistically associated polymorphic positions

17-30 **General Assembly of the MPU**

Wednesday, June 23

Session V **Plant-pathogen interactions**

09.00-09.30 **Invited lecture**
Epaminondas C. Paplomatas
Molecular basis of the interaction of vascular wilt fungi with the host plant

09.30 -09.45 ***L. Mugnai***
Factors associated with symptoms development in grapevine trunk diseases

09.45-10.00 ***Ö.Erincik,*** *M. T. Döken, A. Yildiz*
Host specialization of *Transzchelia discolor* on stone fruits at aecial and uredinial infection stages

10.00 -10.15 ***B. Sharifnabi****, M. Mostafa, A. Esmaeili*
Assessment of the role of NRPS-ABC transporter in pathogenicity of *Alternaria brassicae* using real time – PCR technique

10.15-10.30 ***B. Setti****, M. Bencheikh, J. Henni, C. Neema*
Effect of pea cultivar, pathogen isolate, inoculum concentration and leaf wetness duration on Ascochyta blight caused by *Mycosphaerella pinodes*

10.30-10.45 ***M. Punelli****, Reverberi M., Uva P., Mentzen W., Dolezal A.L., Woloshuk C., Fabbri A.A., Fanelli C, Payne G.A.*
Genes differentially expressed by *Aspergillus flavus* in the interaction with *Zea mays*

10.45-11.00 *I.S. Pantelides,* ***S.E. Tjamos****, I.A. Striglis, I. Chatzipavlidis, E.J. Paplomatas*
Monitoring the interaction of the biocontrol strain *Fusarium oxysporum* F2 with *Verticillium dahliae* on eggplant roots

11.00 - 11.30 Coffee break

11.30-11.45 ***M. Fazli****,*
Co-inoculation of *Ralstonia solanacearum* and *Colletotrichum coccodes* affect more severely potato growth traits.

11.45-12.00 ***K.A.M. Abo-Elyousur***
Induction of defence-related enzymes in tomato plants in response to treatment with fluorescent *Pseudomonas*

12.00-12.15 ***E. Di Nicola-Negri****, L. Salandri and V. Ilardi*
PPV hairpin constructs confer resistance to *Plum pox virus* under biotic stress and different temperatures

12.15-12.30 ***I. N. Boubourakas,*** *A.E. Voloudakis, K. Fasseas, N. Resnick, H. Koltai, P.E. Kyriakopoulou*
Cellular localization of calico variant of *Peach latent mosaic viroid* in peach leaf sections by liquid phase *in situ* RT-PCR

12.30 – 14.00 Lunch

14.00-15.00 Poster sessions IV, V and VI

Session VI **Mycotoxins**

15.00-15.30 **Invited lecture**
Antonio Logrieco
Mediterranean mycotoxin network: ISM and MYCORED initiatives

15.30-15.45 ***Rouissi W.,*** *P. Bertolini, M. Mari*
Effect of *Penicillium expansum* strain R82 liquid culture on postharvest pathogens

15.45-16.00 ***A. Ricelli,*** *Reverberi M., Fabbri A.A., Fanelli C., Donghia A., Ayoub F., and Yaseen T.*
Ochratoxin a contamination of table grapes in Apulia region

16.00-16.15 ***M. Lahrouni****, K. Oufdou, F. El Khalloufi, B. Oudra*
A comparative study of the effect of cyanotoxins on *Rhizobia* isolated from Morocco and their symbiotic association with *Vicia faba*

16.15 - 16.45 Coffee break

16.45-17.00 ***D. Ivić****, B. Cvjetković, M. Peraica, T. Miličević*
Pathogenicity and potential toxigenicity of seed-borne *Fusarium* spp. on soybean and pea

17.00-17.15	***C. Perrone**, R. Rodeva, A.Andolfi, D. Melck, Z. Stoyanova , A.Evidente* Preliminary data on extract phytotoxicity of *Phomopsis foeniculi* from Bulgaria
17.15-17.30	***C. Nobili**, A. Ricelli, M. Reverberi, S. Gatta, V. Scala, G. Aureli, M.G. D'Egidio, A.A. Fabbri, C. Fanelli* Evaluation of susceptibility and tolerance phenotype in *Triticum aestivum* varieties contaminated with two don-producers *Fusarium graminearum* isolates.
17.30-17.45	*R.H. Proctor, F. Van Hove, A. Susca, G. Stea, M. Busman, T. van der Lee, C. Waalwijk, **A. Moretti*** Variation in sequence and location of the fumonisin mycotoxin biosynthetic gene cluster in *Fusarium*

21.00 - 23.00 Social dinner

Thursday , June 24

Session VII	**Control strategies: Pesticides, biological and alternative methods**
09.00-09.30	**Invited lecture** ***Alison Stewart*** Exploiting microbial interactions for plant disease control: a *Trichoderma* success story
09.30-09.45	***A. El Sherif****, F.A. Ismail Amona* Impact of certain fungal filtrates, and soil amendments in comparison with oxamyl on *Meloidogyne incognita* infecting sunflower
09.45-10.00	***M.E. Ehwaeti****, A.S. Almabrok, A.M. Alawami, M.A. Adam* Effects of biofertilizer and mineral potassium on the biochemical compounds of tomato cv. Rio Grande inoculated with *Meloidogyne incognit*a and *Fusarium oxysporum* f. sp. *lycopersici*
10.00-10.15	***E. C. McGawley****, .M. Steckler, N. Nakada* Agri-terra: colloidal ingredient synergy and environmental responsibility
10.15-10.30	***M.P. Aleandri****, G. Chilosi, A. Vettraino, A. Vannini* Isolation and characterization of *Trichoderma* isolates from rizosphere of nursery plants
10.30-10.45	***M. Chattaoui****, A. Rhouma, A. Boudabous, M. Msallem* Biological control of main olive tree pathogens using *Rhizobacteria* and *Actinomycetes*
10.45-11.00	***M. Bouri****, A. Rhouma, A. Boubaker* Efficacy of *Bacillus spp.* in biocontrol of *Agrobacterium tumefaciens*, in plant growth promotion and other beneficial activities

11.00 - 11.30 Coffee break

11.30-12.00	**Invited lecture** ***Matteo Garbelotto*** Controlling SOD in California: successes and failures ten years after the discovery of *Phytophthora ramorum*
12.00 -12.15	***F. Bouazza**, R. Hassiko In vitro* antifungal activity of *Aloe vera* gel *(Aloe barbadebsis* Miller)
12.15-12.30	***M. Baz**, D. Tran, S E. Samri, A. Jamjari, P. Meimoun, M. Barakate, F. Bouteau* Culture filtrate of active *Actinobacteria* against *Pectobacterium carotovorum* subsp. *carotovorum* induces defence reaction in tobacco cell suspensions
12.30 -12.45	***Sallam, N. M. A.*** Evaluation of certain plant extracts against early blight of tomato plants under greenhouse and field conditions
12.45-13.00	***S. Ahmad**, M. Shahzad, Z. Iqbal, Y. Iftikhar* Forecasting and management of O*kra yellow vein mosaic virus* through its vector control in Faisalabad (Pakistan)
13.00 -13.15	***M. R. Safarnejad**, F. Shahriyari, M. Shams-Bakhsh* Cloning and expression of the immunodominant membrane protein (IMP) of Candidatus *Phytoplasma aurantifolia*

13.15 – 14.30 Lunch

1**4.30-15.30**	**Poster session VII**
Session VII	**Control strategies: host resistance**
15.30-15.45	***M. U. Ghazanfar**, W. Wakil, S. T. Sahi* Induction of resistance in chickpea (*Cicer arietinum* L.) against *Ascochyta rabiei* by the application of chemicals and plant extracts.
15.45-16.00	***B. El Yousfi**, R. Jebbouj* An integrated multivariate approach to net blotch of barley: virulence quantification, pathotyping and a breeding strategy for disease resistance

16.00-16.15 ***H. Rahman**, Durreshahwar*
Reaction of two maize populations to S_1 line recurrent selection under leaf blight stress.

16.15-16.30 *H. R. Mirkarimi, **A. A. Moghadam**, J. Mozafari, S. Taheri*
Comparison between methods of potato evaluation for resistance to *Alternaria solani* early blight by greenhouse test and *in vitro* assay.

16.30 - 17.00 Coffee break

17.00-17.15 ***T. Duvnjak**, A. Mijić, A. Sudarić,M. Krizmanić, K. Vrandečić, I. Liović, J. Ćosić*
Sunflower breeding material testing to *Diaporthe helianthi.*

17.15-17.30 ***H. El Bassir**, A. Rhouma , A. Ben dhiab , M. Msallem , M. Chattaoui*
Susceptibility of olive tree hybrids to leaf spot (*Fusicladium oleaginum*)

17.30-18,00 General discussion and closing remarks

Friday, June 25
Full Day Scientific Excursion

PLENARY LECTURE

Eleftherios C. Tjamos

Phytiatry: Priorities and challenges in the Mediterranean basin and worldwide at 21st century

Petria 20 (2), 67-633 (2010)

PHYTIATRY: PRIORITIES AND CHALLENGES IN THE MEDITERRANEAN BASIN AND WORLD WIDE AT THE 21ST CENTURY

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This opening address is referring to the current challenges in the University studies in agriculture in general and in plant protection in particular and focuses mainly on the emerging priority of establishing a new distinct science, the science of **Phytiatry** in the Universities around the globe.

Today the sciences of Medicine in humans and Veterinary in animals cover respective health problems. Similarly, plants have analogous problems and a separate profession is desperately needed worldwide.

Indeed today there is a scientific gap in plant health sciences and this gap has several consequences. So, is Phytiatry a new professional challenge within agricultural and biological sciences? The health/disease duality has been developed alongside human history mainly as a struggle for survival, while pests and diseases of plants may not be as exciting but are extremely crucial for human activities on earth. Several current research disciplines such as Phytopathology, Entomology and Phytopharmacology, hold methodological similarities to conventional medicine, which, thus, allow for correlations among them. Obviously, plant protection and human medical science are based on common scientific principles of modern scientific thought.

Would Phytiatry be a suitable plant care science? What is the rationale?

Why Phytiatry, or Phytiatrie, Fitoiatria, Phytomedicin or Plant Medicine? It became apparent in the scientific community and in the private sector that the currently used term of 'Plant Protection' is narrow and absolute, thus unable to cover the concepts of protection, recovery and therapy in plant and pest disease management. In parallel, significant aspects, related to fundamental or applied research efforts, which contribute to better understanding plant health problems and inventing means or methods of managing them, are not just plant protection. Furthermore, the problems in studying nature, biology, ecology and securing correct identity of the causal agents, pests or plant pathogens, which create vast difficulties in the diagnosticians, must not be considered as plant protection only. The aspects of **comparative symptomatology** in plant diseases or pests leading to the clinical or laboratory plant disease and pest diagnosis are also necessary to deal with pests and parasitic or with the vast number of non-parasitic diseases and plant stress problems. The impact of the use of agrochemicals on agro systems, on soil fauna and microflora, so crucial in world agriculture, are not just plant protection. State vigilance in avoiding dispersal of plant

pathogens or pests around the globe, securing quality of agricultural food and feed (no chemical residues or mycotoxins) and the impact of pest management on the environment are included in the broad concept of the **new science of Phytiatry**.

Without doubt, that lack of attractiveness of our important but individual disciplines, necessitates a revolution in educating students in various plant health disciplines at an undergraduate level. Establishing **Phytiatry** as a University science will be, by far, more attractive comparing with the use of term Plant protection and will elevate the standards of involved researchers and open a broad spectrum of carriers for a **new profession of plant doctor**.

The abundance of quality agricultural products in the markets is partially based on the efforts of scientists working on basic or applied aspects of plant health worldwide. However, the practices of phytopathology, entomology nematology, acarology, herbicide science, phytopharmacology etc. separately are not enough to also be a private profession. We need a broader background and knowledge of all related disciplines to establish a powerful profession. It is evident that the vast science of agriculture desperately needs the establishment of a separate field of plant health sciences called **Phytiatry.** Currently there is an apparent lack of inspiring candidate students to study individual sciences in Phytiatry, due to the uncertainty in obtaining future jobs in limited disciplines (only research centers, few industries and university departments offer limited job opportunities). Thus, I strongly support the idea of educating scientists in the field of plant medicine since several scattered sciences dealing with plant health will come closer and create powerful and synchronous undergraduate programs for plant doctors of preferably a four- to five-year duration. This will also fill the enormous gap of missing specialists in the private sector.

I feel that the International Phytopathological Society, the American Phytopathological Society, the Mediterranean Phytopathological Union, the German Phytomedical Society along with the newly established Hellenic Society of Phytiatry have to exercise their pioneered role and go ahead with such an initiative. Although the late George Agrios, the eminent plant pathologist, writer and university teacher, in Florida, along with Anne Vidaver in Nebraska, were the pioneers in successfully establishing the plant doctor programs at a postgraduate level in the United States it seems that the post graduate studies should come as a step of graduate studies in Phytiatry.

The time had matured to come along with **other related societies** in the United States, in Europe and around the globe to open a broad and fruitful dialogue. University people, could be the leaders in this initiative and bring together all 30–40 different scientific disciplines involved in plant medicine, as indicated by the **German Phytomedical Society** below:

"Disease Monitoring, Disease Diagnosis, Cultivation Practices, Production Systems, Soil Management, Seeds and Plant Propagation, Variety Selection, Stored-Product Protection, Harvest Processing, Plant Protection Strategies, Phytopathology, Phytopharmacology, Plant Virology, Epidemiology, Nematology, Entomology, Weed Science, Horticulture, Agriculture, Forestry, Soil Science, Biometry, Vertebrates,

Mycology, Bacteriology, Technology, Molecular Biology, Breeding, Biotechnology."

This exchange of ideas could help to formalize the new education system, offering a university degree for plant doctors, regardless of plans to work in research, administration, or in the private sector. I am entirely convinced that this initiative, will be a great departure from our current situation, open new job opportunities, and have a great impact on the world of agriculture.

Phytiatry in relevant international scientific societies

Regardless of the existence of hundreds of **international scientific societies** devoted to the plant health sciences, recently new societies use the term Phytiatry or Plant Medicine such as in Germany and Switzerland.

The Swiss Society for Phytiatry: **http://www.sg-phytomed.ch/english/index.html**. The web page of the German Phytomedical Society (DPG): **http://dpg.phytomedizin.org/**

The German Phytomedical Society (DPG) is the largest scientific association in plant production in Germany. The Society is membership-based, and its members are professionals within the entire field of phytomedicine.

Here, it is interesting to see how DPG defines Phytomedicine as the science of plant disorders (whether biotic or abiotic), their diagnosis, management and control. Phytomedicine deals with all infectious agents that attack plants, and also covers damage caused to crops by pests, diseases and weeds. Under our definition, we additionally include abiotic disorders such as drought, frost, flooding, poor drainage, nutrient deficiency, salt deposition and other soluble mineral excesses or wind, which may occur naturally or may be man made. Other examples of man-made 'problems' include soil compaction, pollution of air and soil, salt applications on roads in urban areas, overuse of pesticides, as well as poor education and poor training of people working with plants. The special fields of interest (competences) of the 1,200 individual DPG members clearly reflect the broad scientific range of disciplines and topics encompassed by phytomedicine. In essence, the activities of DPG members are centred on some 20 or so basic disciplines (e.g. Plant disease, Mycology, Plant Virology, Plant Bacteriology, Nematology and Agricultural Entomology). In a multidisciplinary sense, 10 core disciplines emerge, covering important areas such as disease monitoring, diagnosis, plant protection strategies and soil management. The extent of expertise within the DPG membership varies from discipline to discipline, but all areas of phytomedicine are covered. Within the membership, there is a balance between system-oriented, applied approaches to phytomedicine and basic research, which may or may not have direct or indirect application. The former constitute mainly members from applied research and advisory institutions or organisations, who seek to provide or support solutions to plant protection problems, ideally in direct collaboration with advisors (practitioners), growers and agricultural companies. The latter include academic scientists in federal or university research

institutes, whose links to DPG depend largely on their individual interests in plant protection issues. Thus, DPG comprises a community of experts professionally committed to the achievement and preservation of both the healthy plant' and 'healthy plant production'.

Recently **The Hellenic Society of Phytiatry** was established in Greece with the following web page: **http://fytiatriki.gr**

As President of the Hellenic Society of Phytiatry, I sent a letter to the Editor of Phytopathology News published by APS, with over 5000 members. The letter appears in the web page of the Hellenic Society of Phytiatry **http://fytiatriki.gr**

Phytiatry in USA and elsewhere

USA scientistics in Florida and Nebraska established **Phytiatry or Plant medicine** at a Post graduate level. Indeed Phytiatry or Plant medicine is a growing field that started in the University of Florida and has expanded domestically in Nebraska and internationally to Japan, South Korea, Thailand and Egypt. The main purpose was to meet the critical needs of the food industry, plant doctors serve as trained consultants to agricultural firms, liaisons between researchers and producers and educators to the general public.

Information on post graduate programs on Plant Medicine is provided in the following web pages of Florida: **http://dpm.ifas.ufl.edu/ and** Nebraska: **http://dph.unl.edu/**

Phytiatry in Europe

The Agricultural University of Athens in collaboration with the University of Bari, Italy and Plodvil University of Bulgaria have created a TEMPUS INTERNATIONAL JOINT MASTER DEGREE IN PLANT MEDICINE in cooperation with the Universities of Tirana and Korce Albania, Novisad and Belgrade in Serbia, Zagreb in Kroatia, Tetovo and Scopia in FYROM and Pristina in Kosovo.

This is a master degree project started in January 2001. Preliminary information is provided at the web page: **http://serlab.di.uniba.it/tempus/**

SESSIONE 1

Disease epidemiology

ORAL PRESENTATIONS

Petria 20 (2), 67-633 (2010)

THE PLANT PATHOLOGY CONTRIBUTION: A WORLD PERSPECTIVE

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Plant pathology is the study of the causes and effects of plant diseases, but also includes "plant medicine", the science and practice of the diagnosis, treatment and prevention of plant disease. Our discipline has an important role to play, as the world struggles with serious issues associated with the increasing human population (to reach 9 billion people by 2050). Dealing with the associated issues of environment, energy, climate change, biodiversity and dwindling natural resources (e.g. water) "is the great moral, economic and social imperative of our time" (Ban Ki-moon, 2007).

Although world food and fibre production continue to match total demand, almost 1 billion people face chronic malnutrition, and approximately 30% of the world's people lack food security. Non-food uses for crops and productive land (e.g. for biofuel production) increasingly threaten world food supply. Plant diseases also cause substantial reductions in productivity (yield and quality) of food (Strange & Scott, 2005), fibre and timber crops, and severe post-harvest losses of plant products. The overall average yield loss potential (losses without crop protection intervention) is estimated to be 18% for the eight most important food and fibre crops, ranging from 11-12% losses for soybean to 30% losses for potato (Oerke *et al.*, 1994; Oerke and Dehne, 2004). Estimated efficacy of plant disease control measures (Oerke & Denhe, 2004) is generally low, at 32% for fungal and bacterial pathogens and only 13% for plant viruses. Plant pathology, therefore, faces considerable challenges as a contributor in the continued provision of food, clothing and shelter for the world's burgeoning population.

Effective management of plant diseases (the application of "plant medicine") requires sustainable methods to reduce the deleterious effects of plant diseases on crop productivity. We must develop new approaches to effectively control plant diseases, providing solutions which do not harm the environment or human health. Development of effective integrated plant disease management requires multidisciplinary research, beyond the range of traditional plant pathology expertise. Plant breeding, soil science, microbiology, biochemistry, and molecular biology are examples of research disciplines that can assist developing the understanding required for effective disease control. New solutions will increasingly rely on the judicial application of modern biotechnology (see www.isppweb.org, 2000). Collaborative research is essential for development of appropriate, sustainable and effective plant disease management.

Plant diseases will continue to pose problems for production of food crops, for other agrarian activities, and in the world's natural and heritage environments. Our research discipline is therefore an important contributor to human well-being, as the world's population expands, and as efficient, environmentally acceptable crop production is increasingly required (and demanded) by consumers of nutritious, high quality food.

Key words: Plant medicine, Global food security, Integrated disease management, Multi-disciplinary collaborative research.

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MOLECULAR IDENTIFICATION OF NEW NATURAL BEGOMOVIRUS RECOMBINANTS ASSOCIATED WITH TOMATO YELLOW LEAF CURL DISEASE CO-EXISTING WITH PARENTAL VIRUSES IN LEGUME CROPS AND WEEDS IN TUNISIA

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Tomato yellow leaf curl virus (TYLCV; genus *Begomovirus*) is a major plant virus infecting a wide range of crop species worldwide. In Tunisia, the simultaneous presence of the Sardinia (TYLCSV) and Israel (TYLCV) species in the same host plant has recently been described (Pellegrin *et al*., 2008) but their occurrence as recombinants have never been reported.

An extensive survey was conducted by collecting leaves from late field tomato crops, other legume crops and nearby weeds exhibiting severe curling symptoms from the Tunisian Sahel Region and southern Tunisia in 2009.

Genomic DNA was extracted and purified according to Carling (2004). Extracted DNA and DNA of the reference recombinant TYLCV isolate (Accession no. NC_011024) were amplified according to Pellegrin *et al.* (2008). The presence of TYLCSV (366 bp) and TYLCV (750 bp) was revealed.

Samples infected with at least one of these TYLCV species were then tested for the presence of recombinants (RecA and RecB) according to Davino *et al.* (2008, 2009). These conditions enabled production of amplicons that included the intergenic region, (known to be a recombination site in Begomovirus) of ≈570 bp (TY2463/TY247; RecA) or ≈800bp (TY2222/TY255; RecB).

Of the 111 leaf samples collected (fig. 1; fig. 2 A, B), 14 were infected with TYLCV, 9 with TYLCSV and 77 with both TYLCV and TYLCSV (50 tomato, 14 *Solanum nigrum*, 5 pepper, 3 *Vicia faba, 3 Malva parviflora,* 1 *Chenopodium album,* 1 *Lantana camara*). Of the 100 infected samples, 8 tomato and 1 *S. nigrum* yielded a 570-bp product which was also amplified from the reference recombinant A isolate. The 570-bp amplicons from four tomato samples were cloned and sequenced (GU322870-GU322873), and one from one *S. nigrum* sample was directly sequenced (GU322874). The four isolates from tomato samples shared 93% nucleotide sequence identity with TYLCAxV (DQ317696.1) and TYLCMalV (DQ317720.1) species, and the one from *S. nigrum* shared 94% with the TYLCV/TYLCSV recombinant Ragusa (EU719096.1).

These results showed that mixed infection with TYLCV and TYLCSV species was common in tomato, legumes and ware species in the major Tunisian growing areas. Moreover, we report for the first time this new natural recombinant between TYLCV and TYLCSV in Tunisia.

Key words: Begomovirus, TYLCV, Recombinant virus, Tomato

Acknowledgements

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GLOBAL DISTRIBUTION OF THE *mtRFLP4* HAPLOTYPE OF *MYCOSPHAERELLA GRAMINICOLA*

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Previous genetic studies of *Mycosphaerella graminicola* based on RFLP analysis of the mitochondrial genome identified a host-specific haplotype. While this haplotype (*mtRFLP type4*) dominated among isolates collected from durum wheat (*Triticum durum*), it was not detected on bread wheat (*Triticum aestivum*) (Torriani *et al.,* 2008; Zhan *et al*., 2004). Nevertheless, host specialization in the *Mycosphaerella graminicola*-wheat pathosystem is still debated.

This study, addressed the issue of host specificity of *mtRFLP type4* by conducting a large-scale study including 1363 isolates sampled from bread wheat and durum wheat originating from 21 countries. *mtRFLP type4* is characterized by an additional insertion. We tested for the presence/absence of this insertion using a locus-specific PCR amplification (Torriani *et al.,* 2008). mtRFLP type4 was detected on both host species, but with a higher frequency on durum wheat. The distribution of *mtRFLP type4* was limited to *M. graminicola* isolates originating from the Mediterranean region. The highest frequencies were found in isolates from Tunisia (87%) and Algeria (60%). The haplotype was absent in isolates from Europe, Australia, North and South America.

These results suggest that *mtRFLP type4* would be characterized by differential adaptation to the prevailing growing conditions. The relative higher occurrence of the haplotype in North Africa (e.g. Tunisia or Algeria) than elsewhere could be attributed to durum wheat adaptation. The specialized haplotype subsequently spread as indicated by lower frequency of occurrence in the surrounding Mediterranean countries.

Keywords: *mtRFLP4, Mycosphaerella graminicola,* Bread wheat, Durum wheat

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ECOLOGY, EPIDEMIOLOGY AND CONTROL OF *ASPERGILLUS* SPP. IN PISTACHIO ORCHARDS IN GREECE

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Mycotoxin contamination of agricultural commodities is considered as a serious food safety issue worldwide. For this reason strict regulations govern the import and export sales of various food products to minimize the mycotoxins risk for human consumption and animal feed. One of the most carcinogenic mycotoxins is aflatoxin (AF) produced by *Aspergillus flavus* and *A. parasiticus*. During the last years, there were several cases of AF detection above the EU limits in pistachio nuts either produced in Greece or imported from other countries resulting in banning of these products for human or animal consumption. The goal of this study is to evaluate the AF contamination of pistachio nuts in major pistachio production areas in Greece, with varying climatic conditions, and to propose sustainable management strategies. The research is conducted on a large sampling pattern of pistachio nuts and soil collected from different orchards during a period of 3 years. The principle objectives of the project are to: a) assess the geographical and physiological divergence and distribution among *Aspergillus* spp. in pistachio nuts and orchards (Tjamos *et al.* 2006), b) assess the dynamics of the population composition of AF producers during the pistachios growing season, c) determine the AF content in nuts and study the epidemiology of AF contamination in correlation with meteorological data (Cotty and Mellon, 2006; Logrieco *et al.* 2003), d) evaluate novel biocontrol strategies by studying the antagonistic activity of a collection of yeasts (Demakopoulou *et al*., 2008) and atoxigenic *Asperigillus* isolates against *A. flavus* and *A. parasiticus* in laboratory and field experiments (Cotty and Mellon, 2006) and e) evaluate the efficacy of several fungicides in laboratory and field experiments against *Aspergillus*.

Experimental data of the 1st year showed that both *Aspergillus* section *Flavi* and section *Nigri* could be isolated from all parts of healthy and damaged pistachio fruits (hull, shell, nut). A collection of Aspergilli has been created and is currently being analyzed for morphological differences, sclerotium size, AF production and

vegetative compatibility grouping (VCGs) in order to estimate the diversity of the sample population. A better understanding of the population structures of *Aspergillus* spp. will facilitate the development of effective biocontrol strategies. HPLC analysis showed that a very critical step for AF production is maturity since it was the first stage that AF was detected at concentration levels above the limit. In one region, AF concentration was higher at harvest and post-harvest stages showing great variation among different orchards depending on the AF levels at pre-harvest stage, the drying method and the storage conditions. In another region the sampling showed the absence of mycotoxins at harvesting time but their presence during storage conditions but always at low concentrations (Georgiadou *et al.*, 2009).

Keywords: Pistachio, Aflatoxin, Mycotoxins, *Aspergillus,* Biological control

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Petria 20 (2), 67-633 (2010)

POPULATION FEATURES OF *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS* AND EPIDEMIOLOGY OF WALNUT BLIGHT IN ROMAGNA (ITALY)

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Bacterial blight of walnut caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is an emerging disease, which has the potential to severely affect walnut orchards (Mulrean and Schroth, 1981). Symptoms are visible on all aerial parts of the host plant and particularly on leaves and nuts; the disease develops more rapidly during spring, causing spots on leaves and immature fruits, followed by the formation of small cankers on leaf petioles and twigs. Affected fruits fall down throughout the growing season, with a peak from mid-May to mid-June. Primary inoculum is released early in spring, from small overwintering cankers present on twigs. Primary inoculum is spread by wind-driven rain droplets and by pollen.

Our study confirms the spread by pollen, but catkins seems to become infected during their spring development from bacteria oozing out from small twig cankers. Female flowers are not contaminated before pollination, and become infected during pollination and/or during spring rain. The source of primary inoculum appears to be the small overwintering cankers developing near the fruiting buds.

The population structure of a broad collection of *Xaj* isolates, obtained from affected orchards in Romagna, confirms the presence of different genetic groups, identified by rep-PCR (using the REP, BOX and ERIC primers) and by multilocus sequence typing (MLST) and multilocus variable number analysis of tandem repeats (MLVA). Copper resistance was studied on a wide collection of over 150 *Xaj* strains isolated in Romagna walnut orchards during 2007-2009: 83% of the collection strains proved to be tolerant to copper, whereas 36% proved to be highly resistant.

Control strategies are difficult to implement and are based on the timely effective use of copper compounds with an emphasis on spring treatments. The Walnut Blight Forecast Model "Xanthocast", developed in California (Adaskaveg *et al*., 2004), is under evaluation in European walnut orchards. In order to avoid the development of copper resistance, the use of possible resistance inducers is under evaluation, coupled with a reduced use of copper. Glucohumates (active humic and fulvic acids, obtained from leonardite and gluconic acid) were able to control lesion development on walnuts *in vitro* and reduce disease incidence in field experiments.

Key words: *Juglans regia, Xanthomonas arboricola* pv. *juglandis*, Population structure, Epidemiology, Control

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GRAPEVINE AND OLIVE DISEASES IN AUSTRALIA

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Grapevine and olive diseases are caused by several fungal pathogens. They have serious impacts on both fruit yield and quality of wine and olive oil. Several fungi, some of pathogenic importance, were observed on grapevines and olives from different grape and olive growing regions of Australia.

Grapevines: Different parts of grapevine such as trunk, dormant canes, green shoots, leaves, immature, mature and mummified berries of different cultivars such as Chardonnay, Shiraz, Semillon, Cabernet Sauvignon, Pinot Noir and Merlot were examined. The symptoms caused by the different fungi were bleached canes, internal wood rot, dieback, bud necrosis, leaf spots and fruit rots. Fruit rot diseases of high economic importance were grey mould (*Botrytis cinerea*), ripe rot (*Colletotrichum acutatum*), bitter rot (*Greeneria uvicola*), downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*). Phomopsis rot (*P.viticola*), Pestalotiopsis rot (*P. uvicola*), white rot (*Coniella diplodiella*) and Botryosphaeria rot (*B. dothidea*) are rare in Australia and of little economical significance (Sergeeva, 2001). *Rhizopus, Aspergillus, Penicillium* and *Alternaria* spp. were isolated from rotted berries at harvest; however these fungi appear to be secondary pathogens and of minor importance. Other important grapevine diseases are caused by wood infecting fungi which predominantly attack the trunk and canes. Phomopsis cane bleaching occurs in most viticultural regions. *Greeneria uvicola* isolated from canes in New South Wales showed symptoms of dieback. This pathogen was also seen in asymptomatic canes (Sergeeva, 2004). *Colletotrichum* species were isolated from canes. Morphologically distinct taxa of appendaged Coelomycetes have been recognised as occurring on grapevines in Australia. These are *Pestalotiopsis uvicola*, *P. menezesiana*, *Seimatosporium hysterioides*, *Truncatella angustata*, and *Sporocadus rhododendri* (Sergeeva et *al.*, 2005). Botryosphaeriaceae species are recognised as important wood-infecting pathogens of grapevines. The distribution of Botryosphaeriaceae species in vineyards throughout the major winegrowing regions produces a broad range of effects on grapevines that can be potentially severe. These symptoms vary depending on the species of Botryosphaeria infecting aerial parts or through soil–root transmission. Eutypa dieback, caused by *Eutypa lata*, is a major trunk disease of grapevines in NSW and South Australia. Esca disease complex involving several fungi, including *Phaeomoniella chlamydospora* and *Fomitiporia* is rarely observed in Australia.

Olives: Leaves, flowers and fruits of different cultivars such as Nevadillo, Correggiola, FS-17, Picual, Barnea, Frantoio, Manzanillo were examined, Anthracnose,

caused by *Colletotrichum acutatum* and *C. gloeosporiodes* is a common disease particularly in the summer-dominant rainfall regions where fruit rot in ripening olives is a serious problem. Mummified olive fruits were observed when the fruits began to ripen. Some isolates were obtained from olive flowers and leaves (Sergeeva et *al.*, 2008). Cercosporiose (*Pseudocercospora cladosporioides*) causes serious defoliation, although fruit damage may be as important as the leaf infection caused by this pathogen (Sergeeva et *al.*, 2008). The observed symptoms and epidemiology, together with the widespread occurrence of peacock spot of olives caused by *Fusicladium oleagineum*, suggest that it has potential to be an important disease of olives in Australia. *F. oleagineum* and *P. cladosporioides* may occur throughout the year, as young, susceptible olive leaves are always available in these groves. *Neofusicoccum luteum* was occasionally isolated from roting fruits. Minor root diseases caused by soil-inhabiting fungi *Macrophomina phaseolina, Rhizoctonia*, *Fusarium* and species of *Phytophthora* can cause serious diseases in olives such as reduced growth, wilting, root necrosis and, in severe cases, death of the plant. Severity of root disease appears to depend on climate, soil, site and cultural practices. *Verticillium dahliae* was not recorded as a common problem in the project's diagnostic activities. The defoliating strain of *Verticillium* has not been detected in Australia. Olive knot (*Pseudomonas savastanoi*) and bacterial canker (*P. syringae*) were recorded on olives in SA, but do not appear to be a widespread problem.

Key words: Grapes, Olives, Diseases

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Petria 20 (2), 67-633 (2010)

ADDITIONS TO THE RUST MYCOBIOTA OF MARAGHEH AREA, NW IRAN

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Rust fungi (*Basidiomycota, Pucciniales*) are biotrophic plant pathogens with complex and often cryptic life cycles and are among most destructive diseases of orchards and field crops. Rust fungi are unique within the *Eumycota* in many aspects, including the evolution of heteroecism and the numerous (up to six) different spore types that may be produced by a single species. The Pucciniales is the largest order among the *Basidiomycota*, with about 7000 species currently placed in approximately 14 families and 160 genera.

During spring, summer and fall of 2009 different parts of the Maragheh area (including gardens and fields) were regularly visited (usually at monthly intervals from May to December) and the trees, field crops and wild plants were inspected for rust infection. Several rust fungi on *Salix alba*, *Populus nigra*, *Euphorbia seguieriana*, *Tanacetum balsamita* and *Hibiscus syriacus* were collected. The morphology of urediniospores, paraphyses and teliospores was examined using a light microscope and scanning electron microscopy (SEM). For SEM images 5 × 5 mm dry leaf segments carrying uredinia were coated in gold and placed in the low-vacuum, variable-pressure chamber of a Hitachi S3500 SEM and urediniospores were photographed with a digital camera at 3500 × magnification. The spine distances were measured from 50 pairs of randomly chosen adjacent spines in SEM images. For molecular study DNA extractions (referred as samples) were made from urediniospores using the Qiagen Plant DNeasy mini-kit following manufacturer's instructions. Basidiomycete-specific primers ITS1-F and ITS4-B were used for amplification and sequencing of the ITS rDNA region of samples. Amplified products were purified using EXO-SAP-IT PCR cleanup kits and sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit and resolved on an ABI automated DNA sequencer.

The following host-rust combinations were recorded: *Populus nigra-Melampsora allii-populina*, *Salix alba* (isolate 1 & 2)-*Melampsora salicis-albae*, *Salix alba* (isolate 3 &4)-*Melampsora* sp., *Euphorbia seguieriana-Melampsora euphorbiae*, *Tanacetum balsamita-Puccinia balsamita*, *Hibiscus syriacus-Puccinia malvacearum*.

In this preliminary study, results for poplar and willow (isolates 1 & 2) rusts were confirmed by both morphological characteristics and molecular approaches

but in the case of willow (isolates 3 & 4), euphorbia and tanacetum rusts, results of morphological studies were generally not congruent with molecular approaches, so further investigations are being undertaken.

Key words: Pucciniales*, Melampsora, Puccinia,* willow rust, rDNA, *Populus*, willow, *Salix*, Rust fungus

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SESSIONE 1

Disease epidemiology

POSTERS

PETRIA 20 (2), 67-633 (2010)

FUSARIUM POPULATION IN SYRIAN WHEAT SEEDS

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Wheat is one of the main crops in Mediterranean countries and Fusarium Head Blight (FHB) is considered important disease in the Mediterranean basin and worldwide (Stack, 1999; Logrieco *et al*., 2003).

The causal agents of the disease are different *Fusarium* species, responsible for losses in grain quantity and quality as well as mycotoxin accumulation (i.e. deoxynivalenol - DON). Mycotoxin concentration in food and feed is strictly regulated by EU, since high levels are responsible for health hazards to humans and animals (Logrieco *et al*., 2003).

Wheat cultivation in Syria (i.e, year 2007, 1667732 ha) is very important for the Syrian economy. Currently, there are no published records on the epidemiological and etiological aspects of the FHB syndrome.

Based on these premises, we performed *in vitro* analysis on samples of wheat kernels (durum and bread), originated from different Syrian cultivated areas, as seeds are one of the main ways for FHB spread. Four hundred kernels per sample were analyzed following the methodology described by Prodi *et al.* (2009).

Different fungal genera present on the kernels were identified at light microscope according to descriptions by Domsch *et al.* (1980). The *Fusarium* species were morphologically identified (Leslie and Summerell, 2006) and for some of them PCR techniques, using specific primerswere applied to confirm the morphological identification.

The obtained data revealed that *Alternaria* and *Cladosporium* were the most frequent fungal genera isolated in the examined kernel samples while *Fusarium* spp. were present in low percentage. The species found in this study were *F. culmorum*, *F. equiseti-incarnatum* complex, *F. oxysporum* and *F. tricinctum*. The strains of *F. culmorum*, one of the most common worldwide pathogens for FHB, were examined for chemotypes based on the presence of gene for mono- acetylated DON derivatives (3-ADON, 15-ADON) and nivalenol (NIV).

Key words: Syria, Kernels, Wheat, Fusarium

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EFFECT OF RICE-WHEAT SYSTEM ON DISEASES SCENARIO IN MAZANDARAN

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Cultivation of wheat after rice has expanded tremendously in some regions of Mazandaran like Behshahr, Neka and Sari. This system has been associated with several agro ecological changes and pathogen dynamics (Colbach *et al*., 1997; Deacon, 1973). In this system, there is a phenomenal change in the disease scenario of the wheat crop (Hollins *et al*., 1986).

In this study, surveys of wheat crops in Mazandaran Province of Iran were carried out during 2002-2004. In 50 out of 120 fields, diseases like *Alternaria* leaf spot, grain discoloration and Fusarium head blight became the emerging problems of the region, whereas some foot rot diseases like take-all caused by *Gaeumannomyces graminis* var. *tritici,* which is the most prevalent foot and root rot diseases of wheat in the region, was not found due to flooding of rice fields during summer.

Key Words: Alternaria leaf spot, *Fusarium*, *Gaeumannomyces*, Take-all

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PREVALENCE OF *PYRENOPHORA TRITICI-REPENTIS* ON BREAD WHEAT IN MAZANADARAN PROVINCE, IRAN

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Tan spot of wheat caused by *Pyrenophora tritici-repentis*, is becoming more important in some parts of the world (Wright and Sutton, 1990), including Iran, particularly since the wheat variety N-8019 was introduced in Mazandaran Province of this country. The majority of current bread wheat cultivars in the region are susceptible to the disease (Singh and Hughes, 2006; Strelkov *et al*., 2002), and *P. tritici-repentis* can cause severe yield losses. The pathogen survives in wheat stubble and infects new crops.

A survey was carried out in 2008 to determine the distribution and prevalence of tan spot in Mazandaran province including Galoogah, Behshahar, Neka, Sari, Ghaemshahar, Babol, Babolsar and Joibar. Percentage infection in several wheat fields of each area was determined. The results indicated that the disease was widespread throughout the province and was observed on all commercially grown cultivars including N-8019, Daria, Tajan, Milan, Shanghai and Rasool. The highest level of infection was observed on N-8019 in Sari (95%), and Neka (75%), whereas the cultivar Rasool in Behshahar had the lowest incidence level (5%) of the disease.

Key Words: Bread wheat, *Pyrenophora tritici-repentis*, Tan spot

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ALBUGO SPECIES ON WEEDS IN EASTERN CROATIA

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Species of the fungal genus *Albugo* causes white rust or white blister diseases. They are obligatory plant parasites among which some of them are known as crop pathogens (Van Wyk *et al.,* 1999). Different fungal species infect all parts of weeds. Fungal parasitic activity can reduce weed vitality or even cause their decay. Many weed species can be alternative hosts to disease agents of cultivated plants and play an important role in diseases epidemiology (Cosic *et al*., 2008). In the frame of a project supported by the Ministry of Science, Education and Sports Republic of Croatia, the role of weeds in epidemiology of row-crop diseases "weeds mycopopulation" has been studied.

The aim of our research was to identify *Albugo* species that occurred on weeds in eastern Croatia. Weed plants with disease symptoms characteristic for *Albugo* species have been collected since 2001 on location Slavonia and Baranja country (Croatia). They were grown in sunflower, sugar beet, corn and soybean fields. . Morphological characteristics are determined by preparing native preparations taken from fresh material and observation under light microscope conditions. Host tissue containing oospores was soaked in water, and carefully squashed with a needle. Sporangia (conidia) and oospores measurement were carried out using a camera and Olympus DP Soft software.

Identification was based on parasite morphological characters and weed species which was attacked according to descriptions of Choi and Priest (1995) and Branderburger (1985). On *Amaranthus retroflexus* and *Amaranthus hybridus* leaves we determined *A. bliti* (Biv.) Kuntze. The disease appeared as scattered sori restricted to one or a few leaves (local infections) or in the most cases the fungus spread throughout much of a plant parts (systemic infections). *Capsella bursa-pastoris* is host for *A. candida* (Pers.) Kuntze. White to cream-colored, blister-like (pustules) lesions on leaves, stems, floral handle and inflorescences were determined. On *C. bursa -pastoris* local and systemic infections were presented. *Ambrosia artemisiifolia* and *Cirsium arvense* are hosts for *A. tragopogonis* (Person) S.F. Gray. On *A. artemisiifolia* the disease appeared to one or few older leaves or mostly symptom spread on all leaves. Infected plants were smaller than normal and internodes were shortened. On *C. arvense* we noticed only local infections. *Albugo portulacearum* (*Schltdl.*) Kochman & T. Majewski was determined on *Portulaca oleracea* leaves. Depending on the year and location the intensity of white rust diseases vary, while *A. candida* on *C. bursa-pastoris* is widespread and the disease is present every year in relatively heavy intensity. *A. tragopogonis* on *A. artemisiifolia* has been determined only in 2001 and 2002 years. Details about symptoms and morphological characteristics will be presented in the paper.

Key words: *Albugo*, Weeds, Eastern Croatia

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CURRENT STATUS OF POWDERY MILDEW OF SUGAR BEET IN ISFAHAN PROVINCE, IRAN

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Different beet crops such as sugar beet, vegetable beet and forage beet are of economic importantance in Isfahan province, Iran. Powdery mildew disease caused by *Erysiphe polygoni*, *E. betae* and *E. communis* is one of the important diseases of beet crops, which occurs epidemically in sugar beet fields, causing considerable reduction in quality and quantaty of affected crops. For this reason, the beet growing areas which include Isfahan, Semirom, Fraydan and Khorasgan were considered for sugar beet; Falavarjan for vegetable beet; and Ardestan for forage beet. The severity of the disease was assessed at the six growth stages of the crop in ten fields per region based on six distinct scoring scales of 0, 10, 25, 50, 75 and 100 respectively. Results indicated that, the mean severity of infection varied significantly from field to field, region to region and the type of growing beet. The overall severity of infection in sugar beet field in six growth stages was 27.48, 22.66, 29.45, 25.67, 25.32 and 21.16 percent, whereas for vegetable beet, the severity of infection was 44.88, 33.84, 32.27, 44.66, 33.06 and 30.33 percent and for forage beet it was 9.69, 14.14, 10.71, 13.91, 17.83 and 8.54 percent, respectively. The total mean of powdery mildew severity on all types of growing including beets, sugar beet, vegetable beet and forage beet, were 26.86, 23.06, 27.30, 28.88, 25.58 and 19.06 percent respectively. The microscopic studies of the sexual stage of the pathogen indicated the presence of the cleistothecium at the late stages of the growth, including asci and ascospores, confirming the cause of the powdery mildew in these regions.

Keyword: Sugar beet, Vegetable beet, Forage beets, *E. polygoni*, Powdery mildew

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PHYTOPHTHORA NIEDERHAUSERII ON ENGLISH IVY IN ITALY

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English ivy (*Hedera helix*) represents a widely cultivated, highly adaptable ornamental plant. Decline and death of several varieties of ivy have been observed in commercial nurseries located in the Marche region (central Italy) since 2005. Collar and root rot was always found in association with declining and dead plants. A *Phytophthora* species was consistently isolated from the margin of lesions. Colonies on potato dextrose agar (PDA) appeared rosaceous and faintly petaloid, with a snowflake-like pattern and waxy appearance. The isolates produced non-papillate persistent, ellipsoid to ovoid sporangia. All the isolates examined were heterothallic, mating type A1, and produced oogonia with predominantly amphigynous antheridia. The internal transcribed spacer (ITS) of rDNA and cytochrome c oxidase subunit I (*Cox*I) were sequenced and showed 100% and 99% identity with *Phytophthora niederhauserii* sequences retrieved from GenBank for ITS and *Cox*I respectively. Thermophilic isolates able to grow at 37°C were identified. *P. niederhauserii* was reported as a new species in 2003 on *Thuja occidentalis* and *H. helix* plants grown in glasshouses in North Carolina (USA) (Abad and Abad, 2003). Since this initial discovery, *P. niederhauserii* has been reported in different continents on different plant species (Moralejo *et al*., 2009). This pathogen has mainly reported on ornamental potted plants grown in a confined environment such as nurseries and greenhouses (Herrero *et al*., 2008). Though our isolates were obtained from ivy only, numerous ornamental species are affected by this pathogen (Herrero *et al*., 2008), among which *Banksia* spp. (Cacciola *et al*., 2009a), *Callistemon citrinus* and *Cistus salvifolium* (Cacciola *et al*., 2009b) have been reported in Italy. Plant trade can be considered as the principal pathway for the introduction of this invasive and exotic pathogen. These findings suggest that *P. niederhauserii* is a potential threat for the nursery industry and possibly for natural ecosystems.

Key words: *Hedera helix*, Root and collar rot, Nursery, Oomycetes

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EFFECT OF ELEVATED CO_2 AND TEMPERATURE ON INFECTION OF GRAPEVINE BY POWDERY MILDEW UNDER CONTROLLED ENVIRONMENT

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Climate change involves rising of atmospheric CO_2 level and temperature. Carbon dioxide concentration is predicted to reach 730 to 1020 ppm by 2100, due to increasing world population and economic activity (Garrett *et al*., 2006). Plant responses to elevated CO_2 and temperature have been much studied in recent years, but effects of climate change on pathological responses are largely unknown. Increases in CO_2 and temperatures are expected to induce complex effects on plant pathosystems, on host-pathogen interactions, gene expression, plant physiology and population biology (Garrett *et al.,* 2006). Based on a review of literature, Coakley *et al.* (1999) suggested that elevated CO_2 would increase leaf area and duration, leaf thickness, canopy size and density, stomatal density and consequently influence host-pathogen interactions. Among plant diseases, powdery mildews are expected to become more important under temperature increase, which can affect directly or indirectly both hosts and pathogen (Runion *et al*., 2003). In particular, elevated CO_2 would increase canopy size and density and, when combined with increased canopy humidity, would promote foliar diseases such as rusts, powdery mildews, leaf spots, and blights (Chakraborty, 2005).

The pathosystem grapevine (*Vitis vinifera*) - powdery mildew (*Eysiphe necatrix*) was chosen as a model to assess the potential impact of increased CO_2 and temperature on disease incidence and severity. Previous studies simulated future scenarios of downy mildew (*Plasmopara viticola*) epidemics on grape by using simulation models, assessing the potential impact of climate change on the time of first seasonal disease outbreak and project future disease dynamics in the most important grape-growing areas in the world (Salinari *et al.*, 2007).

Grapevine potted plants, belonging to the cv Moscato and Barbera, were grown in phytotrons under 4 different simulated climatic conditions: (1) standard temperature (ranging from 18° to 26° C) and standard CO_2 concentration (450 ppm); (2) standard temperature and elevated CO_2 concentration (800 ppm); (3) elevated temperature (ranging from 22° to 30° C, 4° C higher than standard) and standard CO_2 concentration; (4) elevated temperature and CO_2 concentration. Each plant was inoculated with 2 ml of 10^5 cfu/ml of spore suspension. Disease index and physiological parameters (chlorophyll content, fluorescence, assimilation rate) were assessed.

Results showed an increase of the chlorophyll content with higher temperatures

and CO_2 concentration, to which consequently corresponded an higher fluorescence index. Disease incidence varied according to the different cultivar, but differences were not statistically significant. Our trials indicate that an increase in CO_2 is not increasing powdery mildew incidence, probably due to the increased photosynthetic activity of plants under such conditions. On the contrary, considering that the rising concentrations of CO_2 and other greenhouse gases will lead to an increase in global temperature and longer seasons, we can assume, as also suggested in Coakley *et al*., (1999) and in Garrett *et al.,* (2006) that the rising of CO_2 will allow more time for pathogen evolution and could increase pathogen survival, indirectly affecting an increase in powdery mildew of grapevine.

Key words: climate change, *Vitis vinifera, Erysiphe necatrix*

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BAYOUD DISEASE IN CENTRAL ALGERIA: HISTORY, DISTRIBUTION AND STRATEGIES FOR CONTROL

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The Bayoud disease, a vascular wilt caused by *Fusarium oxysporum* f. sp. *albedinis* (*Foa*), is considered as the main problem of date palm in North Africa. Bayoud has destroyed in one century more than twelve million palms in Morocco (Djerbi, 1983) and three million in Algeria in western and central oases (Brochard and Dubost, 1970; Dubost and Kellou, 1974). In recent years no systematic field investigation has been completed to evaluate the progress of bayoud. In 2008 the oases of Azoua and Bouanji, Algeria, have been surveyed for the presence of symptoms of Bayoud. The first symptom of the disease on each affected plant appears on one or more leaves of the middle crown that wither in a characteristic way: some pinnae or spines situated on one side of the leaf become white; then the disease progresses from the base to the apex of the leaf. The disease advances to the central leaf cluster and the tree dies when the terminal bud is affected. This process may take a few days to several weeks. Wilt and dieback have been observed on 33% of the 987 date palms investigated in 15 plots randomly chosen in the oases. The varieties of date palms Tegaza, Dgel and Tazarzeit were the most susceptible with mortality up to 60%.

Foa has been isolated from soil of plots affected by bayoud, apparently asymptomatic as well as from palm groves co-cultivated with vegetables (tomato, coriander, garlic, onion, lettuce, tobacco).

The density and distribution of *Foa* among fungal populations in soil has been studied and related to the structure of the fungal community. We have developed strategies for controlling bayoud, based on biological treatment and prevention. Efficacy of these methods for protecting date palm seedlings from the disease is discussed.

Key words: Date palm, Wilt, Biological treatment

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ROOT ROT PATHOGENS ON CALAMONDIN GRAFTED ON VOLKAMERIANA LEMON IN SICILY

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During July-August 2009, ornamental plants of Calamondino (*Citrus madurensis* oureiro), grafted on self-rooted Volkameriana lemon (*Citrus volkameriana* Lt. and Pasq.), showed widespread canopy yellowing, reduced development of leaf blade, apical leaf desiccation and death of plants. Feeder roots showed the absence of the cortex, while those apparently healthy, after slight pressure of fingers, easily break cortex showing the white woody cylinder.

Isolation from symptomatic tissues and soil were performed by using the selective media BNPRAH, (Masago *et al*., 1977) and PPA, (Nelson *et al*., 1983) for *Phytophthora* spp. and *Fusarium* spp., respectively. For each sample the percentage of infected root segments (RI), and the inoculum density (ID), of the patogen, expressed as the number of propagules per gram of dry soil (ppg), were determined. The ID was assessed by soil diluition plate method and identification was carried out on the basis of colony morphology and fungal structures, according to Stamps *et al*., (1990) and Leslie and Summerell (2006), and confirmed by comparing ITS sequences for *Phytophthora* and beta-tubulin and translation elongation factor sequences for *Fusarium* with GenBank databases. *Phytophthora nicotianae* and *Fusarium solani* were found associated to 100 and 60% of the samples analyzed, respectively. *P. nicotianae* showed RI values between 10 and 100% and ID values between 2 and 12 ppg. *F. solani* values of RI were between 32 and 50%.

In view of the heavy damage observed in the nursery, (more than 50% of plants showing symptoms), it can be hypothesized that grafting with Calamondin may have lowered the tollerance of Volkameriana lemon to *Phytophthora* root rot (Ippolito *et al*., 1997).

Key words: Feeder root rot, *Phytophthora nicotianae*, *Fusarium solani*, Self-rooted, Volkameriana lemon

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PHAEOACREMONIUM SPECIES ASSOCIATED WITH GRAPEVINE DECLINE IN ALGERIA

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Despite the importance of grapevine cultivation in Algeria, there have been few studies of the diseases that affect this crop. Since the early reports of "apoplexy" by Debray in 1892, and reports of high mortality rates of grapevines (Ravaz, 1905) there have been no other studies until 2003 when a preliminary survey was carried out (Berraf and Peros, 2005). The survey revealed a high percentage of dead vines, and vines affected by either Eutypa dieback or esca (Berraf and Péros, 2005). Several species of *Phaeoacremonium* have been isolated worldwide from grapevines with symptoms of trunk diseases, especially Petri disease in young plants and esca in old vines, considered to be the most destructive decline diseases in grapevine. They are responsible for considerable loss in yield and are the main causes of the shortened production life of vineyards. However, until now there is no information on the species of *Phaeoacremonium* that occur on grapevines in Algeria.

In the present study, 200 grapevines with typical symptoms of esca and dieback in the main grapevine production areas of the north of Algeria were studied. Cross and longitudinal sections of the rootstocks and the stems of each vine were examined and isolations were made from different zones of the necrotic tissue. Small pieces of wood (10×5×5 mm) were cut from the margin of the soft white rot, black line, the sectorial and the central brown zone and the black spots as described by Larignon and Dubos (1997). The pieces were disinfected in calcium hypochlorite, rinsed and then placed on potato-dextrose agar (Difco Laboratories, Detroit, Michigan, USA) plates. After a two months incubation at room temperature and observations carried out every 2–3 days, fungi with colonies corresponding to *Phaeoacremonium* were subcultured on PDA. Isolates were grouped according to their (MSP-PCR) profiles and representatives of each group were selected for sequencing of the β-tubulin and actin genes. Phylogenetic analysis based on partial sequences of the β-tubulin and actin genes placed the isolates in four species, namely *P. aleophilum*, *P. parasiticum*, *P. venezuelense* and *P. hispanicum*. These phylogenetic species were confirmed by comparing morphological features with published descriptions (Mostert *et al*., 2006; Gramaje *et al*., 2009).

Phaeoacremonium species were isolated from 49 of the 200 samples processed. The majority were *P. aleophilum* (67.3%) followed by *P. parasiticum* (16.3%), *P. venezuelense* (14.3%) and *P. hispanicum* (2.1%). *Phaeoacremonium aleophilum* is recognized as the most common species on grapevines worldwide and thus was expected to be the most common one in this study. However, it was interesting to find such a high proportion of *P. venezuelense* since this species has been reported only from Venezuela (Mostert *et al*., 2006). Also of interest was the single isolate of *Pm. hispanicum*, which was described recently and has thus far been found only in Iran and Spain. This work highlights the importance of further studies on this genus on grapevines in Algeria, and indeed indicates that the role played by fungi on the health of Algerian grapevines should be studied in detail.

Key words: Actin, β-tubulin, MSP-PCR, Phylogenetics

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PHOMOPSIS SP. IS ASSOCIATED WITH KIWIFRUIT ROT DISEASE IN ITALY

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Post-harvest diseases of kiwifruit (*Actinidia deliciosa*) cause severe losses during storage, transportation, marketing, and in retail stores or during shelf-life period. Postharvest losses have been often underestimated since a great amount of fruit which seemed healthy in appearance turned out to be decayed after peeling fruit skin.

Phomopsis sp. was found to induce soft rot decay tissue on kiwifruit during storage. Symptoms were as those reported for Phomopsis rot, with inner tissue rot accompanied by tissue disorganization. The brown pubescent skin at the area becomes soft and lighter in color than the adjacent healthy tissue. When the skin is peeled, the affected flesh tissue is usually water soaked, disorganized, soft and lighter green than the healthy one. Rotted fruit often has fermented odor. This symptomatology was typically reproduced only on wound-inoculated fruit. Symptoms, modality of infection reproduced by inoculations, and temperature ranges were similar to those described for *Diaporthe actinidiae* (Kho *et al*., 2005; Lee *et al*., 2001).

Nevertheless, the Italian isolates from rotted kiwifruit were genetically distant from *D. actinidiae* on the basis of internal transcribed spacer (ITS) of rDNA sequence comparison. The Italian *Phomopsis* sp. grouped together with *Phomopsis* sp. group 6 from grape (Niekerk *et al*., 2005), *D. conorum* from Norway spruce, and *P. vaccinii* from blueberry. The genetic and pathogenicity investigations on this pathogen may give additional insights into factors contributing to the disease and may have implications for successful management and control measures. Since postharvest fruit rots occur after harvest, after cool storage, or after cool storage followed by a shelf-life period, kiwifruit should be carefully handled in order to prevent wounds that are conducive to this disease.

Key words*:* *Actinidia deliciosa*, Fruit diseases, Fungi, Fungal diseases, Postharvest diseases

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MAIN OLIVE DISEASES IN MONTENEGRO

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During the last several years a survey of the olive diseases and their harmfulness on olive production in Montenegro has been done.

The most important disease is caused by *Spilocaea oleaginea* (Cast.) Hugh. since the prevailing domestic olive varieties are very susceptible to the parasite. Similar case is with *Camarosporium dalmaticum* (Thüm.) Zachos & Tzav.-Klon. [=*Sphaeropsis dalmatica* (Thüm.) Gigante], which is the next one according to caused damages. The opposite case is with *Pseudomonas syringae* pv. *savastanoi* (Smith) Young that occurs in significant extent only on some susceptible introduced varieties which are not widespread so far. *Pseudocercospora cladosporioides* (Sacc.) U. Braun (=*Cercospora cladosporioides* Sacc.) seems to be dangerous to some introduced cultivars for table use, while *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. causes significant damages affecting both immature and ripe fruits of several introduced and local varieties (Vucinic and Latinovic, 1999; Latinovic and Vucinic, 2002). Other parasites such as *Marthamyces panizzei* (De Not.) Minter (=*Stictis panizzei* De Not.) and *Hysterographium fraxini* (Pers.) De Not. occur sporadically (Mijuskovic, 1999; Vucinic, 1999). Recently *Verticillium dahliae* (Kleb.) on olive cultivar 'Leccino' was established but its importance will be better examined in the future.

Key words: Olive diseases, Montenegro

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PETRIA 20 (2), 67-633 (2010)

SEASONAL VARIATION IN ROOT INFECTION AND POPULATION LEVELS OF *PHYTOPHTHORA* SPP. IN CITRUS NURSERIES IN EGYPT

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Phytophthora root rot is the most destructive disease of citrus production in Egypt (El-Mohamedy, 1998). The pathogen is generally present in citrus nurseries, where potted soil contains the survival propagules that are responsible for its spread in new orchards. This study was aimed at monitoring the seasonal variation of *Phytophthora* spp. in soil and feeder roots in two Egyptian citrus nurseries and to detect and identify the species of *Phytophthora* associated with the disease. Soil and root samples were collected monthly from Sour orange and Volkameriana lemon rootstocks during March-July period. The inoculum density of *Phytophthora* spp. and the percentage of infected feeder roots were calculated using the plate dilution method in conjunction with selective media (Massago *et al.,* 1977).

Phytophthora isolates were identified according to their morphological characteristics and on the basis of the ITS regions of the rDNA. Results showed that different species of *Phytophthora* were isolated from both soil and roots. According to morphological (Stamps *et al*., 1990) and molecular identification, *Phytophthora nicotianae* was the predominant species followed by *P. citrophthora* and *P. palmivora*. In both nurseries, *P. nicotianae* was detected, while *P. citrophthora* and *P. palmivora* were recovered only from one of the two nurseries. The level of population and seasonal variation varied according to the rootstock, environmental condition, and nursery management practices.

Key words: *Phytophthora palmivora***,** Citrus**,** ITS

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Petria 20 (2), 67-633 (2010)

SOME OBSERVATIONS ON TWO MEDITERRANEAN *PINUS* SPECIES FACING THE *HETEROBASIDION IRREGULARE* INTRODUCTION IN ITALY

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The presence of *Heterobasidion annosum* (Otrosina and Garbelotto, 2009), hitherto known as the North American P ISG of *H. annosum* has been well documented in the last few years in Italy (D'Amico *et al.*, 2007; Gonthier *et al.*, 2007). In 2008 the occurrence of an isolate of this species was recorded for the first time on *Pinus halepensis* in Italy (Scirè *et al.*, 2008). A cross pathogenicity test was performed on *P. halepensis* and *P. pinea* using *Heterobasidion* isolates CRA-PAV PF 102 (the only *H. irregulare* individual collected from *P. halepensis* in Villa Doria-Pamphili, Rome, Italy) and CRA-PAV PF 103, a *H. annosum s. s.* isolate, which had been collected from a *P. pinea* tree in the coastal pinewood of Sabaudia (Italy) and was previously confirmed to be pathogenic on *P. pinea* (data not shown). At the end of June 2007, three-year-old seedlings of *P. halepensis* and *P. pinea* (30 replicates) were inoculated with each isolate. A mycelium disc cut from a 10-day-old culture on PDA was used as inoculum. It was placed on a 4 mm diameter wound made on the stem of the seedling 7 cm above the root collar, where the stem diameter was 8-10 mm. As a control, 30 seedlings of each tree species were inoculated with uncolonized PDA discs. All seedlings were incubated in a greenhouse under natural light conditions and 18-28°C. Re-isolation was attempted three weeks later. The stems were cut into 2.5 mm discs, 14 of them above and 14 below the inoculation (70 mm in total). The fragments were incubated on a selective medium (Kuhlman and Hendrix, 1962) and longitudinal growth of the fungus in the stem was determined. Growth data were log-transformed before a two-way ANOVA was conducted, with isolate and host as factors. Moreover, a chi-square test was used to assess significance of presence/absence of infection at the inoculation site only.

The mean growth of the studied isolates was: 12.1 mm and 8.5 mm (*H. irregulare* and *H. annosum,* respectively) in *P. halepensis*, and 32.5 and 33.6 (*H. irregulare* and *H. annosum* respectively), in *P. pinea*. ANOVA showed that differences in growth inside the hosts were not due to different behaviour of the isolates, but rather to host characteristics, and no interaction host/isolate was present. Furthermore, no significant difference was found in susceptibility of the two hosts to colonization by the *H. irregulare* isolate (6.7 and 13.3% of infection failures in *P. halepensis* and

P. pinea respectively) ($\chi^2 = 0.74$; d.f. = 1; p = 0.39). However, the two host species differed in their susceptibility to the *H. annosum* isolate ($\chi^2 = 6.67$; d.f. = 1; $p < 0.01$), with the pathogen failing to colonize 33.3% of the *P. halepensis* vs. 6.7% of the *P. pinea* seedlings.

In conclusion, our data indicate that the host range for *H. irregulare* may be wider than initially thought, and suggest that this pathogen may pose a potential threat to other important Mediterranean conifer species.

Key words: *Heterobasidion annosum*, *Heterobasidion irregulare*, *Pinus halepensis*, *Pinus pinea*

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Petria 20 (2), 67-633 (2010)

PHYTOPHTHORA SPECIES ASSOCIATED WITH CHESTNUT STANDS IN ITALY AND GREECE

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Ink Disease of chestnut (*Castanea sativa* Mill.) represents one of the major threats for agricultural as well as forestry ecosystems, especially since the hypovirulence phenomena reduced the impact of chestnut blight epidemic (Vannini and Vettraino, 2001). It causes yellowing and lightening of crown, flame shaped necroses of young and adult chestnut plants. In Europe *Phytophthora cambivora* and *P. cinnamomi* are the two casual agents of Ink Disease even though other *Phytophthora* species have been reported to coexist with the two pathogens in chestnut soils (Vettraino *et al*., 2005). Several surveys were accomplished to update the distribution map of *Phytophthora* spp. in Greek and Italian chestnut stands. A total of eighteen chestnut stands were investigated for the occurrence of Ink Disease. Soil and tissue samples were collected.

Phytophthora isolates were identified on the base of their morphological and molecular traits (Erwin and Ribeiro, 1996).

A total of ten species have been detected: *P. cactorum, P. cambivora, P. cinnamomi, P. cryptogea, P. gonapodyides, P. megasperma, P. nicotianae, P. plurivora, P. pseudosyringae, P. syringae*. In Italy and Greece *P. cactorum*, *P. cryptogea*, *P. plurivora* were commonly present in chestnut soil samples, while *P. cambivora* was recovered both from soil and tissue. *P. cinnamomi* was isolated only from chestnut soils in Central Italy. To authors knowledge this is the first report of *P. cryptogea*, *P. megasperma, P. nicotianae* and *P. pseudosyringae* in Italian chestnut stands.

The aggressiveness of *P. megasperma*, *P. nicotianae*, *P. pseudosyringae* and *P. syringae* was tested inoculating chestnut seedlings to verify their pathogenicity toward *Castanea sativa*. Involvement of these species in the development of disease is discussed.

Key words: Ink disease, *Phytophthora* distribution, Pathogenicity

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PETRIA 20 (2), 67-633 (2010)

FAGUS SYLVATICA: DIVERSITY AND COMPOSITION OF FUNGAL ENDOPHYTE COMMUNITIES

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Fagus sylvatica L. (beech) is one of the most important forest tree species in Italy. Beech decline has been reported since the mid 1980s in association with climate changes on global and local scale (Danti *et al.*, 2002). *F. sylvatica* is threated by a number of biotic agents that include many pathogenic fungi. Among these *Biscogniauxia nummularia* (Bull.) Kuntze has been associated with severe beech-decline events recorded over the last 20 years in southern Italy (Granata and Whalley, 1994; Capretti *et al*., 2003; Granata and Sidoti, 2004). This fungus causes strip-canker and wood decay in trees that suffer heavy water stress during the growing season (Hendry *et al*., 1998), and spends part of its life cycle in latent form in symptomless host tissues. After a long endophytic phase it may cause charcoal disease in beech trees with cortical lesions that develop into more or less extensive cankers, and accelerate decline and eventually result in death of the tree (Granata and Whalley, 1994; Capretti *et al*., 2003; Granata and Sidoti, 2004).

The aim of this study was to evaluate the composition of fungal endophytic community and the incidence of *B. nummularia* as endophyte and weak pathogen in the beech forest of Monti Cimini area, Viterbo, Italy. From a total of 10 symptomless plants 600 leaves and 150 twigs were collected in two different forest sites, with north and south facing aspect. To study the composition of fungal endophyte communities each sample was split into two parts: one used for direct isolation and morphological studies on PDS medium, and the other one used for DNA extraction for molecular analysis. The percentage of positive isolations was 95%. A total of 26 different endophyte species, belonging to 16 different families, were obtained. *Xylariaceae* was the most frequent family detected. *Biscogniauxia nummularia*, *B. mediterranea*, *Sordaria fimicola* and *Diaporthe phaseolorum* were found to be the main colonizers of twigs while *B. mediterranea*, *Alternaria alternata*, *Apiognomonia errabunda* and *D. phaseolorum* were the main species isolated from beech leaves. Results underlined the different behaviour of fungal endophytes towards a specific plant tissue. This work elucidates the relationship between diversity and richness of fungal endophyte community and *B. nummularia*.

Key words: *Fagus sylvatica*, Beech decline, *Biscogniauxia nummularia*, Fungal endophyte

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Petria 20 (2), 67-633 (2010)

MONITORING OF FUNGAL DISEASES IN NURSERIES IN CENTRAL ITALY

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Soil borne pathogens are considered the main cause of seedling death. Planting infected plants can easily spread the diseases. Due to the increasing incidence of plant decline in the Marche Region, of Italy, a survey was conducted between 2006 and 2008 in 19 nurseries, 18 orchards and six forest sites.

Several plant species showed severe decline symptoms consisting mainly on stunting. Plants of *Quercus ilex, Prunus avium, Photinia fraseri, Olea europaea,* and *Cytisus scoparius* were collected and analyzed for presence of pathogens. Fungal isolates were identified on the basis of morphological characters and analysis of their ITS sequences (Erwin and Ribeiro, 1996; Watanabe, 2002). At least one of the following pathogens was associated with collected plants: *Phytophthora cryptogea*, *P. cinnamomi*, *P. cactorum, Verticillium dahliae, Fusarium oxysporum*. To confirm the pathogenicity of the detected fungi, soil inoculation tests were carried out. Inoculated seedlings developed symptoms similar to those characterized in the collected plants.

These results suggest that infected plants from nurseries used for new plantings in central Italy are important sources of primary inoculum of fungal pathogens associated with root and wilt diseases in the field. Preliminary biocontrol trials have been carried out for control of diseases caused by *V. dahliae* and *F. oxysporum*, and results from these are reported.

Key words: Plant decline, Soilborne pathogens, Biocontrol

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Petria 20 (2), 67-633 (2010)

CURRENT STATUS OF *INONOTUS RICKII* IN THE MEDITERRANEAN AREA

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Inonotus rickii is a pathogen which causes canker and wood-decay on several hardwoods in tropical and subtropical countries. In 1970 it was reported in Morocco and afterwards recorded in several areas in Eurasia, namely, Italy (1985, 2003), Greece and Montenegro (1994), France (1996), Iran (1998), China (1998, 2010), Spain (2002), Portugal (2002), Thailand (2008), Israel (2009). Besides its pileate to ungulate fruiting bodies, this fungus produces anamorph structures (semi-spherical or cushion shaped yellow-brownish, with a reddish brown zonate inner part) identified as *Ptychogaster cubensis*.

Several Italian isolates of *I. rickii* were at first identified on the base of morphological characters of the fungal structures and mycelial cultures (Annesi *et al*., 2003). Sequence analysis of ITS region confirmed the identification (GenBank accession Nos: GU111921/22).

In this study isolates of the fungus collected from diseased trees in Italy (*Acer negundo*, *Celtis australis*, *Albizia julibrissin* and *Sambucus nigra*), in Spain (*A. negundo* and *Platanus* x *acerifolia*) and in Greece (*Robinia pseudoacacia*) were analyzed by Restriction Fragment Length Polymorphism (RFLP) endonuclease digestion patterns (*Alu*I, *Hae*III, *Rsa*I and *Mbo*I) of the ITS region. One isolate collected on *Hevea brasiliensis* in China (Dai *et al*., 2010) and two isolates from Florida (USA) were included in the analysis. The obtained dendrogram shows that *I. rickii* isolates from all the European provenances and from China grouped together whilst the isolates from Florida formed a clearly separate clade. Further investigations are in progress.

I. rickii can cause severe damage in urban tree boulevard, where infected plants show heavy decline symptoms, with sparse foliage and dieback; sometimes, a considerable number of trees have to be cut down. The more damaged host species in Europe seem to be *A. negundo* and *A. julibrissin* (Intini, 2002; Mazza *et al*., 2008), and *C. australis* (Ramos *et al*., 2008). In fact, during surveys carried out in Rome, 12% of boxelder trees (107/887) and 25% of silktrees (56/224) presented reproductive structures of *I. rickii* (Mazza *et al.*, 2008) and in Lisbon, anamorphic fructifications of the fungus were observed on 19% (73/381) of European hackberry trees (Ramos *et al*., 2008).

In conclusion, the results of studies carried out in several European towns demonstrate that this pathogen produces economically important losses when well established within a boulevard. Careful and timely management measures are required to contain fungal spread.

Key words: Wood decay, Canker, Urban tree, *Ptychogaster cubensis*.

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PETRIA 20 (2), 67-633 (2010)

A SURVEY OF NATURAL PLANT COVERS AND OAK FUNGI OF THE SAFEEN MOUNTAIN FOREST IN IRAQ

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This study was conducted to understand the effect of biotic and abiotic factors on the natural forest of the Safeen Mountain in Shaqlawa/Erbil governorate, Iraq. It involves taxonomy and identification of species of forest trees and shrubs; annual, biennial and perennial herbs in four regions. The trees consist of *Quercus aegilops* L. (cupped oak), *Quercus infectoria* Olivier (gall oak), *Pyrus syriaca* Boiss (wild Syrian pear), *Crataegus azarolus* L. (oriental hawthorn = azarole), and *Prunus microcarpa* C.A.Mey (natural plum). Four main tree characteristics, namely: the average length in meters, diameter in cm, number of branches and number of trees for each of the four regions were also studied. Results showed that the average tree length was in the following order for: cupped oak (2.42, 3.83, 3.60, 2.82 m), gall oak (2.20, 0.82, 2.79, 1.54 m), wild Syrian pear (0.63, 0.61, 0, 0.21 m), oriental hawthorn (1.13, 1.61, 1.69, 2.41m) and natural plum (0.89, 0.66, 1.97, 1.1 m). The average tree diameter was in the following order for: cupped oak (4.15, 8.70, 7.73, 5.27 cm), gall oak (4.63, 1.66, 8.69, 3.78 cm), wild Syrian pear (1.46, 3.4, 0, 0.37 cm), oriental hawthorn (2.31 , 5.32, 5.25, 9.43cm), and natural plum (0.77, 0.47, 1.37, 0.95cm). The average number of tree branches was in the following order for: cupped oak (6.05, 6.1, 5.5, 7.35 branches), gall oak (4.15, 4.35, 3.5, 6.61branches), wild Syrian pear (0.57 , 4.27, 0, 0.25branches) , oriental hawthorn (2.12 , 2.87, 3.2, 0.25 branches) and natural plum (9.45, 4.87, 16.75, 18.57 branches). Finally, the average number of trees in the area (20*20)m was in the following order for: cupped oak (31.75, 27.5, 29, 30.75 trees), gall oak (21, 8.5, 8, 13.75 trees), wild Syrian pear (1, 14.5, 0, 1 trees), oriental hawthorn (2.5, 8.5, 1, 3 trees), and natural plum (2, 4, 2.5, 4.5 trees).

The herbs and annual and biennial grasses were represented by 14 annual and 3 biennial plant species: *Trifolium resupinatum, Trifolium lappaceum, Heteranthelium piliferum, Hymenocarpus circinatus, Gagae sp., Aegilops kotschyi, Scorzonera lenata, Anchusa sp, Muscari inconstricum, Taeniatherum crinitum, Poa bulbosa, Echinaria capitata, Avena ludoviciana, Cousonia sp, Anthemis sp, Centauria sp.* Five perennial grasses were represented by: *Tulipa systole, Ranunculus millefolius, Colchicum kotschyi, Hordeum bulbosuum, Astragalus adscendens.* Eleven trees or shrubs were also represented, they included: *Crataegus azarolus L., Rhus coriaria L., Juniperus oxycedrus L., Pyrus syriaca Boiss., Rosa canina L., Prunus*

microcarpa Mey, Crataegus monogyna (Jacq.), *Paliurus spina-christi* (Will.), *Rubus sanctus* (Schreb.), *Anagyris foetids* L., *Prunus amygdaloides* L.

Biotic factors such as fungi affecting oak trees in the Safeen Mountain forest were also studied. Two fungal species were recorded for the first time in Iraq on these trees and identified as *Ganoderma applanatum* and *Crepidotus mollis*.

Key words : Forest protection, Forest fungi

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Petria 20 (2), 67-633 (2010)

INFLUENCE OF CLIMATIC PARAMETERS ON THE SPREADING OF THE HYPOVIRULENT FORM OF CHESTNUT BLIGHT

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In Southern Europe Chestnut blight, caused by *Cryphonectria parasitica*, is one of the most serious diseases of this species. Since 2002 in Tuscany, central Italy, the disease has been regularly monitored by the regional programme META – ARSIA (http://meta.arsia.toscana.it/meta/meta). The work was based on a grid of survey areas equally located on regional country. Their number was proportional to the occurrence of host species, their size corresponded to a square of 400 m^2 (20 x 20m). Each spot was georeferred using a GPS receiver and environmental information were collected in two forms. In the first inventory data were registered (aspect, slope, height, soil), while in the second information on the disease were collected. In the case of chestnut blight the number of different types of canker were monitored in each plot and tree. During this study a total of 1630 chestnut plants were evaluated in the region.

The annual reports on the occurrence of Chestnut blight in Tuscany have shown a progressive decrease of the damage, especially the normal-looking cankers were substituted by healing cankers. Hypothizing a role of site and climatic condition in favouring the positive trend of hypovirulence spread, the evolution of the disease was studied using the 2005 monitoring data and comparing them with climatic data of the areas. The main results showed that the evolution of the disease from damaging Chestnut blight to healing cankers was more pronounced in areas characterized by mild climatic parameters. It is hypothesised that the role of temperature in favouring the transfer the hypovirulence factor (dsRNA) from fungal isolates. Results showed a significant correlation (R^2=0,59; $p<0,05$) among presence of normal–looking cankers and sites where solar radiation was low. In case global warming will confirm its trend, it is possible to assume that the occurrence of normal-looking cankers in Tuscany will be lower in the future.

Key words: Chestnut blight, *Cryphonectria parasitica*, Hypovirulence, Temperature, Monitoring

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GEOSTATISTIC STUDIES OF CITRUS CANKER EPIDEMIOLOGY AND MANAGEMENT USING GEOGRAPHIC INFORMATION SYSTEM (GIS) IN GHIR-KARZIN (FARS-IRAN)

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Citrus bacterial canker disease is one of the most important citrus diseases through out of the world. It has five forms that are caused by three pathovars of *Xanthomonas axonopodis.* The first report of citrus bacterial canker in Fars (Iran) occurred in 2005 in the town of Ghir-Karzin (N 28 32' – 28 54' S 52 6' – 53 13') (Fars-Iran). Physiological, biochemical and pathogenicity tests were carried out to identify and characterize isolates from symptomatic plants. Based on the results of the above tests the isolates were identified as *Xanthomonas axonopodis*. Ghir-Karzin region has 10911 hectares of citrus gardens. Trough Oct and Nov 2006 all the citrus gardens and citrus trees (tree to tree) in Ghir-Karzin region were observed and checked by four special teams, resulting in the identification of 1833 infected key lime (*Citrus aurantifolia*) trees. During 2006 all infected trees in Ghir-Karzin region were marked using GPS (Global Positioning System) and then mapped using the GIS (Geographic Information System) software. The same studies were continued through Oct and Nov 2007 in Gir-Karzin area and data mapped using GIS software. The new studies in 2007 showed that the number of diseased key lime trees increased to 3892 in spite of the application of quarantine principles. Comparison of infection maps in 2006 and 2007 with GIS software were shown to be valuable for epidemiology and management. These maps showed that in spite of the application of strict quarantine principles the infection has developed by two ways:

- Short distance spread; near the diseased trees. This occurred by rain, wind and rubbing between healthy and diseased trees.
- Long distance spread; along the seasonal river course.

Based on GIS maps Ghir-Karzin area have 10911 hectares citrus garden and 162 hectares infected key lime garden (3892 diseased trees) this is equal to 1.5 percent out of all citrus gardens in the region also based on GIS maps, infection can spread easily among adjacent trees and along the river course. So eradication of infected trees or infected gardens, especially the ones located along the river course, is the best way to control citrus canker in new infected area.

Key words: Epidemiology, Management**,** Key lime

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BACILLUS PUMILUS RELATED WITH NECROSIS SYMPTOMS IN *PHASEOLUS VULGARIS* CROPS IN SPAIN

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Since 2003, interveinal yellowing symptoms which develop to chlorotic spots and necrotic areas in bean plants (*Phaseolus vulgaris* L.) have been observed in Southern Spain, disease referred to as "Bean yellowing and necrosis disease". Four pathogenic agents have been related to this disease: Bean yellow disorder virus (BnYDV), *Erwinia persicina*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and recently *Bacillus pumilus*. This latest bacterium has been determined as phytopathogen for the first time in Spain and in Europe in 2009 (Font *et al*., 2009), although it was previously isolated from peach in Egypt causing bacterial blotch (Saleh *et al*., 1997).

The verified implication of *B. pumilus* in the "Bean yellowing and necrosis disease" and given that *B. pumilus* has been proposed as a potential agent of biological control, this study presents the approaches on different aspects of the epidemiology of this bacterium. A pathogenicity assay was performed using two *B. pumilus* isolates (50/08-C1 and 71/08-C2) which were isolated from bean plants cultivar Donna and characterized on the sequence encoding the 16S ribosomic gene. Five weeks after the inoculation of these isolates to bean plants, similar symptoms to those showed by the original plants were recorded and the bacteria were re-isolated from the inoculated plants.

The biological behaviour of different *B. pumilus* isolates (50/08-C1, 71/08-C2, CECT 510 and CECT 5072) and a *B. subtilis* isolate was studied on different hosts, such as bean pods, fruits of peach, potato and carrot. All the inoculated plants of these hosts revealed soft rot. Seed transmission of *B. pumilus* was also verified. To date any commercial product used in ecological agriculture was confirmed as a possible source of inoculum of such organism in the affected greenhouses.

Electron microscopy studies of *B. pumilus* isolates 50/08-C1 and 71/08-C2 showed bacillar morphology and peritrichous flagella, therefore the previous identification of this isolates as members of the genus *Bacillus* was confirmed. The diagnosis and identification of *B. pumilus* has been confirmed using the molecular techniques of SCAR-PCR, and RFLPs (Isenegger *et al*., 2003).

Key words: *Phaseolus vulgaris, Bacillus pumilus,* Spain, Ecological agriculture, Electron microscopy, SCAR-PCR, RFLPs

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Petria 20 (2), 67-633 (2010)

FIREBLIGHT DISEASE IN MOROCCO *(ERWINIA AMYLOVORA)*: IMPORTANCE, GEOGRAPHICAL DISTRIBUTION AND CHARACTERIZATION OF MOROCCAN ISOLATES

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Fireblight disease caused by *Erwinia amylovora* was detected for the first time in Morocco in 2006 and has spread rapidly throughout the most important pome fruit-producing regions (Fatmi *et al*., 2008). Surveys were carried out in these regions to evaluate the current situation of the disease in the country. In 2006, the disease was detected in one farm on pear (*Pyrus communis*), apple (*Malus domestica*), and quince (*Cydonia oblonga*). In 2009, the disease was observed in 71 farms in different counties such as Meknes, El Hajeb, Sefrou, Ifrane, Taounate and Khenifra. In terms of infected acreage, more than 720 ha were recorded in 2009. To date, over 215 ha of pear, apple and quince have been destroyed (dug out and incinerated).

A collection of about 44 isolates obtained from diseased pear, apple and quince trees between 2006 and 2009, was used to investigate the relatedness among the Moroccan isolates and reference strains of *Erwinia amylovora*. All the obtained isolates were identified as *Erwinia amylovora* using morphological, biochemical and serological tests. In addition, classical PCR (Bereswill *et al*., 1992) and Real-time PCR (Lehman *et al*., 2008) as well as pathogenecity were used to confirm the identity of the isolates.

The relatedness among Moroccan isolates of *Erwinia amylovora* was analyzed by rep-PCR (Versalovic *et al*., 1991) using BOX and ERIC primers (McManus and Jones, 1996). According to the obtained results, the Moroccan isolates were grouped in two clusters showing variability among the isolates.

Key words: Fireblight**,** *Erwinia amylovora*, Morocco, PCR, Real time PCR, Fingerprinting, rep-PCR

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Petria 20 (2), 67-633 (2010)

PHYTOBACTERIOLOGICAL INVESTIGATION ON *OLEA* SPP. IN DIFFERENT DISTRICTS OF NEPAL

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Two wild olive species known as *Olea cuspidata* (syn. *O. ferruginea*) and *Olea glandulifera* are naturally present in several Himalayan districts of Nepal. The latter, which distinguishes from *O. cuspidata* for the rosy colour present on the lower leaf surface, is only present in Bajhang District at altitudes ranging from 1530 to 1566 m above sea level (asl). Conversely, *O. cuspidata* is widespread in many Districts including Bajura, Dolpa, Mugu and Humla at altitudes from 1100 to 2500 m asl (Bartolucci and Dhakal, 1999). In Nepal 35 forest types of *O. cuspidata* were identified and recorded under Trans-Himalaya High Alpine Vegetation.

European olive (*O. europaea*) has been introduced in Nepal for the first time two decades ago by private olive growers and planted in Makwanpur and Kavrepalanchok Districts. Furthermore, in the last years, 28 Italian cultivars of olive have been planted in Kathmandu, Bajura and Dolpa Districts during a project (GCP/NEP/056/ITA) entitled "Promotion of olive Production and Consumption in Nepal" financed by Italian Ministry of Foreign Affairs.

Most of the large forests of *O. cuspidata* are present in those Districts.

The aim of our study was to verify whether there was the presence of the etiological agent of olive knot, namely *Pseudomonas savastanoi* pv. *savastanoi,* in those districts where *Olea* species are present. To do this several phytobacteriological surveys were carried out for three consecutive years (2007-2009). The surveys were conducted in spring and autumn, periods when the cambium is active and the climatic conditions are favourable for the pathogen. In each survey, all the *O. europaea* plants were controlled one by one in all the orchards present in different Districts, whereas the plants belonging to wild species were monitored randomly since they are widespread. Particular attention was paid in monitoring the *O. cuspidata* plants present nearby the *O. europaea* orchards.

No bacterial symptoms were found neither on *O. cuspidata* nor on *O. glandulifera*. Same result was obtained from *O. europaea* plants introduced in the last years in Kathmandu, Bajura and Dolpa Districts, whereas olive knots were observed in one of the two commercial private olive orchards established about two decades ago, in Makwanpur District. Samplings were made and bacteria were isolated from the knots (Balestra *et al*., 2009). No symptoms were found in the other private orchard located in Kavrepalanchok District. The pathogen probably arrived together with the plant materials imported from other countries since no necessary control

measures were carried out during importation. The finding of this pathogen must be considered seriously and seeks immediate implementation of control strategies since this pathogen is not present in other areas where *Olea* spp. is present. Good agronomic and cultural practices are strongly recommended to avoid the contamination and consequent dispersion of the pathogen. In particular, the infected materials must be collected and destroyed. If possible, it would be advisable to spray copper compounds on olive plants. Furthermore, particular attention should be paid to avoid the use of infected materials for vegetative propagation.

Key words: *Olea cuspidata*, *Olea glandulifera*, Phytobacteriological survey, Olive knots

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IDENTIFICATION OF VIRULENT POPULATIONS OF ROOT-KNOT NEMATODES IN TURKEY

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Resistant tomato plants carrying Mi-1 gene is effective in controlling the three most common root-knot species, *Meloidogyne incognita*, *M. javanica* and *M. arenaria* (Roberts & Thomason, 1986). However, the resistance conferred by this gene has some disadvantage such as resistance-breaking strains and temperature sensitivity. Occurrence of resistance-breaking root-knot nematode populations was reported in different countries (Eddaoudi *et al*., 1997; Molinari & Miacola, 1997; Ornat *et al*., 2001; Tzortzakakis *et al*., 2005). However, the occurrence of virulent populations overcoming *Mi* gene, in Turkey, has not been reported.

In the present study, 95 populations of *M. incognita, M. javanica, and M. arenaria* were collected from protected vegetable growing areas in the West Mediterranean region of Turkey. The populations were maintained on susceptible tomato cultivar Tueza F_1 in a growth chamber at 25°C. Genomic DNA was extracted from second-stage juveniles. Root-knot nematodes were identified, using species-specific PCR primers, and the virulence was characterized on resistant tomato cultivars Alsancak RN F_1. The results showed that seven populations of *M. incognita* and six population of *M. javanica* were found to be virulent. None of the *M. arenaria* populations was virulent. This is the first report on occurrence of virulent root-knot nematodes populations in Turkey.

Key Words: *Meloidogyne* spp., Tomato, Virulence, Turkey.

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Petria 20 (2), 67-633 (2010)

OCCURENCE OF *WATERMELON MOSAIC VIRUS* AND *PAPAYA RINGSPOT VIRUS* IN ZUCCHINI CROPS IN POLAND

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Watermelon mosaic virus (WMV) and *Papaya ringspot virus* (PRSV) belong to the genus *Potyvirus* and the family *Potyviridae*. WMV is very common in cucurbits worldwide. This virus causes economically important diseases on several horticultural crops, mostly cucurbits and some legumes. PRSV is a pathogen of papaya and cucurbits. The virus is classified into two biotypes namely PRSV-P and PRSV-W (Purcifull, 1984). PRSV-W, which has a natural host range within the *Cucurbitaceae* and is unable to infect papaya, has been described as one of the most important viruses in field-grown vegetables (Tomlinson, 1987). So far, it has been found mainly throughout the tropics and subtropics.

In 2008 and 2009 several samples of zucchini (*Cucurbita pepo* cv.Giromontina) plants suspected of virus infection were collected from three fields in Poland (Kujawsko-Pomorskie region). All samples were analyzed by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) with commercial antiserum for detection of WMV, *Zucchini yellow mosaic virus* (ZYMV), PRSV and *Tomato black ring virus* (TBRV) (DSMZ, Braunschweig, Germany). The presence of WMV and PRSV in tested samples was observed. The leaf extracts from infected plants were mechanically inoculated onto carborundum-dusted leaves of the following indicator plants: *Cucumis sativus, Chenopodium quinoa, Cucurbita pepo, Nicotiana benthamiana, N. tabacum* cv. Xanthi. The symptoms of leaf chlorosis on cucumber and chlorotic lesions on zucchini were observed. Moreover, the presence of WMV and PRSV was confirmed by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from infected leaves using a phenol-chloroform based extraction procedure. RNA samples were tested for presence of WMV with specific primers designed to amplify a fragment of the coat protein gene (Sharifi *et al.,* 2008). The occurrence of PRSV was confirmed by RT-PCR reaction using primers 04-02 and 04-04, which also amplify the coat protein gene (Chin *et al*., 2007). Amplified DNA was gel purified using Qiaex Kit (Qiagen) and cloned into pGEM-T easy (Promega). Overlapping sequences were obtained using universal M13F and M13R primers. The obtained sequences were deposited in the GenBank database under accession numbers GQ927328 and FJ628395. The comparison with PRSV and WMV sequences retrieved from the GenBank database were carried out. The analysis showed that Polish isolates of PRSV shared the highest identity (97 %) with three Australian isolates (U14739, U14740 and U14744). The WMV sequences shared 98% nucleotide sequence identity

with sequences from China and Korea (Accession Nos: EF127832 and AB369278). The occurrence of subtropical viruses like PRSV and WMV in Poland suggested the introduction of new pathogens which are likely to affect zucchini production in this country and beyond.

Key words: WMV, PRSV, ELISA, RT-PCR

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SURVEY OF *TOMATO YELLOW LEAF CURL VIRUS* (TYLCV) AND *TOMATO BUSHY STUNT VIRUS* (TBSV) IN RAZAVI KHORASAN PROVINCE

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Occurrence of symptoms similar to those produced by *Tomato yellow leaf curl virus* and *Tomato bushy stunt virus* in tomato fields in Razavi Khorasan province was the main reason to work on these viruses. About 776 samples with symptoms of above mentioned viruses were collected during summers of 2008 and 2009, and assayed by TAS-ELISA (for the detection of TYLCV) and DAS-ELISA (for TBSV) according to the method by Clark and Adams (1977).

Results showed that 36 samples were infected with TYLCV, but none of them was infected with TBSV. Presence of TYLCV in the region, even at low incidence, could cause severe disease losses in the coming years. Accordingly, annual surveys are necessary to monitor TYLCV spread.

Key words: *Tomato yellow leaf curl virus*, *Tomato bushy stunt virus*, DAS-ELISA, TAS-ELISA.

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OLPIDIUM BORNOVANUS AND *O. VIRULENTUS*: TWO POTENTIAL ROOT PATHOGENS AND VECTORS OF PLANT VIRUSES IN TUNISIA

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Two surveys were conducted in 2007 and 2008 in melon (*Cucumis melo* L.) and tomato (*Solanum lycopersicum* L.) crops grown in plastichouses at Monastir (northwest Tunisia) and Kebili (southeast Tunisia), respectively, to assess the presence and distribution of *Olpidium* spp. Four plastic houses were selected because plants of aforementioned crops showed wilting. Five soil samples were collected from the root zone and used as sources of *Olpidium* spp. All these samples were representative of each region. *Olpidium* spp. were isolated on homologous bait plants (same species that was growing in the sample soil) as described Herrera-Vásquez *et al*. (2009).

Total DNA was extracted from the roots of bait plants and tested by multiplex PCR for the simultaneous detection of *Olpidium* spp. using specific primers based on rDNA-ITS sequences (Herrera-Vásquez *et al*., 2009). Mixed infections of *O. bornovanus*/*O. virulentus* were detected in two melon plants, while a single infection with *O. virulentus* was detected in one tomato plant. No amplicon was produced from melon and tomato healthy roots or water extracts used as negative controls. To confirm the identity of *Olpidium* spp., amplified PCR products were purified and directly sequenced. BLAST analysis of *O. bornovanus* (GenBank Accession No. GU344684) and *O. virulentus* (GenBank Accession No. GU344685) sequences showed 100% nucleotide homology with reference sequences deposited in the NCBI database.

O. bornovanus has been recently reported as a root pathogen of melons (Stanghellini *et al*., 2010). In addition, *O. bornovanus* and *O. virulentus* are economically important because they act as vectors of several destructive plant viruses. *O. bornovanus* is the vector of *Melon necrotic spot virus* (MNSV) (Campbell *et al*., 1995), previously reported in melon from Kebili (Tunisia) (Yakoubi *et al*., 2007), while *O. virulentus* has been recently cited as potential vector of *Pepino mosaic virus* (PepMV) in tomato from Spain (Alfaro-Fernández *et al*., 2009). Additionally, both viruses are transmitted through seeds. Therefore, infected seed may be a concern with regard to long distance spread of the virus and secondary dissemination by the vector and should be considered in disease management strategies. To our knowledge, this

is the first report of *O. bornovanus* and *O. virulentus* as potentials root pathogens and vectors of plant viruses in Tunisia.

Key words: Melon, Tomato, *Olpidium* spp., Bait plants, Multiplex PCR

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OCCURRENCE AND DISTRIBUTION OF *PEPINO MOSAIC VIRUS* STRAINS IN SPANISH TOMATO CROPS

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Pepino mosaic virus (PepMV, genus *Potexvirus*, family *Flexiviridae*) was first reported in Peru and characterized in *Solanum muricatum* (pepino) in 1974 (Jones *et al*., 1980). In 1999 PepMV was reported in tomato greenhouses in the Netherlands (van der Vlugt *et al.,* 2000). Despite strict control measures recommended by the EU, the virus has rapidly spread throughout the major tomato crops (*Solanum lycopersicum* L.) worldwide and PepMV outbreaks are constantly being reported in many European countries, causing severe epidemics in tomato. Since 1999, four different PepMV strains have been described in tomato: PE, EU, US1 and Ch2 (van der Vlugt, 2009), and it is common to find natural mixed infections between PepMV strains. A reliable, sensitive and rapid detection method is of crucial importance for preventing the spread of this virus. We developed a one-step multiplex RT-PCR assay for the detection and differentiation of the PepMV strains (Alfaro-Fernández *et al.,* 2009). Tomato plants showing different symptoms suggestive of PepMV infection were randomly collected during 2000–2009 from different production areas in Spain. Serological and molecular analysis revealed the presence of PepMV in the tomato samples. Molecular analysis of such plants using the multiplex procedure allowed us to identify the presence of all PepMV strains in Spain. In this study, the geographical distribution and the genetic composition of the yearly epidemic outbreaks in Spain are presented.

Key words: Detection, ELISA, Epidemiology, Genotype, Multiplex RT-PCR, PepMV

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WHITEFLY TRANSMISSION TO DIFFERENT HOSTS OF *TOMATO TORRADO VIRUS* (ToTV) AND *TOMATO CHLOROSIS VIRUS* (ToCV)

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Tomato torrado virus (*Torradovirus*, ToTV) and *Tomato chlorosis virus* (*Crinivirus*, ToCV) are two whitefly-transmitted viruses that commonly infect tomato crops in Spain. ToTV was reported to be transmitted by *Trialeurodes vaporariorum* and *Bemisia tabaci* (Pospieszny *et al.,* 2007; Amari *et al.,* 2008), and ToCV is transmitted by three whitefly species: *T. vaporariorum*, *T. abutilonea* and *B. tabaci* (Wisley *et al.,* 1998).

A greenhouse of tomato plants showing typical symptoms of viral diseases was selected and samples were collected and analysed to different viruses, resulting infected with ToTV, ToCV and PepMV. A colony of adults of the whitefly *T. vaporariorum* was collected. This colony was verified to be viruliferous with ToCV and ToTV, by RT-PCR assays using specific primers to both viruses. The colony was released on several species, some of them reported to be hosts of those viruses (Wisley *et al.*, 1998; Pospieszny *et al.,* 2007; Alfaro-Fernández *et al.,* 2009). The list includes different cultivars of tomato, *Nicotiana* spp., *Chenopodium* spp., *Nicandra physaloides* (L.) Gaertn and *Datura stramonium* L.

Different tomato plants were infected with both virus entities; however, not all cultivars tested positive to these viruses. Tomato plants showed typical symptoms of virus infection such as necrosis on the base of the leaflet associated with ToTV-infection, and interveinal yellowing associated with ToCV infection. Other species studied presented virus infection; although only *D. stramonium* expressed clear symptoms of interveinal yellowing which later became necrotic areas.

This study shows the possible transmission of these two viruses, ToCV and ToTV, into new healthy species by the same colony of adults of whitefly *T. vaporariorum*.

Key words: *Solanum lycopersicum*, Transmission, *Trialeurodes vaporariorum*, RT-PCR

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OCCURRENCE OF SEVERAL VIRUSES IN WEEDS ASSOCIATED WITH TOMATO CROPS IN SPAIN.

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During 2007 and 2008, field surveys of greenhouse-grown tomatoes were performed in some of the main production areas in Spain. Surveys were conducted in tomato greenhouses that showed tomatoes symptoms usually associated with viral diseases. Both tomato and weed samples were collected from inside the greenhouses. Serological and molecular analyses were performed on extracts of these samples in order to detect the presence of viruses such as *Cucumber mosaic virus* (CMV), *Pepino mosaic virus* (PepMV), *Potato virus* Y (PVY), *Tomato chlorosis virus* (ToCV), *Tomato infectious chlorosis virus* (TICV), *Tomato mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV), T*omato torrado* virus (ToTV), and *Tomato yellow leaf curl virus* (TYLCV) that commonly caused similar symptoms to those observed in the studied tomato crops. Tomatoes collected in different greenhouses tested positive to the different viruses and sometimes mixed infections were also detected. Weeds of different botanical families surveyed in the same greenhouses of those positive samples of tomato, tested also positive to some of those viruses and more than one virus was sometimes detected in the same natural host. TSWV, TYLCV, ToCV, TICV, PepMV and ToTV were reported to infect several weeds which serve as potential virus reservoirs in Spanish tomato crops (Jordá *et al.,* 2000; Jordá *et al.,* 2001; Font *et al.,* 2004; Córdoba *et al.,* 2004; Alfaro-Fernández *et al.,* 2008). Arable weeds usually present in tomato greenhouses are known to be potential reservoirs of several viruses and their vectors. Natural hosts could play a critical role in epidemiology as virus sources. Control measures such as elimination of these plants from inner and outer borders of greenhouses are required for managing virus epidemics.

Keywords: Natural hosts, *Solanum lycopersicum*, Virus reservoirs, Survey

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CURRENT STATUS OF *PEPINO MOSAIC VIRUS* IN ITALY

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Since its appearance in Europe (van der Vlugt *et al*., 2000), *Pepino mosaic virus* (PepMV- genus *Potexvirus*) has become a growing concern among plant virologists and stakeholders of the tomato industry. Until now, PepMV is regulated by a temporary EU decision (Commission Decision 2004/200/EC) that is directed to prevent introduction of PepMV by tomato seed into the European Union. Surveys on tomato production premises in all the Member States are officially supported at European level (PEPEIRA – www.pepeira.wur.nl) to definitely assess the economical importance of PepMV.

In Italy, first PepMV outbreak occurred in a greenhouse in Sardinia, in 2000 (Roggero *et al*., 2001); but the disease was efficiently eradicated. Monitoring and surveys to control tomato seedlings, plants and fruits production and imported seeds established the PepMV absence in Italy until the end of 2007 when the virus was detected in an important tomato growing area in Sicily (Davino *et al*., 2008). The disease caused severe symptoms on fruits with high quality and yield losses. In 2009, an investigation was carried out and the present work reports the results obtained.

Spread of PepMV occurred throughout the Ragusa province (South Sicily). The virus appeared again in Sardinia where it was found in one greenhouse, only. Multiple symptoms has been observed on leaves and fruits. Numerous isolates were collected and analysed by real-time RT-PCR assay for virus genotyping. Only Ch2 genotype has been identified. Complete nucleotide sequences of two Sicilian PepMV isolates and the Sardinia one using RT-PCR-based genome walking strategy. The complete genome sequence of the first isolate reported in Italy in 2000 (Sardinia) was obtained and compared with the isolate recently collected from the same area. Pairwise comparisons of the Italian PepMV genomes with other published PepMV full sequences showed that the average highest nucleotide sequence identity was of 98.7%, to that of Ch2 strain. Italian PepMV isolates shared around 78% nucleotide sequence identity to both reference European and US1 strains. Phylogenetic analyses with various gene products confirmed Ch2-clade clustering of our isolates. The only

difference has been obtained in TGB2 region as the new Sardinian isolate clustered alone in comparison to the other Italian isolates.

The recent reappearance of PepMV represents a serious threat for Italian tomato industry. Infected seeds (Hanssen *et al*., 2010) appears to be the most probably pathway for the new outbreaks occurred in Sicily and Sardinia. All year round production of indoor tomato in a very crop specialized area is certainly responsible for a rapid spread of PepMV in Sicily. Establishment of a safety programme is in progress to prevent new PepMV outbreaks in other regions and to effectively manage the disease where it appears to be established (Tomassoli and Faraglia, 2001).

Keywords: PepMV, Tomato, Occurrence, Virus genotyping

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HIGH INCIDENCE OF *FREESIA MOSAIC VIRUS* IN PROTECTED CULTIVATIONS OF FREESIA IN SOUTHERN ITALY

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Freesia mosaic virus (FreMV) was detected during autumn-winter 2009 in protected cultivations of freesia in Campania region (Southern Italy) using a combination of enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Symptoms elicited by FreMV in two imported freesia varieties, Cassis and Champagne, consisting mainly of chlorotic intervenial coalescing areas becoming later necrotic. Overall, the symptoms observed resembled those previously described in freesia cultivations in Northern Italy (Vaira *et al.,* 2006) and Europe (Casper and Brunt, 1971). In addiction, floral scapes were also deformed, while necrotic patches were often observed on floral stipules and sepals. Flowers colour alterations were also noted in some cases. Apparently all the cultivations inspected were affected by this symptomatology.

Preliminary electron microscope observations of leaf-dips, prepared from crude sap of ten symptomatic plants, revealed the presence in all the samples of filamentous and flexuous virus-like particles, with a modal length of about 820 nm. The same plants reacted positively in DAS-ELISA tests against commercial antisera to FreMV. The identity of the virus was also confirmed by sequencing the RT-PCR product of about 1600 bp obtained by using a potyviruses universal primer set (Chen *et al*., 2001). Sequence comparison of the putative coat protein (CP) gene of the FreMV field isolate (named Ca-1) with those available at GenBank, showed 96% nucleotide identity with the FreMV described in USA in *Spiranthes cernua* (Guaragna *et al*., 2006). Dot-blot hybridization assays with a FreMV specific digoxigenin-labelled riboprobe was also used to identify the virus directly from crude saps obtained from dormant bulbs.

The virus was detected in all the bulbs tested (n. 55) demonstrating the high incidence of FreMV in vegetatively propagated material introduced in Italy. In all the samples tested FreMV was apparently found alone and not in mixed infections with other viruses (i.e. *Ophiovirus*, *Varicosavirus*) as described previously in Northern Italy (Vaira *et al.,* 2006). Nevertheless, further analyses on a large number of samples are in progress in order to verify the association of FreMV with other viruses.

This is the first report of a so high incidence of FreMV in protected cultivations of Southern Italy and this finding confirms once again the poor phytosanitary status of the vegetatively propagated material introduced in Italy, suggesting that more severe phytosanitary controls should be adopted.

Key words: FreMV, Potyvirus, Phytosanitary status, Vegetatively propagated material

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PRESENT STATUS OF *PLUM POX VIRUS* IN THE MAIN STONE FRUIT GROWING AREAS OF CAMPANIA REGION (SOUTHERN ITALY)

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Plum pox virus (PPV), the causal agent of sharka disease is the most devastating virus of stone fruits. The disease is highly detrimental because it reduces fruit quality and may cause dropping of immature fruit (Dunez and Sutic, 1988; Nemeth, 1994). PPV strain identification is useful for controlling virus spreading and breeding programmes and it is generally associated with PPV epidemiological studies. For this reason, it is important to know the distribution of the virus and the different strains occurring (Pasquini and Barba, 1994). The main objective of the present study was to provide new data on spread of PPV strains in Campania region (Southern Italy), the region with the highest production of apricot in Italy (about 35% of national production with four thousand hectares of cultivated orchards).

During spring-summer 2009, an extensive PPV survey in Campania region was conducted to determine the virus incidence and identify its strains. A total of 378 symptomatic leaf samples consisting, respectively, of 211 apricot (55.82%), 47 plum (12.43%), 108 peach (28.58%) and 12 sweet cherry (3.17%) were analyzed by ELISA using a commercial PPV polyclonal antiserum. The highest incidence of PPV infection was found in apricot (24.6% of samples), in particular from cultivations located in Naples province. The sequences of approximately 1600 pb fragment, comprising the 3' end of the NIb gene, the coat protein (CP) gene and the 3' untranslated region of PPV genome, from 14 apricot and 1 plum PPV-infected plants, were determined. Phylogenetic relationships, as well as *in silico* restriction patterns obtained after virtual digestion with *Rsa*I endonuclease of the CP gene sequences, indicated that both PPV-D and PPV-M strains were present in the region. In particular, 12 PPV-D isolates and 2 PPV-M isolates. In one apricot orchard, located in Naples province, the virus D and M strains were both present. Nevertheless, the PPV-Rec strain was not identified so far among many samples from the same cultivation.

In contrast to North of Italy, where the efficiently aphid transmitted PPV-M strain was the main virus strain (Di Terlizzi and Boscia, 2006), the present study showed that both PPV-D and PPV-M were present in Campania and in particular the D strain was the most widespread in the region, especially in apricot orchards. A 3 year of sampling and molecular characterization of a larger number of PPV isolates, will

provide more detailed information on distribution of PPV strains in the Campania region.

Key words: Sharka, PPV strains, Sequencing, Stone fruits, Epidemiology

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FIFTY YEARS INVESTIGATING *CITRUS TRISTEZA VIRUS* IN ITALY

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Many diseases of phytoplasmas, uncultured bacteria, viruses or viroids have caused and continue to cause severe damage to citrus orchards worldwide. *Citrus tristeza virus* (CTV) and Huanglongbing (yellow dragon disease = citrus greening disease) caused by *Candidatus* Liberibacter spp. are particularly destructive.

Over 100 million trees on sour orange rootstock have been destroyed in Brazil, Argentina, California, Florida, Spain, and Venezuela by CTV. The virus continues to spread into new areas. The most efficient damaging aphid vector *Toxoptera citricidus* Kirk. of CTV has been discovered in new areas such as Portugal and Spain.

It would be a grave threat to the Italian citrus industry if this vector reached Italy and spread rapidly through citrus orchards as has happened with other insect vectors. Once present only in Asia and Africa, Huanglongbing (citrus greening disease) is now found in North and South America where it is causing enormous damage in Brazil and Florida.

In 1956, Russo reported that all the Mediterranean countries including Italy were at a state of alert for the citrus tristeza disease – it could seriously damage the Mediterranean citrus industry given that the rootstock is sour orange and considering that at that time the virus was infecting orchards worldwide through Meyer lemon and the Satsuma mandarin.

Russo carried out the first tests on 4 Meyer lemons in Acireale (Catania) and Palermo, and on 3 Satsumas in Acireale and 2 in Paternò (Catania). The virus was detected on the lemons and Satsumas in Acireale (Russo, 1956). In 1967, the virus was detected on 8 Satsuma at Muravera in Sardinia (Servazzi *et al*, 1967) and at Monasterace in Calabria on Meyer (Catara, 1968).

The severe crisis in the citrus industry gave rise to illegal importation of different species and along with them new pathogens. In 1974 in Catania, Satsuma budsticks were found infected with CTV imported from Japan. From 1982 to the present new cases have been on the rise: Golden Buckeye sweet orange, Ceylon

lemon, Marsh seedless grapefruit and Wase Satsuma have been found infected in the same orchard in Calabria where 15 years earlier the Meyer was infected. In the two years following, 200 more plants were found infected by new serological CTV test.

From 1991 to 1994, some CTV-infected plants of Alemow, Shamouti and Valencia sweet orange as well as Marsh seedless grapefruit and Long-Yauzhai Satsuma were identified in Catania Province. From 1995, the number of infected plants started increasing with the discovery of 10,000 calamondin and 4,000 Otahete Rangpur lime cultivated in various Tuscan nurseries. From 1996 to present, the number of plants infected with CTV is so numerous to be countable.

Among the first were more than 15,000 Fortune mandarin and various New Hall, Navelina e Valencia sweet oranges, Satsumas and grapefruit discovered around Syracuse. Some years later, CTV was found on thousands of field plants around Catania Province on Tarocco Comune and Navelina sweet orange (Davino *et al.*, 2004).

Almost at the same time, CTV infections were found in Apulia on Navelina and in Rosarno (Calabria) on Fortune and Satsuma. Tests carried out in the infected areas showed that the virus vector was aphids (over 90% *Aphis gossypii* Glover).

Molecular investigations in the three regions showed that the strains in these citrus areas were different; among them one isolate (CTV-SY 568), found on Tarocco Comune sweet orange was very severe one isolate was very severe and has been consedered on of the of most destructive and easily transmitted even by *A. gossypii*.

Of over 60,000 tested plants, about 30,000 were infected. Therefore, CTV is diffused in Italian citrus orchards and is spreading to new areas through infected material and above all by aphids.

Based on bibliographic references of the world's major citrus areas, all Meyer plants around the world are CTV infected as they originate from budsticks imported into the United States from China in 1908. The situation is different for Satsuma which is very common throughout China and has become widespread around the world since it is grafted onto trifoliate orange rootstock and does not show CTV symptoms.

Key words: Citrus, *Aphis gossypii*, Diffusion

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SESSIONE 2

Diagnosis

of plant pathogens

ORAL PRESENTATIONS

PETRIA 20 (2), 67-633 (2010)

DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF WHITEFLY-TRANSMITTED VIRUSES WHICH AFFECT SOLANACEOUS AND CUCURBIT CROPS IN THE MEDITERRANEAN REGION

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Whitefly-transmitted viruses cause devastating crop losses worldwide. Two genera of whiteflies transmit virus diseases, *Bemisia tabaci* and *Trialeurodes* spp. *B. tabaci* is by far the most important virus vector. Several biotypes of *B. tabaci* were reported (Perring, 2001). Since the mid 70s, a rapid spread of biotype B over large geographic areas was observed, with a simultaneous increase in expansion and severity of new viral diseases, which sometimes progressively displaced the predominant virus species. About 90% of the whitefly-transmitted viruses belong to the genus *Begomovirus* and 6% to the genus *Crinivirus*; while the remaining percentage belongs to other genera (Jones, 2003). Therefore, this report will focus on begomoviruses and criniviruses.

The genus *Begomovirus* (family *Geminiviridae*) is the most economically significant group of plant viruses and probably the largest, it includes over 132 approved species (Fauquet and Stanley, 2005). The genome of *begomoviruses* is composed of circular ssDNA, it can be either monopartite or bipartite (called DNA A and DNA B). In the Euro-Mediterranean region (Euro-Med) and in several other regions, the most important disease of solanaceous crops is tomato yellow leaf curl disease (TYLCD) which induces yield losses of up to 100% when tomato plants are infected at an early stage of development. TYLCD may be caused by nine TYLC virus species (Fauquet and Stanley, 2005). In the Euro-Med region the most important species are Tomato yellow Leaf curl virus (TYLCV, formerly TYLCV-Israel), Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl Malaga virus (TYLCMalV) (Anfoka *et al.*, 2008). More recently, Squash leaf curl virus (SLCV) and Watermelon chlorotic stunt virus (WmCSV) have also been detected on cucurbits crops in the Mediterranean region, with disease incidence between 95-100% in several fields in Egypt, Greece, Iran, Israel, Jordan and Lebanon (Ali-Shtayeh *et al.,* 2010).

The genus Crinivirus (family *Closteroviridae*) has a genome composed of two linear, positive sense, ssRNAs, that are both needed for infectivity and are separately encapsidated. This genus includes eight recognized species that are transmitted by whiteflies, *B. tabaci* and/or *Trialeurodes* spp. (Martelli *et al.,* 2005). In the Euro-Med region, the most important viruses detected on solanaceous crops are Tomato chlorosis virus (ToCV) and Tomato infectious chlorosis virus (TiCV). On cucurbits, Cucurbit yellow stunting disorder virus (CYSDV) is widely spread in several Euro-

Med countries with yield losses ranging between 30-50% and has displaced Beet pseudoyellows virus (BPYV) in some European countries (Celix *et al.,* 1996; Abou-Jawdah *et al.,* 2000).

Several biological, microscopic, serological and molecular techniques were used for diagnosis or detection of these viruses, of particular importance in the history of detection of geminiviruses is the use of heterologous monoclonal antibodies and the use of degenerate primers in PCR-RFLP tests (Deng *et al.,* 1993). Recently, a Rolling Circle Amplification (RCA) technique has been developed as an important tool for detection and molecular characterization of circular DNA viruses and is more sensitive than polymerase chain reaction (Haible *et al.,* 2006). For criniviruses, early detection relied on dsRNA analysis and the use of degenerate oligonucleotide primers corresponding to conserved sequences of the heat-shock protein 70 homologue (HSP70h). These primers were used in RT-PCR of total RNAs or dsRNAs extracted from tissues infected with BPYV, CYSDV, Lettuce infectious yellows virus (LIYV), and other closteroviruses. The amplified cDNAs were cloned, sequenced and used in nucleic acid hybridization tests with radioactive or non-radioactive labeling (Celix *et al.*, 1996). The sequence of the whole DNA A is used for molecular characterization and phylogenic studies of begomoviruses. For criniviruses and closteroviruses, phylogenetic analyses were recently carried out on the amino acid sequences of the RNA dependant RNA polymerase (RdRp) (Coutts and Livieratos, 2003) and the nucleotide sequences of the HSP70h gene (Martelli *et al.,* 2005).

Solanaceous and cucurbit crops in the Euro-Med region are under constant threat to attack by newly introduced pests including begomoviruses, criviruses or other whitefly-transmitted causal agents. An example is the recent introduction to Lebanon of SLCV, WmCSV and tomato purple leaf disorder (TPLD), a disease transmitted by whiteflies. Over hundred whitefly transmitted viruses were reported in other regions, some should be considered as quarantine pests, and included in EPPO list1 after an appropriate risk assessment. The development of new methods like the RCA would greatly improve and facilitate virus detection and diagnosis.

Key words: *Bemisia tabaci, Vector, Trialeurodes, Begomovirus, Crinivirus*

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MULTIPLEX DIAGNOSIS OF VIRAL AGENTS OF TOMATO BY DNA MICROARRAY TECHNOLOGY

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Tomato (*Solanum lycopersicum* L.) is a worldwide-cultivated vegetable crop that is affected by several viruses that induce significant economical losses. Therefore, the detection and identification of the viruses and their strains affecting tomato crop is of critical importance to plant virologists in general and to plant quarantine and certification programs (worldwide) in particular. Tomato viruses and virus like pathogens are inherently diverse groups and do not share nucleotide sequences. Therefore, it is highly important to use multiplex methods for their detection and differentiation, as the demand of globalization of trade requires pathogen-free propagation material.

Detection and diagnostic methods to evaluate the sanitary status of tomato seed, seedlings, field plant or viruliferous vectors may include biological indexing, electron microscopy, antibody-based methods, including enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and/or microarrays (Esteban *et al.,* 2010).

ELISA and RT-PCR are the most common and widely used techniques for routine screening of pathogens. Nevertheless, they have limitations such as the restricted number of viruses detectable in a single assay. Symptomatic tomato samples may often contain several pathogens, making these techniques time-consuming, and labor intensive.

Among the latest and potentially useful diagnostic techniques for a high multiplex detection is DNA microarray as it provides the highest capability for parallel yet specific testing which can be used to detect individual plant viruses or combinations of many plant viruses and/or virus like pathogens (Barba and Hadidi, 2007).

In a previous paper (Tiberini *et al*., 2009 a,b) we described the efficacy of a DNA microarray chip for simultaneous multiple detection, differentiation and/or genotyping of the following tomato virus species, including their strains or isolates: *Impatiens necrotic spot virus*, *Tomato spotted wilt virus, Cucumber mosaic virus*, *Pepino mosaic virus*, *Potato virus Y*, *Tomato infectious chlorosis virus, Tomato chlorosis virus*, *Tobacco mosaic virus, Tomato mosaic virus, Tomato yellow leaf curl virus* and *Tomato yellow leaf curl Sardinia virus.*

By using Combimatrix (Mukilteo, WA, USA) platform, it was possible to synthesize more than 500 unique viral oligonucleotides designed against new viral

pathogens not previously considered for a total of 37 tomato viruses and 6 viroids. Genus-level specific probes for 18 different genera were now included in the chip.

Validation of the microarray was performed by the detection of 20 tomato viruses and 1 viroid in green tomato samples with single and multiple infection. Each sample, in single virus infection and/or in mixed infection, was tested using a target preparation protocol, including an indirect labelling step and avoiding any amplification step. Hybridizations using single stranded cDNAs, obtained using a retro-transcription step with random hexamer and labelled with both Cyanine (Pasquini *et al*., 2007) were performed. Most of the evaluated specific 40-mer oligonucleotide probes were able for detecting and genotyping the considered virus and/or viroid. This microarray–based detection method allows simultaneous multiple detection and genotyping of major economically important tomato viruses and their strains. In addition this chip can be considered feasible, friendly-used and economically convenient, because can be re-used at least 8 times.

Keywords: Microarray, Tomato, Tomato viruses, Multiple detection, Virus genotyping

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ONE-STEP RT qPCR ASSAY FOR THE DETECTION AND QUANTIFICATION OF *GRAPEVINE FANLEAF VIRUS*

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Grapevine fanleaf virus (GFLV) is the causal agent of the fanleaf degeneration disease, which confronts grape growers worldwide. The genome of GFLV is composed of two single-stranded positive-sense RNA molecules, RNA1 (7342 nt) and RNA2 (3774 nt), with typical *Nepovirus* structure, each encoding a polypeptide (Andret-Link *et al.*, 2004).

The use of healthy propagation material, free of viroids, viruses and bacteria is an important strategy for disease control in viticulture. Correct diagnosis is essential for the production of certified pathogen-free propagation material and for the effective control of GFLV spreading.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is the standard procedure for GFLV diagnostics allowing virus detection directly in grapevine extracts, but lacking the sensitivity required for the detection of low virus concentrations occurring, i.e. in latent infections, at defined points within the season, such as, late summer and autumn (Cepin *et al.*, 2009; Rowhani *et al.*, 1992) and when detecting the virus in nematodes.

In order to eliminate false-negative results expected in the above mentioned samples, and to better characterize natural GFLV isolates, new molecular methods have been developed in recentyears, including a few conventional RT-PCR methods which allow detection of a limited number of GFLV genotypes, as well as a TaqMan chemistry based RT-qPCR method, developed by Osman and Rowhani (2006). Up to now the genetic variability of GFLV isolates has been analyzed mainly at the RNA2 level (Pompe Novak *et al.*, 2007), of which the $2C^{CP}$ gene has been the most extensively characterized, and therefore most, if not all, of the existing molecular assays are targeted to the $2C^{CP}$ gene.

In this study, a TaqMan® MGB-probe-based one-step RT real–time PCR (RT-qPCR) assay was developed for the specific detection of *Grapevine fanleaf virus* (GFLV), targeting the $2A^{HP}$ gene, which corresponds to the most conservative region of the GFLV RNA2 molecule. The assay specificity was evaluated on GFLV isolates from a wide range of geographical locations including USA, France, Italy, Spain and Slovenia and also on all other viruses infecting grapevines, as well as on healthy plants. The sensitivity of the developed assay was approximately 1000-fold higher

than the sensitivity of the conventional ELISA test. Lower concentrations of GFLV, with C_q values up to 38, could also be reliably detected by RT-qPCR.

The newly developed method offers a fast, reliable, specific and sensitive identification test for GFLV, easily applicable for high-throughput diagnosis of GFLV in different types of plant material including dormant phloem scrapings. Complementary to ELISA or other methods it can also be used for relative quantification of GFLV virus. The quantitative nature of the assay was demonstrated by monitoring the seasonal variation of the GFLV amount present in the grapevine phloem samples.

Keywords: *Grapevine fanleaf virus*, RT-qPCR, Grapevine, Specificity, Sensitivity.

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MULTIPLEX REAL-TIME RT-PCR FOR DETECTION OF THE MOST IMPORTANT GRAPEVINE VIRUSES

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Diagnosis of grapevine viruses is very important in clonal sanitary selection and certification schemes of propagation materials. Serological tests (ELISA) and PCR-based techniques are generally used for detection of the most dangerous viruses, which are also listed in the EU Directive No. 2005/43/CE. The aim of this work was to develop reliable, sensitive and fast molecular assays for the detection of some of the most important viruses of grapevine, based on multiplex real-time RT-PCR.

Real-time RT-PCR assays were developed for the following viruses: *Grapevine leafroll-associated virus* 1, 2 and 3 (GLRaV-1, 2 and 3), *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine virus A* (GVA), *Grapevine fleck virus* (GFKV) and *Rupestris stem pitting-associated virus* (GRSPaV). Two primers and a hybridization probe specific for each virus were designed on the basis of the nucleotide alignment of the genome sequences available in GenBank. A total of approximately 150 vine samples, which were infected with different viruses and different variants of the same viruses, and a few control healthy grapevine plants, which were obtained from *in vitro* propagation and maintained in screenhouse, were tested. The results of singleplex real-time RT-PCR tests were compared with those obtained from ELISA and conventional RT-PCR assays with different primer pairs.

The real-time singleplex RT-PCR assays developed for the 8 viruses showed to be generally more sensitive than the ELISA or the conventional RT-PCR assays. Only a very few samples, known to be infected by rare divergent viral variants which are not yet molecularly characterized, were negative in the real-time assay.

Two multiplex real-time RT-PCR assays were designed for clonal sanitary selection purposes, allowing the simultaneous detection of ArMV, GFLV, GFKV, GRSPaV, and GLRaV-1, 2, 3, GVA, respectively. Multiplex real-time RT-PCR was also developed for the detection of ArMV, GFLV, GLRaV-1, GLRaV-3 and GVA in certification procedures. The multiplex assay results on vine samples always agreed with singleplex results.

The limits of detection (LOD) of singleplex and multiplex PCR assays were also determined using the target amplicons cloned into plasmids.

In conclusion, the multiplex real-time assays developed in this work proved to be a useful tool for the diagnosis of grapevine viruses, as they are more sensitive and faster than the conventional diagnostic methods and they allow reducing reagent and cDNA template consumption with respect to singleplex assays.

Key words: Diagnosis, Multiplex real time RT-PCR, Viruses, *Vitis vinifera*

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A RAPID AND ACCURATE METHOD FOR DETECTION OF CITRUS VIROIDS IN NORTHERN IRAN

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Mazandaran province is a major citriculture region in northern Iran. Most citrus plants in this region are infected by viroids and trees may be infected by one viroid or more (Bove, 1995). In most cases, the infected trees are asymptomatic because sour orange, the predominant rootstock used in Mazandaran, does not show symptoms of viroid infection. Detection of viroids through biological indexing on sensitive indicator plants followed by sequential polyacrylamide gel electrophoresis (sPAGE) is standard but it is time consuming and requires plants to be kept at optimum conditions. We applied the optimized method for citrus viroid detection that it was first developed for plant viruses' dsRNA detection, based on the specific column chromatography with CF-11 cellulose powder in presence of ethanol (Rezaian *et al*., 1990).

During 2006 to 2008, leaf samples from citrus trees with suspicious symptoms of exocortis and cachexia diseases (such as bark scaling and stem-pitting) were collected. Each sample was extracted by this procedure and the nucleic acid extracts were analyzed by 1% agarose gel electrophoresis. Specific bands of the two viroids with molecular sizes of 7000-7800bp (of the viroid circular form) and 300-400bp (of the viroid linear form) were detected every seasons and warm seasons, respectively. The dsRNA nature of the bands was confirmed by nucleases treatments (DNaseI and RNaseA) and 2M LiCl extraction method (Dodds and Bar-Joseph, 1983). Viroid entity of each nucleic acid sample was identified on the basis of the size of RT-PCR amplification products, using specific primers to cachexia and exocortis viroids (Almeyda *et al*., 2007; Alvarado-Gomez *et al*., 2000). Each primer set specific for cachexia or exocortis viroid, amplified viroid- specific 300-400bp fragments.

Thus, the CF-11 column chromatography provided a rapid, accurate and efficient detection method of citrus viroids in northern Iran. This is the first report of citrus viroids detection by the optimized method.

Keywords: CF-11 column, Citrus viroids, Cachexia, Exocortis, RT-PCR

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EPPO's DIAGNOSTIC ACTIVITIES

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Since 1998, EPPO has established a work programme in the area of diagnostics to harmonize procedures across the region. The different activities conducted in the framework of this programme are presented.

Diagnostic protocols

In 1998, a programme was initiated to develop diagnostic protocols for as many as possible of the pests of the EPPO A1 and A2 lists (Zlof *et al.*, 2000). The preparation of protocols involves close collaboration between different Panels composed of diagnostic experts: the Panels on Diagnostics (coordination role), Bacterial Diseases, on Nematodes, on Certification of Fruit Crops and the European Mycological Network. Each first draft is prepared by an individual expert according to a common format and should contain all the information necessary to detect and positively identify a particular pest. The draft is then reviewed by relevant Panels. 92 diagnostic protocols for specific pests and 3 horizontal standards have now been approved as (see www.eppo.org). 15 protocols are in different stages of preparation.

A survey on the use of the protocols was conducted in 2008 on a selection of 58 protocols in all disciplines of plant health diagnosis (Petter and Suffert, 2010). Laboratories registered in the EPPO database on Diagnostic Expertise (see below) were asked to indicate the number of samples that they tested in 2007 and which test they used. From this survey it can be concluded that many of the tests for detection mentioned in EPPO diagnostic protocols are widely used in laboratories in the EPPO Region.

Accreditation and quality management

In 2003, a separate Panel was created to develop standards on quality assurance (two standards have been developed so far OEPP/EPPO, 2007 and 2010). A joint communiqué between EPPO and EA (European Co-operation for Accreditation, the European network of nationally recognised accreditation bodies) states that "*EA will recommend that assessors from Accreditation Bodies take note of EPPO documents when evaluating plant pest diagnostic laboratories".* It is also envisaged to create a database where validation data from laboratories could be shared between EPPO countries. EPPO also organized two workshops on quality assurance in 2007 and 2009, to allow experts to share their experience on quality assurance and accreditation.

EPPO database on diagnostic expertise

In 2004, EPPO Council stressed that the implementation of phytosanitary regulations for quarantine pests was jeopardized by decreasing knowledge in plant protection. The Panel on Diagnostics proposed that an inventory should be made

of the available expertise on diagnostics in Europe. The database on Diagnostic Expertise was created (Roy *et al.,* 2010) to allow identification of experts who can provide diagnosis of regulated species and those who can help in the identification of new or unusual species. EPPO member countries were contacted and as of May 2010, 70 laboratories from 25 countries have provided data corresponding to more than 500 experts). These results are available in a searchable database on the EPPO website. The database can also help national accreditation bodies identify auditors for pest diagnostic laboratories for accreditation.

The EPPO Secretariat considers that these initiatives and future plans will aid the optimization of diagnostic activities in laboratories in the EPPO region.

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DIAGNOSIS OF DATE PALM DISEASES CAUSED BY *THIELAVIOPSIS PARADOXA* (DE SYNES) HÖHN

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Date palm (*Phoenix dactylifera* L.) is a perennial tree grown in permanent or semi-permanent systems. It is extensively cultivated for its edible fruits but also used as an ornamental tree in public parks, houses, and alongside roads. Date Palm trees could be affected by over 30 different arthropods and 20 diseases (Carpenter, 1978; Djerbi, 1983). Among them *Thielaviopsis paradoxa* (De Synes) Hõhn, anamorph of *Ceratocystis paradoxa* (Dade) C. Moreau, is a dematiaceous hyphomycete with cosmopolitan distribution which infects a broad range of palm trees such as date palm, coconut, oil palm, Washingtonia palm, Canary Island palm and royal palm (Elliott *et al.*, 2004). Besides palms, the fungus was previously recorded on pineapple, sugar cane, bananas and figs (Elliott *et al.*, 2004). On date palm, the fungus causes a variety of disease syndromes known as black scorch; terminal bud rot (Fool disease-Madjnona in Arabic); trunk dry rot; stem bleeding; fronds scald; fruit rot and neck bending (Abbas *et al.*, 2003).

Symptom development, field diagnosis, laboratory identification of the fungus and environmental factors affecting the disease were examined in this study. The study also revealed that the fungus occurs on date palm trees wherever are cultivated under stress conditions. All palm species are considered to be potential hosts of the fungus.

Additionally, the study unveiled that the fungus enters the date palm trees through wounds made during pruning or removing the offshoots. On the diseased palms, the fungus produces volatile substances, specifically ethyl acetate and ethyl alcohol, which often give the rotted tissues a fermented fruit odor. Moreover, the high level of salinity in water, tended to increase the infection of date palm trees by *T. paradoxa* which can be efficiently spread by air, insects and irrigation water .

Key words: Fungal diseases, *Phoenix dactylifera*

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INFLUENCE OF DIFFERENT NUTRIENT MEDIA ON GROWTH OF *PASSALORA FULVA IN VITRO*

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Tomato Leaf Mold caused by *Passalora fulva* (Cooke, Braun, Crous) (synonym: *Fulvia fulva* (Cooke, Ciferri)) is an important problem in tomato greenhouse production in Croatia. Diseased leaves were collected during 2006 and 2007 years from seven localities. Influence of nutrient media on isolation and development of mycelium and conidia of *P. fulva* were studied by growing the fungus on three different media: PDA (potato dextrose agar), tomato-agar and fruit-agar. For morphological studies 7-day old monosporic cultures were used.

According to de Vries (1952), Schubert and Braun (2005) and Stergiopoulos *et al.* (2007) PDA is recommended for isolation of *P. fulva*. In our research PDA was not suitable for that purpose. One of the biggest problems was the appearance of fast growing saprophytes such as *Cladosporium cladosporioides* (Fres.) de Vries, *C. herbarum* Penzig, and *Fusarium* spp.

In our research tomato-agar (fresh or frozen tomato leaves - v. Belle, bacteriological agar and distilled water) was the best medium for isolation, inoculum production and determination of morphological characteristics. Contamination by bacteria or saprophytic fungi was rare.

Fruit-agar (multivitamin juice, bacteriological agar, distilled water and $CaCO_3$) also gave very good results for isolation and study of morphology characteristics. Contamination with saprophytes was very rare. Because of that tomato and fruit agar are suitable for long term investigation.

Regardless of medium type the first colonies formed 7 to 10 days after inoculation and they always had characteristic spotted growth. Statistically significant differences in conidial dimensions between eight investigated isolates were determined on PDA and tomato agar. The most intensive conidial germination and hyphal swelling were detected on PDA. Conidial germination on tomato and fruit agar were slow and rare.

Key words: Tomato, *Passalora fulva*, Nutritient media

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IDENTIFICATION OF ROOT-KNOT NEMATODE SPECIES FROM THE NORTH KORDOFAN AREA, SUDAN, BY MORPHOLOGY, ESTERASE PHENOTYPES AND RAPDS

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Root-knot nematodes (*Meloidogyne* spp.), one of the most economically important plant pathogens, are a destructive pest of tomato (*Solanum lycopersicum* L.) that reduces production in infested areas and is difficult to manage. Over 80 species have been described, but more than 90% of the estimated damage worldwide is considered to be caused by: *M. incognita* (Kofoid and White, 1919) Chitwood, 1949; *M. javanica* (Treub, 1885) Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949; and *M. hapla* Chitwood, 1949 (Netscher & Sikora 1990; Siddiqi, 2000). Identification of the species has been based on morphological characters, biochemical and molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (ALFP) (Stanton *et al*., 1997; Semblat *et al*., 1998).

This study was conducted to identify the root-knot nematode populations found in North Kordofan State using morphological, biochemical and molecular characters. The morphology of the perineal patterns of the root-knot nematode adult females, obtained from infected plants, collected in five locations in North Kordofan State (Bara, Bashiri, Alhumara, Almolbas and Alhaegena), showed that the dominant species was *M. javanica*. The phylogenic tree obtained by analysis of the esterase isozyme and RAPD fingerprinting, performed on single females using different primers, revealed that all populations were *M. javanica*.

Key words: Esterases, *Meloidogyne javanica*, Morphology, RAPD, Sudan

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SESSIONE 2

Diagnosis

of plant pathogens

POSTERS

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COMPARISON OF MOLECULAR DIAGNOSTIC METHODS FOR THE IDENTIFICATION OF THE QUARANTINE PATHOGEN *TILLETIA INDICA MITRA*

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Tilletia indica Mitra is a seed-borne quarantine plant pathogen fungus agent of the Karnal bunt of wheat. The fungus, which originally spread from the Asian and later in the American Continent, is actually inserted in the A1 list of European and Mediterranean Plant Pathology Organisation (EPPO), since, the introduction in European Union, where the pathogen is absent up today, is considered of high risk (Directive 2000/29/CE).

Methods for diagnosis have been developed by EPPO (OEPP/EPPO, 2007)) in order to check the unwelcome introduction in the above countries of the pathogen. However, the morphological identification of *Tilletia indica* has to be considered difficult for the potential co-presence of other *Tilletia* species especially as the content of teliospores, available in the seed samples under diagnosis, is poor. Since ryegrass seeds and wheat seeds are usually transferred by ship, contamination of *T. walkeri* on wheat seeds is a possible event. Moreover, the incapacity to well-distinguish the two species by morphological criteria for those samples which have a poor teliospore content could cause misidentification and/or false alarmism for the presence of *T. indica*.

Different molecular methods were developed (Pimentel *et al*., 1998; Frederick *et al*., 2000), and introduced in the PM 7/29 protocol**.** However, the above methods need DNA extracted from mycelium, but the low percentage of germination because of the dormancy and the poor content of teliospores which can frequently result from the standardize 50 grams seed sample used for the "washing test" are factors which may negatively affect the identification of species.

A very recent and promising one-tube fluorescent assay by Real Time PCR was developed for multiplex DNA identification of *Tilletia* species by Mui-Keng Tan *et al*., (2009). The above method and some of the EPPO methods were tested on teliospores and/or DNA of *T. indica* and *T. walkeri* in order to compare the ability of the different protocols. The results in term of identification of species, advantages and limits of each protocol are showed and discussed. Emphasis and discussion is more focalised upon the use of Tan *et al*. protocol for the detection of the two species in seed samples with low-teliospore content by performing the analysis directly on a crushed single-spore as starting material without performing any DNA extraction procedure.

Key words: *Triticum* spp., *Lolium* spp., Real Time PCR, Karnal or Partial Bunt of Wheat, Identification.

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FUSARIUM WILT OF LETTUCE: IDENTIFICATION OF *FUSARIUM OXYSPORUM* F.SP. *LACTUCAE* RACE 1 WITH TWO DIFFERENT PCR ASSAYS

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Fusarium oxysporum f.sp. *lactucae* the causal agent of fusarium wilt of lettuce, *Lactuca sativa*, has recently been included into the EPPO alert list.

Determination of the *forma specialis* and the races with traditional methods is very difficult, while the molecular methods are faster and specific (Lievens *et al*., 2008).

At the Plant Protection Service of Emilia-Romagna Region, two molecular methods proved to be very useful to detect *Fusarium oxysporum* f.sp. *lactucae* race 1 from culture, vegetable material and seeds.

PCR-RFLP, amplification of IGS (intergenic spacer region) sequences (Mbofung *et al*., 2007), sufficiently variable to allow discrimination among lettuce isolates from other *formae speciales,* has been used. Race 1 has been identified by specific restriction enzymes.

Such a molecular test is sensitive and specific and it has currently been employed in our laboratory since 2007 for disease diagnosis and seed monitoring.

Another molecular marker, the genomic region between the insertion of long terminal repeat retrotrasposon copies, has been used in diagnostic assay for *Fusarium oxysporum* f.sp. *lactucae* race 1 strains. It is based on the inter-retrotrasposon amplified polymorphism (Pasquali *et al*., 2007). The specific PCR product is a small fragment of 183bp that will be employed for the identification of the organism on infected plants and lettuce seed.

Key words: *Fusarium oxysporum* f.sp. *lactucae*, *Lactuca sativa*, PCR-RFLP, Intergenic spacer region, Tasposon, Diagnostics.

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Petria 20 (2), 67-633 (2010)

CHARACTERISATION OF *FUSARIUM OXYSPORUM* F. SP. *CICERIS* IN ALGERIA BY RAPD MARKERS

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The chickpea and bean are the two species of legumes grown in most countries of the Maghreb area (Maatougui *et al.*, 1996). In Algeria, *Fusarium oxysporum* f. sp. *ciceris* (FOC) is one of the most damaging chickpea diseases (Bouznad *et al.*, 1998).

This study is based on the molecular characterization by RAPD markers, using two primers OPF 10 and OPI 01, and 11 isolates of FOC designated as follows: K-93, E-00, IT-03, SS-03, I-99, S-93, O-93, O-10/01, O-04/02, and O-10/02 G-93, obtained from different surveys of the main chickpea areas in Algeria. Genomic DNA of each fungal isolate was extracted from 4 g of fresh ground mycelium. Aliquots of samples were analyzed on 1% agar gels in Tris-acetate EDTA buffer (40mM Tris-acetate and 1mM EDTA, pH 8.0) to estimate the concentration and quality of the DNA. Samples were diluted with sterile water to a final concentration of 50 ng/µl.

RAPD reactions were carried out with 2, 10-mer oligonucleotide primers corresponding to the PROLIGO OPF-10 (**5'-**GGAAGCTTGG-**3'**) and OPI-01 (**5'-**GGAAGCTTGG-**3'**), primers. Each reaction mixture (25 µl) consisted of 0.5 µM of primer, 200 µM of each dNTP, and 2.5 µl of 10×reaction buffers, 2U of *Taq* DNA polymerase, 1.5 mM $MgC1_2$ and 50 ng of fungal DNA. Amplifications were performed in a thermocycler PTC 100 (Peltier Thermal Cycler), programmed for 4 min of denaturation at 94 °C, followed by 30 cycles of 1 min of annealing at 37 °C, 3 min of extension at 72 °C and denaturation for 1 min at 94 °C. The final cycle consisted of 1 min of annealing followed by 6 min at 72 °C to produce fully double-stranded DNA fragments. Amplification products were separated by electrophoresis on 1.4% agar gels at 50 V for 10 min followed by 1 h 30 min to 95 V, stained with ethidium bromide and visualized under UV light. (Jiménez-Gasco *et al.*, 2001).

Two primers, one from set OPF and another from set OPI, produced consistent, informative polymorphisms in the RAPD analyses. A binary matrix of combined data from two primers for the 11 FOC isolates was prepared by scoring bands for presence or absence, DNA bands of the same mobility were assumed to be identical. The M.V.S.P. 3.12 (Multi-Variate Statistical Package) was used to cluster the isolates by an unweighted paired group method with arithmetic averages (UPGMA), based on Jaccard's similarity coefficient.

The DNA extracts of isolates amplified with two primers OPF-10 and OPI-01, produced 9 and 15 bands respectively. Each of the two primers yielded specific profiles for each isolate or group of isolates. Thus, both isolates IT-03 and S-93 gave the same profile with primer OPF-10. Furthermore, the primer OPI-01 also generated one profile for isolates S-93, O-04/02 and O-93, whereas the same primer did not

amplify the isolates E-00, I-99. This result revealed a large polymorphism, where no fragment was common for all isolates. However the profile shows some isolate-specific fragments, but, some fragments are shared by several isolates. UPGMA cluster analysis of the RAPD data distinguished one cluster that shared about 55% similarity with three isolates (SS-03, O-93 and S-93) where the last two are closer to each other than the first.

This study showed a remarkable polymorphism among isolates studied. Statistical analysis methods used, allowed us to distinguish one group of isolates that contained isolates SS-03, O-93 and S-93, which induced yellowing symptoms . The remaining isolates have a specific behavior. This suggests a polymorphism within this group ofFOC isolates and consequently the great variability of this special form of *Fusarium oxysporum*.

Key words: Chickpea, *Fusarium oxysporum* f.sp. *ciceris*, RAPD markers

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Petria 20 (2), 67-633 (2010)

DEVELOPMENT OF A DNA-BASED MACROARRAY FOR MULTIPLEX DETECTION OF SOIL-BORNE FRUIT TREE PATHOGENS

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In recent years the European Community, followed by each of the Member States, has developed and published technical rules for the marketing of the propagation material of fruit, ornamental and horticultural plants, with the aim to favour trade, harmonize the system in Member States, guarantee quality and health and prevent introduction and spread of harmful organisms. To this scope, the Italian Ministry of Agriculture published the "Technical guides for the production of certified fruit plant propagation materials" in which it is stated that the production of propagation material of pome fruit tree has to be performed on soils free from the following pathogens: *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae.* At present, no published simultaneous diagnostic method is available for this set of pathogens to certify the absence from soil, even if single molecular diagnostic methods for some of them are reported in the literature. Here we describe the ongoing work for the development of a multiplex method for the detection of the above-mentioned pathogens from soil with a single analysis, useful to ease the certification of propagation material of fruit trees. For this purpose, a macroarray system (Lievens *et al.,* 2006; Zhang *et al.,* 2007) was chosen because this technology permits a high level of multiplexing and is essentially an open system that allows the addition of new targets. With this technology, oligonucleotides detectors are immobilized on a solid support, such as a nylon membrane, and used for the specific detection of the microorganisms. The DNA to be tested is amplified, labelled and then hybridized to the membrane under stringent conditions. If possible, all oligonucleotide detectors should be designed within the internal transcribed spacer region (ITS) of the ribosomal DNA, because of its sequence variability and availability in GenBank databases. The final aim of this study is the set up of a DNA extraction method from soil, the choice of methodology and equipment for macroarray, the identification of specific probes for each fungal species and the development of optimal parameters for hybridization and detection.

For DNA extraction from soil, the UltraClean Soil DNA Kit of Mo Bio Laboratories was chosen but still need optimization, especially as regards the nature

of the soil and DNA yield. For each fungal species under study, the knowledge and the state of art is different: for *V. dahliae* and *V. albo-atrum* oligo detectors already exist in the literature (Lievens *et al*., 2003) and have been used to validate the method; for *P. cactorum*, four oligo detectors have been designed on the ITS region that should be able to distinguish *P. cactorum* from other fungal and *Phytophthora* species, especially from the very closely related *P. hedraiandra*. The other fungal species needed more studies on molecular genetic variability because few data are available in GenBank. For this reason, different isolates of each species were collected and their ITS region sequenced. The aim is to obtain one or possibly two oligonucleotides detector for each fungal species with uniform hybridization kinetics (around 55°C by the nearest-neighbor method) for spotting on the membrane.

Key words: Diagnosis, Macroarray, Soil-borne pathogens, Pome fruits.

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PETRIA 20 (2), 67-633 (2010)

MOLECULAR DETECTION OF *PHOMA TRACHEIPHILA* IN PLANT TISSUES AND ANALYSIS OF GENETIC DIVERSITY OF ISOLATES FROM DIFFERENT CITRUS SPECIES IN TUNISIA

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Phoma tracheiphila (Petri) Kantschaveli & Gikashvili is a destructive vascular pathogen of citrus, particularly lemons (*Citrus limon* (L.) Burm. f.), but the fungus has also been reported on many other *Citrus* spp. (Perrotta and Graniti, 1988). *P. tracheiphila* is a major threat to lemon in the Mediterranean and Black Sea region and is considered as a quarantine pest in the A2 list by the EPPO and in the A1 list by most other regional plant protection organizations (EPPO/OEPP., 2005). Since its first observation in limited citrus areas in the northern part of Tunisia in 1960, the disease has become endemic in many orchards and spread to the major producing regions of the country (Hajlaoui *et al.*, 2008).

Although highly sensitive and reliable PCR techniques were developed for earlier pathogen detection and monitoring for epidemiological and breeding studies (Ezra, 2007; Licciardello, 2006; Balmas *et al*., 2005), current diagnosis of the disease in Tunisia continues to be mainly based on observation of symptoms and isolation of the pathogen and therefore do not allow early detection.

This study focused on genetic diversity of a collection of *Phoma tracheiphila* isolates recovered from different orchards and host species to determine the major profiles allowing the assessment of the pathogen downward progression from inoculated point on Citrus leaves.

Genetic variability of 58 isolates of *P. tracheiphila* including four Italian isolates assessed by ITS-RFLP molecular markers revealed that Tunisian isolates of *P. tracheiphila* are homogenous and are genetically similar to the Italian isolates. Development of this pathogen is likely clonal under Mediterranean conditions and should be taken into account for the development of management strategies based on resistant varieties.

This result made possible the molecular assessment of the pathogen migration in sour orange vessels using only one representative virulent isolate. PCR showed that within 10 dpi (days post inoculation) fungal DNA was detected 10 cm from inoculation point and at 30 dpi fungal DNA was present in the stem vessels at a distance of 50 cm and more from inoculation point. *P. tracheiphila* basipetal translocation is very quick and is prior to symptom expression. Consequently, diagnosis of mal secco based on typical symptoms as shedding of leaves, wilting and the salmon pink discoloration of

wood is not appropriate for early detection and limiting the spread of the disease and must be replaced by a molecular technique that allows the detection of the infection in asymptomatic plants in nurseries.

Key words: Mal secco, *Phoma tracheiphila*, PCR, ITS-RFLP, Early detection

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Petria 20 (2), 67-633 (2010)

DETECTION OF *BOTRYTIS CINEREA* LATENT INFECTION IN STORED TABLE GRAPE

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Botrytis cinerea Pers. is regarded as one of the most important postharvest fungal pathogen causing significant losses in a wide range of crops, but particularly in grapes (*Vitis vinifera* L.). Invasion by the fungus may occur by active penetration or passive ingress through wounds, as well as *via* floral parts (petals, stigmas, styles, or stamens), where it remains dormant until ripening, when it resumes activity resulting in bunch rot (Prusky, 1996). Under conditions prevailing during storage, shipment and marketing, such latent infections may account for a high percentage of berry infections (Nair *et al.*, 1995). Control of *Botrytis* on harvested crops has relied mainly on preharvest chemical fungicides, however, the severe restrictions and regulations imposed on postharvest chemical treatments have made the future of many of these chemicals uncertain (Droby and Licher, 2007). To develop better and more efficient methods for controlling *Botrytis* storage rot, an early detection of the pathogen in the field and/or during storage is essential.

In the present investigation two traditional strategies and a molecular method were utilized to detect *B. cinerea* latent infections in stored table grape berries. Traditional strategies were based on the induction of tissue senescence to activate latent infections on surface sterilized berries and consisted of: i) paraquat application or ii) fruit freezing for 2 hours at -20°C. Apart from being less harmful, freezing was more effective than paraquat in showing up latent infections of *B. cinerea.* Using this strategy it was assessed that the tested grape genotypes varied in their resistance to infection, nevertheless, in all of them infection was mainly located in the berry-pedicel attachment zone.

A new molecular method based on real-time quantitative PCR (qPCR) was developed using the Taqman chemistry and exploiting variable intergenic spacer (IGS) regions of the ribosomal DNA (rDNA). The method proved to be highly specific and sensitive enabling the quantification of as little as 100 fg of *B. cinerea* DNA. Furthermore, it also incorporated the detection of a gene from the plant host in order to compensate for variations in extraction efficiency and enable more accurate quantitative analyses.

Freezing and qPCR assays were utilized to detect infection of *B. cinerea* in grape berries naturally infected or artificially inoculated at the berry-pedicel attachment zone. Freezing and qPCR results were always in accordance, reflecting the fact that the actual disease severity was detected. However, qPCR assay was more effective in detecting latent infection at an early stage of development, leading to a new capability for investigating infection processes *in-planta,* and particularly for detecting and quantifying the pathogen prior to the development of any symptom.

The combined ability of the two assays to early detect *B. cinerea* in berry and to monitor fruit colonization, provides a resource for informing disease management decisions and to study mechanisms of disease resistance.

Key words: Freezing, Real-time PCR, *Botrytis cinerea*, Latency, Quantification

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PETRIA 20 (2), 67-633 (2010)

EFFICACY OF DIFFERENT BAITS TO ISOLATE AND QUANTIFY *PHYTOPHTHORA* SPECIES IN CHESTNUT SOIL SAMPLES

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The genus *Phytophthora* is known to be potentially harmful to woody plants in natural forest and hardwood plantations. Some species are host specific such as *Phytophthora quercina* on *Quercus* (Jung *et al*., 2000). Other *Phytophthora* species are associated with decline of a broad range of hosts in forest stands, natural environment and plantations. Several *Phytophthora* species have been associated with Ink Disease, which is one of the main threats to sweet chestnut in Europe (Vettraino *et al*., 2005). *Phytophthora cambivora* and *P. cinnamomi* are the only two species that have been isolated from necrotic chestnut tissues. The roles of other *Phytophthora* species need to be clarified even if their pathogenicity on chestnut seedlings has already been reported in the literature (Vettraino *et al*., 2001). Early diagnosis is a crucial point to define the disease control strategies. The detection of *Phytophthora* species by baiting is a simple and sensitive method for monitoring the pathogens in soil (Cooke *et al*., 2007).

This study was undertaken to elucidate the efficacy of different baits to isolate and quantify the presence of *Phytophthora* species in chestnut soil samples. We developed a method for the isolation and quantification of species in this genus. *Azalea*, *Castanea* and *Rhododendron* leaves, and *Carnation* petals were tested as baits in soil. The baits proved to be selective towards the different species ($P>0.05$). *Carnation* petals and *Rhododendron* leaves were more effective than the other baits for the isolation of rare *Phytophthora* species (*P. megasperma*, *P. nicotianae*, *P. pseudosyringae* and *P. syringae*). *Phytophthora cryptogea* was isolated only with *Azalea* and *Castanea* leaves. In a sensitivity test chestnut leaves and *Carnation* petals detected a higher percentage (more than 22%) of *Phytophthora* species after a baiting period of one week. The baiting method described here will be useful for monitoring *Phytophthora* species in chestnut soil samples.

Key words: *Phytophthora* spp., Baiting, *Castanea sativa*

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PETRIA 20 (2), 67-633 (2010)

DETECTION OF *BISCOGNIAUXIA NUMMULARIA* IN ASYMPTOMATIC BEECH TREES OF THE ITALIAN APENNINE MOUNTAINS

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Biscogniauxia nummularia (Bull.: Fr.) Kuntze is a fungus that causes strip-canker and wood decay on European beech trees (*Fagus sylvatica* L.) when they are subjected to environmental stress (Capretti *et al*., 2003). The fungus is also able to live in host tissue without showing any symptoms, i.e. surviving as an endophyte.

In this study we evaluated the usefulness of TaqMan real-time PCR to assess the incidence of the latent phase of the fungus, i.e. in symptomless host tissue of apparently healthy beech trees, in two different forests located in Tuscany and Latium (northern and central Apennines). One-two-year-old twigs were collected from asymptomatic individuals adult trees throughout the four seasons. Samples were used both for isolation and DNA extraction following the method described by Luchi *et al.* (2006).

Real time PCR detected the pathogen in > 50% more samples than classical reisolation. Furthermore, *B. nummularia* DNA content varied between the two sampling areas as well as among seasons and ranged between 10^{-2} to 10^{6} pg/µg total DNA.

B. nummularia occurred more frequently in areas of central Italy characterized by milder temperate climate than in the northern Apennines, suggesting similarities to *B. mediterranea*, the causal agent of charcoal disease on oak species (Luchi *et al.*, 2005), which is also harmful to trees suffering from water stress.

Key-words: *Fagus sylvatica*, *Biscogniauxia nummularia*, Early detection, Real-time PCR, Symptomless tissue

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Petria 20 (2), 67-633 (2010)

REAL-TIME PCR ASSAY TO DETECT *PHYTOPHTHORA* SPECIES FROM PLANTS, SOIL AND WATER

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Species in the genus *Phytophthora*, occurring in terrestrial and aquatic habitats, are well known as primary parasites of fine roots and collar rots of plants (Moralejo *et al.*, 2009). Among the diagnostic tools developed in plant pathology real-time PCR has proved to be an efficient method to detect and quantify *Phytophthora* species in different environments. In this study a real time PCR assay, using SYBR Green chemistry, was optimized to detect and quantify *Phytophthora* spp. from different samples, namely a) bark collected from symptomatic chestnut tree (*Castanea sativa*) infected with *P. cambivora*; b) sterilized soil, inoculated with *P. cambivora*; c) *Vicia faba* seedlings inoculated with *P. cinnamomi* and *P. cactorum* isolates; d) sterilized water samples inoculated with *P. cinnamomi* and *P. cactorum* strains.

DNA was extracted from fungal mycelium, plant material and water as described by Luchi *et al.* (2005). DNA from soil samples was extracted with the FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. A real time PCR assay was developed, using primers described by Schena *et al.* (2008). A genus-specific primer was used to detect *P. cinnamomi* and *P. cactorum*, while *P. cambivora* was detected with species-specific primers. Their specificity was firstly tested on DNA extracted from pure cultures of *Phytophthora* spp. and *Pythium*. By using species-specific primers with real time PCR, it was possible to detect the presence of *P. cambivora* in chestnut and soil. *Phytophthora cambivora* DNA was detected in bark samples after nested PCR. Also inoculated soil showed the presence of *P. cambivora* DNA (10^4 pg/μg of total DNA extracted).

The sensitivity of the method during the early stage of colonization was shown by detecting *P. cinnamomi* and *P. cactorum* DNA in seedlings after 3, 5 and 10 days after-inoculation. The DNA of pathogens was quantified and ranged from 10^3 to 10^4 pg/g. It was also possible to detect the occurrence of *P. cactorum* and *P. cinnamomi* DNA in water. Presence of pathogens was: 10^2 pg/μg and 10^3 pg/μg respectively. The sensitiveness and specificity of real-time PCR were also confirmed in infected plants, water and soil, showing its ability to detect small amounts of *Phytophthora* DNA from different samples.

Key words: *Phytophthora*, Real-time PCR, SYBR Green, Collar rot

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Petria 20 (2), 67-633 (2010)

IDENTIFICATION DIFFERENT POPULATIONS OF *HETERODERA SCHACHTII* IN IRAN WITH BASED OF MORPHOLOGY AND PCR-RFLP

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Heterodera schachtii, the sugar beet cyst nematode, is an economically important pest of sugar beet widespread in most European countries, USA, Middle East and other parts of world (Sharma, 1998).This nematode is very important in Iran and cause serious yield reduction and decreases sugar content of sugar beet wherever the crop is grown (Mehdikhani *et al.*, 1996). *Heterodera schachtii* is very similar to *H. betae* in the morphology of vulval cone of cyst and morphometrics of second-stage juvenile (J2) and, in some *H. schachtii* populations, these characters are so variable that an accurate identification is difficult. DNA-based methodologies have been used as an alternative for taxonomy and diagnostic of plant-parasitic nematodes. It was demonstrated that the DNA technique based on ITS-PCR-RFLP is useful for separating *H. schachti*i from other species of this genus (Tanha Maafi *et al.*, 2003)

During 2009-2010, 250 soil and root samples were collected from sugar beet fields in Khorasan province, Iran. The cysts were extracted by a combination of Cobb's sieving and decanting method and sugar flotation method. The morphological and morphometrical characters of 150 *H. schachtii* populations were studied (Mulvey & Golden, 1982). For each population, vulval cones of several cysts were mounted in glycerin and J2, from the same cysts, were killed by gentle heat, fixed in TAF and transferred to glycerin. The specimens were examined and measured with a light microscopy. Among the 150 populations, 20 populations with high variation in morphological characters were selected for the molecular studies. DNA was extracted from J2 and eggs using the methodology described by Joyce *et al.* (1994). Each PCR product was digested with the restriction enzyme MvaI. Procedures for obtaining PCR amplified products and endonuclease digestion were repeated at least twice.

The restriction patterns obtained with MvaI were similar in all the 20 *H. schachtii* populations which means that molecular intraspecific polymorphism was not detected in these populations. Our results confirm that ITS-PCR-RFLP is a reliable character to identify *H. schachtii* populations with morphobiometrical variability.

Key words: ITS-PCR-RFLP, Morphobiometry, Sugar beet cyst nematode

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Petria 20 (2), 67-633 (2010)

GENE-SEQUENCE ANALYSIS FOR THE MOLECULAR DETECTION OF *PSEUDOMONAS SYRINGAE* PV. *ACTINIDIAE*

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Severe damages on kiwifruit, caused by bacterial canker, occurred in central Italy in the last three years. The causal agent of the disease is the bacterium *Pseudomonas syringae* pv. *actinidiae*, recorded in Italy on *Actinidia deliciosa* cv. Hayward in 1994, observed also in China, and causing important economic losses in Japan and South Korea. In the last few years, many serious damages were observed on yellow kiwifruit (*A. chinensis* Planchon) cultivars Jin Tao and Hort 16A (Balestra *et al*., 2008; Ferrante e Scortichini, 2009). The detection of this *P. syringae* pathovar is mainly based on traditionally techniques, followed by identification of pure cultures by rep-PCR or sequencing of 16S rDNA. Molecular detection by PCR amplification was reported by Koh and Nou (2002) (KN-PCR) and, recently, by Rees-George *et al.* (2010) (RG-PCR). Both these methods, were found not to be specific, producing an amplicon of the same size with *P. s.* pv. *theae* (Rees-George *et al,* 2010). Moreover, KN-PCR was reported to give false positive signals also with strains of *P. s.* pv. *tomato* and *P. s.* pv. *syringae,* and produced an aspecific amplicon with *P. s.* pv. *papulans* (Rees-George *et al.,* 2010). On the other hand, *P. s.* pv. *actinidiae* is reported to be genetically related to *P. s*. pv. *theae* and to *P. avellanae* also, all belonging to the genomospecies 8 *sensu* Gardan *et al.* (1999); Gardan *et al*. (1999) reported that genomospecies 3, which *P. s.* pv. *tomato* belongs, and 8 were not clearly distinguished by ribotyping; furthermore, a multilocus sequencing typing (MSLT) analysis based on seven housekeeping genes, grouped together *P. s*. pv. *actinidiae*, *P. s*. pv. *tomato* and *P. s*. pv. *theae*. To find new specific DNA marker for a specific PCR-based detection of *P. s*. pv. *actinidiae,* a gene-sequence analysis was performed in this study. In consideration of the high relationship of this pathovar with other *P. syringae* pathovars, this investigation was focused, other than on highly conserved genes, also on genes potentially involved in the interaction with the host plant. The following genes were investigated: *avrD*, *hrpW*, *hrpL, rpoD*, 16SrDNA, on representative isolates of *P. s*. pv. *actinidiae*, *P. s.* pv. *theae, P. s*. pv. *tomato* and *P. avellanae;* the sequence of the 492 bp amplicon of Kou and Nou (2002) was also analyzed. The comparison among these nucleotide sequences, each other and with known NCBI GenBank sequences, confirmed the high genetic correlations among the cited bacterial species. The nucleotide identity varied from 85% to 100% among the different hortologs, highlighting their potential use as specific markers. This investigation enabled us to develop a duplex-PCR able of differentiating *P. s*. pv. *actinidiae* from *P. s.* pv. *theae, P. s*. pv. *tomato* and *P. avellanae.* This method is being

evaluated by testing different *P. syringae* pathovars, different species and genera of plant pathogenic bacteria, and also naturally infected kiwifruit plants.

Keywords: *Actinidia deliciosa, A. chinensis***,** bacterial canker**,** molecular detection

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PHYTOPLASMA DETECTION BY LNA PROBE-BASED REAL TIME-PCR IN POTATOES

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Phytoplasmas are unculturable, wall-less prokaryotes that cause disease in many plant species world-wide. Different phytoplasmas have been associated with diseases of potatoes, including virescence, 'witches' broom and the two quarantine diseases: purple top wilt and stolbur. Following classification based on RFLP analysis of the 16SrRNA gene sequence, potato-associated phytoplasmas were found to belong to 16SrI, 16SrII, 16SrVI, 16SrXII groups and to the proposed specie *'Candidatus* Phytoplasma americanum' (Lee *et al*., 2006). More recently, a 16SrIII group phytoplasma in Montana (Lee *et al*., 2009) and the '*Candidatus* Phytoplasma australiense' in New Zealand (Liefting *et al*., 2009), have been reported. Due to the wide diversity found in phytoplasmas affecting this host a detection method which is specific, yet sensitive and reliable is required. Phytoplasma detection using the available universal primers designed from the 16SrRNA gene, produced many false positives resulting from the presence of other bacteria naturally present in the potato samples analyzed. Once sequenced these bacteria were found to be close relatives of phytoplasmas, on the basis of their 16SrRNA gene. A similar approach based on nested-PCR improved the specificity of this diagnostic test but with inconsistent results using different primer combinations.

As a consequence, an alternative approach based on the use of locked nucleic acid (LNA) probes and real-time PCR was investigated. The chemistry of LNA probes offers advantages of improved specificity and sensitivity over conventional DNA probes (Costa *et al.*, 2004; Josefsen *et al*., 2009). The detection assay developed using this approach has been trialled with 100 potato samples and improvements in specificity, repeatability, and sensitivity were all evident when compared against results obtained using conventional PCR. This is the first report of use of LNA probe in Real Time PCR as diagnostic tool for phytoplasmas.

Key words: Locked Nucleic Acid, Real time-PCR, *Solanum tuberosum*, phytoplasma

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Petria 20 (2), 67-633 (2010)

DETECTION AND RELATIVE QUANTIFICATION OF '*CANDIDATUS* PHYTOPLASMA PRUNORUM' BY SPOT REAL-TIME RT-PCR TAQMAN ASSAY

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'*Candidatus* Phytoplasma prunorum' is associated with the quarantine phytoplasma European stone fruit yellows (ESFY) disease that generally induces yellows, tree decline or die-back and vegetative disorders with typical symptoms such as an early bud break and leaf rolling on most of wild and cultivated *Prunus* species. ESFY is mainly known in Europe, but has also been reported in Turkey. It was first described in Italy as a decline of Japanese plum (*Prunus salicina*).

'*Ca.* P. prunorum' causes substantial economic loss due to the decline and death of the infected trees. Due to the high efficiency of its natural vector *Cacopsylla pruni* and the use of not symptomatic infected plant material, '*Ca.* P. prunorum' can spread rapidly in stone fruits cultivated areas. In apricot orchards the number of infected trees can double in few years (Ramel and Gugerli, 2004).

In order to reduce ESFY spreading, a specific, sensitive molecular diagnostic method is needed to early identify and then remove infected plants.

Symptoms variability related to season, host plant and presence of asymptomatic infected plants make the symptomatic detection of '*Ca.* P. prunorum' unreliable. Up to now several specific molecular assays have been published based on DNA extraction protocols and nested or Real-Time SYBR Green PCR methods

In the present work plant sap, obtained grinding 1.0 g of plant material on appropriate buffer, was spotted on Nylon membrane discs for rapid extraction (25 minutes) of nucleic acids (Osman and Rowhani, 2006).

Using sequence alignment of the 16S rRNA gene region of nine different phytoplasma strains (Baric and Dalla Via, 2004) an assay based on specific '*Ca*. P. prunorum' primers and MGB probe was designed. A previously published assay (Osman *et al.,* 2007) was slightly modified in order to design a control assay using sequences of plant 18S ribosomal RNA.

Experiments conducted on 10 selected samples analyzed in presence and absence of Reverse Transcriptase on DNase treated and untreated nucleic acid extractions revealed high abundance of phytoplasma RNA instead of DNA suggesting that methods based on Reverse Transcription PCR reach the maximum efficiency for phytoplasma detection.

One hundred two among plum, apricot and peach samples were analyzed using Spot Real-Time RT-PCR assay and all results were consistent with those obtained using the well established previously described method (Angelini *et al*., 2001) with some modification.

In conclusion, a rapid, sensitive and reliable diagnostic method based on Spot Real-Time Reverse Transcription - PCR TaqMan assay has been developed for '*Ca.* P. prunorum' detection and relative quantification.

Key words: *'Candidatus* Phytoplasma prunorum*'*, ESFY, Reverse-Transcription, Real-Time PCR

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Petria 20 (2), 67-633 (2010)

TOMATO YELLOW LEAF CURL DISEASE ASSOCIATED WITH *BEGOMOVIRUSES* AND WHITEFLY VECTORS IN GREECE AND CYPRUS

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Tomato yellow leaf curl disease (TYLCD) is considered to be one of the most important and devastating viral diseases of tomato and other cultivated crops in many agricultural systems around the world (Czosneck and Laterrot, 1997). Several virus species, vectored by the sweet potato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in a persistent manner, have been shown to be associated with the disease, all assigned to the genus *Begomovirus*, family *Geminiviridae*. In Europe and the Mediterranean region, two are the most common *Begomovirus* species involved: *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Accotto *et al*., 2000), transmitted by the B and Q biotypes of *B. tabaci*. Though these virus species cause indistinguishable symptoms, their differentiation is of high importance because TYLCV is more aggressive, causes greater economic losses and has a broader host range (Martinez-Culebras *et al*., 2001). On the other hand, *B. tabaci* biotypes are morphologically indistinguishable, lacking clear-cut differentiating morphological characters in either the pupae or the adults (Brown *et al*., 1995).

The epidemiology and characterization of begomoviruses involved in TYLCD and biotypes of the vector *B. tabaci*, was studied in Greece and Cyprus during 2006-2009. Two real-time TaqMan® PCR assays were developed and optimized for the rapid, simultaneous identification of TYLCV/TYLCSV, as well as for the multiplex detection of the B and Q biotypes of the *Bemisia tabaci* complex. More than 8000 samples of different cultivated plants (including tomato, bean, pepper) and weeds were analyzed together with approximately 1300 adult *B. tabaci* from 9 and 5 districts of Greece and Cyprus, respectively.

Results showed that TYLCV was widespread on tomato crops and weeds in the mainland of Greece, the islands Crete, Rhodes and Cyprus. In Greece, TYLCSV was only found on Peloponnese (mailand) and Crete in a very low incidence whereas TYLCV was also reported to cause leaf crumble symptoms on bean plants. In Cyprus, TYLCV was detected in 461 samples of 50 different species belonging in the families of Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Euphorbiaceae, Geraniaceae, Leguminosae, Malvaceae, Orobanchaceae, Plantaginaceae, Primulaceae, Solanaceae, Umbelliferae and Urticaceae. Molecular

identification of *B. tabaci* biotypes showed that Q was the only biotype found in the mainland of Greece and the island Crete, whereas biotype B was only reported on Rhodes Island. In the Cypriot *B. tabaci* populations both B and Q biotypes co-exist with biotype B being more widespread in the island. Phylogenetic analysis of the mtCOI DNA sequences corroborated the identity of the B and Q biotypes 100% of the time and the haplotypes were grouped in the major North African-Mediterranean-Middle Eastern clade of the *B. tabaci* complex (Papayiannis *et al*., 2009).

Key words: TYLCV, TYLCSV, *Bemisia tabaci*, Biotypes, TaqMan PCR

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Petria 20 (2), 67-633 (2010)

UNIVERSAL PRIMERS BASED ON CONSERVED REGION OF COAT PROTEIN FOR THE DETECTION OF WHITEFLY TRANSMITTED BEGOMOVIRUSES

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Diseases caused by whitefly transmitted begomoviruses result in huge yield losses in foodfiber and vegetable crops in tropical and subtropical regions. The begomoviruses have characteristic twinned particle morphology (18x30mm), encapsidate a circular single stranded DNA genome of ~ 2.7kb, and are all transmitted by only one species of the vector *Bemisia tabaci* Genn. All the begomoviruses share around 60% identity in the coat protein region and based on coat protein gene comparison, could be clearly distinguished into Old World and New World begomoviruses. Within the coat protein region, from nucleotide 4 to 279, is variable resulting in a diverse N' terminal region; however in the C' terminal region, all the viruses share 75.3 % sequence identity.

Universal primers to detect all begomoviruses was designed based on multiple alignment of sequences of begomoviruses infecting tomato, cotton, bhindi and other weed hosts in India. DNA was extracted from different samples of begomoviruses-infected plants from different regions, and tested in PCR using the primers (ToLCPF/ ToLCPR:

AAGATATGGATGGATGAGAAC/ACATAATTATTAACCCTAACAA). In normal PCR, the primers could detect six tomato leaf curl viruses occuring in India (*Tomato leaf curl New Delhi virus, Tomato leaf curl Palampur virus, Tomato leaf curl Gujarat virus, Tomato leaf curl Bangalore virus, Tomato leaf curl Joydebpur virus, Tomato leaf curl Karnataka virus*), two cotton leaf curl viruses (*Cotton leaf curl Rajasthan virus*) and the yellow vein and the yellow mosaic viruses occurring in *Acalypha, Xanthium, Ageratum* spp.

Further validation of these primers in confirming the detection of begomoviruses in large number of hosts is in progress. This may fund its application in molecular epidemiology of important diseases.

Key words: Begomoviruses, Whitefly, Leaf curl

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PETRIA 20 (2), 67-633 (2010)

OPTIMIZATION RT-PCR DETECTION OF *TOMATO INFECTIOUS CHLOROSIS VIRUS* AND *TOMATO CHLOROSIS VIRUS*

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Tomato infectious chlorosis virus (TICV) and *Tomato chlorosis virus* (ToCV) are two whitefly-transmitted viruses, members of the genus *Crinivirus*, family *Closteroviridae*. First identified on tomato (*Solanum lycopersicum*) in the USA in the mid 1990s (Duffus *et al*., 1996; Wisler *et al*., 1998), TICV and ToCV rapidly spread worldwide as they been reported in many temperate areas of Europe, the Mediterranean basin, North America, South Africa and Asia. In Italy, TICV and ToCV appeared in 2001, in Liguria (Vaira *et al.*, 2002) and in Sardinia, Sicily, Apulia (Accotto *et al.*, 2001), respectively, and now they are in all regions where tomato is grown indoors. Both viruses induce similar symptoms (interveinal yellowing, red or brown necrotic flecking and brittleness on older leaves) that can be often confused with nutritional deficiencies or physiological disorder. This factor might have led to underestimation of the incidence of the disease. TICV and ToCV have been included in EPPO A2 list, thus the development of a validated diagnostic protocol is needed. The routine diagnosis of TICV and ToCV is currently done using molecular tools, mainly RT-PCR. The most commonly primers target is a highly conserved protein – the heat shock related protein (HSP70) - encoded by RNA 2 - ORF 1 of all criniviruses.

Recently, surveys were carried out in different Italian regions with the aim to verify and investigate the spread of the two viruses in protected crops. The uncertain data obtained routinely by testing symptomatic samples using RT-PCR, revealed the need for a more suitable and sensitive test. Therefore, in the present study several primer sets reported by different authors were evaluated for their efficacy in routine diagnostic assays of both viruses.

For each virus, ten isolates from naturally infected tomato leaves, collected in four different Italian regions, were selected. Five specific primer sets, each for TICV and ToCV, designed in different genomic regions (HSP70, coat protein - CP, diverged coat protein - CPd), were evaluated. Specificity, sensitivity and repeatability were determined. Under our laboratory conditions, the primer sets showed some differences concerning these performance characteristics. As result, an universal protocol of one step RT-PCR was optimized providing accuracy (specificity/sensitivity) and repeatability for detecting TICV and ToCV in naturally infected samples.

Keywords: Crinivirus, TICV, ToCV, Diagnostic protocol

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PETRIA 20 (2), 67-633 (2010)

QUALITATIVE AND QUANTITATIVE DETECTION OF *POLISH PEANUT STUNT VIRUS* STRAINS USING RT-PCR AND REAL-TIME PCR TECHNIQUES

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Peanut stunt virus (PSV) belongs to the *Cucumoviridae* family. It is one of the most important pathogens of legumes all over the world. The genome of this pathogen consists of three single RNA components and two subgenomic RNAs encoding 2b and coat protein (CP). In some cases there is also a fifth component designated satellite RNA (satRNA) (Militao *et al*., 1998). RNA1 and RNA2 encode proteins that are compounds of the replication complex; RNA3 encodes the viral CP (Karasawa *et al*., 1992). The main role of this protein is viral particles encapsidation, but it is also important in viral replication and distribution.

Among the molecular diagnostics approaches prevail those based on viral CP characteristics are prevail. Mainly due to their high sequence conservation that can be utilized in identification as well as in comparative alignments and phylogenetic applications (Hull, 2002).

Our goal was to develop a specific and efficient protocol for the qualitative and quantitative detection of PSV in infected plants. We studied five Polish strains: PSV-P (from yellow lupine), -Ag (form celery), -G (from pea), -RobRos and -SA6 (from *Robinia pseudacacia)*. First, specific primers complementary to the viral CP were designed on the basis of the alignment of gRNA sequences obtained from the Polish PSV strains deposited in the GenBank. Total RNA extracted from *Nicotiana benthamiana* plants inoculated with afore-mentioned strains of PSV, was used as a template. RT-PCR reaction was carried out followed by its optimization. As a result we could obtain the specific product approximately 150bp in length and reaction proved to be robust, efficient and suitable for all studied strains.

To increase the detection sensitivity, we developed also a real–time RT-PCR protocol with SybrGreen dye using the same primers. To estimate the lowest concentration of target that is still detected and the efficiency of the reaction, serial dilutions of the analysed RNA template were used. Amplification was efficient even if 10000-fold dilution of template RNA was used.

Performed diagnostic tests have shown very high sensitivity and efficiency. Therefore, they can be applied for virus diagnostics, examination of disease development in different plant hosts, as well as for estimation of the degree of accumulation of viral particles in the host.

Key words: PSV, CP, Detection, Real-time PCR

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DETECTION OF THE POLISH ISOLATE OF *SUGARCANE MOSAIC VIRUS* (SCMV) USING A REAL-TIME RT-PCR ASSAY

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Maize mosaic is the most widespread viral disease of maize in Europe. *Sugarcane mosaic virus* (SCMV) is one of causal agents of this disease (Fuchs, 2004). In Poland SCMV was first reported in 2006 (Trzmiel *et al.,* 2006). Since then the virus has been regularly detected in maize cultivars. Infected maize plants were confirmed in the western (Wielkopolska), in the southern (Lower Silesia and Małopolska), in the south-eastern (Podkarpacie and Lubelszczyzna) regions of Poland.

SCMV is a member of the *Potyviridae* family and the *Potyvirus* genus. It infects many species of the *Poaceae* family. It is transmitted by aphids, mechanical inoculation and by seeds (Persley, 1980). Chemical control of SCMV is not possible due to the non-persistent mode of virus transmission by aphids. The breeding and cultivation of resistant varieties have proved to be the most promising and only possibility of an effective control.

Viral quantification is an essential tool for the study of breeding for resistant plants. A real time quantitative reverse transcription polymerase chain reaction (QRT-PCR) is a technique that provides accurate and reproducible quantification of gene copies. This method monitors fluorescence emitted during the reaction. An increase of fluorescence is associated with the concentration of RT-PCR product in the reaction. The method can be useful to examine disease development in different varieties.

The main aim of this investigation was an identification of the Polish isolate of SCMV by the real time RT-PCR and optimization conditions of the reaction.

The reaction was done using Brilliant II SYBR Green QRT-PCR Master Mix Kit, 1-Step (Stratagene) following the manufacturer's instruction. Total plant RNA from 100-120 mg of fresh infected maize leaves was extracted by RNeasy Mini Kit (Qiagen) according to the procedure supplied by the producer. The extraction was done about twelve days post inoculation. The concentration was determined in The Thermo Scientific NanoDrop 1000 spectrophotometer**.** The specific primers pair were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3-www.cgi) for amplification of 249 bp fragment of coat protein gene. The presence of an expected RT-PCR product was analyzed also by agarose gel electrophoresis.

The real time RT-PCR assay could be used as an excellent diagnostic tool for SCMV detection in maize and different host plants.

Key words: *Sugarcane mosaic virus* (SCMV), Real Time RT-PCR, Detection of viruses

Acknowledgements

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IDENTIFICATION AND MAPPING OF POTATO CYST NEMATODES IN CYPRUS USING MULTIPLEX TaqMan® REAL-TIME PCR AND GEOGRAPHICAL INFORMATION SYSTEMS

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Potato cyst nematodes (PCN), *Globodera pallida* (Stone) Behrens and *G. rostochiensis* (Wollenweber) Behrens are considered to be responsible for major losses in several potato growing areas of Cyprus. To alleviate losses, growers make excessive use of nematicides having a negative impact on both the environment and human health. The present study aims towards the integrated management of PCN through the utilisation of potato varieties resistant to particular PCN species and biotypes.

The incidence and prevalence of PCN species was investigated in a large potato production area located in the eastern part of the island, using a previously described PCR assay (Bulman and Marshall, 1997). A multiplex TaqMan® real-time fluorescent PCR assay was developed and evaluated, for the high-throughput discrimination and identification of the two *Globodera* species. The assay was successfully used to detect and quantify the number of eggs in infested fields. Results confirmed the presence of both *G. pallida* and *G. rostochiensis* in the island. *G. rostochiensis* was more prevalent in the largest part of the surveyed area (Xylophagou, Liopetri, and Ormideia), whereas *G. pallida* was more frequently detected only in an area of 20 km² in Sotira. At the moment, the determination of PCN biotypes is being investigated through greenhouse pathogenicity tests using a set of differential hosts.

In order to map the distribution of the two species, the coordinates of the surveyed fields were recorded at the time of sampling using Global Positioning System equipment. The use of Geographical Information Systems will assist in understanding the distribution of PCN and in the development and optimisation of an integrated pest management system.

Key words: *Globodera* spp., Real-time RT-PCR, Taqman probes

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SESSIONE 3

New or unusual disease reports

ORAL PRESENTATIONS

EMERGING PLANT DISEASES: THE EPPO PERSPECTIVE

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Human societies have throughout their histories faced the emergence of new plant diseases which damaged crops or the environment. In plant pathology, the classical example remains the disastrous consequences of the introduction of potato late blight which caused famine in Ireland in the 1840s and now causes problems in potato production worldwide. In more recent history, many new plant diseases have emerged in different parts of the world, and this phenomenon seems to have accelerated. Although there is no agreed definition of what is an emerging plant disease, it can correspond to an already known disease whose incidence or geographical distribution is notably increasing but it may also be caused by newly described pathogens. The causes of plant disease emergence are multiple and quite complex, but it is generally accepted that human activities (e.g. trade of plants, accidental introduction of vectors, modifications of agricultural practices or land use) play an important role.

In the European and Mediterranean region, agriculture is an economically important sector covering a large variety of plants which are subject to an ever increasing trade and at the same time potentially threatened by a wide range of pests and diseases. Therefore, it is essential for Plant Protection Services to avoid the introduction and spread of new pests via commercial exchanges. Over the years, EPPO has made recommendations to its fifty member countries on phytosanitary measures which should be implemented to avoid the introduction of damaging pathogens (e.g. *Xanthomonas citri* pv. *citri, Liberibacter* species associated with citrus huanglongbing which are currently emerging in the Americas) or to prevent further spread of diseases which already occur in the region (e.g. *Citrus tristeza virus, Plum pox virus*). However, these existing phytosanitary measures can be challenged by the emergence of new diseases. In the EPPO strategy, it is felt essential to assess the risks associated with emerging diseases and, whenever appropriate, to propose management measures (i.e. restrictions on trade) against them. EPPO has elaborated a Pest Risk Analysis (PRA) scheme which will be presented. When new diseases are emerging, it is also important to provide early warning to Plant Protection Services so that they can put into place import inspections and surveillance programmes on their territories. Since 1998, EPPO has set up an Alert List on its website (www.eppo.org) to provide data on emerging diseases (e.g. '*Candidatus* Phytoplasma solanacearum', *Chalara fraxinea, Fusarium oxysporum* f.sp. *lactucae, Phytophthora*

kernoviae, Pseudomonas syringae pv. *actinidae,* viroids of solanaceous plants, new tomato viruses). Some of these emerging pathogens may later be submitted to a PRA and eventually be recommended for regulation as quarantine pests. When a quarantine status is considered appropriate for an emerging pathogen, EPPO Standards can also be developed in order to provide guidance on diagnostics, certification schemes, eradication and containment programmes.

Key words: Emerging diseases, Pest Risk Analysis, Alert systems

DETECTION OF A NEW DSRNA VIRUS IN KERGUELEN ISLANDS NATIVE APIACEAE *AZORELLA SELAGO*

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Symptomatic and asymptomatic *Azorella selago* were collected from the spontaneous flora of Kerguelen Islands. Lyophilised leaves materials from ten samples were regrouped and used for dsRNA extraction to allow the detection of any virus presence. Two bands, probably corresponding to dsRNA, were revealed in agarose gel. cDNA fragments were synthesised using random primers and used as template to amplify the whole genome with WGA Kit (GenomePlex Complete Amplification kit, Sigma-Aldrich). PCR products were purified, cloned into pGEM-T Easy vector and sequenced. BLASTx analyses revealed a clone with viral sequence (418 nt) presenting an identity of 51% (67% of similarity) with the RdRp domain of the P122 fusion protein of *Southern tomato virus* (STV), defining a new taxon related to the *Totiviridae* and *Partitiviridae* families (Sabanadzovic *et al*., 2009). The characterization of the 3'-end of the viral genome was pursued, and confirmed the genomic organization. The C-terminal part of the deduced protein (602 aa) showed 49% identity with the fusion protein of STV. The size of the 3' non- coding region was similar in both viruses (ca. 110 nt). Despite these features, the determination of the 5' part of the genome would allow us to precise the genomic organization of this new virus and hence its taxonomical position.

To ensure the origin of this new virus (plant or fungi), samples were tested for the presence of fungi by molecular test using the broad-spectrum fungal primers, ITS-1/ITS-4. These investigations revealed that the presence of the virus is not related to any fungi infecting the *A. selago* samples. In order to precise the prevalence of this virus in *A. selago*, specific primers were designed and used to screen 10 samples. Specific RT-PCR analysis revealed that 4/10 samples were infected with this new virus.

These preliminary results suggest that a new plant virus infects the genus *Azorella*, a native plant of Kerguelen Islands. This virus belongs to the dsRNA viruses, is related to STV, a new dsRNA virus taxomy that constitutes the transition between *Partitiviridae* and *Totiviridae* families (Sabanadzovic *et al*., 2009).

Key words: dsRNA, Plant virus, *Azorella*, Whole genome amplification, Diagnosis.

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FREQUENT ALTERATIONS IN SICILIAN OLIVE-YARDS: FIRST PATHOGENICITY TESTS

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In the last years, an undescribed decline of *Olea europaea* L., has been frequently detected in several Sicilian olive-yards. Particularly, symptomatic plants showed more or less extensive foliar chlorosis, sometimes associated with necrotic irregular marginal or apical spots. At the same time, the young twigs showed apical defoliation and, in some cases, cortical necrosis and withering.

In order to ascertain the nature of symptoms and their evolution in field, an etiological and epidemiological investigation in two olive-yards, similar for cultivar and agricultural management and different for altitude, was carried out for two consecutive years (2007-2008).

During spring, summer and autumn of each year, samples of symptomatic leaves and twigs were collected and subjected to isolation tests. Fungal colonies growing out were singly sub-cultured on MEA and identified on the basis of both macro- and microscopic features. Furthermore, seasonal epidemiological investigations were carried out to evaluate both incidence and severity of leaf and branch symptoms as well as their evolution. The fungal pathogen genera *Diplodia* (Moral *et al*., 2008; Thomidis and Michailides, 2008), *Phoma* (Tosi and Zazzerini, 1994), *Septoria* (Frisullo *et al.*, 2002) and *Stemphylium* (Llorente and Montesinos, 2006) were more o less frequently associated to symptomatic leaves and twigs in both countries, thus used for Koch's postulates. Particularly, fungi were tested by inoculation of healthy olive plants (cv. Biancolilla) grown under controlled conditions.

For each isolate, single spore cultures were prepared and utilized for pathogenicity assays. The inoculum was placed both on leaves, after pricking with sterile pins, and twigs, after removing the bark. Fungi were inoculated both singly and in combinations, in order to assess their possible synergic action. All plants were watered periodically and daily controlled; re-isolation from symptomatic tissues were carried out.

The decline symptoms showed a different evolution depending on sampling site and season. In the hilly olive-yard either incidence and severity of symptoms often reached higher values than in the flat olive-yard. The former site was characterized by high temperatures and low rainfall, which may have determined a higher stress on plants. In both sites the results of isolation assays showed the association of fungal pathogen genera to symptomatic plants, mainly to chlorotic leaves. With regards to pathogenicity tests, only a few inoculations were successful: *Stemphylium* alone and

all fungal combinations caused symptoms. First symptoms appeared 7 days after inoculation, mainly on leaves. Symptoms consisted in chlorotic areas on leaves and darkening of internal tissues on twigs. All fungi were re-isolated from inoculated leaves and twigs. No alteration was detected on control plants. Consequently, these fungi might be thought as weak pathogens of olive-tree, and thus able to induce symptoms only if present together on the same stressed plant.

Therefore, on the basis of our preliminary results, a complex syndrome of *O. europaea* L. could be hypothesized, as being caused by a complex of fungi on plants whose defence is weakened by a-biotic stress factors.

Key words: *Olea europaea*, Decline, Weak pathogens, Sicily

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SNOW MOLDS AND SCLERODERRIS CANKER ON *PINUS NIGRA SUBSP. PALLASIANA* ON THE DEDEGÜL MOUNTAIN IN TURKEY

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Herpotrichia juniperi (Duby) Petr., *Neopeckia coulteri* (Peck) Sacc. (syn: *Herpotrichia coulteri*), *Phacidium infestans* P. Karst., as well as small tree type and alpine biotype of *Gremmeniella abietina* (Lagerb.) M. Morelet var. *abietina* are snow dependent parasitic fungi that grow and attack conifers during dormancy at low temperatures under snow cover. These fungi affect survival and growth of their hosts and cause significant losses in nurseries, plantations and natural forests, where sufficient snow cover is present until spring (Sinclair *et al.,* 1987; Marosy *et al.,* 1989; Laflamme, 2002). These fungi have been reported from many European countries and North America on a broad spectrum of coniferous hosts, including mainly *Abies, Picea* and *Pinus* species (Sinclair *et al.,* 1987). In general, they have the potential to alter the characteristics of plant populations and communities at high altitudes (Hinker *et al.*, 2008). However, records for Turkey, where the ecological condition on some mountainous regions are obviously favourable for these fungi, are inadequate.

The occurrence of these snow related fungi on *Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe within a protected natural mountain forest in western Turkey was investigated on east and north facing slopes of Mt. Dedegül, where a transition between the Mediterranean and continental climates prevails, on pure *P. nigra* and mixed *P. nigra, Cedrus libani* A. Rich and *Abies cilicica* Carr. stands with natural regeneration dynamics.

The occurrence of snow molds, as either blackish brown mycelium binding needles and twigs together, which is characteristic for *H. juniperi* or *N. coulteri* (Simms, 1967), and weathered needles bearing apothecia of *P. infestans,* was assessed during field trips. Needles and twigs infected with *H. juniperi* or *P. infestans* were collected for further identification. Occurrence of fruit bodies of *G. abietina* on dead shoot samples collected during the field surveys was investigated in the laboratory.

Based on our first observations in the survey area these fungi could be the causal agents of the severe damage detected in the field. However, other factors, such as insect damage or grazing could also have played an important role in the mortality, either individually or in combination. Further studies are needed to investigate the disease severity in the area.

Key words: *Herpotrichia juniperi*, *Neopeckia coulteri, Phacidium infestans*, *Gremmeniella abietina*, Turkey

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FIRST REPORT OF THE NEW CAUSAL AGENT OF CONCAVE GUM DISEASE ON THOMSON NAVEL ORANGE IN NORTHERN IRAN

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Mazandaran province with about 90000ha citrus planting area is a major citriculture region in the north of Iran. Thomson Navel sweet orange is a predominant citrus scion on sour orange rootstock that interests more than 70% of the plantings. During years 2007 to 2008, some of the Thomson Navel trees showed symptoms similar to those of the concave gum disease, including bark gumming on upper parts of the bud union, exuded through tiny cracks in the bark at summer season, conspicuous broad concavities of various sizes on trunk or limbs with concentric gum deposits present in the layers of wood beneath.

Most trees were moderately stunted, without any psorosis type bark scaling. The young and mature leaves showed oak-leaf patterns. Sampling was done from the infected citrus leaves and nucleic acids (dsRNAs) were purified by the column chromatography method, using CF-11 cellulose powder in presence of ethanol (Rezaian *et al*., 1990).

The purified nucleic acids solution was analyzed by 1% agarose gel electrophoresis and band with molecular size of 300bp was detected in the gel. The dsRNA nature of the band was confirmed by nucleases treatments (DNaseI and RNaseA) and 2M LiCl extraction method (Dodds *et al*., 1983). The purified nucleic acids solution was used as a template of RT-PCR amplifications, by using specific primers for Cachexia and Exocortis viroids (Almeyda *et al*., 2007; Alvarado-Gomez *et al*., 2000). Only with the primers set for Cachexia viroid the expected 300bp fragment was specifically amplified. So far, viral agents have been suggested as the cause of all concave gum diseases of Navel orange in Iran and many citriculture regions in the world (Bove, 1995). This is the first report of detection of the causal agent of concave gum disease with viroid origin in the north of Iran.

Keywords: Cachexia viroid, CF-11 cellulose powder, dsRNA, Navel orange

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SESSIONE 3

New or unusual disease reports

POSTERS

PETRIA 20 (2), 67-633 (2010)

FIRST RECORD OF GRAPEVINE DIEBACK DISEASE IN NORTHERN JORDAN

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The study was carried out in 29 randomly selected vineyards cultivated with Daraweeshy, Khudary and Zainy cultivars in Ajloon province. Vineyard age ranged from 4 to 32 years. All vines were growing by the crawling training system. Disease incidence and severity were determined. Chips approximately 0.5 cm long each were excised from the margin of discolored sapwood of diseased branches or trunks and surface disinfected in 0.5 % NaOCl for 3 min. After drying on sterilized filter paper, segments were placed in 9 cm Petri dishes with potato dextrose agar amended with 100 µg/ml streptomycin sulfate, 50 µg/ml chlortetracycline HCL and 5 µg/ml pentachloronitrobenzene. Plates were incubated at 24º C. Fungi were identified according to Glawe and Rodgers (1984) and Mckemy *et al*. (1993). Infected grapevines were inspected during winter for the presence of perithecia.

Early foliar disease symptoms in Kafranjah and Listeb appeared as new branches reached 40 cm in length in early spring as small yellow, cupped leaves and tattered margins, clusters on affected shoots had a mixture of both large and small berries. When an infected trunk is cross sectioned, a wedge shaped zone of dead tissue can be seen. *Eutypa maura* was isolated from all vines with disease symptoms. Colonies on agar media were at first white and cottony cream-colored in reverse, with no fruiting structures. Exposing the culture plates to 12hr light/dark regimes for 8 weeks promoted conidial formation. Long single–celled conidia typical of *Eutypa* measuring 18-45 X 0.8-1.5 µ were observed.

Large areas of stromatic tissue formed on the surface of dead branches remained in the vineyard from last pruning. After the loose bark had fallen off, black pear shaped perithecia measuring 250 µ in width X 700 µ in length with asci were seen in high numbers. Asci were transparent and borne on pedicles with an apical pore. Ascospores were pale orange-yellow, allantoid in shape with an average length of 10.4 µ and average width of 2.5 µ. *E. maura* was reported for the first time in Jordan and in neighboring countries. *Phialophora melinii* as well as *Phoma syriaca* which cause black mold disease of grapevines were reported in southern Syria (Mousli and Al-Ahmad, 2000). *Eutypa lata* was reported in Palestine (Carter, 1991).

Only one 4 year old vineyard was disease free, the others had disease indices ranging from 5 to 75% in 5 and 32 year old vineyards respectively. Symptoms developed later in the season in Ibeen, where disease incidence and severity reached 58.9% and t 23.1%, respectively in mid July. Disease symptoms developed much

earlier in the other three locations and increased up to 6 fold in Kafr-anjah from mid May to mid June. Older vineyards were more severely infected than the younger ones. Ibeen had the highest disease incidence. Previous reports have shown that the incidence of *Eutypa* increased with age of the vineyard (Munkvold and Marois, 1995). The average length of the dead arm was 53.5 to 104.9 cm which depended on disease severity and plant vigor. The maximum length of the dead arm reached 183 cm where the disease severity was the highest in Ibeen. These were annual branches with a diameter ranged from 1-1.7 cm. The lowest disease intensity was obtained from the youngest vineyard in Ajloon.

Key words: *Eutypa*, Grapevine, Wilt, Dieback**,** Perithecia

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PETRIA 20 (2), 67-633 (2010)

OCCURRENCE OF THE QUARANTINED PATHOGEN *MONILINIA FRUCTICOLA* ON ITALIAN FRUIT

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Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. In the European countries brown rot of peaches and nectarines is caused by two fungi, *M. laxa* and *M. fructigena. M. fructicola* is commonly present in Asia, North America and Australia and it is a quarantined pathogen in Europe. During a survey carried out in Piedmont (Northern Italy) during 2008, *M. fructicola* was detected on the nectarines originated from two orchards (Pellegrino *et al.,* 2009). Brown rot symptoms appeared on the fruit during storage, starting 3 weeks after harvest. Preliminary morphological identification of fungi resembling *M. fructicola* was confirmed by multiplex PCR using a common reverse primer and three species-specific forward primers obtained from a sequence characterized amplified region (Côté *et al.,* 2004) and a product of 535 bp, diagnostic for the species *M. fructicola*, was obwith SCAR primers. The BLAST analysis of the amplified sequence (Accession No. FI569728) showed 96% similarity to the sequence of a *M. fructicola* isolated from Canada (Gen Bank Accession No. AF506700). Moreover, two sequences obtained through the amplification of ribosomal region ITS1-5.8S-ITS2 (White *et al.,* 1990), showing 100% similarity to the same ribosomal sequence of *M. fructicola*, were deposited in GenBank (Accession Nos. FJ411109 and FJ411110). Pathogenicity tests confirmed the virulence of the isolates. This is the first report of the quarantined fungus *M. fructicola* in Italy. A careful monitoring in the orchards of the region surrounding the area where the first isolates were found is currently carried out. The new pathogen, probably introduced together with propagation material, could spread in other fruit producing regions of Italy. New integrated control strategies, including biological control and new chemicals applied in preharvest, are needed to effectively control *M. fructicola* on stone fruit.

Key words: *Monilinia fructicola*, Multiplex PCR, Nectarine, Ribosomal DNA, Stone fruit

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PETRIA 20 (2), 67-633 (2010)

COLLETOTRICHUM ACUTATUM AS CAUSAL AGENT OF OLIVE ANTHRACNOSE IN AUSTRALIA

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Two anamorphic species of *Colletotrichum*, *C. gloeosporioides* (Penz.) Penz. & Sacc. and *C. acutatum* J. H. Simmonds, were reported to be associated to olive anthracnose, however the latter species prevails in areas where the disease occurs epidemically. *C. acutatum* is a species complex comprising nine (A1-A9) distinct molecular groups (Sreenivasaprasad and Talhinhas, 2005; Shivas and Tan, 2009). Recently, two new species have been described, *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov., which correspond to the A3 and A2 molecular groups respectively, whereas *C. acutatum sensu stricto* would correspond to the A5 group (Shivas and Tan, 2009).

In the present study a population of olive isolates of *C. acutatum* from diverse olive-growing areas of Australia was characterized by amplifying and sequencing the ITS2 region and a region of the b-tubulin gene 2 comprised between exon 5 and 7. The ITS2 region was amplified and sequenced by using the universal primers ITS3 and ITS4, while the b-tubulin gene was amplified and sequenced by using degenerate primers designed by aligning and comparing more that 1000 GenBank available sequences. Other olive isolates of *C. acutatum* from Italy and Portugal as well as isolates of various geographic origins and hosts were included in the study.

ITS and b-tubulin gene 2 sequences obtained in the present study were compared with homologous regions of reference isolates utilized by Shivas and Tan (2009). Sequences were aligned using ClustalX and introduced to TOPALi (http://www.topali.org/) for phylogenetic analysis with the MrBayes 3 method.

Phylogenetic groups based on the analysis of the ITS2 region corresponded to those previously reported by Sreenivasaprasad and Talhinhas (2005) and by Shivas and Tan (2009). Most olive isolates from Australia clustered in the *C. acutatum* group A5. As opposed, isolates from Portugal as well as isolates from strawberry of worldwide origin clustered with the new specie *C. simmondsii* (formerly *C. acutatum* group A2) while olive isolates from southern Italy clustered with the *C. acutatum* group

A4. Phylogenetic groups were supported by high (0.7-1.0) bootstrap values. Similar results were also obtained by the analysis of the b-tubulin genes 2, although, due to the short fragment shared (approximately 250 bp) between sequences examined in the present study and those reported by Shivas and Tan (2009), a less accurate separation was possible.

Results of the present study corroborates the hypothesis that the A4 molecular group of *C. acutatum* represents another still undescribed species (Cacciola *et al*., 2007).

Key words: Olive anthracnose*, Colletotrichum* spp.*,* ITS regions, b-tubulin gene 2

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FIRST REPORT OF *VERTICILLIUM DAHLIAE* ON OLIVES IN MONTENEGRO

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Olive is one of the most important fruit crops in Montenegro and covers around 3200 ha. There are approximately 420 000 olive trees, located along the Adriatic seacoast (Miranovic, 2006). Although these are mostly old olive orchards, there are recently many attempts for improving olive production by renewing and introducing new olive variety.

A survey of olive orchards throughout the Montenegrin seacoast in spring of 2006 revealed the occurrence of wilted olive young trees of cultivar "Leccino" in the Bar region. Affected stems and leaves lost their greenish hue and become light brown in colour, and the leaves curled downward. Cross sections of diseased branches revealed darkening of xylem tissue. Wilting was progressive over the course of the growing season. A fungus was consistently isolated in May on potato dextrose agar (PDA) from xylem tissue of symptomatic branches at the margin between discoloured and healthy-looking tissue. Identification was made after incubation period at 25 ºC in darkness for 10-15 days. Morphological features of the obtained isolates (verticillately shaped conidiophores and abundant production of microsclerotia) corresponding to the description of *Verticillium dahliae* (Kleb.) (Colella *et al*., 2004).

Pathogenicity test was done according to Koch's rules. Artificial inoculations of two-year-old healthy olive plants (cultivars "Leccino" and "Manzanilla") have been performed to verify the hypothesis that the fungus is the cause of the disease. Inoculations were made by watering olive plants with suspensions of fresh conidia (from fungal colonies cultured on PDA) diluted in sterile distilled water. Olives treated in the same way with sterile distilled water were used as a control (Lachqer *et al*., 2002; Pace-Lupi *et al*., 2006). All plants were kept under the same controlled conditions (temperature 25 ± 1°C and adequate humidity). During subsequent weeks, first chlorosis of the leaves and then wilt of the whole inoculated plants began to appear. Symptoms were absent on the control plants. At the end of June, *V. dahliae* was reisolated on PDA from all inoculated plants, while all attempts to isolate the pathogen from control plants were unsuccessful.

This is the first report of olive verticilliosis in Montenegro.

Key words: Olive verticilliosis, Montenegro

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH *ROSMARINUS OFFICINALIS* IN IRAN

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Rosmarinus officinalis (rosemary) is a woody, perennial herb species of the family Lamiaceae, with fragrant evergreen needle-like leaves, native to the Mediterranean region. Rosemary is a plant with potential medicinal use that contains a number of biologically active compounds, including antioxidants such as carnosic acid and rosmarinic acid. Extracts of this plant have also been used as an antioxidant food additive (Krause & Ternes, 2000). Several plant-parasitic nematodes have been reported associated with rosemary (Imaz *et al*., 2002). This study was undertaken to provide information on the nematofauna associated with rosemary in Iran.

During 2008-2009, a survey was conducted on the rosemary plants in Mashhad, Iran and fifty soil samples were collected. Nematodes were extracted from soil by Jenkins (1964) method, and processed to be transferred to glycerin by De Grisse (1969). The identification of nematode species was based on morphological and morphometrical characters (Firoza and Maqbool, 1994; Handoo *et al*., 2007). Plant-parasitic nematodes were detected in soil samples. Ten species belonging to four genera were characterized and identified: *Boleodorus thylactus, Helicotylenchus pseudorobustus, H. californicus, H. plumaria, H. nigereinsis, Merlinius microdoratus*, *M. indicus*, *Psilenchus minor*, *P. hilarulus,* and *Psilenchus sp*.

Helicotylenchus pseudorobustus was the most widely distributed species.

Helicotylenchus plumaria, H. nigriensis, M. indicus and *M. microdurus* are recorded for the first time from Iran.

Key words: Iran, Plant-parasitic nematodes, *Rosmarinus officinalis*

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PANTOEA ANANATIS – A NEW PATHOGEN OF MAIZE PLANTS IN POLAND

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In the last few years, thanks to continuous development of bioenergy sector, maize (*Zea mays*) has become one of the most important crop in Poland.

It is also known that in favorable environmental conditions maize can be affected by numerous bacterial pathogens (Lamka *et al.*, 1991; Smidt and Vidaver, 1986; Lindow *et al.,* 1982; Ribeiro *et al.*, 1977; Dickey *et al.*, 1987; Goszczynska *et al.* 2007; Rosen 1922).

However in Polish scientific literature there is almost no publication concerning bacterial diseases of maize. In this study the problem of bacterial diseases of maize in Poland was investigated

The collected samples of maize plants displaying symptoms corresponding to those described for leaf spot Disease of maize were analysed for the presence of the bacterial pathogen *Pantoea ananatis* using biological, biochemical, serological and molecular tests. Pathogenicity of the tested bacterial strains was evaluated. All tested strains have fulfilled Koch`s postulates. Performed biochemical, serological and molecular tests confirmed the presence of *P. ananatis* affecting maize in Poland.

Keywords: Bacteria, Detection, Plant pathogen

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FIRST REPORT OF *XANTHOMONAS* ON *VALERIANELLA LOCUSTA*

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Corn salad (*Valerianella locusta* (L.) Laterr) is an important ready-to-use packaged salad. In 2009 the total Italian corn salad production was of 142.491 tons; in Salerno (Southern Italy) 12.500 tons were produced (ISTAT, 2009).

In the summer of 2009, in a farm in Pontecagnano (SA), a new disease was observed in the field. The plants showed necrotic spots on the basal leaves, localised along the leaf margins, symmetric as to the central leaf vein. The disease caused severe yield losses and downturn in production.

Yellow bacterial colonies were constantly isolated from leaves of different valerianella samples on general medium (Nutrient Agar, NAG) and then even on semi-selective media mCS20ABN (Anonymous, 2005). The colonies were mucoid, yellow, convex, circular with regular shape on NAG; they produced a clear halo on mcs20Abn due to the starch hydrolysis. These colonies had typical *Xanthomonas* morphology. No colonies of *Acidovorax valerianellae* were isolated from the samples.

Pathogenicity test confirmed that these *Xanthomonas* strains were the causal agent of the disease. The strains caused a weak hypersensitivity reaction on tobacco, they hydrolyzed starch and utilised aesculine. They were identified as *Xanthomonas campestris* pv *campestris* with 97% similarity by sequencing of 16S–23S rDNA intergenic spacer according to Goncalves and Rosato protocol (2002).

The host range of these *Xanthomonas* strains was assayed and results showed that they were not pathogenic for cauliflower, wild rocket and tomato.

In conclusion, the new *Xanthomonas* strains were identified as *Xanthomonas campestris* pv *campestris* by molecular analysis, but they failed to infect cauliflower in *in-vivo* test. In order to better characterise the corn salad isolates further tests and phylogenetic analysis are in progress.

Key words: *Valerianella locusta, Xanthomonas*

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FIRST REPORT OF PHYTOPLASMA DISEASE IN CELERY AND PARSNIP IN SPAIN

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During 2008, a new disease outbreak occurred in several celery (*Apium graveolens* L.) and parsnip (*Pastinaca sativa* L.) fields located in the South-East of Spain. The affected celery plants exhibited severe stunting and yellowing, proliferation and shortened of the petioles, which appeared moderately to severely curved and twisted. In this area the production of celery was very affected during 2008, and high populations of psyllid species (2/3) and several leafhopper species (1/3) were observed in the affected fields. In the same area, plants in several parsnip fields showed symptoms of yellowing and proliferation of leaves and small roots, deformation, reduction, and early senescence of roots. Celery and parsnip fields were infested by psyllids and leafhoppers.

Symptomatic celery and parsnip plants were collected and analysed by DAS-ELISA with polyclonal antibodies for *Celery mosaic virus* (CeMV), *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV) and by nested-PCR (Lee *et al*., 1994) using phytoplasma ribosomal DNA-universal primer pairs (Gundersen and Lee, 1996) or P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) followed by R16F2n/R16R2 (F2n/R2) (Gundersen and Lee, 1996). Serological analysis only revealed CeMV infection of some celery plants. The presence of phytoplasma in celery and parsnip plants in the field was confirmed by amplification of 1.2 kb bands in nested-PCR reaction. Restriction fragment length polymorphism (RFLP) analysis of DNA products obtained from nested-PCR with the endonucleases *Alu*I, *Kpn*I, *Mse*I and *Rsa*I revealed the presence of two different phytoplasmas belonging to 16S rRNA group I (Aster yellows phytoplasma) and 16S rRNA group XII (Stolbur phytoplasma) in celery and parsnip, respectively (Lee *et al*., 1994; Schneider *et al*., 1995).

To our knowledge, this is the first detection in Spain of aster yellows and stolbur phytoplasmas infecting celery and parsnip crops, respectively.

Key words: *Apium graveolens*, *Pastinaca sativa*, Phytoplasmas, Stolbur, Aster yellows, Nested-PCR, RFLP

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"*CANDIDATUS* PHYTOPLASMA ASTERIS" ASSOCIATED WITH RICE DISEASE IN SPAIN

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Rice (*Oryza sativa* L.) is one of the vital world food crop and constitutes the basis of the economy in many countries. In Spain, rice grown in the Mediterranean coast is one of the main traditional crops. In fact, the 15% of the national production of rice is grown in Valencia region, where this cereal is the main ingredient in the local cooking.

During 2008, symptomatic and asymptomatic rice plants were collected from Pego marsh (Valencia). Symptoms of affected rice plants included general yellowing and stunting, which reminesce phytoplasma diseases. Total DNA extraction was performed from collected samples and later analyzed by nested PCR using ribosomal universal primers pairs P1/P7 (Deng and Hiruki, 1991) and R16F2n/R2 (Gundersen and Lee, 1996). PCR products of 1.2 kp obtained from symptomatic rice after the second amplification were analyzed by RFLP (Restriction Fragment Length Polymorphism) with different restriction enzymes. RFLP analyses showed that the phytoplasmas were related to the 16SrI group (Aster yellows group) (Lee *et al*., 1993; Lee *et al*., 2000).

To evaluate nucleotide identity with reference sequences, one amplicon was purified and directly sequenced. BLAST search analysis showed a 99% identity with those of '*Candidatus* Phytoplasma asteris' (16SrI group) associated with aster yellows diseases (Lee *et al*., 2004). To our knowledge, this is the first report of '*Ca.* Phytoplasma asteris' in rice in Spain.

Key words: *Oryza sativa*, Nested PCR, RFLPs, Aster yellows

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OLIVE LATENT VIRUS-1 INFECTING TOMATO PLANTS IN POLAND

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Different isolates of *Olive latent virus-1* (OLV-1, genus *Necrovirus*, family *Tombusviridae)* have been obtained form *Olea europaea* L. trees in Italy, Jordan and Portugal (Gallitelli and Savino, 1985; Félix and Clara, 1998), from citrus trees, in Turkey (Martelli *et al.,* 1996) and from tulips in Japan (Kanematsu *et al.,* 2001).

The virus has isometric particles ca. 30 nm in diameter and a monopartite, positive sense ssRNA genome ca. 3700 in size (Grieco *et al.,* 1996). OLIV-1 has a narrow host range and its detection in tomato plants was a reason to have a closer look at its biological and genetic features.

In Poland, OLV-1 (CM1) was isolated from the greenhouse tomato plants with necrotic spots on leaves. Virus was maintained in *Nicotiana benthamiana*. The CM1 isolate was mechanically inoculated on the different plant species. Inoculated plants were assayed for the presence of the virus by RT-PCR reaction. DAS-ELISA was carried out according to the standard protocol, using antisera to Tobacco necrosis virus (TNV), Tomato aspermy virus (TAV) and Tomato torrado virus (ToTV). Nucleic acid was extracted from purified viral preparation and purified viral RNA was mixed with random hexamers (Novazym) and incubated for 10 min at 70 °C. Reverse transcription was performed according to the manufacturer's instructions. The products obtained were then purified, cloned and sequenced. Nucleotide and amino acids sequence data were analyzed using BioEdit software. The sequence results obtained allowed the design of specific primers: OLVF 5' TAGTTAAGTATACGAATAACA 3' and OLVR 5'AATCTGGTGTTGGGTCCACT 3'. The amplified product of the expected size of 1200 bp was cloned, sequenced and deposited in GenBank under accession number GU326337.

Mechanical inoculation experiments onto indicator plants showed that *N. benthamiana* reacted with local, necrotic lesions. Among tested plant species only *N. benthamiana* and *N. occidentalis* were infected systemically. The virus did not react with antisera to TNV, TAV and ToTV.

The CP region of the CM1 isolate consisted of 810 nucleotides encoding 270 amino acids. Sequence identities of tomato CM1 isolate with these of olive and citrus isolates were 91.8% and 89.5%, respectively. The CM1 isolate shared 92.5% sequence identity with the tulip isolate (Pare-P). Furthermore, the CM1 isolate, similar to the citrus isolate, produced systemic symptoms after mechanical inoculation on *N. benthamiana*. By contrast, the GM6 isolate only produced local symptoms on *N.*

benthamiana. These differences could be related to the amino acid changes found in the CP protein, which is involved in long distance movement. Interestingly enough, the CM1 isolate was more similar to the GM6 and Pare-P isolates than to the citrus isolate. The analysis of the whole CP region revealed the presence of few amino acids changes which are unique for the CM1. It seems that nucleotide sequence differences observed between Polish and other OLV-1 isolates up to date might lead to changes in the biological properties of the virus, with major epidemiological consequences, including the appearance of resistance-breaking strains or having a broader host range.

Key words: *Olive latent virus-1*, ELISA, random hexamer, RT-PCR

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FIRST FINDING AND MOLECULAR CHARACTERIZATION OF *ALFALFA MOSAIC VIRUS* INFECTING *ORIGANUM VULGARE* IN ITALY

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Origanum vulgare L. (family *Lamiaceae*) is a perennial herbaceous species, originating from Mediterranean regions and Asia, widely cultivated as culinary herb and for therapeutic purposes.

During spring-summer 2009, 3-4% of potted plants, randomly distributed in protected nurseries of Albenga area (Liguria region), were noted to be stunted and showing a bright yellow leaf mosaic ("calico" type). Mother-plants growing in the same nurseries for cutting production were symptomless.

Preliminary electron microscopy observations of leaf-dips prepared from symptomatic and asymptomatic plants, revealed the presence of bacilliform virus-like particles in only symptomatic samples.

Symptoms observed on herbaceous hosts mechanically inoculated with crude sap of a single affected plant (isolate Orv-1) were: chloro-necrotic lesions in *Chenopodium murale*, *Beta vulgaris*, *Phaseolus vulgaris* and *Vigna unguiculata*; systemic mosaic in *Capsicum annuum;* necrotic local lesions followed by systemic necrosis in *Solanum lycopersicun*; systemic mosaic and necrotic line-patterns in *N. tabacum* "Samsun". The virus was serologically identified as an isolate of *Alfalfa mosaic virus* (AMV) by applying DAS-ELISA technique and molecularly using AMV-specific oligonucleotides pair, designed to retrotrascribe and amplify the coat protein (CP) gene. Restriction profile obtained after *Bam*HI digestion of the putative CP gene amplicon, identified Orv-1 as an AMV subgroup I isolate (Parrella *et al*., 2000). A large set of oligonucleotides were used to amplify and sequence the whole RNA3 segment. Sequence analysis revealed that Orv-1 RNA3 was 2038 nucleotides in length, containing two open reading frames (ORFs) identified by sequence comparisons as coding the putative movement proteins (MP) and the CP. The first ORF consisted of 867 residues, coding for the 31 kDa putative MP, the second consisted of 657 residues, coding for the 24 kDa putative CP. Orv-1 RNA3 showed the highest percentage of identity with the RNA3 of the 425 Madison isolate (Acc. n. K02703) also belonging

to AMV subgroup I. Phylogenetic relationships of Orv-1 isolate with members of AMV subgroups I and II, clearly showed that both CP and MP nucleotide sequences of this *O. vulgare* isolate clustered within the subgroup I, confirming preliminary results obtained by restriction analysis of the CP gene. Further works are in progress in order to sequence the entire genome of the Orv-1 AMV isolate.

Although AMV was already reported to infect *O. vulgare*, first in Argentina (Feldman and Gracia, 1977) and then in New Zealand (Fletcher, 1987), to our knowledge this is the first record of AMV infecting this aromatic plant in Italy. This finding confirms an increasing diffusion of AMV in Liguria region, especially in aromatic crops.

Key words: AMV, Viral genome, *Origanum vulgare,* Molecular characterization

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FIRST REPORT IN LEBANON ON DETECTION OF TWO WHITEFLY TRANSMITTED CUCURBIT VIRUSES AND THEIR MOLECULAR CHARACTERIZATION

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During the late cropping season, farmers in Lebanon complained about severe infections by viruses with unusual symptoms especially on squash. Field visits showed the prevalence of leaf curling on squash and yellowing on melon and watermelon. Surveys were conducted in cucurbit fields over two years. Serological techniques were used for detection of *Cucurbit yellow stunting disorder virus* (CYSDV) and molecular techniques, PCR using a universal primer pair for detection of several begomoviruses, supplemented with rolling circle amplification (RCA). CYSDV was detected in most samples.

Squash leaf curl virus (SLCV) was detected mainly in squash in the late cropping season in samples collected during October – December from all the regions surveyed. *Watermelon chlorotic stunt virus* (WmCSV) was detected in cucumber, melon, watermelon and squash in South Lebanon but not in North Lebanon.

The full genomes A and B of SLCV and WmCSV were sequenced. Many melon and watermelon samples with yellowing symptoms gave negative results in PCR tests. Further analysis proved that the universal primer pair PAL1v1978 / PAR1c496 used to detect begomoviruses failed to detect several isolates of WmCSV. Therefore, new primers were designed and used for the specific detection of either WmCSV or SLCV. A multiplex PCR protocol was developed for detection of SLCV and WmCSV. Mixed infections with two or three whitefly transmitted viruses were very common.

Key words: Begomoviruses, SLCV, WmCSV, Squash, Melon, Watermelon, Cucumber

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PETRIA 20 (2), 67-633 (2010)

IDENTIFICATION AND SOME PROPERTIES OF *WHEAT DWARF VIRUS* AFFECTING CEREALS IN SYRIA

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Field survey was conducted during the 2008/2009 growing season, covering five regions of Syria: northern (Aleppo and Idleb), central (Homs), coastal (Lattakia and Tartus), eastern (Al-Raqa, Dear Al-Zor and Al-Hasskah) and southern (Dara'a and Sweida). A total of 938 wheat (*Triticum aestivum*) and 971 barley (*Hordeum vulgare*) samples with typical symptoms of viral infection (dwarfing, yellowing, stripping, reddening and stunting) were collected from 103 fields (45 wheat and 58 barley). All samples were tested for the presence of six viruses by the tissue-blot immunoassay (TBIA) (Makkouk and Kumari, 1996) at the Virology Laboratory of ICARDA, Aleppo, Syria, using the following polyclonal antibodies: *Barley stripe mosaic virus* (BSMV), *Barley yellow dwarf virus-PAV* (BYDV-PAV), *Wheat streak mosaic virus* (WSMV), *Barley yellow striate mosaic virus* (BYSMV), *Maize streak virus* (MSV) from the Virology Laboratory at ICARDA, and *Wheat dwarf virus* (WDV) provided by DSMZ (AS # 0216), Germany. Serological results indicated that BYDV-PAV was the most commonly encountered virus (20%) followed by BYSMV (1.2%), WDV (1.1%), BSMV (0.6%) and WSMV (0.5%), whereas MSV was not detected. BYDV-PAV and WSMV were detected in five regions of Syria, whereas BYSMV was detected only in southern region and WDV was found only in one village in Al-Hasskah governorate (eastern region) with mean relative occurrence of 16.3% (26.1% on wheat and 6.5% on barley).

Samples that reacted with WDV antiserum were transmitted from infected plants to healthy plants of *Avena sativa*, *Bromus rigidus*, *Dactylis glomerata*, *Festuca elatior*, *Hordeum vulgare*, *Triticum aestivum* and *Triticum turgidum* using four different leafhopper species collected from wheat and barley fields, in a persistent manner. Results indicated that only the leafhopper species, *Psammotettix provincialis* Ribaut (Homoptera: Cicadellidae), transmitted WDV from infected barley plants to barley and *A. sativa* under experimental conditions; where up to 95% of the barley plants were infected.

Total DNA was extracted from six WDV positive samples (3 wheat and 3 barley) and tested by PCR using WDV primer set described by Oluwafemi (2006). All six samples generated amplicons around the expected size (~ 253 bp). Comparing the sequence of a Syrian barley isolate to other WDV isolates showed a 98% similarity to isolate of Barley dwarf virus-Iran and 92-93% similarity to most European WDV

isolates.

WDV has been reported to infect cereals in few countries in West Asia and North Africa (Turkey, Tunisia and Morocco), and causes economic losses on wheat in many countries in Europe (e.g. Sweden). WDV is persistently transmitted by leafhoppers (*Psammotettix alienus* Dahlbom) only to a wide range of cereals and wild grasses. Two strains of WDV are known, one that primarily infects wheat and another that infects barley (Vacke *at al*., 2004). To our knowledge, this is the first report of WDV infecting wheat and barley in Syria, and the first report of *P. provincialis* as a WDV vector worldwide.

Key words: WDV, Syria, TBIA, PCR, *Psammotettix provincialis* Ribaut

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PETRIA 20 (2), 67-633 (2010)

FIRST REPORT OF *BARLEY YELLOW DWARF VIRUS* AND *MAIZE DWARF MOSAIC VIRUS* IN JORDAN

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Cereals (wheat, barley, and maize) are important field crops grown in Jordan. The total area planted to wheat and barley was estimated in 2008 at 16,500 ha. In the same year, Jordan imported 1065 thousand tons of wheat and 662 thousand tons of barley (Anonymous, 2008).

Although cereals are of great importance, little is known about the viral diseases affecting cereals in Jordan. Therefore, this study has been done to detect and characterize the most prevailing cereal viruses at the molecular level. To achieve this goal a total of 41 wheat, 20 barely and 210 corn samples were collected from different locations in Jordan where cereals are grown. Total RNA was extracted from symptomatic leaves using SV total RNA isolation system (Promega) and cDNA was synthesized using first strand cDNA synthesis kit (Fermantas). Samples were analyzed by PCR and multiplex PCR using oligonucleotide primers specific for *Barley and Cereal yellow dwarf viruses* (B/CYDVs), *Soil-borne wheat mosaic virus* (SBWMV), *Wheat spindle streak mosaic virus* (WSSMV), and *Wheat streak mosaic virus* (WSMV), *Wheat yellow mosaic virus* (WYMV), *Barely mild mosaic virus* (BaMMV) and *Maize dwarf mosaic virus* (MDMV). Data of PCR analysis showed that 4 wheat samples collected from Irbid region were mixed infected with BYDV-PAV, -MAV and -SGV viruses and 26 samples of maize were infected with MDMV. Amplified PCR fragments were cloned into pTPCR vector and sequenced. Sequence analysis showed that BYDV-PAV from Jordan shared high (99%) nucleotide identity with isolate 05GG6 (EU332311) and 06KM14 (EU332332) from China. High degree of nucleotide identity (97%) was also observed between the Jordanian BYDV- MAV and 05GG6 isolate (EU332311) from China. The sequence of BYDV-SGV had 97% nucleotide identity with isolates ASL-1 from Germany, 0109 and 129 from USA.

In 2010, a nationwide survey will be conducted to investigate the spread of detected viruses in more cereal growing regions and to study the occuerence of other cereal viruses previously reported to occur in the Middle East. Up to our knowledge this is the first report on the occurenec of BYDV-PVA, -MAV and -SGV and MDMV in Jordan.

Key words: Cereals, Viruses, Jordan, PCR

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PETRIA 20 (2), 67-633 (2010)

FIRST REPORT OF CSNV IN IRAN AND OCCURRENCE OF SOME VIRAL DISEASES OF ORNAMENTAL PLANTS IN MASHHAD REGION, IRAN

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Several viral diseases have been thoroughly described in ornamental plants. Some of them reported from Iran such as *Tomato spotted wilt tospovirus* (TSWV), *Impatiens necrotic spot tospovirus* (INSV), *Arabis mosaic virus* (ArMV), *Carnation mottle virus* (CarMV), *Tobacco streak ilarvirus* and *Cucumber mosaic cucumovirus* (CMV). During the spring and summer of 2008, samples of ornamental plants (*Dianthus*, *Chrysanthemum, Iris, Petunia, Rosa* and others) with mosaic, leaf chlorosis, leaf malformation, yellowing, mottling, ring spots, and dwarf symptoms were collected from greenhouses and landscapes. The samples were tested for the presence of CarMV, *Chrysanthemum stem necrosis virus* (CSNV), *Turnip mosaic virus* (TuMV), and ArMV with DAS-ELISA and for INSV using TAS-ELISA. ELISA results showed that the samples reacted positively with antibodies of CarMV, CSNV and INSV, but not with antibodies for any of the other viruses listed above. This is the first report of CSNV in Iran.

Key words: *Carnation mottle virus*, *Chrysanthemum stem necrosis virus*, *Impatiens necrotic spot virus*, ELISA

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PETRIA 20 (2), 67-633 (2010)

MOLECULAR DETECTION OF *TOMATO SPOTTED WILT VIRUS* INFECTING *CACTUS PEAR* IN SPAIN.

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Cactus pears (*Opuntia ficus-indica* (L.) Mill.) are native only to the Western hemisphere, however, they have been introduced to other parts of the globe as Australia and the Mediterranean Basin and flourishes in areas with a suitable climate. Prickly pears are grown wild by the side of farms, beside tracks and other otherwise non-cultivable land. Opuntia spreads into large clonal colonies, which contributes to the fact that it is considered a noxious weed in some places (Griffith, 2004). During August 2007, unusual symptoms suggesting virus infection were observed in fruits and cladodes of cactus pear plants in the Eastern coastal areas of Spain coinciding with high infestations of *Frankliniella occidentalis* (Pergande). Chlorotic mottle and/or mosaic symptoms in fruits were accompanied by the uneven ripening and fruit malformation.

Cladode and fruit samples from symptomatic plants were analyzed by DAS-ELISA against *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV). Infection with TSWV was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and sequence analysis. A fragment of 275 nt of the RNA dependent RNA polymerase gene was analysed. Blast analysis of the nucleotide sequence obtained from one infected plant revealed 97% nucleotide identity with the TSWV isolate obtained in Greece from artichoke (Accession no. AM940436). This *Tospovirus* is transmitted in a persistent manner by several *Thysanoptera* species and by grafting. Even though the vector species are present in the cactus pear growing areas around the world, further studies are needed to clarify the efficiency of the different transmission ways. Although TSWV has a wide plant host range occurring worldwide (Brunt *et al.,* 1996), to our knowledge, this is the first time TSWV has been detected infecting cactus pear plants.

Keywords: *Cactaceae*, RT-PCR, Thrips, TSWV

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PETRIA 20 (2), 67-633 (2010)

CITRUS LEAF BLOTCH VIRUS: MOLECULAR CHARACTERIZATION AND PHYLOGENIES OF ITALIAN ISOLATES

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Citrus leaf blotch virus (CLBV), the type species of the genus *Citrivirus* (family *Flexiviridae*), has filamentous particles 960 nm long containing a single-stranded, positive-sense RNA, 8747 nt in length. The viral genome consists of three open reading frames (ORFs) which encode a polyprotein involved in replication, the movement protein (MP) and the coat protein (CP), respectively. CLBV was first isolated from naturally infected Nagami kumquat (*Fortunella margarita* Lour. Swingle) in Corsica in 1984 (Navarro *et al.*, 1984), later it was found associated with bud union crease in Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) and citrumelo (*C. paradisi* x *P. trifoliata*) (Galipienso *et al.*, 2001). CLBV was also reported from different citrus varieties in Japan, Australia, Florida and Spain. More recently, it was found in Nagami kumquat (Guardo *et al*., 2007a) and Calamondin (Guardo *et al.,* 2007b) in Italy.

In 2007 and 2008, surveys in nurseries and private collections in Sicily and Calabria were made to monitor the spread of CLBV on Nagami kumquat and Calamondin trees. Surveys were carried out using one step RT-PCR with specific primers (Vives *et al.,* 2002) to amplify part of the virus coat protein (CP). Each positive sample was analysed by Single-Strand Conformation Polymorphism (SSCP) and samples with different electrophoretic pattern were cloned and sequenced. Sequence analyses were carried out by CLUSTALW program and compared with the virus isolate CLBV SRA-153 from Corsica (GenBank Accession No. AJ318061). All isolates from the same area showed the same SSCP pattern and three new isolates were deposited in GenBank: ISA 9-ME-I (EF203230), ISA 10-CT-I (EU877531), ISA 11-T-I (FJ449705). The three isolates (ISA 9-ME-I, ISA 10-CT-I, ISA 11-T-I) shared 97%, 98% and 99% identity, respectively, with the CLBV SRA-153 isolate. The high identity of ISA 11-T-I with CLBV SRA-153 suggests a common origin and indicates that the sequences of Italian CLBV isolates are highly conserved.

Phylogenetic analyses of the cDNA Italian CLBV sequences compared with 16 isolates from Spain, Japan, USA, France, Australia and New Zealand were conducted using MEGA4 by "neighbour-joining" methods with 500 replicates of bootstrap consensus tree. Evolutionary distances were composed using the Maximum Composite Likelihood method and are in the units of the member of base substitutions per site.

Results showed three main clusters, one of which includes ISA 11-T-I and CLBV SRA-153 isolates.

Another cluster comprises the other two Italian isolates (ISA 10-CT-I and ISA 11-T-I isolates). The others isolates, except one from Australia, are distributed in a third cluster without a geographic distribution. These results suggest a low genetic variation between isolates of *Citrus leaf blotch virus* from different geographical origin probably due to recent dispersion by infected budwood.

Key words: RT-PCR, *Flexiviridae*, Phylogenetic analyses

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SESSIONE 4

Variability of plant pathogens

ORAL PRESENTATIONS

THE IMPORTANCE OF BEING CORRECT: WHY THE RIGHT FUNGUS NAME MATTERS

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The name that is applied to a species is the key to all the information that is available on it, including its hosts, behaviour, geographical range and means to control its activities. This information is relevant only if the name is correct. Ideally the name should also indicate the relationship of any given fungus to all others. Unfortunately this is not always true because of the way in which species have been defined in that past. As new information becomes available it is inevitable that some species will be transferred to other genera, or may be relegated synonymy with other species. The changes that systematic mycologists apply to genus and species names of fungal pathogens are a constant irritation to plant pathologists. However, such changes are based on careful study and they are introduced with the aim of helping rather than hindering the work of a pathologist.

Systematics is a dynamic science. New methods for classification and for studying the relationships of fungi are evolving resulting in more accurate and precise concepts to define a taxon. The early mycologists used a simple hand lens, and then later they had microscopes to study, classify and identify their fungi. The transition from hand lens to microscope revealed greater detail and more characters to use in species definitions. These extra details resulted in the recognition of more species and genera. In addition to morphological traits, some fungal species were defined on the basis of the host they were found on. The concept of host association as a defining character resulted in a proliferation of species names, and was often applied indiscriminately. Today, sequencing and comparison of portions of the genome are used to characterize fungi and determine species concepts and relationships at all taxonomic levels. Often the relationships revealed by sequencing correlate surprisingly well with the relationships revealed by microscopy. On the other hand, sequence comparisons show relationships that could not be determined from morphological studies. One major advantage of sequence comparisons is that the phylogenetic relationships of fungi can be determined; another is that a fungal isolate can still be classified and identified even if it fails to produce any diagnostic morphological characters in culture.

Soon after sequencing and sequence comparisons became a routine procedure, databases became available for researchers to deposit their data. The number of entries in these databases grew at an incredible rate and became major sources of information. Today we routinely isolate DNA, sequence an appropriate portion of the genome and do a BLAST search of the International Nucleotide Sequence Database (INSD; GenBank, EMBL, and DDJB). However, identifications based on BLAST

searches can be very misleading because the names applied to fungal sequences are notoriously wrong. In the data sets studied by Bridge *et al*. (2003), species annotations of up to 20% of the sequences available in the Fungi subset of the EMBL sequence database may be unreliable. According to Nilsson *et al.* (2006) 10–21% of the INSD ITS sequences have incorrect or unsatisfactory taxonomic annotations. These aspects are illustrated with examples from the Botryosphaeriaceae and *Diaporthe*.

Key words: Fungi, Systematics, Taxonomy

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GENETIC VARIATION OF *HETEROBASIDION ABIETINUM* POPULATIONS: DIVERSIFICATION ACROSS THE SOUTH EUROPE AND MEDITERRANEAN BASIN

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Heterobasidion abietinum is a fungal pathogen that causes root rot in *Abies* species (Capretti *et al.* 1990). In Italy and in central Europe the fungus mainly attacks *Abies alba*. In the past few years the fungus has also been found on other hosts and in other geographical regions, such as on *A. pinsapo* in southern Spain (Sanchez *et al.*, 2007), which is considered the western limit of the distribution of the fungus, and on *A. nordmanniana* in Turkey and the Caucasus (Doğmuş –Lehtijärvi *et al.*, 2006), which represent the eastern border of the distribution of the fungus. To investigate the genetic diversity of *H. abietinum*, isolates collected from southern Europe and the Mediterranean basin were analysed using minisatellites (DAMD-M13) and microsatellites (RAMS) (Vainio and Hantula, 1999). These markers were used to generate a *H. abietinum* haplotype network using NETWORK 4.5.1.0 software (Fluxus Technology Ltd., 2008),

The haplotype network showed genetic variation in *H. abietinum* populations. Isolates collected from Spain (*A. pinsapo*) had the highest significant ($p < 0.05$) divergence in comparison to the other European provenances. Smaller differences were found among European populations, excluding the Spanish one.

In conclusion, this study shows that *H. abietinum* distribution in Europe is strongly associated with the history of *Abies* spp. re-colonization after glaciations and also that the isolation of *A. pinsapo* in the refuge area of Southern Spain prevented gene flow between this and other H. abietinum populations.

Key words: *Heterobasidion abietinum*, M13, RAMS, Fungal population

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TOWARDS MEANINGFUL SPECIES DEFINITIONS IN *DIAPORTHE* AND *PHOMOPSIS*

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Since the description of *Diaporthe* in 1870, species in this genus have been described mainly on the basis of host association, contributing to an extensive proliferation of species names. However, in 1933 Wehmeyer revised the genus based solely on a study of teleomorph morphology of herbarium specimens. As a consequence, he placed many names in synonymy, thus reducing 650 species to about 70. In fact, recent studies have recognised that host association is of minor importance in the taxonomy of *Diaporthe* and its *Phomopsis* anamorphs (Rehner & Uecker, 1994). Not only are species non-host specific but more than one species can occur on a single host. On the other hand, morphological characters are now known to be unsuitable for species definition because of their plasticity and overlap between different species (Santos & Phillips, 2009). Thus, a morphological species definition is difficult to apply to these fungi.

Currently, species in these genera are based mainly on molecular phylogenies, especially those derived from sequences of ITS region of the rDNA, following a Phylogenetic Species Concept (PSC) (Santos & Phillips, 2009). The EF1-α gene has also been widely used to infer phylogenies in phytopathogenic fungi including *Diaporthe* and *Phomopsis* (e.g., van Rensburg *et al*., 2006). Moreover, several authors have defended the use of mating-type (*MAT*) sequences in the phylogenetic resolution of fungal species over other genomic regions such as ITS (Du *et al*., 2005). Biological Species Recognition (BSR) has not been applied to *Diaporthe*, presumably because the teleomorph has been induced in culture for relatively few species. Furthermore, some species are known to be self-fertile and thus a BSR cannot be applied to them.

Recently, we sequenced the ITS region of about 70 isolates, including ex-type isolates, belonging to more than 30 species. The same isolates were selected for sequencing and phylogenetic analysis of other genomic regions such as part of the EF1-α and *MAT* genes. Surprisingly, species delimitations in the ITS tree were strikingly different from those defined by other genes. In contrast, the species boundaries represented in EF1- α and *MAT* trees were very similar. Mating experiments were performed with some isolates to assess their biological species boundaries. Interestingly, the biological species matched perfectly the phylogenetic species present in EF1- α and *MAT* trees, whereas biological species were split into several phylogenetic species in the ITS phylogram.

The PSC is particularly useful in organisms where sexual reproduction is

difficult or impossible to evaluate, thus preventing the implementation of a Biological Species Concept (BSC). In sexual reproducing microorganisms, such as some phytopathogenic fungi, the ability of isolates to mate might be particularly relevant as it can determine genetic exchange between pathogens and potential spread of pathogenicity traits. Therefore, a BSC can certainly be relevant in these organisms. Our results indicate that biological and phylogenetic species definitions can be reconciled, provided the studied genes are chosen carefully.

Finally, although morphological characters are generally not helpful in the definition and distinction of species, the morphology studies performed within some biological and phylogenetic species showed that morphology should not be neglected.

Key words: *Diaporthe*, *Phomopsis*, Teleomorph, Mating-types, ITS, EF1-α, Phylogeny, Systematics, Taxonomy, Morphological Species, Phylogenetic s pecies, Biological species

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IDENTIFICATION OF TWO GENOTYPES OF LEVEILLULA POWDERY MILDEWS ON SUNFLOWER *(HELIANTHUS ANNUUS)* BASED ON ITS SEQUENCES

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At least three powdery mildew species, including *Golovinomyces cichoracearum*, *Podosphaera xanthii* and *Leveillula taurica* have been reported on sunflower (Braun 1995, Chen *et al*., 2008). *L. taurica* has been reported on hosts belonging to many different and phylogenetically unrelated plant families (Braun, 1995). However, strains of *L. taurica* from different host families are morphologically uniform. This species is considered to be a complex of species (Braun, 1987, 1995; Khodaparast *et al*., 2001, 2007). On the other hand, in some cases one host plant genus may be infected by more than one species of the genus *Leveillula*. However, identification of specimens at species level is difficult, due to the degree of morphological similarities between species. As a solution rDNA diversity has recently been used for phylogenetic analysis of several specimens in *Leveillula* including more than 35 specimens of *L. taurica* (Khodaparast *et al*., 2001).

In Iran sunflower powdery mildew is caused by two types of anamorph, which are characterized by their primary conidial morphology. In the first type primary conidia are subcylindrical with parallel sides that are pointed towards the apex, which is typical for some *Leveillula* species such as *L. picridis*. This type of conidial morphology has already been recorded for some collections on *Helianthus annuus*, *Medicago sativa*, *Vicia variabilis* and others (Khodaparast *et al*., 2001, 2007). In the second type of collections on sunflower, primary conidia are more or less lanceolate, which is typical for *L. taurica*. We sequenced ca 600 bp of the rDNA ITS region for three *Leveillula* specimens from *H. annuus* and compared them with several already published sequences from the genus *Leveillula*. The rDNA ITS sequences of these specimens showed that sunflower in Iran is infected by at least two different genotypes of *Leveillula* that are differentiated morphologically as well as phylogenetically.

Key words: Powdery mildew, Erysiphaceae, ITS, Phylogeny, rDNA

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CHARACTERIZATION OF *FUSARIUM LATERITIUM* ISOLATES IN NUT GREY NECROSIS DISEASE OF HAZELNUT

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Fusarium lateritium is a globally distributed pathogen. It has been reported on numerous hosts mainly woody and fruit trees, where it causes wilt, tip or branch dieback, and cankers. In Italy, this pathogen has been reported recently as the causal agent of nut grey necrosis (NGN) disease (Santori *et al.*, 2010) and twig cankers (Belisario *et al*., 2005) on hazelnut (*Corylus avellana*). The disease caused on hazelnut fruit was named NGN because of the symptoms observed on the affected fruit (Belisario *et al*., 2003, Santori and Belisario, 2008). Symptoms appear as brown-greyish necrotic spot/patch on nut and bracts, and sometimes on petiole (Belisario *et al*., 2003). Since its first occurrence in 2000, a great attention has been given to NGN due to its damaging nature in causing severe fruit drop (up to 60%). Morphological studies combined with inter-simple-sequence-repeat (ISSR) profile analysis, and sequence analysis of translation elongation factor 1-a (TEF-1a) gene were carried out to resolve relationships among 32 *F. lateritium* isolates from NGN affected hazelnut fruit, 14 from other substrates and 8 from hosts other than hazelnut. Based on colony color, *F. lateritium* isolates from hazelnut showed dark greyish-olive colonies differing from the orange-yellow group of all isolates from other hosts.

Generally, isolates from NGN affected fruit differed from all others in failing to produce sporodochia on carnation-leaf agar (CLA). A relationship between hazelnut twig cankers and NGN occurrence was suggested by ISSR analysis. Cankers may represent a source of inoculum to NGN for the production of sporodochia in correspondence to lesions (Belisario *et al.* 2005; 2009). In contrast to differences in morphological and biological characteristics, the molecular marker used here (TEF-1a) failed to resolve a clear phylogenetic structure in the *F. lateritium* population with respect to the NGN isolates. The morphological and biological variations shown by NGN *F. lateritium* isolates combined with a certain degree of genomic variation, suggest that an evolutionary process may be in progress, which could stabilize over time the *F. lateritium* NGN isolates into a resolved population specific for hazelnut and/or hazelnut fruit.

Key words*:* Fungi, Morphology, Phylogeny, Nut disease, *Corylus avellana*

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INVESTIGATING THE PHYLOGENETIC SIGNAL IN PHATHOGENECITY PHENOTYPES OF *FUSARIUM* SPP. ON CITRUS SEEDLINGS

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Phylogenetically-based statistical methods have been advocated for comparative biology studies to overcome the problem of non-independence of character values on the phylogeny tips (taxa). The effect of phylogeny on phenotypes has been tested as presence of phylogenetic signal, which is the tendency for related species to resemble each other. Whether this assumption is valid or not for a given set of data, this is an empirical question that could be tested by statistical method introduced by Blomberg *et al.* (2003).

Phylogenetic analysis of 68 strains of *Fusarium* spp. from IAMB collection was made previously based on sequence analysis of β-tubulin and α-elongation factor loci, from which 13 strains were chosen for further characterization of their pathogenicity phenotypes on citrus seedlings (Spina *et al.* 2008; Balech *et al.* 2008). We have applied Blomberg *et al.* (2003) statistical method to investigate the presence of phylogenetic signal in the pathogenicity characters given. Contrast values were calculated for characters values for the phylogeny tips and then simple randomization procedure tested the null hypothesis of no phylogenetic signal. Results revealed that, calculated probabilities of not rejecting null hypothesis p(H0), failed to proof significant presence of phylogenetic signal, at threshold $p(H0) \leq 0.05$ for any of the 7 characters tested, although at higher threshold, for instance $p(H0) \leq 0.1$, some characters showed tendency for phylogenetic effect.

This could be attributed to the nature of the phenotype characters tested which is the pathogenicity, or due to reasons related to the molecular marker used to build the phylogenetic analysis. Pathogenicity is a behavioural character that shows great plasticity, imposed by nature of this complex interrelationship between the pathogens, the hosts, and the environment. In addition, molecular markers used in this study were encoding for fundamental proteins (house-keeping genes) in fungal biological structure and function. It could be presumed that the time frame considered for variation in these molecular markers is different, and particularly much longer, when compared to the fast rate of evolution of pathogenicity traits.

Key words: *Fusarium* spp., Phylogenetic signal, Citrus

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MORPHOLOGY ENUMERATES RESTING SPORES IN COLLECTIONS OF *SPONGOSPORA SUBTERRANEA* SPOROSORI

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Plasmodiophorid sporosori are aggregations of resting spores, and the sporosori of *Spongospora subterranea* are the most complex of this group of obligate biotrophic Cercozoan pathogens (Karling 1968). *S. subterranea* causes the economically important diseases powdery scab of potato and crook root of watercress (Merz & Falloon 2009). Resting spore enumeration in *S. subterranea* is not possible using culturing techniques. Morphological methods were used to count resting spores in sporosori of *S. subterranea* f. sp. *subterranea* aiming to assist quantification of inoculum for plant pathology studies and disease prediction in the field.

Numbers of resting spores in sporosori were estimated using light microscopy for detailed examination of sporosorus form. Individual sporosori were measured, their volumes (prolate spheroids) were calculated, and a morphologically derived multiplication factor (Falloon *et al.,* 2007) was applied. Numbers of resting spores were determined for 30 sporosorus collections from potato tuber lesions from 11 different countries from Europe, the Americas, Asia, Africa and Australasia. Scanning electron microscopy was also used to indirectly measure resting spore viability in 25 individual sporosori, by counting resting spores which had released zoospores after exposure to host (tomato) roots in a laboratory bioassay (Merz, 1989).

Mean numbers of resting spores per sporosorus for the different collections ranged from 199 to 713, with mean numbers differing for collections within countries ($P < 0.001$), but not between countries ($P > 0.10$). Counting resting spores that had released zoospores after exposure to host roots indicated that proportions of viable resting spores in sporosori were highly variable (2 - 51%, overall mean = 19.6%).

This study has provided methods for determining *S. subterranea* inoculum potential, to assist epidemiological studies and practical powdery scab risk assessment for this economically important disease.

Key words: Microscopy, Powdery scab, Potato, *Solanum tuberosum*

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POINTS MUTATION IN *ERYSIPHE NECATOR CYP51* GENE

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Previous studies showed that a single-point mutation in the *CYP51* gene, coding for a cytochrome P450, is responsible for a high level of resistance to sterol 14a-demethylation inhibitor fungicides (DMIs) in *Erysiphe necator* (Délye *et al*., 1997a; 1997b).

Allele-specific PCR amplification of *E. necator* DNA was performed using the primers MUT1 (5'-AATTTGGACAATCAA-3') and U14DM (5'-ATGTACATTGCTGACATTTTGTCGG-3'), designed on the region of the *CYP51* gene where the point mutation (A495T) responsible for high resistance to DMIs occurs (Délye *et al.*, 1997b). The MUT1 and U14DM primers were used to screen 50 isolates sampled from seven different vineyards. Only four isolates (X109, X112, X113 and X115) carrying the point mutation in the *CYP51* gene, hence yielding a DNA band of expected size, were detected.

The normal response to tebuconazole, established for 20 fungal isolates through an *in vitro* bioassay, was EC_{50} = <0,1-3 mg ml^{-1} and MIC = 1-6 mg ml^{-1}. The isolates X112 and X115 and the isolate X104, not carrying the mutation, were less sensitive than normal (EC_{50} = 6-10 mg ml^{-1} and MIC = 10 mg ml^{-1}), while the isolate X109, although carrying the point mutation, was normally sensitive (EC_{50} = 0.3 mg ml^{-1} and MIC=3 mg ml^{-1}).

The primer pairs C14 (5'-TAAGGTAGTATTGAGGCGGG-3') and C14R (5'-TTCTAACCCTAACACCTGCC-3') that amplify the whole *CYP51* gene were used in PCR with the four strains. Amplified DNA was cloned, sequenced and aligned with the nucleotide sequence of the whole *E. necator CYP51* gene (1,755 nt) available in GenBank (accession number U83840, http://www.ncbi.nlm.nih.gov/Genbank/) (Déley *et al*., 1997a). The alignment confirmed that the obtained sequences were actually of the *CYP51* gene and that the strains X109, X112 and X115, but not X104, showed the point mutation A495T. Additional point mutations at different positions on the *CYP51* sequence were detected in X112 (G136T, A344G and G1753T), in X109 (A733G), in X115 (C1304), and in X104 (A732C).

Our results suggest that the point mutation A495T in the *CYP51* gene is not constantly associated with resistance to DMIs in *E. necator.* Further studies are in progress to explore the molecular basis of resistance to DMIs of the grape powdery mildew fungus *E necator.*

Key words: *Erysiphe necator*, *CYP51* gene, Fungicide resistance, DMI fungicides

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MOLECULAR CHARACTERIZATION OF *PYRENOPHORA TRITICI-REPENTIS* RACES IN SYRIA USING AFLP TECHNIQUE

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Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr) is a common disease on wheat responsible for economic losses in some wheat growing areas worldwide. The study aimed to use AFLP technique determine variation between Syrian isolates. 29 Ptr were obtained from the durum wheat growing provinces in Syria (Aleppo, Homs, Hama, Hassakeh, Lattakia, Tartous, Idlib). Their colony morphology on Potato Dextrose Agar were investigated. To identify the different races causing tan spot, AFLP templats were prepared by the digestion of Ptr DNA with *Eco*RI and *Mse*I restriction. A total of 745 AFLP polymorphic bands were obtained using 3 primer combinations. The results showed that AFLP technique could determine the genetic variation in the Ptr population. This variation was low (9.87%) between sites within the same district, but was high (90.12%) within the same site. UPGMA cluster analysis jointly with PCoA analysis helped to show the high variation within Ptr population as well as the possible similarity of some groups. Genetic similarity between some Ptr isolates was found between different geographical locations. This technique will be helpful to researchers studying the genetic variation in the Ptr populatin.

Key words: *Pyrenophora tritici-repentis*, AFLP, Culster, Genetic Variation

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PLASMID PROFILES OF *PSEUDOMONAS SYRINGAE* PV. *MACULICOLA* AND CLOSELY RELATED PATHOVARS

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Pseudomonas syringae pathovars *maculicola* (*Pma*), *tomato* (*Pto*), *anthirrhini* (*Pat*), *lachrymans* (*Pla*) comprise a genomospecies (Gardan *et al*., 1999). These pathovars were indistinguishable using standard techniques such as host range and temperature response (Wiebe and Campell, 1993). AFLP and RAPD have been used to differentiate between *Pma* and *Pto* (Clerc *et al*., 1998). Characterisation of plasmid profiles is one of the direct but partial genomic analysis methods (Louws *et al*., 1999).

Genetic relationship between strains of *Pseudomonas syringae* pathovars *Pma*, *Pto*, *Pat*, *Pla,* and *coriandricola* (*Pcr*) was investigated by native plasmid profiles analysis. The results revealed inter- and intra-pathovar diversity. Great variation in plasmid content of *Pma* strains and its related groups ranged from 7 to 398 kb for some *Pma* strains and to plasmidless in other *Pma* and *Pat* isolates. The plasmid profile analysis classified most of the *Pma* strains into six different groups designated as A, B, C, D, E and F. Strains of *Pto* showed a greater genetic diversity among all strains tested. The remaining *Pma* together with *Pto*, *Pla*, *Pcr* and *Pat* strains were unique.

Key words: Plasmid profile, *Pseudomonas syringae*, Pathovars

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FINGERPRINTING METHODS (AFLP, MLST) FOR IDENTIFICATION AND CHARACTERIZATION OF PECTOLYTIC, SOFT ROT CAUSING BACTERIAL STRAINS FROM SYRIA IN COMPARISON TO STRAINS FROM WORLDWIDE ORIGIN

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A collection of 30 pectolytic enterobacterial strains was sampled from potato fields in Syria between years 2002-2004. The strains were characterised using biochemical tests, including their ability to utilize pectin on CVP medium, and virulence assays on potato tubers, pepper slices and tomato plants. For 64 strains, including 34 reference strains of *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*), *P. atrosepticum* (*Pba*), and *Dickeya* species *(Dickeya* spp.) (provided by Julius Kühn-Institute, DSMZ-Germany and SCRI-UK) Fatty Acid Methyl Ester analysis (FAME), metabolic fingerprinting using 95 carbon sources (GN Biolog assay), and PCR using different primers for the *Pel* genes family and the intergenic spacer region ITS (Toth *et al*., 2001) were previously performed.

Two DNA fingerprinting approaches were applied on these 64 strains, Amplified Fragment Length Polymorphism (AFLP) and Multi-locus Sequence Analysis (MLSA). The AFLP-analysis was based on 9 primer combinations and produced approx. 1000 clearly scorable DNA-fragments. Reproducibility was tested and confirmed on a selection of 10 different strains. The banding patterns were evaluated in the FAMD 123 software, using Jaccard similarity index to compute pairwise distances. As a test for the reliability of the dendrograms bootstrapping was performed using 1,000 replicates and revealing six consensus AFLP phylogenetical trees. By this analysis the 64 strains could be divided into four main clusters: cluster I contains all *Dickeya* strains including the two Syrian *Dickeya* strains, cluster II contains all *Pba* strains, one *Pba* Syrian strain, cluster III contains 7 *Pcc* strains, and the fourth cluster contains 27 Syrian strains. The fourth cluster could be further divided into four subclusters: subcluster A contains 20 Syrian strains interfering with five *Pcc* strains; subcluster B contains 3 Syrian and 3 *Pcc* strains. Subclusters 3 and 4 each contain two Syrian strains, which are distinct from the entire Syrian strain population.

MLSA, as a sequence-based approach, depends on the information of the nucleotide sites for multiple house-keeping loci. We amplified fragments of eight

conserved gene (*acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, *rpo*S and *proA*) from each of the 64 strains. Overall 496 amplified products were sequenced and used to build up a pairwise and multiple alignments with clustal W implemented in the Mega 4 software. The strains were clustered in 9 neighbour-Joining (NJ) trees. The topology of the dendrograms from the multi-locus sequencing using the eight partial genes showed a very high similarity to the dendrograms obtained by the AFLP method. Using both methods the 20 Syrian strains were grouped in a distinct cluster including 3 to 5 *Pcc* strains. In trees based on individual sequences, the rare appearance of single strains in clusters of a different species may represent the results of horizontal gene transfer between these taxa.

Three-hundred and five sequences for 44 taxa of *Pectobacterium* and *Dickeya* species (Ma *et al*. 2007), were available from the NCBI GeneBank, and included to construct one NJ tree for a total of 108 taxa. As a result the 20 *Pc* Syrian strains were placed into clade II and indicate that *Pc* strains divided into five distantly related genetic clusters, three of which contains the *Pc* Syrian strains which are also supported by AFLP analysis.

Key words: Soft rot bacteria, MLST, AFLP, NJ trees, Bootstrapping

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MOLECULAR INVESTIGATION ON GENETIC VARIABILITY OF *CITRUS TRISTEZA VIRUS* ISOLATES RECOVERED IN CALABRIA (SOUTHERN ITALY)

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Citrus tristeza virus (CTV) is becoming a major threat to the citrus industry in Italy, where almost all citrus trees are grafted on the susceptible sour orange rootstock. Recently, new and alarming outbreaks of the virus have been identified in different areas of Calabria (southern Italy) (Albanese *et al.,* 2010). Preliminary analyses conducted using the selective monoclonal antibody (Mab) MCA13 (Permar *et al*., 1990) revealed the presence of both MCA13-reactive and non reactive CTV strains.

Further investigations were then conducted to assess the genetic and biological variability existing in the CTV population spreading in the region. Five representative CTV-infected samples collected from citrus cultivars located in different orchards were subjected to molecular characterization. Molecular tests included: single strand conformation polymorphism analyses (SSCP) of the *p20* and coat protein (CP) genes multiple molecular markers analysis (MMM) (Hilf *et al.*, 2005) and nucleotide sequence analysis.

SSCP analysis of the *p20* gene showed the presence of at least four different SSCP patterns, denoted as type I, II, III, IV (Ferretti *et al.,* 2009), revealing that genetic diversity exists in the population analyzed. Similar results were obtained with SSCP analysis of the CP gene.

MMM analysis showed that 2 samples reacted with the VTPOL and T3K17 markers and 3 with the T30POL marker, indicating they contained VT, T3 and T30 genotypes, respectively. These results were in agreement with those obtained using the Mab MCA13. The two MCA13-reactive samples were positive for the VT and T3 MMM markers whereas the three MCA13 non-reactive samples showed positive reactions only with the T30 markers.

Amplified cDNA fragments of both CP and *p20* genes were cloned and three recombinant clones sequenced. Nucleotide sequences of the *p20* gene obtained from isolates showing the SSCP profile type I, associated with the MCA-13 reactive samples (Ferretti *et al.,* 2010), shared 98% homology with the Argentinean isolate C315-16 (AY962338), phylogenetically related to the severe reference isolates VT and SY568 (Iglesias *et al.,* 2005). More than 99% of nucleotide sequence identity

was found among the isolates sharing the same SSCP profile type I. Similar identity (99%) was found among the isolates showing the SSCP profiles 'type II and III' and the reference isolate T30 from Florida (EU937520); whereas, the isolates sharing the SSCP profile 'type IV' showed 99% of identity with the Italian isolates DS1-SR from Sicily (AY263360) and CTV-DS4CZ from Calabria (DQ325521).

Phylogenetic analysis of the nucleotide sequences of the CP gene proved that the MCA13-reactive isolates were in the same main clade with the severe SY568 and VT strains, but were phylogenetically distinct (only 93% identity); higher identity (98%) was found with CTV isolates K1-76 (EU579411) and P13 (GQ475569) previously described in Egypt and India, respectively.

The data presented show that genetic diversity occurs among the isolates recovered in Calabria. Since this diversity is associated with MCA13 reactivity and with the presence of VT/T3 genotypes, vigilance must be continued to limit the spread of such strains.

Greenhouse biocharacterization of these diverse field isolates is presently underway to confirm their biological activities.

Key words: CTV, Characterization, Genetic diversity, SSCP analysis, MCA13.

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OCCURRENCE, DISTRIBUTION AND CHARACTERIZATION OF *CITRUS TRISTEZA VIRUS* (CTV) IN OMAN

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Citrus is among the top-ranked worldwide fruits in terms of planted area and in production quantity. The annual citrus production in 2007 was estimated at 100 million tons, from a total area exceeding 8 million hectares (FAO, 2009). Citrus species, mainly lime, orange, grapefruit and sweet lime are among the top fruit crops in production in Oman. They rank fourth after date palm, banana and mango, with a total production of 6000 tons in 2007.

Among several viruses which infect different citrus species, *Citrus tristeza virus* (CTV), the causal agent of citrus tristeza disease, is the most important and serious (Herron *et al.,* 2005). This virus has killed millions of citrus trees in Brazil and the USA, especially those which have been grafted onto sour orange rootstocks. Symptoms of the disease vary according to citrus species, viral strain and rootstock used for grafting. Symptoms include mild vein clearing, stem pitting, slow decline and sudden decline of affected trees. The negligible amount of data available on CTV and citrus tristeza disease in Oman is a barrier to the establishment of future management programs for the disease. This study was therefore established to investigate the prevalence, natural host range and strains of CTV in Oman.

The study was conducted from 2008 to 2009. Over 250 citrus samples were collected from about 80 citrus orchards from all over the country. The samples included lime, grapefruit, sweet orange, sour orange and mandarin. They were analyzed for the presence of CTV using enzyme linked immunosorbent assay (ELISA). Further analysis of some of the samples was done using reverse transcriptase PCR (RT-PCR) as described by Huang *et al*., (2004) using primers HCP1, HCP2, CP3 and CP4 in order to discriminate the virus strains.

RT-PCR was more sensitive than ELISA in detecting low concentrations of the virus. Severe and mild strains of CTV were found present in all surveyed regions. Infection of lime and mandarin with CTV was more prevalent compared to other citrus species. Although CTV was detected in many citrus trees and seedlings, no virus symptoms were apparent on most of these plants.

Key words: *Citrus Tristeza Virus*, RT-PCR, ELISA

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MOLECULAR CHARACTERIZATION OF *OLIVE LEAF YELLOWING ASSOCIATED VIRUS* ISOLATES

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Olive leaf yellowing associated virus (OLYaV) was reported associated with a yellowing disorder affecting olive trees (Savino *et al.,* 1996). Gel electrophoretic analysis of RNA extracts from olive plants with yellowing symptoms revealed a complex double stranded RNA (dsRNA) pattern with the larger dsRNA band of about 15kb. On this basis , OLYaV was classified in the family *Closteroviridae* but with an unassigned genus because of lack of sufficient information on its genome (Martelli *et al*., 2002).

Currently, there is information on sequence of t OLYaV genome which consists of *ca* 5500 nt longthat comprises *RNA dependant RNA polymerase (RdRp), Open reading frame 1 and 2 (ORF1-2)*, putative *heat shock protein 70 (HSP70),* putative *heat shock protein 90 (HSP90)* genes and some not-transcribed regions. This sequence was obtained from only one virus isolate.

In 2006 a single strand conformation polymorphism assay based on 383 bp of the putative *HSP70* of 30 OLYaV isolates showed an unexpected genetic variability among the isolates ranging from 1 to 23%, allowing the identification of three distinct groups of the virus (Essakhi *et al*., 2006).

In this work, in order to understand and verify the molecular variability and to have more information on OLYaV genome, various primer pairs were designed to amplify the five known open reading frames and the not-transcribed regions of 18 OLYaV isolates.

Obtained results showed a different behavior among the 18 OLYaV isolates, confirming the existence of almost two different variants of this virus. Some of the obtained amplified fragments were sequenced and their multiple alignments showed a variability ranging from 2 to 10% among them. Phylogenetic analysis performed on the three conserved genes (*RdRp, HSP70, HSP90)* demonstrated that OLYaV seems more distant to the genus *Closterovirus* than expected, suggesting its classification in a new genus within the family *Closteroviridae*.

Key words: OLYaV, *Closteroviridae*, Molecular characterization

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ANALYSES OF MOLECULAR VARIABILITY OF THE CAPSID PROTEIN OF *APPLE CHLOROTIC LEAF SPOT VIRUS*

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Apple chlorotic leaf spot virus (ACLSV) (genus *Trichovirus;* family *Betaflexiviridae*) (Carstens, 2010) is distributed worldwide in *Rosaceae* fruit trees such as pome fruits (apple, pear, quince) and stone fruits (plum, peach, apricot, almond, cherry). ACLSV is an important and damaging latent virus causing significant losses in apple, an important temperate fruit crop in many countries. ACLSV infection may reach up to 80-100% in many commercial apple cultivars with yield losses of 30-40% have been reported (Nemchinov *et al.,* 1995; Cembali *et al*., 2003). The virus is latent in most apple cultivars, but in sensitive varieties malformation and reduction in leaf size and chlorotic rings or line patterns are common. The severity of symptoms elicited by ACLSV infection depends largely on plant species and virus strains (Németh, 1986).

ACLSV has been widely studied in many countries. However, in India it was characterized for the first time, at the molecular level, as late as in 2007. Preliminary field surveys in major pome and stone fruit growing states from 2007-2009 reported an incidence as high as 80-90% in apple orchards alone, based on ELISA results and pointed the prevalence of this pathogen in apple, an important commercial crop of the hill states of India: Himachal Pradesh (HP) and Jammu & Kashmir (J&K). The virus was also detected in almost most of the pome and stone fruits grown in the region. Other important apple viruses such as *Apple mosaic virus* (ApMV), *Apple grooving virus* (ASGV) and *Apple stem pitting virus* (ASPV) were also detected but, ACLSV was the most widespread.

It was of interest to know if mixed cropping of pome and stone fruits, mixed infection and prevalence of old cultivars of apple and their use as mother stocks over the years have generated variability or evolution through recombination in the virus. The coat protein has been reported as the most conserved part of ACLSV genome and thus used for variability analysis. The complete sequences of capsid protein gene of twenty-six isolates of ACLSV from India were determined using primers specifically designed for complete coat protein amplification (Accession numbers AM490253 and AM490254). The isolates were obtained from various pome (apple, pear, quince) and stone (plum, peach, apricot, almond, Wild Himalayan cherry) fruit trees from different locations in states of HP and J&K, India.

The phylogenetic relationships of Indian coat proteins of ACLSV with all the available complete and partial sequence of ACLSV pome and stone fruit isolates from

the world was examined to detect possible heterogenity. Comparison was also done with previously characterized other species of the genus *Trichovirus*. All the Indian-ACLSV CP isolates showed sequence identity of 91-100% and 86-100% at the amino acid level with each other and isolates from other countries, respectively. Only the recently sequenced TaTao isolate of ACLSV from peach shows about 70 % sequence identity with most of the ACLSV isolates at the amino acid level.

Multiple alignments of all Indian ACLSV-CP isolates indicated variation at seventeen amino acid positions. A classification based on covariation of these amino acids can divide the Indian isolates into two groups, biological properties of which need to be ascertained. The highest degree of variability was observed in the middle portion with 9 amino acid substitutions in contrast to the N-terminal and C-terminal ends which were maximally conserved with only 4 amino acid substitutions.

Recombination detection program analysis (RDP3 ver.2.7) done for all the Indian isolates and available complete coat protein sequences of other countries has provided no significant evidence of recombination. However, only one recombination among Indian isolates was detected.

Key words: ACLSV, Coat protein, India, Phylogenetic analysis, Recombination, Variability

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EXPLOITATION OF GENETIC VARIABILITY IN THE POTYVIRUS HELPER COMPONENT PROTEINASE FOR CONSTRUCTING AND ANALYZING A COMPLEX NETWORK OF HIGHLY STATISTICALLY ASSOCIATED POLYMORPHIC POSITIONS

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Alignment of homologous proteins typically result in two kinds of regional patterns: a) strictly conserved positions in which one amino acid appears consistently and b) polymorphic positions in which more than one amino acid appear with varying frequencies. At polymorphic sites, at least some mutations may be concurrent (Korber, 1993), and in the simplest form, a specific amino acid in one position will appear in combination with a certain amino acid in another, implying a kind of association. Such residues are considered to be co-evolving, and their relationship may contain structural and/or functional information (Pollock *et al.*, 1999).

The genus *Potyvirus* consists of species with flexuous filamentous particles. Their genomes consist of a ssRNA of positive polarity encoding a polyprotein precursor cleaved into ten mature proteins by *cis* and *trans* catalysis by three virus encoded proteinases. One of the proteins, the helper component (HCPro), is a multifunctional molecule (Rojas *et al.*, 1997) involved in several biological processes including aphid transmission, RNA-silencing suppression, systemic movement and virus replication. Functionality is achieved by interactions of HCPro with other viral and possibly host molecules. A number of polymorphic positions in this protein have been found by side directed mutagenesis to be involved in functionality.

In this work, a combined statistical and bioinformatic methodology was applied for examining the associations among polymorphic positions in HCPro and identifying their possible functional patterns. Assigning the polymorphic positions as vertices and the identified associations among them as links, the relevant graph was constructed. The relationships among positions in HCPro displayed a complex topology with a small number of vertices dominating a large number of associations (Manoussopoulos and Zevlekari, 2009). The node degree did not fit the Poisson distribution, suggesting a non-random process in the allocation of associations to vertices. In contrast, the degree distribution of this network fits adequately a power law, implying a B-A type network (Barabasi and Albert, 1999). Interestingly, some of the most connected nodes in the network were known functional positions, whereas, some others were found in the vicinity of functional sites, suggesting an association of over connectivity to functionality.

These findings signify the importance of exploitation of genetic variability in viral proteins for identifying intramolecular relationships among polymorphic positions and constructing the relevant networks. Graph theory has been rapidly advanced in the last decade with many applications in biology, and intramolecular network analysis. It may provide a powerful means for understanding virus evolution and functionality.

Key words: *Potyvirus, Helper Component, Intramolecular network, Complex network*

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SESSIONE 4

Variability of plant pathogens

POSTERS

PETRIA 20 (2), 67-633 (2010)

STUDY OF *IN VITRO* GROWTH AND PATHOGENICITY OF SOME ISOLATES OF *FUSARIUM* SPP.: CAUSAL AGENT OF *FUSARIUM* HEAD SCAB AND ROOT ROT OF WHEAT

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Fusarium head blight (FHB), also know as ear blight or scab, *Fusarium* foot rot and *Fusarium* seedling blight of wheat are commonly caused by *Fusarium culmorum*, *F. avenaceum* (*Gibberella avenacea*), *F. poae*, *F. graminearum* (*Gibberella zeae*) and *Microdochium nivale* (fries) (*Monographella nivalis*), formely known as *F. nivale* (Parry *et al*., 1995). Head blight or head scab is usually preceded or accompanied by seedling blight, foot rot and root rot (Agrios, 2005). FHB has received significant attention in recent years because of the impact that infection may have on yield, mycotoxin contamination of grain and the lack of preharvest fungicides for disease control (Brenan *et al*., 2003).

Studies on the effect of temperature on *in vitro* growth of Algerian isolates of *Fusarium* spp. obtained from wheat collar and spike showed that the optimum growth was at 25°C for all isolates belonging to the four species of the *Fusarium* genus namely *F. lateritium*, *F. culmorum*, *F. graminearum* and *F. solani*. However *F. moniliforme*, *F. avenaceum* and *M. nivale* showed an optimum temperature for growth of 20°C. Similar results were obtained by Brenan *et al*. (2003) for *F. graminearum*, *F. culmorum*, *F. avenaceum* and *M. nivale*, where the optimum temperature for the growth of *F. graminearum* and *F. culmorum* was 25°C, while that for *F. avenaceum* and *M. nivale* was 20°C.

Among the species studied, the *F. culmorum* isolates showed the highest rates of growth at all temperatures tested: 15, 20, 25 except at 30°C where *F. solani* isolates showed the highest rates of growth. Furthermore the growth rate of all species studied increased between 20 and 25°C, and decreased between 25 and 30°C with no growth of any isolate at 35°C.

Pathogenicity tests were carried out by soil inoculation and evaluated by the severity of disease at the collar level estimated by a disease scale ranging from 0 to 3. In these tests the highest disease index (2.89) was induced by *F. graminearum* isolate (FG04-08) obtained from a diseased collar, followed by *F. moniliforme* isolate (FM 01-07) from a wheat spike (disease severity score of 2.75), *F. culmorum* (FC01-08) from an infected collar (disease severity of 2.4) and *M. nivale* isolate (MN 01-08) from a diseased collar (disease severity index 1.3). A range of disease severities was found among *F. graminearum* and *F. culmorum* isolates. *Fusarium graminearum* and *F. culmorum* isolates obtained from diseased collars caused disease severity indices higher than the isolates obtained from spikes.

Results obtained in this study showed that there is no correlation between in *vitro* growth and pathogenicity of *Fusarium* spp. isolates used in this study. Walker *et al.* (2001) reported the existence of both direct and indirect relationships between *in vitro* growth rate and *in vivo* pathogenicity among *F. graminearum F. culmorum* and *F. avenaceum* isolates. The *F. avenaceum* isolate exhibited the lowest *in vitro* growth rate and in the greenhouse had the lowest disease score, in contrast a *F. culmorum* isolate had the second lowest *in vitro* growth rate but caused the highest disease score. Bai and Shaner (1996) also reported a direct relationship between *in vitro* growth rate and *in vivo* pathogenicity of *F. graminearum* isolates. Furthermore it was shown in this study that the *Fusarium* species that induce head scab of wheat are also aggressive on root and collar of wheat.

Key words: Agressiveness, *Fusarium* spp.*, In vitro* growth, Wheat

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SEQUENCE VARIATION OF THE rDNA ITS REGIONS WITHIN AND BETWEEN OF *RHIZOCTONIA* SPP. FROM RICE IN GUILAN PROVINCE, IRAN

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The *Rhizoctonia* sheath blight disease complex, comprising *R. solani* anastomosis group AG1-IA and *R. oryzae-sativae* AG-Bb (teleomorphs: *Thanatephorus cucumeris* and *Ceratobasidium oryzae-sativae,* respectively) causes significant yield losses in rice in Asia (Ou, 1985). *Rhizoctonia oryzae-sativae* while not considered to be as important as *R. solani*, has been reported on rice in regions where sheath blight frequently occurs (Johanson *et al*., 1998). Both species are distributed within paddy fields in Guilan province and caused yield loss.

In recent years, rDNA-ITS sequence analysis seems to be the most appropriate method for comprehensive classification of *Rhizoctonia* spp. Mazzola *et al.* (1996) revealed the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) have been used successfully to generate specific primers capable of differentiating many closely-related fungal species. Gonzalez *et al*. (2001) pointed out using phylogenetic analysis of ITS sequences that there are at least 12 monophyletic grouping within *Ceratobasidium* and *Thanatephorus*. Although severalAG *Ceratobasidium* may be more closely related with some AG from *Thanatephorus*, these relationships were not as strongly supported by bootstrap analysis. Johanson *et al*. (1998) designed specific primers using ITS sequence for distinguishing the *Rhizoctonia* spp on rice. Kuninaga *et al.* (1997) expressed that sequence of ITS rDNA regions of *R. solani* may be a valuable tool for identifying AG subgroups of biological significant.

This study was carried out for molecular identification and phylogenetic analysis of *Rhizoctonia* species recovered from rice plants in paddy field in Guilan province. In this study, 11 isolates from *R. oryzae-sativae*, 10 isolates from *R. solani* and one isolate from *Sclerotium hydrophilum* were used to phylogenetic analysis. Data of our isolates and isolates that were obtained from GenBank were analyzed together in neighbor joining (NJ) and maximum-parsimony (MP) trees.

DNA sequence analysis revealed that all of the isolates tested formed two distinct clades with above 90% bootstrap support. The sequence homology in the ITS regions for each species was high. All sequences from *R. oryzae-sativae* were similar. A few base substitutions were found for *R. solani* isolates. Our results confirmed both species, *R. solani* AG1-IA and *R. oryzae-sativae* collected on rice, make well supported monophyletic species. Moreover, phylogenetic analysis indicated that *S. hydrophilum* is closely related to binucleate *Rhizoctonia* species (*R. oryzae-sativae*) rather than other *Sclerotium* or *Rhizoctonia* multinucleate taxa.

Key words: Iran, rDNA ITS regions, *Rhizoctonia* spp., Sequence variation.

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MORPHOLOGICAL CHARACTERIZATION OF MOROCCAN POPULATION OF *PYRENOPHORA TERES* OF BARLEY TO ESTABLISH THE GENETIC DIVERSITY

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Net blotch, caused by *Pyrenophora teres*, is a prominent foliar disease of barley *(Hordeum vulgare* L.) and is responsible for large economic losses (40%) in most of barley growing areas in Morocco (Bentata *et al.*, 2006). In order to establish the degree of genetic diversity within the Moroccan population of *P. teres* in space, we carried out the morphological characterization (Frazzon *et al*., 2002).

The purpose is to place the Moroccan isolates in various groups according to their mode of culture. Each mode of culture is represented by five parameters: diameter, importance of mycelial growth, texture, color and importance of sporulation. The study of the similarity (Rohlf, 1997) among the 51 Moroccan isolates of the collection with a level of similarity of 0.9 gave 11 groups.

Taking account this variability, it is necessary to proceed to seek a resistant germplasm to a large range of isolates of *Pyrenophora teres*.

Keys words: Moroccan population, *Pyrenophora teres,* Genetic diversity, Barley

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MORPHOLOGICAL AND HOST RANGE STUDIES OF *UROMYCES VICIAE-FABAE* AS A HETEROGENEOUS SPECIES

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In this investigation host range and spore morphology of faba bean rust *Uromyces Viciae-fabae* were studied. Three *Vìciae fabea* bulks (Barekat, Saraziry, Shakh Bozi) and 4 pea, *Pisum sativum* bulks (Green Agro, Atrilo, Dorango, Mr. Big), which are commonly grown in Iran, were inoculated with urediniospores in a greenhouse.

These spores were collected from rust-infected faba bean plants. Disease symptoms were recorded 12 days after inoculation. Infection type (IT) of the entries, recorded according to Stakman's method, indicated that all broad bean and pea bulks were susceptible to the rust isolate. Disease severity (DS) and latent period (LP) were also studied.

Lens culinaris and *Vicia sativa*, *Vicia villosa* and *Lathyrus sativa* which have been reported as hosts of this rust, were inoculated by 3 methods: dusting, brushing and mist spraying. However, no rust symptoms developed on any of these plants. Morpholgical studies on photomicrograph and scanning electron micrograph showed that uridiniospore have fine echinulations and smooth teliospores. Morphological studies of urediniospores of this isolate from broad bean and pea as well as urediniospores of herbarium specimen of this rust on lentils revealed no significant differences for the parameters of spores length, width, number of germ pores and their position. Also teliospores of this isolate from the faba bean and pea hosts were identical in morphological features. Analysis showed that there are significant differences between teliospores of faba rust isolate and those of the lentil isolate in terms of spore length, apex length and pedicel length.

The results of this study and reports of other researchers indicate that isolates of this rust differ not only in their host specificity but also in spore morphology. Based on the results of this study and reports in literatures, this rust, *Uromyces viciae-fabae,* is composed of a species complex which may need to be revised.

Key words: Rust, Faba bean, Pea, Teliospore, Uridiniospore

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IDENTIFICATION OF CUCUMBER POWDERY MILDEW AGENTS AND DETERMINATION OF THEIR RACES IN EAST AZERBAIJAN PROVINCE, IRAN

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Podosphaera xanthii and *Golovinomyces cichoracearum* are the two main species causing powdery mildew of cucumber worldwide. Cucumber powdery mildew and the fungal species involved in this disease have poorly been studied in East Azerbaijan province of Iran. In the present study, 37 cucumber plants samples with powdery mildew symptoms were collected from Tabriz, Ahar, Sarab, Basmenj, Shabestar and Karkaj, during August and September in 2007. All collected species were identified based on conidial and cleistothecial morphology (Braun, 1987; Cook *et al*., 1997). In total, 48 isolates were studied, of which 37 (77%) were identified as *G. cichoracearum* and 11 (23%) as *P. xanthic*.

In order to determine the race of isolated species, pure and single clones were produced by inoculating a susceptible cucumber cultivar (MP73). Spores of each isolate were inoculated by brush onto the adaxial surface of the leaves in a greenhouse at 20–24°C with 70% humidity. Inoculated plants were enclosed in plastic covers to avoid cross contaminations. Symptoms developed on the adaxial surface of leaves in inoculated plants after one week. A set of differential melon cultivars (Iran H, PMR5, PMR45, Vedrantais, Edisto47, Nantais oblog, Mr-1, PI124112, PI414723 and WMR29), were used to determine the race of species.

Leaf disks of 9 mm in diameter were removed from leaves with a cork borer and were placed into Petri dishes (15 cm in diameter) containing 0.16 % water agar amended with 25 µg/ml of Carbandasim 60%. Carbandasim was used to prevent early senescence of the leaf disks. Inoculum was prepared by rinsing powdery mildew conidia from the leaf surface with water containing 0.01% Tween 20. Leaf disks were inoculated with 10 µl of conidial suspension (2×10^4 conidia/ml) of *G. cichoracearum* or *P. xanthii* on the adaxial side of each leaf disk (Cohen 1993). After inoculation, the Petri dishes were placed in an incubator at 24°C and 70% humidity at a light intensity of 100 $\mu Em^{-2}\ s^{-1}$. After 15 days races were determined based on presence or absence of symptoms on differential host. Pitrat chart was used to evaluate and score the results (Pitrat, 2006).

Based on the results, from 37 isolates of *G. cichoracearum*, 26 isolates were determined at the race level and of these 19 isolates were identified as race 0 and 7 isolates determined as race 1. Among 11 isolates of *P. xanthii*, 8 isolates were

identified as race 0. However for the remaining 11 isolates of *G. cichoracearum* and 3 of *P. xanthii*, results were not conclusive because of overlapping between differential set of cultivars. Analysis of Variance and mean comparison (P=5%) was performed by using MSTATC software.

Resistance assessment of the differential lines to different isolates showed that "Iran H" with 15.17 % mean infection was determined as susceptible and "Edisto 47" with 1.56 % mean infection was determined as resistant to *G. cichoracearum*. MR-1, PMR 45, PI 414723 and Nantais oblog were completely resistant and no infection was observed when inoculated with *G. cichoracearum*. For the other species isolates, in comparison to Vedrantais (with 7.5 % mean infection) "Iran H" (with 15.72 % mean infection) was also determined as susceptible to *P. xanthii*. The other lines were not infected and were considered resistant to *P. xanthii*.

Key words: Cucumber, Powdery mildew, *Golovinomyces cichoracearum*, *Podosphaera xanthii*, Race determination

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STUDY OF VARIATION OF *ALTERNARIA* SPECIES CAUSING EARLY BLIGHT DISEASE ON TOMATO PLANTS IN ISFAHAN, IRAN USING MORPHOLOGICAL CHARACTERS AND MOLECULAR IGS MARKER

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Alternaria is a common fungal genus with several species pathogenic or saprophytic on different hosts. This fungus causes diseases or produces host specific toxins on many plants and crops in the field or in storage. Tomato early blight is one of the most important diseases caused by *Alternaria* species (Chaerani and Voorrips, 2005).

To identify the casual agent of early blight in tomato fields in Isfahan province (IRAN), tomato leaves and fruits were collected from different fields. Samples showing early blight symptoms were surface sterilized and transferred on PDA media. A total of 200 fungal isolates showing *Alternaria* characteristics were purified, transferred on PCA medium (Simmons, 2007) and incubated under a photoperiod of 8 hr light and 16 hr dark. Using a taxonomic key for *Alternaria* species, nine species were identified: *A. alternata*, *A. infectoria*, *A. arborescens*, *A. dumosa*, *A. radicina*, *A. petroselini*, *A. tomaticola*, *A. merytae* and *A. mimicula*. *A. infectoria* was the most common species. This is the first report of *A. tomaticola*, *A. merytae* and *A. mimicula* in Iran.

Pathogenicity tests were performed in a greenhouse using a complete randomized block design with 6 replicates. Rutgers tomato cultivatr was used in this test. The results showed that *A. petroselini* was the most pathogenic species on tomato.

To study the genetic variation and molecular identification of the *Alternaria* species, PCR-RFLP analysis was conducted using GVA30 and IGS27 specific primers of the IGS region (Ma and Michailides, 2007). The PCR products were digested using *Dra*I, *Hind*III and *Eco*RI restriction enzymes. Scorable bands ranged between 2200 bp and 2900 bp. Based on observed polymorphism there was a considerable variation among the species. The results revealed that in spite of morphological differences between *A. alternata* and *A. tomaticola*, a high similarity exists in IGS region for these two species. In the other species, genetic similarity ranged from 43% to 68%.

Key words: Early blight, IGS, Genetic variation, Tomato

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GENETIC CHARACTERIZATION OF *PHYTOPHTHORA NICOTIANAE* BY ANALYSIS OF MITOCHONDRIAL DNA

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Phytophthora nicotianae is a polyphagous pathogen infecting more than one thousand different species of host-plants and causing serious economic losses worldwide. Despite the importance of this species, current knowledge of its genetic variability is mostly restricted to populations from tobacco. In previous studies, Random Amplified Polymorphyc DNAs (RAPDs) and Amplified Fragment Length Polymorphysms (AFLPs) were used as genetic markers for characterizing *P. nicotianae* isolates from tobacco (Zang *et al.*, 2003; Lamour *et al*., 2003) but comparing results from one laboratory to another has proved problematic. Simple Sequence Repeats (SSRs), also known as microsatellites, are powerful markers for fingerprinting studies; however, their application is strongly limited by the need for previous sequence information to design primers for amplification of specific loci (Schena *et al.*, 2008). On the other hand, phylogenetic studies of *Phytophthora* have been based on conserved genes that generally are unsuitable to characterize intraspecific variability (Blair *et al*., 2008).

In the present study, a new approach based on the analysis of very variable mitochondrial intergenic regions was applied to study intraspecific variability in *P. nicotianae*. Two variable intergenic regions flanked by genes *Trny* and *Rns* (Trny/Rns) and by genes *Trnw* and *Cox2* (*Trnw*/*Cox2*) were identified by comparing the whole mitochondrial genomes of *P. infestans* (NC_002387, AY894835, AY898627, AY898628), *P. ramorum (*DQ832718) and *P. sojae (*DQ832717), and were amplified and sequenced using primers designed on flanking conserved genes.

Selected regions showed a variable length, comprised between 429 and 444 bp (Trny/Rns) and between 313 and 373 bp (*Trnw*/*Cox2*), when amplified and sequenced from a population of 53 *P. nicotianae* isolates from different geographical regions and hosts. Sequences were aligned using ClustalX and introduced to TOPALi (http://www.topali.org/) for phylogenetic analysis with the MrBayes 3 method (Milne *et al.*, 2009). The analysis of the Trny/Rns region enabled the identification of 17 different haplotypes and 6 uniform clusters indicating some specific associations among

genetic groups and hosts. In particular, the great majority of isolates from citrus were included in a single cluster, irrespective of their geographic origin. Similar results were also obtained with the *Trnw*/*Cox2* region although this latter region enabled a less accurate discrimination among isolates. In particular 7 different haplotypes and 5 uniform clusters were identified with this gene.

Results of the present study demonstrate that variable mitochondrial intergenic regions are a suitable genomics platform to study phylogenetic relationships within *P. nicotianae*. This method could be extended to other *Phytophthora* species as it doesn't require previous DNA sequence information of the microorganism to be analyzed. Moreover it could be implemented by the identification and analysis of other variable mitochondrial and/or nuclear genomic regions. An additional major advantage is that DNA sequences obtained with this method can be deposited in GenBank thus providing an easily and freely available molecular database.

Key words: *Phytophthora nicotianae*, Intraspecific variability, Phylogenetic relationships, Mitochondrial DNA

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VARIABILITY IN A *PHYTOPHTHORA CINNAMOMI* POPULATION FROM SOUTHERN EUROPE

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Phytophthora cinnamomi is known as an important pathogen of agricultural and forest plants, and causes severe losses in walnut (Belisario *et al*., 2006), chestnut (Robin *et al*., 2006) and oak stands (Vettraino *et al*., 2002). Though several *Phytophthora* spp. are known to attack walnut, *P. cinnamomi* is thought to be the most virulent and widespread in southern Europe (Belisario *et al*., 2006). Its occurrence is also frequent in nurseries on several ornamental plants such as *Viburnum* spp. (Belisario *et al*., 2004). Since isolates were from different environments (nursery, forest stand, orchard) and from fairly distant hosts, the genotypic variation was examined to know more about population structure. Sixty three isolates of *P. cinnamomi* from Italy, France and Spain were analyzed using amplified fragment length polymorphisms (AFLP) and inter-simple-sequence-repeat (ISSR) profile analysis to determine the genetic variability and the existing genetic relationships among isolates. A total of 48 isolates were collected from walnut trees, 4 from viburnum plants, and 3 and 8 from oak and chestnut stands respectively. Genetic dissimilarity matrixes were obtained in this study by means of Jaccard coefficient and UPGMA method. Pairwise tests detected significant differences among hosts. The sub-population of isolates from Spain clustered in a tight group by ISSR profiles analysis. French *P. cinnamomi* isolates from walnut appeared to be evenly distributed, though a certain degree of similarity was present between French and Venice's walnut isolates, and this may be due to the origin of the propagation material grown in Venice which was imported from French. The AMOVA analysis of the isolates collected from walnut trees in Italy and in France showed that the majority of the genetic diversity was distributed within populations (92.55%) and only 7.45% was among populations. Presently, no works has been done on the genetic variability of European *P. cinnamomi* population, and in previous works results on *P. cinnamomi* genetic variability were discordant. South African and Australian *P. cinnamomi* populations were represented with an extremely low level of genetic distance (Linde *et al.,* 1999). In contrast, a high level of genetic diversity was found among *P. cinnamomi* isolates from Papua New Guinea (Old *et al.,* 1984). The nature of the *P. cinnamomi* populations here investigated, revealed by AFLP and ISSR analysis, might be a consequence of the trade of plants within Europe from common nurseries. Further investigations will be addressed to determine the origin and nature of the extensive genetic variation revealed by AFLP and ISSR

analysis providing additional insights into factors contributing to the disease which may have implications for successful management and control measures.

Key words: Tree decline, Genetic variability, Oomycetes, Nursery disease

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VARIABILITY OF *PHOMOPSIS* SPP. ON CROATIAN INDIGENOUS GRAPEVINE CULTIVARS

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Phytopathogenic fungi of the genus *Phomopsis* are causal agents of Phomopsis cane and leaf spot of grapevines, which in Croatia is known as black spot. In recent years, knowledge about the etiology of the disease has been updated with information that fungus *Phomopsis viticola* should not be regarded as the only cause of the disease, since some other species are considered as possible causal agents. There are 16 species of *Phomopsis*/*Diaporthe* reported from grapevine among which for four species (*P. viticola, P. vitimegaspora, P. amygdali* and *D. viticola*) various degree of pathogenicity on grapevine has been proven (Mostert *et al*., 2001; Niekerk *et al*., 2005).

In order to obtain isolates of *Phomopsis* spp./*Diaporthe* spp. and determine their taxonomic identity and variability on Croatian indigenous grapevine cultivars, during winters of 2008 and 2009, grapevine cane samples from various grape growing regions of Croatia were collected. After establishment of pure cultures, phenotypic characterization (morphological characters and vegetative compatibility grouping) and DNA sequence analysis (ITS-1,5.8S,ITS-2) of the isolates was performed and their taxonomic status was determined, which was followed by testing of their pathogenicity on grapevine (Schilder *et al*., 2005). Additionally, *in situ* observations have been made regarding the sensitivity of grapevine cultivars to *P. viticola*.

Within a large collection of isolates from all vine-growing regions of Croatia and on all indigenous cultivars, the almost exclusive majority were identified as *P. viticola* and proved to be highly pathogenic on grapevine. Also, considerable level of variability of these isolates with regard to belonging to 9 different vegetative compatibility groups, was found, which emphasizes the variability of *P. viticola* on grapevine in general.

Based on *in situ* observations, indigenous cultivars of grapevine differ with regards to sensitivity to *P. viticola*, with cultivars Plavac mali, Škrlet, Žilavka, Debit and others being sensitive, while Plavina, Babić, Žlahtina and others were relatively resistant.

Key words: Grapevine, Indigenous cultivars, *Phomopsis viticola*, Variability

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CHARACTERIZATION OF FUNGAL PATHOGENS ASSOCIATED WITH ROOT DISEASES OF CITRUS IN OMAN

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Citrus species suffer from infection by different plant pathogenic fungi. Soil-borne fungal pathogens are probably the most serious. Phytophthora gummosis and foot/root rot is considered the most serious root disease of citrus in the world (Graham and Menge, 2000). This disease has been reported to occur in most citrus growing regions, affecting several citrus species including lime, sweet lime, orange and grapefruit (Graham and Menge, 2000). The disease has been reported to be caused by *Phytophthora palmivora*, *P. nicotianae* and *P. citrophthora*. However, other pathogens such as *Fusarium* species have been reported to produce similar symptoms (Timmer, 2000). Several fungi are also reported to be associated with citrus diseases such as dry root rot, sudden death and branch diebacks, including *Fusarium, Phomopsis*, *Botryodiplodia* and *Ceratocystis* species (Timmer *et al*., 2000). Little is known about the fungi associated with root diseases of citrus in Oman, about the incidence of these diseases in different parts of Oman or about the most commonly affected citrus species. This is a barrier to the establishment of effective management strategies.

This study was conducted to characterize the most common pathogens associated with root diseases of citrus in Oman. The survey covered five different regions. Isolations were carried out from roots, shoot bases and stems of healthy and diseased citrus trees which included acid lime, sweet lime, mandarin, grapefruit, sour orange and others. Identification of fungal pathogens was based on morphology and sequences of the internal transcribed spacer region of the ribosomal DNA (ITS rDNA) as described by Al-Sa'di *et al*. (2007). Root rot, gummosis, stem girdling and slow and rapid decline were found to be the most common diseases associated with citrus trees in Oman. Different species of *Fusarium*, *Pythium, Phytophthora, Lasiodiplodia, Phoma* and *Rhizoctonia* were isolated from diseased citrus trees. *Fusarium* species (mainly *F. solani*) were found to be the most common species associated with declining trees, followed by *Lasiodiplodia theobromae*. Further studies are in progress to characterize pathogenicity of the isolated fungi and oomycetes.

Key words: *Phytophthora*, Lime, ITS rDNA

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CHARACTERIZATION OF *FOMITIPORIA MEDITERRANEA* POPULATIONS ASSOCIATED WITH HEARTWOOD ROT OF CITRUS IN SOUTHERN ITALY

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In a recent survey of citrus orchards in the most important citrus growing regions of Italy, i. e. Apulia, Basilicata, Calabria and Sicily, *Fomitiporia mediterranea* was found to be the fungal species associated most frequently with heartwood rot of living citrus trees. Heartwood rot is a chronic disease occurring rather frequently on old trees in most citrus growing areas of the world. The causal agent of this wood decay was initially identified as *Fomes applanatus* (Pers.) Wallr. (Childs, 1953) and subsequently as *Phellinus punctatus* (Ippolito *et al.*, 1998), but more recently it was referred to *F. mediterranea*, the same species associated with esca disease of grapevine (Fischer, 2002; Elena *et al.*, 2006). Although heartwood rot caused by *F. mediterranea* is not a limiting factor to the citrus industry, it causes a deterioration of orchards since affected trees show concentric cankers of trunk and branches, a progressive decline in vigour, reduction of fruit production and sudden collapse of branches. Moreover, affected branches are more prone to break. Typically resupinate fruiting bodies of the pathogen are usually but not always associated with the cankers and wood rot. In Sicily, the disease was found to be very common on old trees of lemon as well as 'Moro' and 'Ovale' sweet orange, with frequencies of up 80% of symptomatic trees in an orchard, while it was less frequent on old trees of 'Valencia' and 'Tarocco' sweet orange, suggesting differences in susceptibility among cultivars. In Apulia and Basilicata, it was found also on 15–20-year old trees of 'Clementine' mandarin, probably as a consequence of frost injury.

Isolates of the pathogen were obtained from both fruit bodies and symptomatic wood on potato-dextrose-agar or a selective medium (Kuhlman and Hendrix, 1962) and were identified by amplifying and sequencing the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) using the universal primers ITS1 and ITS4. Isolates from grapevine and olive were used as a reference. DNA sequences were compared with those available in GenBank (BLAST analysis). ITS sequencing showed the presence of few polymorphic bases and enabled the identification of three different haplotypes. DNA sequences of the *F. mediterranea* isolates from citrus did not differ from the sequences of the *F. mediterranea* isolates from grapevine and olive.

Key words: Citrus, Wood rot, *Fomitiporia mediterranea*

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VIRULENCE OF *BOTRTYS CINEREA* STRAINS ISOLATED FROM CV MOSCATO IN NORTHERN ITALY

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Populations of *Botrytis cinerea* have been recently characterized taking into account the presence of transposable elements, namely *Boty* and *Flipper* (Giraud *et al.,* 1999). In French vineyard, the *transposa* strains, carrying both transposable elements, were predominant at harvest, while the *vacuma* isolates lacking both transposons, were more frequent in early phenological stages (Martinez *et al.,* 2005). *Transposa* strains have smaller macroconidia, are more frequently resistant to vinclozolin and diethofancarb, exhibit slower rates of mycelial extension when grown on highly nutritive agar media at different favourable temperatures and more virulent on berries (Giraud *et al.,* 1997). Researches carried out in other countries showed different composition of *B. cinerea* populations isolated from different vineyards (De Miccolis *et al.,* 2004; Vaczy *et al.,* 2008), but no further information on the effect of transposable elements on virulence was available. The aim of the present investigation was to evaluate the virulence of *B. cinerea* strains, carrying or lacking transposable elements, isolated from cv Moscato in northern Italy on grapevine and *Nicotiana clevelandii* leaves and on cv Moscato berries.

A total of 390 *B. cinerea* strains were isolated from cv Moscato vineyards, treated or untreated during dormancy, at the beginning of berry touch, at veraison and at harvest. All the strains were characterized for the presence of transposable elements. Their virulence was assessed on leaf disks of cv Cabernet Sauvignon and *N. clevelandii* and on berries of cv Moscato collected at the beginning of berry touch, at veraison and at harvest.

The majority of the isolated strains did not contain any transposable elements, while 31% of strains carried both *Boty* and *Flipper* transposable elements and were classified as *transposa* strains, 15% carried only *Boty* element, 2% the only *Flipper.* No differences in virulence were detected neither on *V. vinifera* and *N. clevelandii* leaves, nor on berries collected at beginning of berry touch, veraison and harvest, between *transposa* strains, strains carrying the only *Boty* or the only *Flipper* element, and *vacuma* strains. The differences were not significant also when comparing *vacuma* strains with strains carrying at least one transposable element.

From the present results indicate that transposable elements did not influence the virulence of *B. cinerea* strains.

Keywords: Transposable elements, *Boty, Flipper,* Leaves, Berries

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MATING TYPES AND FUNGICIDE SENSITIVITIES OF CYPRUS POPULATIONS OF *PHYTOPHTHORA INFESTANS*

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Potato late blight (PLB) caused by the oomycete *Phytophthora infestans*, is an extremely destructive disease of potato, causing significant crop losses worldwide. The occurrence of sexual reproduction and the increased aggressiveness of *P. infestans* globally in the last decades, combined with increasing cases of reduced fungicide efficacy due to resistance development, make the management of PLB more challenging than ever before (Mizubuti and Fry, 2006). Despite the importance of potato production to the economy of Cyprus and the huge impact of PLB to the crop during "epidemic" years, studies regarding the aforementioned attributes of local populations of *P. infestans,* and their potential implications to disease management, are missing.

During 2009 and 2010, blighted potato leaves were sampled from 91 commercial fields throughout the major potato growing areas of Cyprus and 270 isolates of *P. infestans* were obtained. Mating type was determined using known A1 and A2 tester isolates of *P. infestans* on V-8 agar media (Perez *et al.,* 2001). Both A1 and A2 mating types were isolated in both years. About 56.3% of the isolates collected in 2009 and 52.4% in 2010 were of the A1 mating type, suggesting that A1 and A2 in Cyprus are at a 1:1 ratio. This is the first report for the presence of both mating types of *P. infestans* in Cyprus.

The sensitivity to the phenylamide fungicide metalaxyl-M was also examined for a subset of 253 isolates of the pathogen. Based on the radial mycelial growth of the isolates on V-8 agar, amended with 0, 5, and 100 μg/ml metalaxyl-M, the assayed isolates were characterized as resistant (R), intermediate resistant (IR), or sensitive (S) (Perez *et al.,* 2001). Among the tested isolates 162 were R (64%), 76 IR (30%), and 15 (6%) were S to metalaxyl-M. It was also noticeable that the metalaxyl R isolates were of the A1 mating type, while the other two sensitivity groups (IR and S) were characterized as A2.

Using the serial dilution method, fungicide sensitivities were also determined for cymoxanil, another commonly used fungicide against PLB. The calculated sensitivities of a group of 40 *P. infestans* isolates, in terms of EC_{50} values, ranged between 0.51 and 0.77 μg/ml. Thus, no resistance was evident for this active ingredient.

Key words: Metalaxyl, Cymoxanil, Fungicide resistance

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POPULATION STRUCTURE OF *CRYPHONECTRIA PARASITICA* IN IRANIAN CHESTNUT FORESTS

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Chestnut blight disease caused by *Cryphonectria parasitica* (Murill) M. E. Barr, is one of the major diseases of chestnut (*Castanea* spp.) and has caused serious damage in the orchards and in forests since its introduction in Iran (Kazempour *et al*., 2006). The diversity of vegetative compatibility (vc) types and distribution of mating types in *C. parasitica* are major factors affecting the success of biological control of chestnut blight by transmission of double-stranded RNA (dsRNA) viruses that cause hypovirulence (Heiniger & Rigling, 1994).

Seven sites from Shaft (Visrud, Taleghan and Babarekab), Lahijan (Shahbalutmahaleh and Gharibabad), Rezvanshahr (Doran) and Rasht were selected for investigating the occurrence and frequency of vegetative compatibility groups. To evaluate population structure of *C. parasitica* four sites in two main growing regions include Shaft (Visrud, Taleghan and Babarekab) and Rezvanshahr (Doran) were selected.

VCG were assessed according to the mycelial-barrage response on PDA (Powell 1995). Among 272 evaluated isolates, four Iranian VCGs namely IR-1 to IR-4 were detected. Diversity of VC groups at individual localities varied between one and two groups. IR-1 was the dominant VCG present at five populations, comprising 63.2% of all isolates, and IR-4, had the lowest frequency (3%) and occurred in a single locality. IR-1 was the dominant group in Taleghan and Babarekab with 50 (7%) and 31 (3%) respectively. IR-3 comprised 88% and 12% of all isolates in Shahbalutmahaleh and Doran respectively.

Cryphonectria. parasitica isolates were crossed with each of the two mating type testers M1115 (*MAT*-2) and M1297 (*MAT*-1) of *C. parasitica* on autoclaved pieces of *Castanea sativa* stems (Rigling, 2006; Bissegger *et al.,* 1997). According to the result *MAT* idiomorphs (*MAT*-1 and *MAT*-2) were determined in the majority of isolates (72.3%). One idiomorph in each evaluated sites was detected, and 20.4% of isolates were not able to produce perithecia. Moreover, 7.4% of isolates were sexually compatible with both mating types and produced perithecia with both testers.

Key words: *Cryphonectria parasitica*, Vegetative compatibility groups, MAT, Epidemiology

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INTRASPECIES GENETIC DIVERSITY IN *MELOIDOGYNE JAVANICA* ISOLATES FROM TOMATO IN IRAN

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The root-knot nematode (RKN), *Meloidogyne javanica*, is widely distributed among tomato (*Solanum lycopersicum*) production areas in Iran, where infestations are often associated with yield reduction. RKN have different spectra of virulence towards host resistance genes, therefore, is important to know the genetic diversity present in the populations. The analysis of random amplified polymorphic DNA (RAPD) based on the polymerase chain reaction (PCR) amplification of DNA segments, using oligonucleotide primers, has been used to detected genetic variability in RKN (Blok *et al*., 1997; Baum *et al*., 1994). The goal of this study was to evaluate the genetic diversity among populations of *M. javanica* from northern Khorasan province, Iran.

During 2009-2010, 21 root samples were collected from infested tomato fields in northern Khorasan province, Iran. Pure populations were obtained from single egg masses, handpicked from infected tomato roots, that were inoculated in tomato Red Claude cultivar plants. Two months after the inoculation, adult females and egg masses were collected from each pure population. Perennial patterns of adult females were cut in 45% lactic acid mounted in glycerin. Second-stage juveniles (J2) hatched from the egg masses were fixed in TAF and transferred to glycerin. Genomic DNA was extracted from 30 egg mass, with a phenol-chloroform based protocol RAPD reactions were carried out with 10 random primers described by Silva, 1990. For each pure population, a data matrix of ones and zeros was constructed based on the presence or absence of each RAPD marker. Genetic distances and cluster analysis by the unweighted pair group method using arithmetic averages (UPGMA) were obtained.

The morphology of the perennial patterns of adult females and the morphometrical characters of J2 did not revealed polymorphism among the *M. javanica* populations. RAPD analyses resulted in 175 DNA fragments and 85 bands revealed polymorphism. The separation of the populations in three clusters was achieved with an average of 91%. Cluster one with 7 populations, cluster two with 9 populations and cluster three with 5 populations. Intraspecific genetic diversity was not detected among these *M. javanica* populations.

Key words: *Meloidogyne javanica*, RAPD, *Solanum lycopersicum*

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SIDEROPHORES PRODUCTION AND PATHOGENICITY OF SOFT ROTTING STRAINS OF *ERWINIA*

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Iron is one of the most important micronutrients that is essential for growth, metabolism and survival of nearly all living systems. So, each organism has one or more mechanisms for obtaining iron of which siderophores are the most important mechanism. Siderophores have been considered as a virulence factor of some plant pathogens such as *Erwinia* spp. (Enard *et al*., 1988).

In this study, sixteen soft rotting local bacterial strains isolated from diseased vegetables of different plant species and identified in other studies as *Erwinia* spp. (El-Hendawy *et al*., 2002; El-Hendawy *et al*., 2006), were used. They were characterized by analysis of cellular proteins and cellular fatty acids. All sixteen strains were able to produce a yellow halo when grown on chrome azurol S (CAS) agar medium (Alexander and Zuberer, 1991) indicating the production of siderophores. Analysis of siderophores produced by the most potent strains, *Erwinia chrysanthemi* (*Echr*) strain Car1B, *Erwinia carotovora* subsp. *carotovora* (*Ecc*) strain Cab21C, *Ecc* strain pep7C and *Erwinia carotovora* subsp. *atroseptica* (*Eca*) strain pepX, was carried out. The results revealed that the strains produce both catechol and hydroxamate siderophores. Pathogenicity of the sixteen strains was tested on different plant species related to their original hosts to determine whether if they are restricted to their hosts or they can extend to other plants. Six weeks old seedlings were inoculated with $0.7x10^6$ cells through the stem or $1x10^7$ cells through the leaf. Results were recorded 7 days after inoculation. Differences in pathogenicity as well as the host range were detected not only between different strains but also from the same strain according to the inoculated plant species. Interestingly, the results obtained from the pathogenicity tests revealed that there is a correlation between host range, severity of disease symptoms and siderophore production by *Erwinia* strains, which may indicate a role of siderophores as a virulence factor.

Key words: Soft rot, *Erwinia*, Siderophores, Pathogenicity, Virulence

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COMPARISON OF 16S rDNA ANALYSIS AND REP-PCR GENOMIC FINGERPRINTING FOR MOLECULAR IDENTIFICATION OF *ERWINIA PERSICINA* ASSOCIATED WITH BEAN YELLOWING AND NECROSIS DISEASE IN SOUTHERN SPAIN

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Four pathogens are known to be associated with bean yellowing and necrosis disease affecting common bean plants (*Phaseolus vulgaris*). They are: *Bean yellow disorder virus*, *Erwinia persicina, Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and *Bacillus pumilus*. Interveinal yellowing disease symptoms which develop to chlorotic spots and necrotic areas on infected leaves have been observed in southern Spain since 2003. Bacteria isolated from symptomatic samples from Almeria were characterized using the general properties of the family *Enterobacteriacea*e. Ribosomal RNA-based approaches have been applied normally to bacterial classification and identification. The gene encoding 16S rRNA (Edwards *et al*., 1989) was amplified by PCR then sequenced from two bacterial isolates from Spain and the sequence showed 99% nucleotide identity with different strains of *Erwinia persicina* (González *et al*., 2005). To identify the bacteria infecting common bean plants in southern Spain, a large number of samples were collected from affected common bean plants grown in greenhouses. Specific primers were designed from the 16S rDNA sequences of *Erwinia persicina* isolated from affected bean. Symptomatic samples collected from southern Spain were tested by PCR assays with these specific primers. PCR products were sequenced and showed high nucleotide identity with the 16S rDNA sequences of other *Erwinia* species (*Erwinia persicina*, *Pantoea agglomerans*, *Erwinia aphidicola*, etc.). To discriminate among *Erwinia* species, a molecular variability study was performed based on the use of Enterobacterial Repetitive Intragenic Consensus (ERIC) primers (Versalovic *et al*., 1994). The use of repetitive DNA sequences such as ERIC, is frequent for bacterial classification and allows differentiation at the species, subspecies and strain levels. In ERIC assay, *Erwinia* sp. isolates from symptomatic samples and *Erwinia* species reference strains were included in this study. ERIC-PCR was carried out as described by Versalovic *et al*. (1994). Computer-assisted analysis of the ERIC-PCR fingerprints showed that *Erwinia* sp. strains, isolated from affected fields and sequences of the ribosomic gene 16S rRNA, previously characterized molecularly as *Erwinia persicina,* were easily distinguished from *Erwinia persicina* reference cultures and it was more closely related to *Erwinia aphidicola* reference

cultures. Our results confirm that sequence analysis of 16S rDNA may not be reliable enough to discriminate within closely related species, although 16S rRNA sequences were a good indicator for analyzing phylogenetic relationships of bacteria (Vandamme *et al.,* 1996). Recently, *Erwinia aphidicola* was reported to infect bean and pea crops in Spain based on PCR analysis with primers designed for *dnaJ*, *recA* and *gapDH* genes (Santos *et al.,* 2009).

Key words: *Phaseolus vulgaris, Erwinia* spp., ERIC-PCR, DNA fingerprinting

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GENETIC AND PHENOTYPIC DIVERSITY OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* STRAINS, CAUSING THE APICAL NECROSIS OF MANGO

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Bacterial apical necrosis of mango (NAM), elicited by *Pseudomonas syringae* pv. *syringae* (Pss) limits fruit production in southern Spain and Portugal (Cazorla *et al*., 1998). Sprays of copper compounds (mainly Bordeaux mixture) are the typical treatment used for the control of the NAM (Cazorla *et al*., 2006). However, their efficacy is often limited, and it could be related to selection of copper-resistant strains of Pss. The copper resistance genes has been cloned and characterized from Pss strains, and they are mainly associated to native plasmids. Among the plasmids described in Pss strains, a 62 Kb plasmid that contains homologous sequences to copper resistance genes (*cop*ABCD operon) is broadly detected (Cazorla *et al*., 2002).

In this work we performed the characterization of copper resistance genes. Hypothesis about acquisition of resistance copper determinants is supported by high diversity in these genes. In order to demonstrate this hypothesis, a study of sequence on the *rep*A gene was performed, suggesting a possible common origin of the 62 Kb plasmids.

Simultaneously, an epidemiological study of Pss strains to establish the origin and distribution of these pathogenic bacteria is being studied. Different phenotypic and genetic techniques (Gutiérrez-Barranquero *et al.*, 2008) were used to evaluate a selection of representative Pss strains isolated from mango tissues. AP-PCR (arbitrarily primed) has been used to carry out epidemiological studies. Four different primers set (ERIC1-ERIC2, BOXA1R, GTG-5 and CAG-5) were selected to analyze 125 Pss strains from different seasons and locations (including mainland Spain and Canary Islands, Portugal, Italy and Israel).

To complete the study on phenotypic variability of this phytopathogenic bacterium, different analysis have been carried out: antibiotic resistance, copper resistance assays and dot-blot hybridization, and bioassays to determinate the production for the principal antimetabolite toxins: mangotoxin, phaseolotoxin, coronatine and tabtoxin (Arrebola *et al.*, 2003).

The results confirmed the high diversity among Pss strains isolated from

mango. The most resolving of the genetic techniques to assess diversity was the AP-PCR, using the ERIC primers set. The antibiotic resistance pattern is also heterogeneous, but not for copper resistance, mostly associated with 62 Kb plasmids. These techniques could be used for future epidemiological studies to determine the source of the isolates and their distribution.

Key words: *Pseudomonas syringae*, Genetic diversity, Mango, Apical necrosis

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GENETIC VARIABILITY OF *PSEUDOMONAS SAVASTANOI* POPULATIONS FROM DIFFERENT HOSTS AND DIFFERENT GEOGRAPHYC AREAS

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The bacterium *Pseudomonas savastanoi* causes knot disease on plants belonging to different families: Oleaceae (*Olea*, *Jasminum*, *Forsythia*, *Phillyrea*, *Ligustrum*), Fabaceae (*Retama*), Rhamnaceae (*Rhamnus*), Myrtaceae (*Myrtus*) and Apocynaceae (*Nerium oleander*) (Janse, 2006). Despite many efforts in the attempt to discriminate *Pseudomonas savastanoi* pathovars, until now only pv *fraxini* appears unequivocally distinguishable from the others (Janse, 1991; Sisto *et al*., 2007). The aim of this work is to evaluate the relative weight of the environment and of the host plant on the selection of the bacterial genotypes through a comparison among strains belonging to different pathovars isolated from two narrow geographic areas.

Olive and oleander strains from the province of Florence (Central Italy) and olive, oleander and myrtle strains from Rhodes (Greece) were compared by means of different genetic fingerprinting analysis (Rep-PCR, tDNA-ILP e AP-PCR).

Rep-PCR (ERIC e REP primers) and tDNA-ILP-PCR profiles did not allow to distinguish strains from different hosts or geographic origins. Instead AP-PCR banding patterns were more informative and when the profiles were subjected to UPGMA clustering analysis, the bacterial populations from the province of Florence and from Rhodes were split into two different clusters. Moreover, within each cluster, 100% of the strains isolated in the province of Florence and 78% of those isolated from Rhodes, could be grouped according to the host of provenance.

Furthermore profile analysis showed that the level of polymorphism in Rhodes bacterial population was higher than that observed for the population from the province of Florence. Despite Rhodes is an island where exchange of plant material is thought to be limited, it seems that here the environmental conditions are more favorable to the bacterium, which maintain a higher genotypic variability degree and host range. Indeed in Rhodes *P. savastanoi* was also found on myrtle, a plant species only rarely reported as its host.

In the province of Florence, although this is not a delimited region as Rhodes island, a lower degree of polymorphism was observed. In this case olive and oleander strains' profiles were more similar to each other than those of strains from the same hosts coming from Rhodes. It seems that this geographic area, the northern boundary of olive orchards in Italy, has selected only few genotypes enabled to adapt and spread on respective hosts.

Key words: *Pseudomonas savastanoi,* Genetic fingerprinting, Environmental effects.

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GENETIC VARIABILITY OF BOIS NOIR PHYTOPLASMA ISOLATES IN PIEDMONT (NORTH-WESTERN ITALY)

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Phytoplasmas belonging to the 16SrXII-A taxonomic subgroup (Stolbur group, "*Candidatus* Phytoplasma solani") are associated with several diseases of wild and cultivated plants such as grapevine Bois noir (BN). The cixiid planthopper *Hyalesthes obsoletus* Signoret is the principal vector of BN phytoplasma (BNp) to grapevine in the Euro-Mediterranean Region. Biological and genomic variability have been widely described among "*Ca.* P. solani" isolates from different areas and host plants, but low variability was evidenced following PCR-RFLP analysis of 16SrRNA gene (Cimerman *et al.*, 2009). Non ribosomal genes (*tuf*, *vmp1, secY)* are currently used as genetic markers for a finer discrimination of phytoplasmas within the 16SrXII-A subgroup (Fialova *et al.*, 2009; Pacifico *et al.*, 2009).

In this work, PCR-RFLP analysis of *vmp1* gene was used to investigate the genetic diversity of BNp isolates collected in eight BN-affected vineyards of Piedmont Region in 2007, 2008 and 2009. Weed and grapevine samples were collected in different moment of the vegetative season (spring, summer and autumn). BNp infection was checked by RT-PCR with Stolbur-specific primers (Margaria *et al.*, 2009). BNp isolates from different cixiid species sampled in the same vineyards were also included in the analysis.

BNp infection was detected in about 60 % and 50 % of weed and grapevine samples, respectively. *Vmp1* fragment of different sizes were amplified from weed, grapevine and insect BNp isolates. Several *vmp1* types were detected following the digestion with *Rsa*I or *Alu*I endonucleases, confirming the presence of BNp variants in north-western Italian vineyards (Pacifico *et al.*, 2009). In each vineyard, the BNp population was represented by a complex of different *vmp1* types, indicating the presence of high genetic variability of the pathogen during the three years.

Key words: PCR-RFLP, Bois noir, Grapevine, *Vmp1*

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MOLECULAR PROPERTIES OF AN APULIAN ISOLATE OF GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS FROM *VITIS VINIFERA*

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Grapevine rupestris stem pitting-associated virus (GRSPaV, genus *Foveavirus*, family *Betaflexiviridae*) is known as one of the most common viruses of grapevine, widely distributed in many, if not all, grape-growing areas of the world (Meng *et al.*, 2007; Lima *et al.,* 2009). It is associated with disorders known as rupestris stem pitting (RSP) and vein necrosis (VN).

Following the first description of the virus (Zhang *et al.*, 1998) molecular investigations have led to the complete sequencing of six viral strains denoted GRSPaV-1 (AF057136), GRSPV (AF026278), GRSPaV-SG1 (AY881626), GRSPaV-BS (AY881627), GRSPaV-SY (AY368590) and GRSPaV-PN (AY368172).

We now report the full-length genome sequencing of a new viral isolate from a *Vitis vinifera* accession of cv. Moscato Giallo (GRSPaV-MG), from Apulia (southern Italy).

To this aim, viral dsRNA extracts from cortical scrapings of dormant canes were used as templates for RT-PCR amplification of several fragments spanning the whole genome. Upstream and downstream primers were designed by aligning previously published GRSPaV nucleotide sequences. All PCR amplicons obtained, were cloned using the Strataclone kit (Stratagene, USA) and the resulting plasmids were submitted to automated sequencing.

The complete sequence was 8,722 nt long, excluding the polyA tail, and had a 43% GC content. ORF finder analysis (NCBI Web server) showed that the structural organization of the genome of our viral isolate was identical to that of previously investigated GRSPaV isolates, as it comprises six putative open reading frames (ORFs) plus a 5' and 3' non-coding regions (NCRs) of 60 nt and 139 nt, respectively.

In particular, ORF 1 codes for a polypeptide of 2,161 aa, putatively identified as the replicase, gene whereas ORFs 2, 3 and 4 code, in the order, for three proteins (221 aa; 116 aa; 80 aa) recognized as the triple gene block (TGB) of foveaviruses.

ORF 5 codes for the 259 aa capsid protein (CP) and is followed by ORF 6 which significantly overlaps the CP gene and codes for a protein of 119 aa in size. This latter 3' most ORF, putatively coding for a protein with nucleic acid binding properties, had previously been detected in other members of the family *Flexiviridae* (Zhang *et al.*, 1998; Gentit *et al.*, 2001; Lima *et al.,* 2009).

Assembled sequence data were analysed using the BioEdit 7.0.9 program (Ibis Biosciences, USA) in comparison with the published sequences of the six GRSPaV genomes. Sequence identity among isolates at the nucleotide level ranged from76% (strain GRSPaV-PN) to 94% (strain GRSPaV-SG1). Identities at the amino acid level substantially confirmed these values, for they ranged from 85 to 94%.

The close relationship with strain GRSPaV-SG1 was confirmed when each single ORF was analysed. Nucleotide sequence identity between our isolate and GRSPaV-SG1 ranged from 96% for the most conserved ORF5, to 94% for ORF 1, which appeared to be the most variable, in accordance with earlier reports (Lima *et al*., 2009).

The present data confirm the widespread presence in southern Italy of GRSPaV isolates with significant phylogenetic relationship with GRSPaV-1, as ascertained in a recent survey (Morelli *et al.,* 2009).

Key words: *Vitis vinifera*, GRSPaV, *Foveavirus*, *Grapevine rupestris stem pitting-associated virus*

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CHARACTERIZATION OF MEDITERRANEAN *CITRUS TRISTEZA VIRUS* ISOLATES BASED ON ANALYSIS OF RESTRICTION PATTERNS AND SEQUENCES OF THEIR COAT PROTEIN GENES

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Citrus tristeza virus (CTV), the most serious viral pathogen of citrus, is usually present in field trees as a mixture or complex of isolates that produce a variety of symptoms in different citrus hosts. Depending on virus strain and on the species or scion-rootstock combinations, CTV may cause three distinct syndromes named tristeza or quick decline (QD), stem-pitting (SP) and seedling yellows (SY). The virus is reported from almost all Mediterranean countries and is a serious threat to their citrus industries due to the predominance of the CTV-susceptible sour orange as rootstock. The ability to control disease damage depends to a large extent on the CTV incidence and on the virus strain and citrus varieties predominant in each region. Therefore typing of prevailing CTV strains is a key element for predicting disease impact and devising appropriate control strategies suitable to specific regions. At the same time studies of genetic structure and diversity are important in understanding the evolutionary factors shaping CTV populations (Moreno *et al*., 2008). The finding that sequence differences of CTV isolates are associated with specific biological activities has led to the development of several molecular methods of CTV strain characterization, in order to identify mild from virulent strains.

The present study analyzed the genetic variability of field CTV isolates from several countries of the Mediterranean Basin based on RT-PCR amplification patterns with genotype-specific multiple molecular markers (Hilf and Garnsey, 2000) and on restriction fragment length polymorphism (RFLP) (Gillings *et al*., 1993) and sequence analysis of the coat protein (CP) gene.

The results showed high CTV isolate variability between an within different countries exist. The mild T30 genotype is quite common and genetically stable, associated with symptomless or very mild symptom affected trees. The incidence of VT genotype is equivalent to the incidence of VT and T30 genotype mixtures; these isolates caused severe fiels symptoms (decline, sweet orange stem pitting). The T36 genotype was assigned to 2 isolates from Italy and Albania, which caused typical symptoms of sweet orange quick decline.

RFLP profile, performed on the amplified CP gene of symptomatically and geographically different isolates, using *Hinf*I and *Rsa*I as restriction enzymes, revealed considerable polymorphisms among isolates and confirmed that *Hinf*I could efficiently discriminate between mild and severe strains. All the isolates, except the

mild isolates, contained a thymidine at position 371 of the CP nucleotide sequence corresponding to phenylalanine at amino acid position 124, and therefore reacted with MCA-13 antibody.

The phylogenetic tree generated using CP nucleotide sequence of the Mediterranean isolates and the sequence of well-characterized CTV isolates produced several main clusters. The most striking aspect is that mild isolates as well as sweet orange SP and SY isolates clustered into two main groups, while decline and QD isolates are geographically separated, although they are from similar hosts and cause similar symptom phenothypes. The data may suggest that CTV population of T36 genotype evolve under a different selection pressure compared to CTV population of T30 genotype and VT genotype.

Key words: *Citrus tristeza virus*, Isolates, CP gene, RFLP, Phylogenetic analysis

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MOLECULAR CHARACTERIZATION OF PPV ISOLATES FROM EGYPT

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Sharka, caused by *Plum pox virus* (PPV), is the most damaging disease of stone fruit trees, reducing fruit quality and yield (Cambra *et al.,* 2006). PPV is easily transmitted by aphids and by vegetative propagation. Despite the considerable efforts made in many countries, sharka disease has been reported in most important *Prunus* cultivated areas.

No curative actions currently exist against sharka. Moreover, aphid vectors control is ineffective. Control of PPV is based essentially on early identification and elimination of infected trees in the field, and on the use of resistant germplasm .

PPV was first reported in Egypt in 1987 (Dunez, 1988) and the well characterized El Amar strain has been so far identified only in that country on apricot plants.

During the 2009 growing season, a total of 100 leaf samples [from peach (69), apricot (12) and plum (17)] from symptomatic plants were collected from orchards and nurseries in Alexandria governorate, Egypt and then tested using both serological (ELISA) and molecular (RT-PCR) methods. Sixteen and 20 samples were infected, respectively, by DASI-ELISA using the PPV universal monoclonal antibody 5B (Agritest, Italy) and by RT-PCR analysis using primers pair P1/P2 (Wetzel *et al.*, 1991). Results obtained showed, within the collected samples, a high incidence of PPV infection on plum (50%, 9/18 plants) compared with incidence of 17% (2/12) and 13% (9/69) obtained from apricot and peach plants analyses, respectively.

The last 593 nucleotides at the 3' end of PPV RNA, including portion of the coat protein gene and the complete 3' untranslated region, were amplified and sequenced from all infected samples.

Nucleotide sequence analysis of all PPV isolates investigated in the present work revealed the identification of PPV Dideron strain. In samples analyzed.

High sequence identity, ranging from 98.3% to 100%, was observed within all Egyptian isolates and no relationships between host species and sequence identity were observed.

All Egyptian accessions showed high phylogenetic distance against the recombinant isolate "Serbia-MI" from Serbia and Montenegro (92.6% to 93.8% of sequence identity), against PPV-Marcus isolates (93.9% to 95.4%) and against PPV-El

Amar isolates (92.9% to 94.1%). The highest sequence identity was detected against one PPV-D East-European isolates from Belarus (99.2% to 99.7%) and against PPV-D isolates from USA (98.0% to 99.5%), Chile (98.0% to 99.3%) and Germany (97.5% to 99.5%).

Our results show high incidence of PPV-D infected plants in orchards and nurseries in Alexandria governorate. PPV-f Dideron strain is considered the least epidemic among PPV strains due its low transmission efficiency by aphids, suggesting a large spread of PPV-infected material used for plant propagation. For this reason an implementation of certification schemes is necessary in Egypt in order to guarantee the production and the employment of virus-free propagating material.

Key words: *Plum pox virus*, Sharka, Egypt, Molecular characterization

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GENETIC VARIABILITY AMONG ISOLATES OF A CARLAVIRUS FROM CAPER

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Caper (*Capparis spinosa* L.) is a native plant to the Mediterranean basin but grows widely in various regions in the world. Currently, Morocco and Turkey are the main producers but caper pharmacology and cosmetic properties as well as its use as spice are known and appreciated in many European, Mediterranean and overseas countries. In Italy, specialized caper cultivations were developed in several minor Sicilian islands (Aeolien Archipelago, Pantelleria, Ustica and Linosa) in the last century to produce immature flower buds and caper berries for human consumption.

C. spinosa subsp *spinosa* var *canescens* and *C. spinosa* subsp. *rupestris* are reported as naturally endemic species in Sicily (Fici and Gianguzzi, 1997). In particular, different biotypes with different morphological characteristics were identified in Sicilian islands. In all these locations, caper is suffering a progressive decline (Infantino *et al*., 2007). Our previous studies identified three viruses associated with caper decline; a carlavirus provisionally considered as *Caper latent virus* (CapLV), a rhabdovirus provisionally considered as *Eggplant mottle dwarf virus* (EMDV) and, in Salina and Lipari only, *Cucumber mosaic virus* (CMV) (Tomassoli *et al.*, 2006). Among these viruses, the carlavirus was the most recurrent without eliciting any apparent symptoms in plants. CapLV was identified for the first time in 1987 in a caper plant in Apulia (South Italy) and it was assigned to *Carlavirus* genus (*Flexiviridae* family) according to its morphological, biological and physicochemical properties and serological affinity to *Helenium virus S* (Gallitelli and Di Franco, 1987). A preliminary molecular characterization assigned all the virus isolates recorded in Sicilian islands to the genus *Carlavirus* (Tomassoli and Tiberini, 2006), although, they seem serologically unrelated to the previously identified CapLV. Therefore, we provisionally denote our isolates CapLV-Sicily. In this paper we report the results of their molecular characterization by sequencing 700 nts of ORF1 of carlavirus genome.

Phylogenetic analysis was performed with PAUP 4.0 (Swafford) to assess the relationship between the CapLV-Sicily isolates and the sequences of other carlaviruses published in the GenBank. Sequence homology with other members of the genus *Carlavirus* was in the average of 74%, which indicates that viruses isolated from

caper are distinct from other carlaviruses sequenced so far. The analysis was extended to isolates from other Mediterranean countries showing that all partially sequenced isolates clustered into two different clades. On the basis of results available so far we can speculate that they had a different phylogenetic evolution, probably reflecting differences in caper biotypes as well as their spread from native location to different areas.

Key words: Caper, Carlavirus, Nucleotide sequence, Genetic variability

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GENOME ANALYSES OF *CARNATION MOTTLE VIRUS* IRANIAN ISOLATE (FUM2)

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Carnation mottle virus (CarMV) is the type member of the genus *Carmovirus* within the family *Tombusviridae* of plant viruses. It is a 30 nm icosahedral plant virus consisting of a single-stranded, positive-sense 4.0 kb RNA. Sequence analysis of the CarMV genome revealed the presence of five open reading frames (ORFs): p27, p86 p7, p9, p38.

In this research, nucleotide sequence of 2 segments [involve 4 genes of an Iranian isolate of CarMV (FUM2)] was determined and compared with already available sequences of CarMV on the basis of nucleotide sequences.

The nucleotide sequence of FUM2 isolate have been submitted to the NCBI nucleotide sequence database and have been assigned the accession numbers GU229739 for p7, p9 and partial CP genes and GU229740 for p86 gene of CarMV.

During the winter and spring of 2008, about 450 leaf samples of carnation with leaf yellowing, mottling, leaf malformation, and dwarf symptoms were collected from greenhouses in Mashhad and Chenaran regions. The samples were tested for the presence of CarMV, using DAS-ELISA, according to the method described by Clark and Adams (1977).

Total RNA was extracted by RNX™ (-Plus) solution (CinnaGen Inc., Iran) from infected plants that confirmed positive by DAS-ELISA. RNA was used for RT–PCR reactions. AccuPowerR RT Pre Mix Kit (Pioneer Inc. Korea) was used for synthesis of cDNA and PCR amplification. RT-PCR assay amplified two DNA fragments approximately 1037bp and 676 bp.

PCR products were sequenced by MWG Biotech Pvt. Ltd. (Germany). The determined sequences of FUM2 isolate were analysed with those of previously reported 23 CarMV strains, using Bioedit software (Fullversion 7.0.9.1).

A Neighbour-joining method of MEGA 3.1 was applied to construct un-rooted trees for 3 genes (p7, p9 and partial p38). Analysis of the phylogenetic tree showed that our isolate is close to the Shanghai isolate (China).

Key words: *Carnation mottle virus*, RT-PCR, Phylogenetical position, Iran, Carnation

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GENETIC DIVERSITY OF *MELON NECROTIC SPOT VIRUS* AND *OLPIDIUM* ISOLATES FROM DIFFERENT ORIGINS

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Melon necrotic spot virus (MNSV), a species of the *Carmovirus* genus in the *Tombusviridae* family, is an endemic virus in greenhouse and open field crops of melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] worldwide (Hibi and Furuki, 1985). In nature, MNSV is transmitted by the chytrid fungus *Olpidium bornovanus* (Campbell *et al*., 1995) and through seeds (Herrera-Vásquez *et al*., 2009). This fungus plays an important role in melon seeds by acquiring and transmitting the virus to plant roots to not only initiates primary infection, but also to continue secondary infection cycles (Campbell *et al*., 1996).

The geographic incidence, genetic diversity and phylogenetic relationships of MNSV and *Olpidium* isolates were studied in three cucurbit species from several Latin American and European countries on different collecting dates. One hundred and twelve cucurbit samples were collected from different locations of Brazil, Guatemala, Honduras, Mexico, Panama, Spain, Tunisia and the USA over a 10-year period (1999–2008). Root samples were collected from field-grown cucurbit crops (cucumber, melon and watermelon) plants which showed MNSV-like necrosis symptoms, as well as wilting and plant death (collapse), and were used as sources of both MNSV and the *Olpidium* species.

Of the 112 cucurbit samples analysed, 69 from Guatemala, Honduras, Mexico, Panama and Spain were DAS-ELISA-positive for MNSV. *O. bornovanus* and *O. virulentus* infections, and MNSV infections mixed with these *Olpidium* species, were observed for all these countries.

Twenty-nine MNSV isolates from all the origins where the virus was detected were selected and amplified by RT-PCR. The resulting RT-PCR of the p29, p89, p7A, p7B and p42 proteins was used to estimate the genetic diversity and the phylogenetic relationships of the MNSV population. The sequences obtained in this study were compared with the MNSV sequences of the NCBI database, and three groups were recovered by nucleotide composition according to geographical origins: the EU-LA

genotype group (with two subgroups: EU and LA, European and Latin American isolates, respectively), the JP melon genotype group (Japanese melon reference isolates) and the JP watermelon genotype group (Japanese watermelon reference isolates). The genetic diversity in the entire p7A and p7B proteins of MNSV suggests that these coding regions are under strong selective pressure.

Additionally, the rDNA-ITS region was analysed in 40 *O. bornovanus* and *O. virulentus* isolates associated with each geographical location and host examined. Phylogenetic analysis showed two groups for each *Olpidium* species, and these groupings were related to the host from which they were originally isolated.

As serious outbreaks of MNSV have been associated with new strains of the virus and the *Olpidium* species, and have occurred at various sites in the world, the accurate identification of virus strains and *Olpidium* species, as well as the determination of their genetic variation, are the first necessary steps to be taken in order to design effective disease control strategies.

Key words: *Carmovirus*, Chytrid, Cucurbits, Fungal vector, rDNA-ITS region, Viral proteins

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CURRENT MOLECULAR VARIABILITY OF *TOBACCO MILD GREEN MOSAIC VIRUS* IN PEPPER

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In the last years *Tobacco mild green mosaic virus* (TMGMV) was reported infecting pepper species (*Capsicum annuum* L. and *Capsicum chinense* L.) crops in different countries: Korea, Venezuela (Córdoba *et al*., 2006), Panama (Herrera-Vásquez *et al*., 2008) and Tunisia (Font *et al*., 2009).

During 2008, pepper crops from different Spanish areas were affected by TMGMV causing severe problems such as yield and fruit quality reduction. To evaluate the molecular variability of the viral genome and the phylogenetic relationships among different isolates, eight Spanish isolates were collected in 2008 from three important pepper production regions (three from Murcia, two from Pontevedra and three from Bizkaia) and analyzed by RT-PCR with specific primers which amplified a partial fragment of the coat protein gene (Cohen *et al*., 2001).

One Tunisian isolate collected in 2008 and different isolates published in the GenBank database from different geographical origins were included in the analyses. Phylogenetic analyses among the nucleotide (nt) and amino acid (aa) sequences of coat protein (CP) of the studied TMGMV isolates were estimated using the neighbor joining method. Percentages of similarity/identity showed a low variability in the partial CP gene of TMGMV isolates studied. TMGMV isolates clustered together in two main groups, hereafter referred to as European and Asiatic groups. However five subgroups could be observed where Spanish isolates clustered in the same subgroup. The identity/similarity among the aa predicted sequences ranged from 98% to 100%. Genetic distances for each pair of isolates were estimated by Kimura's two-parameter method and ranged from 0.000 a 0.023. The degree of selective constraint on a coding region can be estimated by the ratio of nucleotide diversity values at non-synonymous to synonymous sites. Pair-wise genetic differences at the synonymous (d_S) and non-synonymous (d_{NS}) nucleotide positions were estimated according to the method of Pamilo and Bianchi (1993) and Li (1993). The calculated ratio d_{NS}/d_S was low (0.088), suggesting that the studied fragment of the coat protein was under negative selective pressure, and that genome region studied not this under significant selection processes. Selection by factors such as the interaction of the virus with host plants, and random

genetic drift may in fact reduce genetic diversity in populations (García-Arenal *et al*., 2001).

Low variability and the phylogenetic tree obtained might be supported by the founder effect hypothesis that is colonization of new areas occurred from a single viral origin, and later this virus is long-distance disseminated by mechanical inoculation or seed transmission (Córdoba *et al.,* 2006).

Key words: Pepper, Disease, Population diversity, Sequence

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GENETIC VARIABILITY OF *PEANUT STUNT VIRUS* STRAINS IN POLAND

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Peanut stunt virus (PSV) is a serious pathogen of legumes and other important crops occurring worldwide. It is a member of *Cucumovirus* genus from the family *Bromoviride*. PSV has a positive single-stranded tripartite genome, where RNA1 and RNA2 encode the replicase complex and RNA3 encodes movement (3a) and coat protein (CP). In addition, PSV has two subgenomic RNAs transcribed from the negative strands of RNA 2 and 3. Its virion may also be associated with the fifth component, designated satellite RNA (satRNA) that could modulate severity of disease symptoms. Strains of PSV have been classified currently into four subgroups: I (eastern) and II (western) both occurring in different parts of the world, III, reported in China and IV, found recently as a common subgroup parasitizing *Robinia* plants. Classification is based on the homology of nucleotide sequences, mainly RNA3 strand. First report on the presence PSV-P in Poland was published in 1983. Then several additional strains were collected (PSV-Ag, PSV-G, PSV-RobRos, PSV-SA6).

In the present study molecular characteristics of five known Polish strains was carried out. Virions were isolated from infected plants and RNA was extracted. RT-PCR amplification of coat protein or other ORFs sequences was performed followed by sequencing. Obtained results were the basis for comparative and phylogenetic studies. We also tested the possibility of all analyzed strains to support satRNA.

Previous genetic analyses of the PSV-P revealed that this strain does not belong to any known subgroups, but is related to subgroup I (Obrepalska-Steplowska *et al*., 2008). PSV-Ag, and PSV-G strains also can not be classified as subgroup I members, but their relationship with other subgroup I strains and PSV-P is equally distant, indicating on the significant heterogeneity among the subgroup I. On the other hand, PSV-RobRos and PSV-SA6 strains isolated from *Robinia pseudacacia* were found to be 95% identical to other subgroup IV members, confirming higher homogeneity of strains isolated from this plant in Europe (Kiss *et al*., 2009).

Key words: *Peanut stunt virus*, Phylogeny, Molecular characteristics

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MOLECULAR ANALYSIS OF THE POLISH ISOLATE OF *TOMATO TORRADO VIRUS* -ROS ToTV, SERIOUS PATHOGEN OF TOMATO CULTIVATION

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Tomato torrado virus (ToTV) has been identified in the last several years and now it has been reported in Europe, Middle America and Oceania. *ToTV* was classified to the new family *Secoviridae*, in the new genus *Torradovirus* (Sanfacon *et al*., 2009). The virus infects mainly tomatoes (*Solanum lycopersicum*) but it occurs also in weeds that might be considered as its natural reservoir. ToTV is transmitted by *Trialeurodes vaporariorum* and *Bemisia tabaci*. Classical symptoms of "torrado disease" manifest with stunting, malformations, mosaics, necrotic spots and leaf necrosis resulting in the death of the whole plant. Genome of ToTV consists of two (+)ssRNA strands. RNA1 encodes ORF1 for a protein of unknown function and a polyprotein with the domains important for viral replication. RNA2 encodes a large polyprotein that carries the information for movement (MP) and the coat protein (CP). The latter consists of three subunits.

In Poland, the newly identified virus of tomato plants was first reported in 2005, but the first sequencing of this pathogen was performed for Spanish isolate in 2007 (Verbeek *et al.,* 2007). So far, in our country three isolates of this pathogen were identified: Wal'03 (Budziszewska *et al*., 2008), Kra (2007) and Ros ToTV (2007).

In this study we concentrated on the sequence and phylogenetic analysis of Ros ToTV isolate. First, viral particles were isolated from virus-infected plants. Then RNA was phenol/SDS-extracted and ethanol precipitated (Sambrook *et al*., 2001). Afterwards, series of RT-PCR reactions were carried out with specific primers designed on the basis of ToTVPRI (Spain) sequences deposited in the GeneBank database. Products of the RT-PCR reactions were subsequently cloned, followed by sequencing and comparative analyses in BLAST software. The phylogenetic studies were performed by using MEGA 4.0 software (Tamura *et al.,* 2007), on the basis of ToTV sequences available in databank. The nucleotide comparison of available sequences of RNA1 and RNA2 indicated high similarity to isolates from Spain (PRI, CE, CAN from Canary Islands) and Hungarian (H2) amounting sometimes to even 99% of nt identity. Phylogenetic analysis confirmed these results showing high genetic correlation between Ros isolate and isolates from afore-mentioned countries,

as well as very short distance with Kra isolate. Moreover, we observed that Kra isolate of ToTV was more virulent than both, Wal`03 and Ros isolates. Therefore comparative genetic analysis needs to be performed, because the slight differences in genome sequence might be responsible for a varying virulence potential of three considered isolates of ToTV. Further studies will concentrate on determination of virulence domains within characterised ORFs of ToTV genome and whether they are related to changes in amino acids composition and distribution. Additionally, exact determination of conserved domains within the genome of the Polish isolates of ToTV will give basis to design sensitive protocols, based mostly on RT-PCR, applicable in the ToTV diagnostics in plants.

Key words: *Tomato torrado virus* (ToTV), Phylogeny, Virulence

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DIVERSITY OF THE POLISH *ZUCCHINI YELLOW MOSAIC VIRUS* ISOLATES

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Zucchini yellow mosaic virus (ZYMV), a member of the family *Potyviridae* and genus *Potyvirus,* causes yield losses in cucurbit crops worldwide. The virus is highly infectious and can be transmitted not only by aphids but also mechanically or by infected seeds (Schrijnwerkers *et al.*, 1991). Differences among ZYMV isolates in their host range, pathogenicity, serological and molecular characteristics have been defined worldwide. Virus isolates were collected from naturally infected zucchini (Zuy and Zug) or cucumber plants (Cu) in Poland. The virus in the sap from symptomatic plants was mechanically transmitted on test plants including *Cucurbita pepo* cv giromontiina "Astra Polka", *C. maxima, Cucumis melo, C. sativus, C. pepo* cv giromontiina, *Citrullus lanatus, C. pepo* cv patissonina and *Nicotiana benthamiana.* The presence of ZYMV in the original host or in experimental test plants was checked by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercial polyclonal antiserum (AS-0234; DSMZ, Braunschweig, Germany). In addition, the RT-PCR using M4 and Sprimer primers was performed to amplify two different parts of the genome, namely the gene of the coat protein (CP) and the gene of nuclear inclusion protein b (Nib) with function of polymerase (Chen *et al*., 2005). The obtained RT-PCR products were cloned and sequenced. The sequences of the CP gene were deposited in the GenBank database under the accession numbers: EU561043 (Cu), EU561044 (Zuy) and EU561045 (Zug). Sequence analysis was carried out in order to investigate the genetic diversity between the Polish ZYMV isolates and establish their molecular relationships to the previously characterized ZYMV isolates from different parts of the world.

All diseased plant material used in this study showed positive reaction in ELISA test, only with the antiserum against ZYMV. The host range and the severity of the systemic symptoms induced by Cu, Zuy, and Zug isolates on the tested plants were different. The most aggressive was Zug isolate which induced stunting and severe malformation of leaves. The biological differences between the Polish ZYMV isolates were confirmed by phylogenetic analyses. Amplification of the 3'end region of the RNA resulted in a RT-PCR product of approximately 1700 bp for all three samples. The nucleotide and deduced amino acid sequences of two genes, CP and Nib, were compared. We included the sequence of 22 additional ZYMV isolates for which this genomic region was available. The separation of two groups, A and B could be observed. Within group A, isolate Cu was most closely placed to the Central European strains, however strains from Israel and Japan are separated. Isolate Zuy

was also in group A, but formed a common branch with two Chinese isolates, Ningbo and Shangyu. Finally, isolate Zug clustered with another Chinese isolate (Shandong) within group B, where a Vietnamese isolate form a separate branch on the phylogenetic tree. Our results confirm that the Polish isolates of ZYMV are both biologically and genetically diverse.

Key words: Diversity, RT-PCR, ZYMV.

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SESSIONE 5

Plant-pathogen interactions

ORAL PRESENTATIONS

MOLECULAR BASIS OF THE INTERACTION OF VASCULAR WILT FUNGI WITH THE HOST PLANT

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The development of dynamic molecular tools during the last decades has offered new possibilities to study the interactions of plant pathogenic fungi with the host-plants allowing new insights in the identification and functional analysis of pathogenic determinants and the respective host defence responses of this pathogen-host interplay (Tudzynski and Sharon, 2003). However, among the pathogens studied so far the vast majority of fungi are of airborne nature and only limited research has been devoted to soilborne vascular wilt pathogens, mainly due to their distinct life-style. Among the genes targeted are those involved in perception and transduction of environmental signals by the pathogen and the subsequent degradation of plant cell wall, but also genes implicated in early stages of plant recognition and defence responses caused by the pathogen invasion. Fungal genes involved in signalling are G protein coupled receptors (GPCR), heterotrimeric G proteins, adenylate cyclase, mitogen activated protein (MAP) kinases, cAMP dependent protein kinase A (cPKA). Experimental data in the literature report that inactivation of G protein genes in the vascular wilt pathogen *Fusarium oxysporum* or MAP kinase genes in both *F. oxysporum* and *Verticillium dahliae* resulted in reduced pathogenicity of mutated strains; however, there is still limited information on the interaction between signalling components and signal transmission in vascular pathogens (Jain *et al.*, 2003; Delgado-Jarana *et al.*, 2005). Recently, we disrupted the β subunit of G protein and the catalytic protein kinase A signalling genes in *V. dahliae* and demonstrated their crucial role in virulence and physiology of this important vascular wilt pathogen (Tzima *et al.*, 2009b; 2010).

Specifically, deletion mutants (70*ΔGb* and 70*ΔPKA*) showed reduced pathogenicity on appropriate hosts and decreased ethylene production, which were more drastic in the *Gb* mutants. Moreover, both mutants intensively produced microsclerotia. In addition, 70*ΔGb* mutants germinated faster and presented a vertical rather a radial growth pattern on agar media. Furthermore, experimental evidence of the present work indicated a possible interaction between *Gb* and *PKA,* regulating virulence, physiology and development in *V. dahliae*, since overexpression of the *PKA* gene in 70*ΔGb* mutants restored the radial wild type growth, germination and conidiation. The resulting mutants, although unable to produce microsclerotia, were capable of causing typical disease symptoms on tomato plants.

In another approach aiming to study the role of various determinants in pathogenicity of vascular wilt pathogens, we disrupted the sucrose non-fermenting

protein kinase (*SNF1*) gene (Ospina-Giraldo *et al.*, 2003) in three *V. dahliae* races and revealed its implication in virulence and expression of genes involved in cell wall degrading machinery of this pathogen (Tzima *et al.*, 2009a). *VdSNF1* mutants of the defoliating and the non-defoliating strains did not cause any visible symptoms on cotton plants while mutants of race 1 were almost avirulent on tomato and eggplants. Specific cell wall degrading enzymes (CWDEs) were not activated in the resulting mutants after induction. Growth of the mutants was significantly reduced on pectin and galactose, while on glucose, sucrose and xylose they grew similarly to the wild type and ectopic strains. Using fluorescent microscopy, tomato stem cross sections at the cotyledon level showed reduced xylem vessel colonization of an *EGFP* transformed race 1 mutant strain compared with the wild type, which was further confirmed by quantification of fungal biomass in roots, stems and cotyledons by Real-Time PCR.

Finally, we provided insights into the role of the necrosis and ethylene inducing protein (*VdNEP*) gene (Wang *et al.*, 2004) in virulence of *V. dahliae* by over expressing it in wild type strains (Tzima *et al.*, 2009c). In pathogenicity assays on cotton plants, increased necrosis symptoms were observed in plants inoculated with *VdNEP* overexpressing mutants of the cotton defoliating and non-defoliating pathotypes, compared to the wild type strains. Moreover, a TRV-expression vector of *VdNEP* was constructed and transient expression of *VdNEP* in tomato plants caused typical necrosis symptoms.

Concerning the second factor of this interaction, the host plant, we demonstrated that ethylene (ET) perception and signalling play an important role in the host defence responses to *V. dahliae* infection through pathogenicity experiments on mutated *Arabidopsis thaliana* or tomato plants (Pantelides *et al.*, 2010a). Impaired perception of ET via *ETR1* was shown to play a crucial role in defence against *V. dahliae*, as *Arabidopsis thaliana etr1-1* (ET receptor mutant) plants expressed reduced foliar symptoms and pathogen colonization (as revealed by quantitative Real-time PCR analysis) of their vascular system, whereas salicylic acid, jasmonic acid or other ET-deficient mutants showed symptoms at the wild type level.

Microarrays and Real-time PCR analysis of the expression levels of defence related genes, revealed differential transcriptional changes of the *etr1-1* compared to wild-type and *ein4* (ET receptor mutant) plants, in response to *V. dahliae* infection. The activation and increased accumulation of the *PR-1*, *PR-2*, *PR-5* (PR proteins), *GSTF12*, *GSTU16* (glutathione-S-trasferases), *CHI-1*, *CHI-2* (chitinases) and *Myb75* genes, observed in *etr1-1* plants after *V. dahliae* inoculation, indicate that the defence response of *etr1-1* plants is dependent on a set of defence genes activated upon pathogen attack.

To investigate whether impaired perception of ethylene also affects the resistance of tomato plants against *V. dahliae*, a *Tobacco rattle virus* (TRV) based virus induced gene silencing (VIGS) system was employed, to knock down the *LeETR4* (that encodes an ethylene receptor in tomato plants) gene expression in tomato plants (Pantelides *et al.*, 2010b). Similar to the results on *Arabidopsis etr1-1* mutants, the pathogenicity experiments revealed that Verticillium disease severity in the ethylene

insensitive *Never ripe* (*Nr*) mutant plants (*Nr* mutation disrupts a tomato homologue of *Arabidopsis ETR1* mutant) and *ETR4*-silenced plants was statistically reduced compared to wild-type and control plants, respectively. Reduction in symptom severity in the *Nr* plants was associated with significant reduction of the fungal biomass in the vascular tissues of the *Nr* plants compared to wild-type plants as revealed quantification of *V. dahliae* by qPCR, suggesting that loss of function of the Nr receptor results in increased disease resistance.

We are currently fortifying our efforts in studying the interactions of the vascular wilt pathogen *V. dahliae* with the host plant targeting to identify and analyse molecular determinants of this interplay.

Key words: cAMP dependent protein kinase A, Ethylene perception, Virus induced gene silencing (VIGS), *Fusarium oxysporum*, G proteins, MAP kinases, Necrosis and ethylene inducing protein (*VdNEP*), Pathogenicity genes, Sucrose non-fermenting protein kinase (*SNF1*), *Verticillium dahliae*

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"LEAF STRIPE DISEASE" AND THE ROLE OF PHYTOTOXIC METHABOLITES IN GRAPEVINE TRUNK DISEASES

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Trunk diseases of grapevine have recently gained an increasing importance in all wine producing countries. Among them esca has a most relevant importance in the Mediterranean countries and in some others grapegrowing countries in the world (Mugnai *et al*., 1999). Many achievements have been made in the last 15 years on understanding its aetiology and epidemiology, that lead to propose to separate the wood decay, which originated the name 'esca', caused by *Fomitiporia mediterranea,* from a different disease, also known as 'young esca', well distinguished from the decay even if often caused by vascular fungi such as *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. These two fungi showed to be the main agents of the typical interveinal tiger-like leaf necrosis. On the base of this symptom the name 'grapevine leaf stripe disease' was recently proposed for young esca (Surico, 2009), where the typical foliar symptom are attributed to vessel obstruction and/or to the activity of phytotoxic metabolites produced by the two fungal agents in the wood and carried to the foliage by the plant sap (Surico *et al.*, 2008). Phytotoxic metabolites were also recently identified in the culture filtrates of other grapevine wood agents, i.e. some Botryospheriaceae species causing dieback, cankers, and characteristic wedge-shaped necrosis in the arms and trunks of grapevine plants (Úrbez-Torres *et al.,* 2008).

Specifically, *P. chlamydospora*, *P. aleophilum* and several Botryosphaeriaceae species (including *Neofusicoccum parvum*) have been shown to produce lipophilic low molecular weight compounds (scytalone, isosclerone, melleins, etc.) and high-molecular weight phytotoxins, these latter appearing to be exo-polysaccharides (EPSs) in preliminary chemical investigations. In this communication the chemical and biological characterization of the EPSs produced by *P. chlamydospora* and *N. parvum* are reported, including the evaluation of their phytotoxic activity on host and non-host plantlets.

Key words: Leaf stripe disease, Esca, *Phaeomoniella chlamydospora,* Botryosphaeriaceae, Phytotoxins

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HOST SPECIALIZATION OF *TRANSZCHELIA DISCOLOR* ON STONE FRUITS AT AECIAL AND UREDINIAL INFECTION STAGES

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Tranzschelia discolor (Fckl.) Tranz. & Litv. is an important rust fungus found throughout the world wherever *Prunus* species (stone fruits) are grown, including Turkey. This fungus attacks almost all stone fruits and causes infections mainly on leaves but it also affects fruit and twigs on peaches. Premature leaf fall caused by leaf infection is one of the most damaging symptoms of the disease (Ogawa *et al.*, 1995).

Tranzschelia discolor is a heteroecious rust fungus requiring two unrelated hosts in order to complete its life cycle. *Prunus* spp. are known as primary hosts whereas some members of the Ranunculaceae are alternate hosts for the fungus. *Tranzschelia discolor* develops five different spore stages during its life cycle, only two of them (aeciospore and urediniospore) are responsible for infections on stone fruits. Aeciospores are produced on alternate hosts and act as primary inoculum, whereas urediniospores produced on stone fruits in multiple cycles throughout the season are able to re-infect stone fruits.

Reports from published studies caused confusion over the host range and the level and extent of physiologic specialization of *T. discolor*. In California, cross inoculations with urediniospores from plum, peach and prunes caused infection only on the species from which inoculum was collected (Bolkan *et al.*, 1985). On the other hand, in Australia, cross inoculations of urediniospores between prune and peach caused infection in both species (Kable *et al.*, 1986). Five physiological races at aecial stage of *T. discolor* causing infection on almond, apricot, peach, plum and Japanese plum were reported in Israel (Sztenjnberg and Afek, 1979). On the other hand, Linfield and Price (1983) reported that inoculation of aeciospores from *Anemone* spp. onto almond, cherry plum, european plum, bullace (damson) and sloe resulted in infections only on european plum.

Rust has been observed on plum, almond, peach and apricot, but not cherry in Aydın Province. Preliminary studies indicated that rust infection on *Anemone coronaria* appears to be very high in spring in the region. In this study, host specialization of *T. discolor* at aecial and uredinial stages on *Prunus* species in the Aydın Province was investigated. Different aeciospore inocula were collected from the alternate host, *A. coronaria,* at ten different locations of Aydın Province in early spring and were inoculated to 1–2 years old nursery stocks of plums, peaches, apricots, almonds and cherries in growth chamber conditions. Rust pustules developed on plums at 15–21 days after inoculations, but not on peaches, apricots, almonds

and cherries. Urediniospore inocula were collected separately from naturally infected plums, apricots, peaches and almonds and were inoculated to plum, peach, apricots, almond and cherry in a series of cross-inoculations. Rust pustules developed only on the plants inoculated with the urediniospores from the same plant species. Results of this study indicate that there is host specialization of *T. discolor* at aecial and uredinial infection stages in Turkey.

Key words: Rust, *Tranzschelia discolor*, Stone fruits, *Anemone coronaria*

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ASSESSMENT OF THE ROLE OF NRPS-ABC TRANSPORTER IN PATHOGENICITY OF *ALTERNARIA BRASSICAE* USING REAL TIME – PCR TECHNIQUE

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The fungus *Alternaria brassicae* (Berk.) Sacc. is the causal agent of gray leaf spot on Brassica plants, including canola and other cruciferous plants (Parada *et al.,* 2007). The pathogen affects all aerial plant parts, reducing the photosynthetic area and accelerating senescence and defoliation and finally becomes a seed-borne pathogen.

It has been reported that *A. brassicae* produces four cyclic depsipeptide phytotoxins belonging to the family of compounds named destruxins. Destruxin B is the major phytotoxin produced by this pathogen *in vitro* and *in planta*. Bains and Tewari (1987) reported that the degree of susceptibility to the toxin correlates with the degree of susceptibility to the pathogen and classified destruxin B as a host specific toxin. However, Buchwaldt and Green (1992) suggested that both host and non-hosts of *A. brassicae* are sensitive to destruxin B (Parada *et al.,* 2007).Very little information is currently available concerning the pathogenicity determinants produced by *A. brassicae*. A nonribosomal peptide synthetase (NRPS) gene named *AbrePsy1* was identified (Guillemett *et al.,* 2004). Structural analysis of *AbrePsy1* revealed four complete elongation modules, two of which have epimerization domains. In the vicinity of *AbrePsy1,* a second gene named *AbreAtr1*, which encode an ATP binding transporter, was identified. The expression kinetics of these two physically clustered genes on the *A. brassicae* genome, encoding a NRPS and ATP – binding cassette (ABC) transporter, respectively, were determined during the infection process. Increased expression of *AbrePsy1* and *AbreAtr1* was observed during host – plant infection (Guillemett, 2004).

No information is available on the relation between degree of *AbrePsy1* and *AbreAtr1* genes transcription with metabolite production and pathogenicity. In this research work, we studied the transcription profile of *AbrePsy1* and *AbreAtr1* genes in six isolates of *A. brassicae* using real time PCR and biochemical techniques.

The isolate extracts from Fries culture medium were obtained and partially purified. Comparison of the results from transcription profiles of the *AbrePsy1* and *AbreAtr1* genes with toxicity test of secondary metabolites and degree of pathogenicity on canola plants revealed the positive correlation between symptom development, metabolite production and transcription of the two genes. According to the results of this research and the previous works by (Guillemett 2004) and (Parada *et al.,* 2008),

the role of the clustered genes of NRPS and ATP – binding cassette (ABC) transporter in canola was elucidated.

Key words: *Alternaria brassicae*, Destruxin B, Real time PCR, NRPS- ABC transporter

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EFFECT OF PEA CULTIVAR, PATHOGEN ISOLATE, INOCULUM CONCENTRATION AND LEAF WETNESS DURATION ON ASCOCHYTA BLIGHT CAUSED BY *MYCOSPHAERELLA PINODES*

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In Algeria, little information is available on factors affecting disease severity (DS) and yield losses caused by *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr on peas (Bouznad,1998). According to Van der Plank's theory, quantitative information concerning the effect of environment on specific disease components should be useful in estimating the quantitative impacts of plant genotypes with partial resistance on the rate of disease progress in the field. The occurrence of this disease has increased in the recent years due to the presence of high inoculum left in the field which then spread by wind and splash rain from one area to another.

The materials used in these tests, Pea plants cv. Onward and cv. Merveille de Kelvedon (MK) were used in all experiments. The two cultivars are the most commonly cultivated varieties in Algeria, the first one being highly susceptible and the second one moderately resistant to the blight. As fungal material, two isolates of *M. pinodes*, md0202 and tn0203 were used in the study. The isolates came from two localitions in the Chellif region and presented respectively a low and a high score of aggressiveness on 'Onward'.

For the leaf wetness duration (LWD) study, two weeks old plants of 'Onward' and 'MK' were sprayed to run-off with a conidial suspension of $4x10^6$ spores ml^{-1}. The pea seedlings were then subjected to LWD of 6, 12, 24, 48 and 72 h. During the wet period, plants were covered with clear polyethylene bags sprayed inside with distilled water. The unbagged plants were considered as unexposed to a wet period. At the end of this period, seedlings were uncovered and kept in glasshouse where temperature ranged from 15 to 25°C. The inoculum concentration **(**IC) effect was investigated on fifteen days old (three leaf stage) pea plants of 'Onward' and 'MK'. Plants were inoculated by spraying to runoff with a suspension containing $2.5x10^3$, $4x10^4$, $3.5x10^5$, $4x10^6$, and $5.2x10^7$ spore ml^{-1}.

The variance analysis showed that IC, cultivar and isolate had significant effects on IP (incubation period), LP (latent period) and DS ($P<0.001$). Furthermore, interactions between cultivar and IC were significant for DS, IP and LP. The shortest

period (mean: 3.3 days, sd: 0.52) was noted with $5.2x10^7$ concentration, with the couple tn0203-Onward and the longest IP (mean: 8 days, sd: 0.86) was observed with $2.5x10^3$ in the couple md0202-Onward. ANOVA showed that LWD, cultivar and isolates had significant effects on DS, IP and LP ($P<0.05$). For DS and IP, a significant difference between cultivars x LWD was also noted. LWD of 6 h was sufficient to initiate symptoms formation on leaves of both the susceptible and the moderately resistant cultivars. Furthermore, the lowest that permitted the observation symptoms was of 2.5×10^3 spores ml^{-1}. Both the IP and LP were affected by IC. The interaction between the cultivar x isolates was also significant for DS and LP. However, no interaction between isolate x LWD or between isolate x cultivar x LWD was observed. Comparatively, many previous studies have provided the LP for different species of Ascochyta blight. This was of 5 days for *A. rabiei* on chickpea (Trapero-Casas and Kaiser 1992), 6 days for *A. fabae* f.sp. *lentis* (Pederson and Morrall, 1994, Allard *et al*. 1993) and 8 to 10 days for *A. fabae* on fabae bean (Wallen and Galway, 1977).

Key Words: Ascochyta blight, Pea, Spray inoculation

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GENES DIFFERENTIALLY EXPRESSED BY *ASPERGILLUS FLAVUS* IN THE INTERACTION WITH *ZEA MAYS*

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Aflatoxins are carcinogenic fungal secondary metabolites produced by *Aspergillus flavus* and other closely related species. Levels of aflatoxins in feed and food commodities are strictly regulated by many countries because of the health hazard. Many internal and external factors, such as nutrition, environment and interaction with the host (e.g. *Zea mays*) affect aflatoxin biosynthesis. In relation to this, we hypothesise 3 phases (saprophytic, chemotrophic, pathogenic) in the interaction between the host and the pathogen. Our experimental set was aimed to reproduce *in vitro* and *in vivo* these different phases. The saprophytic phase was mimicked by growing *A. flavus* on dead kernels of maize. In the chemotrophic phase, the fungus was grown in an Erlenmeyer flask with a minimal medium in which was submerged a dialysis membrane containing viable maize kernels, previously wounded. The host-chemical diffusible compounds should be able to elicit fungal responses related to host perception. The final phase, the pathogenic, was performed by wound inoculating *A. flavus* onto maize ears in the field. We carried out a comparison of the expression profiles, using a custom Affymetix GeneChip microarray containing all the predicted *A. flavus* genes, at the different experimental conditions for identifying trends in gene expression associated with the different phases of *A. flavus* infection of *Zea mays* (OBrian *et.al.,* 2003).

A comparative pathway analysis was performed to highlight the molecular mechanisms of the fungus-host interaction. The results highlight many significant differences in expression profiles during the comparison between pathogenic and saprophytic phases, in particular it is evident an up-regulation of aflatoxin biosynthetic process, cell wall degrading enzymes, oxidative stress related pathways and G protein coupled receptors. The comparison between the chemotrophic phase and the *blank* (a flask with a minimal medium inoculated with the fungus) shows an up-regulation in the response to oxidative stress and carbohydrate transport and a down-regulation in DNA replication and RNA processing.

Further investigation will be carried out in order to individuate a subset of

gene preferentially expressed in the chemotrophyc phase and during the maize kernel contamination. In particular a reverse-genetic or RNAi approach will be performed for understanding the key processes in the interaction of *A. flavus* with the maize kernels.

Keywords: *Aspergillus flavus*, *Zea mays*, Saprophytic phase, Chemotrophic phase, Pathogenic phase, Affymetix GeneChip microarray

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MONITORING THE INTERACTION OF THE BIOCONTROL STRAIN *FUSARIUM OXYSPORUM* F2 WITH *VERTICILLIUM DAHLIAE* ON EGGPLANT ROOTS

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Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, is a devastating disease of a wide range of plant hosts. Since there are no chemical means to control the pathogen, management strategies are focused on preventive measures. In a previous study, the efficacy of a non pathogenic *Fusarium oxysporum* strain, F2, isolated from a suppressive compost amendment, was shown to reduce Verticillium wilt symptom development in eggplants (Malandraki *et al.,* 2008). The goal of the present study was to monitor the interaction of F2 with *V. dahliae* on the rhizosphere of eggplants to gain insights into the mode of action of this biocontrol agent. To visualize their presence on the root surface of eggplants, the F2 and *V. dahliae* isolates were transformed with the *eGFP* and *DsRed2* reporter genes, respectively. In addition, the endophytic presence of both fungi was monitored by qPCR analysis. It was shown that F2 colonizes the root surface along the intercellular junctions excluding *V. dahliae* from the same ecological niche. qPCR analysis revealed that application of F2 reduced the levels of *V. dahliae* biomass colonising the vascular tissues, and this reduced the disease severity. In a split root experiment, F2 was not able to trigger the defence mechanisms of eggplants against *V. dahliae*. Therefore, competition for space and/or nutrients on the root surface was considered the main mechanism of action of F2 against *V. dahliae* (Pantelides *et al.,* 2009).

Keywords: Biological control, *DsRed2*, *eGFP*, Vascular wilts

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POTATO GROWTH TRAITS ARE SEVERLY AFFECTED BY CO-INOCULATION OF *RALSTONIA SOLANACEARUM* WITH *COLLETOTRICHUM COCCODES*

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Interactions between fungal and viral pathogens for disease symptom expression have been reported (Bateman, 1961; Beniwal and Gudauskas, 1972; Farley and Lockwood, 1964; Mullen and Bateman, 1975). For example, symptom expression in tomato plants infected by *Verticillium dahliae* or *Fusarium* spp. may be modified by the presence of *Tobacco mosaic virus* (Thanassoulopoulos, 1976). Pathogenicity of *Ralstonia solanacearum* and *Meloidogyne javanica* in the presence of both pathogens is more severe than each pathogen alone (Sitaramaiah and Sinha, 1984). Potato plants are infected by many fungi, bacteria, viruses and phytoplamas as well as by one viroid. The two most important diseases of potato in Hamedan province, Iran, are the black-dot disease caused by *Colletotrichum coccodes* and bacterial wilt disease caused by *Ralstonia solanacearum*. In this study interactions between the two pathogens were investigated on three potato cultivars namely: Agria, Boren and Diamant by two inoculation methods. Potato stem and root length, wet and dry weight and root colonization rate with *C. coccodes* sclerotia were measured for evaluation of disease severity. Potato cultivar Agria was less susceptible to *R. solanacearum* infection than *C. coccodes*. *R. solanacearum* increased *C. coccodes* severity on potato plants although there were no significant differences between Cc and Rs + Cc treatments. Persistenc of *C. coccodes* in plants enhanced the severity of pathogenicity of *R. solanacearum*. When the two pathogens infest the soil and infect potato tubers at the same time, potato wet root weight decreased by 61/39% and 85/64%, respectively. Root colonization of potato cultivar Agria by *C. coccodes* sclerotia was scored as 50 – 70% (Tsror (Lahkim), 2004). Pathogenicity of *C. coccodes* or *R. solanacearum* on potato cultivar Boren was almost equal. Co-inoculation of potato plants by the above-mentioned fungus and bacterium showed pathogenicity synergistic effect. Potato growth factors in the presence of both pathogens decreased in potato cultivar Boren than in potato cultivar Agria. As a result, potato cultivar Boren was probably more resistant than potato cultivar Agria to both pathogens. This claim was confirmed by low levels of root colonization of potato cultivar Boren by *C. coccodes* sclerotia . In all cases, resistance of potato cultivar Diamant to both pathogens was more than the other cultivars especially as it

was partially resistant to bacterial wilt disease. Totally, potato stem and root length and their weights decreased in the presence of the two pathogens in comparison to control plants of all cultivars. Co- inoculation of potato plants with *C. coccodes* and *R. solanacearum* in all cases increased plant susceptibility to both pathogens and decreased plant growth factors. The highest level of susceptibility to both pathogens was found in potato cultivar Agria, followed by cultivar Boren and cultivar Diamant. Potato cultivar Diamant showed relative resistance to *R. solanacearum.* No significant differences were observed between the two inoculation methods, but tuber inoculation apparently was more destructive.

Key words*:* Interaction, Potato cultivars, Soil inoculation, Tuber inoculation

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INDUCTION OF DEFENSE-RELATED ENZYMES IN TOMATO PLANTS IN RESPONSE TO TREATMENT WITH FLUORESCENT PSEUDOMONADS

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This study investigated the induced defense responses and protective effects against tomato wilt caused by *Ralstonia solanacearum* (RS) by application of fluorescent pseudomonas (isolate Pf2). Soil treatments of tomato plants with isolate Pf2 significantly reduced disease severity of bacterial wilt on tomato caused by RS compared to infected control. The effect of Pf2 to induce resistance in tomato plants against Rs was investigated in greenhouse conditions. Changes in the activities of Polyphenoloxidase (PPO), ß-Glucosidase (ß-GL) and Peroxidase (PO) activities on tomato after application of Pf2 and inoculation with RS were studied (Ojalvo *et al.*, 1987; Baysal *et al*., 2005). In physiological studies on tomato plants significant changes in the activities of PPO, ß-GL and PO were found after Pf2 treatment. In uninoculated plants all enzymes increased by 44%, 45% and 26.2% respectively after 4 and 6 days after application. Moreover in inoculated plants all enzymes increased to 113%, 130% and 101% respectively after 4 and 6 days application (Abo-Elyousr, 2006).

Keywords: Peroxidase, SAR, Polyphenoloxidase, ß-Glucosidase, Tomato wilt, *Ralstonia solanacearum*

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PPV HAIRPIN CONSTRUCTS CONFER RESISTANCE TO *PLUM POX VIRUS* UNDER BIOTIC STRESS AND DIFFERENT TEMPERATURES

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The most devastating viral disease affecting *Prunus* species is sharka caused by *Plum pox virus* (PPV), a single stranded positive sense RNA Potyvirus.

Seven PPV strains have been characterised (M, D, Rec, EA, W, C and T) but D, M and Rec are the most important from an agronomical point of view.

Gene silencing in plant, among other functions, acts as a defence mechanism against viral infections. Double stranded RNA (dsRNA) triggers degradation of homologous RNAs in the cell.

In order to obtain resistance to PPV infection, four PPV-M derived gene constructs (UTR/P1, P1/HCPro, HCPro e HCPro/P3) based on the hairpin RNAi technology have been developed. In *Nicotiana benthamiana* model plants all the constructs were able to induce immunity to the transgene-homologous PPV isolate (Di Nicola-Negri *et al*., 2005; Ilardi *et al*., 2007).

The production of plant resistant to a wide range of PPV isolates is essential for effective control of the virus. R1 *N. benthamiana* plants were challenged with nine PPV isolates belonging to M, D, Rec, C and EA strains collected from *Prunus* species in different geographic areas (Greece, Bulgaria, Italy, Hungary, ex Czechoslovakia and Egypt). All the lines were resistant to the PPV-D, M and Rec isolates. Moreover, the line 6 transformed with the UTR/P1 sequence was also resistant to the distantly related PPV-C and PPV-EA strains (Di Nicola-Negri and Ilardi, 2006).

To verify whether abiotic stress, such as variations of temperature, can affect UTR/P1 construct ability to induce PPV resistance, PPV infection tests were performed at different temperatures (15°C, 25°C and 30°C) on UTR/P1 R2 homozygous plants. Results showed that resistance to PPV conferred by RNA silencing of PPV UTR/P1 sequences is not temperature dependent.

As plant viruses evolved proteins to suppress RNA silencing, mixed infection with PPV-M and: 1) *Cucumber mosaic virus* (CMV); 2) PPV-C or 3) *Potato virus Y* (PVY) were performed to verify whether the presence of functional heterologous viral suppressors can affect PPV-M resistance.

Plants harbouring the hairpin constructs are resistant to PPV-M in mixed infection with viruses expressing 2b or HC-pro suppressors.

All these data strongly suggest that the UTR/P1 construct can induce PPV resistance also under different temperatures and biotic stresses.

Key words: Sharka, Silencing, RNAi, Silencing suppressor, Temperature

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CELLULAR LOCALIZATION OF CALICO VARIANT OF *PEACH LATENT MOSAIC VIROID* IN PEACH LEAF SECTIONS BY LIQUID PHASE *IN SITU* RT-PCR

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Peach latent mosaic viroid (PLMVd) is a member of the family *Ansunviroidae* and infects mainly peach (*Prunus persica*) as a complex mixture of variants, most of which consist of 335-338 nucleotides (Flores *et al*., 2003). Some of these variants possess an insertion of 12 to 13 nucleotides that folds into a hairpin capped by a U-rich loop and are responsible for an albino-variegated phenotype called peach calico (PC) (Ambros *et al*., 1998).

In order to study the biology of infection by PLMVd, a sensitive method for its cellular localization is needed. The *in situ* Sybr Green reverse transcription-polymerase chain reaction (RT-PCR) amplification method has been applied for studying indigenous plant gene expression (Gal *et al*., 2006). We have utilized this technique for cellular localization of PLMVd. The samples used in this study were the calico and non-calico PLMVd infected peach leaf tissue. Healthy peach leaf tissue served as negative contol. All peach leaf sections were FAA-fixed and pre-treated with pepsin and DNase I before Sybr Green RT-PCR was performed. All steps of the method were carried out in liquid phase (in 0.2 ml PCR tubes) except for the final step of signal detection.

Epifluorescence microscopy was used to observe the signals emitted by PLMVd or *rbcL* (served as the positive internal control gene). A bright signal deriving from the amplified products was observed in peach palisade leaf parenchyma cells with sub-cellular localization of the PLMVd signals in chloroplasts, the organelles where it is known that PLMVd replicates and accumulates. No background auto-fluorescence signal was observed in the calico infected albino plant tissue. On the other hand, a strong background fluorescence signal was observed in the green (non-calico) peach PLMVd infected tissue, presumably due to chlorophyll auto-fluorescence (data from observations before the application of Sybr Green RT-PCR). However, upon prolonging the washing period of leaf sections, in washing buffer, the background

fluorescence signal was reduced and as a result the quality of the pictures after Sybr Green RT-PCR improved significantly.

In conclusion, the liquid phase *in situ* Sybr Green RT-PCR was sensitive in chloroplast localization of PLMVd, with low signal background, and short specimen processing time.

This technique does not require specialized equipment and is relatively inexpensive when compared to similar *in situ* localization methods. For these reasons, it is recommended for cellular localization analysis of subcellular plant pathogens. To our knowledge, this is the first report of using liquid phase *in situ* RT-PCR for viroid localization in infected cells.

Key words: In situ RT-PCR, Calico, Peach latent mosaic viroid, Sybr Green

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SESSIONE 5

Plant-pathogen interactions

POSTERS

EFFECT OF SEEDBORNE *FUSARIUM VERTICILLIOIDES* ON CORN SEED GERMINATION AND SEEDLING GROWTH

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Corn seeds can be often contaminated by *Fusarium verticillioides* worldwide. The presence of this fungus can lead to seed germination failure, seedling diseases or to symptomless infection of growing corn seedling and later on kernel. Since seeds are considered important *F. verticillioides* inoculum sources for the subsequent corn plant and ear contamination, the aim of this work was to investigate *F. verticillioides* contamination pattern in seedlings belonging to four hybrids, Arma, Kubrick, Tucson and Costanza grown in controlled conditions.

Thirty seeds per hybrid were surface sterilized in sodium hypochlorite (7%), placed in sterile filter paper in plastic cups and incubated under 12 dark/light cycle at 22 °C for 10 days. The isolation was carried out on the whole seedling, by dividing each organ in small fragments (5×5 mm) incubated on acidified potato dextrose agar at 25 °C. The identification of *F. verticillioides* was carried out using the biological species concept proposed by Leslie (1991). Fumonisin FB_1 production was assessed using the method described by Glenn and co-workers (2008).

High germination rates were recorded for all hybrids except for Kubrick. Isolation frequency levels (IFs) of *F. verticillioides* in seeds were high for all hybrids except for Tucson seeds, contaminated mainly by *Trichoderma* spp. All the seeds unable to germinate were colonized by *F. verticillioides* mycelium. Healthy seedlings, processed at three unfolded leaves stage (GS 13), were heavily contaminated by *F. verticillioides* except for Tucson seedlings. Contamination with *F. verticillioides* did not cause any disease in maize seedlings. Asymptomatic seedlings developed a wide adventitious root system and the average length of the primary radicle was 10 cm. Arma primary radicle showed the longest average primary roots, while the shortest ones were detected on Kubrick seedlings. No significant correlation was found between radicle length and IFs detected in roots. Seedling development was similar in all hybrids except from Kubrick, which produced shorter epicotyls. IFs assessed in seedling fragments was very high in all below-ground organs and decreased in leaves. Tucson hybrid seedlings were less contaminated than others in all considered tissue fragments. All the isolates were able to produce FB_1 and moreover the great majority of strains synthesized, after seven days of incubation at 27 °C, more than 100 mg/g of FB_1. These results suggested that seedborne *F. verticillioides* does not suppress seedling growth, but causes systemic colonization of the entire plant, and moreover this population is mainly constituted by strong FB_1 producers.

Keywords: Pathogenicity, Hybrid, Fumonisin

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EVALUATION OF SOME WHEAT GENOTYPES IN THE SEEDLING STAGE TO WHEAT YELLOW RUST DISEASE

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Yellow (Stripe) rust disease agent caused by *Puccinia striiformis* f.sp. *tritici* is one of the major wheat diseases in moderate and cold areas of Iran. In this study seedling of 62 promising lines from 4 different trials for moderate and cold areas (18 lines from ERWYT-M-85-6, 16 from ERWYT-M-86-7, 18 from ERWYT-C-85-86 and 10 from ERWYT-C-86-87) and 72 advanced lines from 2 trials for moderate and cold areas (36 lines from ARWYT-M, 36 from ARWYT-C) were tested against the predominant race in Iran 166E6A+*Yr27*. Yellow rust spores were multiplied and purified on the susceptible wheat line Bolani. After incubation, plants were kept in dark room at 10º C for 24 hours, then plants were exposed to 16000 lux light intensity for 16 hours of photoperiod at 17-18°C.

Infection types (IT) as described by McNeal *et al.* (1971) and based on 0-9 scale were recorded 14 and 17 days after inoculation. ITs: 0 (fleck), and 1 to 6 or combination of these ITs were considered as low infection type (LIT) indicating resistance, and ITs 7 to 9 showed high infection types (HIT) and therefore classed as susceptible.

Most of lines included in cold area trials are either resistant or partially resistant to yellow stripe rust whereas trials for moderate area contained mainly susceptible lines. These results showed that the chance to select resistant wheat varieties is greater within ARWYT-C and ERWYT-C-85-6 trials.

Keyword: Wheat, *Puccinia striiformis* f.sp. *tritici,* Resistance

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FUNGI ASSOCIATED WITH CORN SEEDLINGS IN THINNED PATCHES OF CORN FIELDS IN DASHTNAZ OF MAZANDARAN

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Seedling abnormality is an important disease of corn in some parts of the world (Greaney and Machacek, 1942; Khonga and Sutton, 1988; Sutton, 1982). Based on an inspection carried out in early May 2008 by the Dashtnaz Agricultural Organization in Mazandaran province of Iran, patches showing thinning were observed in some corn fields. Seedlings either failed to emerge or there was emergence of plants with poor color, slow growth, wilting and withering of the leaves, followed by collapse of the plants. Symptoms were scattered or in small patches. Samples of weak seedlings were collected from the aforementioned patches. The samples were plated on potato dextrose agar, rosebengal agar and malt agar media after surface sterilization with sodium chloride. *Fusarium moniliforme*, *F. oxysporum*, *F. graminearum*, *Pythium* spp., *Trichoderma harzianum*, *T. viride* and *Rhizoctonia solani* were found associated with the syndrome. Among them, *Pythium* and *Fusarium* were the most common fungi associated with seedlings of corn in this study.

Key words: *Fusarium*, *Pythium, Rhizoctonia, Trichoderma*

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VARIATION IN SENSITIVITY TO TAN SPOT IN COMMERCIAL BREAD WHEAT IN MAZANADARAN PROVINCE OF IRAN

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Tan spot caused by *Pyrenophora tritici-repentis* has become one of the major disease of wheat since new resistant varieties to wheat yellow rust i.e. N-8019, have been introduced in Mazandaran province of Iran. Genetic resistance is the appropriate and best method for controlling the disease (Ali *et al*., 2008; Forrer and Hecker, 2003; Singh *et al*., 2008). In this study N-8019, Daria, Tajan, Milan, and Rasool lines and cultivars of bread wheat were evaluated against tan spot in greenhouse conditions, under artificial inoculation in 2007 in order to compare their reaction against the disease.

The plants were inoculated by spraying with spore suspension at a concentration of 2000 spores per ml, 22 days after seeding. The inoculated plants were kept at 25°C, with 90% humidity for 48 h. Other plants from each line or cultivar were not inoculated, and kept as control in the same conditions. After 48 hours all the plants were placed in the greenhouse at 25°C.

All plants were scored by visual comparison using a Standard Grading Scale. If 70% or more of inoculated plants of a particular line have ratings ≤ 3, the line is considered as resistant, whereas if more than 30% of the plants of a particular line have a ratings ≥ 4, the line is considered as susceptible. Evaluation of the reaction of the bread wheat varieties showed that line N-8019 was susceptible, however cultivars Rasool and Milan were resistant, and Daria, Tajan, Shanghai and Shiroudi were intermediate.

Key Words: Bread *Pyrenophora tritici-repentis*, Tan spot, Wheat

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PATHOGENIC SPECIALIZATION OF *PYRENOPHORA TRITICI-REPENTIS* (DIED.) DRECHS. ON *TRITICUM DURUM* DESF. AND *T. AESTIVUM* L. VARIETIES GROWN IN ALGERIA

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The Tan spot of wheat is a fungal disease caused by *Pyrenophora tritici-repentis* (Died.) Drechs. whose anamorph is *Drechslera tritici-repentis* (Died.) Shom. It has a wide host range, including 37 species of grass (Krupinsky, 1992). Wheat, with its two subspecies (*Triticum aestivum* L. and *T. durum* Desf.) remains the preferred host (Hosford and Morrall, 1975). However, fungus infection is more important on durum wheat varieties than on common wheat, which can be attributed to the parasitic specialization of this fungus on these two species.

For this reason, we performed a pathogenicity test of two fungus isolates (I1. BD and I2.BT) obtained from two different species of wheat, on a range of wheat and barley varieties cultivated in Algeria: durum wheat (Vitron, Chen's, Waha, Moroco10), common wheat (H97813, Hidhab, Anza, Mexipak) and a variety of barley (Saida). The test was carried out in order to ascertain the presence or absence of specialization within the same culture, to assess the resistance level of these varieties and the level of aggressiveness of the two tested types of isolates (from common and durum wheat).

The test consists of a cross-inoculation of both types of isolates on different varieties, selected in a completely randomized arrangement with 4 iterations. The inoculation was performed on seedlings at the 3-4 leaf stage, which were placed in a saturated humidity chamber at 21°C for 48 hours with 16 hours light and 16 hours of darkness.

Two types of ratings were made 9 days after inoculation, the percentage and the severity of infection is estimated using a scale from 1 to 5 (Lamari and Bernier, 1989) which excluded the percentage of infected leaf surface.

This test showed different behavior for barley and the two wheat species and it was very resistant to both isolates of *P. tritici-repentis*. Compared to the two wheat species (durum and common), the variance analysis of the disease severity did not show a significant effect. On the other hand, for percentages of infected plants, this analysis revealed a highly significant difference (the percentage of infected durum wheat plants was higher than that of the common wheat plants). This analysis also showed a significant difference between the two fungus isolates tested, the I1. BT isolate (obtained from common wheat) infected more plants than the I2.BD isolate (obtained from durum wheat).

The results of this test revealed that there was no pathogenic specialization on common and durum wheat species, since both were sensitive to both types of isolates, and had the same disease severity. The large number of infected durum wheat plants compared with common wheat plants can be attributed to the degree of co-evolution of the association durum wheat-*P. tritici-repentis*, that the very high specificity of host-parasite relations finds its origin in the co-evolution of these in the same biotype (Tugayé, 2001).

Key words: *Pyrenophora tritici-repentis*, Parasitic specialization, Markers, Species, Durum wheat, Common wheat

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THE EFFECTS OF *STAGONOSPORA NODORUM* ON DURUM WHEAT KERNELS: PRELIMINARY DATA ON ANALYSIS OF PROTEINS

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Stagonospora nodorum blotch (SNB), caused by the fungus *Phaeosphaeria nodorum* (E. Müll.) Hedjar. (anamorph *Stagonospora nodorum* (Berck.) E. Castell. & Germano) is a serious wheat disease occurring all over the world. The pathogen attacks epigeous parts of the plant, with direct damage on kernel quality causing heavy yield losses (Eyal *et al*., 1987). In Italy the disease has been observed in the most important cereal growing areas (Pasquini *et al*., 2002; Iori *et al*., 2003).

The aim of this preliminary study was to compare the effect of *Stagonospora nodorum* on electrophoretic protein pattern durum wheat (cv. Simeto) according to the following tests: shrivelled kernels from artificially infected plants in field plots directly analyzed and after incubation period on water agar; finally the sound kernels of the same variety infected in laboratory and then incubated on water agar plates.

An isolate of *Stagonospora nodorum* obtained from durum wheat was used for the spore suspension ($1x10^6$ spore ml^{-1}) utilized for artificial inoculation. Conidial suspension was applied on the plants at ear emergence. Seed samples were harvested at maturity. Shrivelled field seeds were directly tested while other shrivelled seeds were incubated for six days on water agar plates, crushed in liquid nitrogen and freeze-dried. Moreover sterilized sound kernels were inoculated in laboratory with the aforesaid conidial suspension and incubated on water agar plates for a period of six days. These last seed samples were taken at different days, crushed in liquid nitrogen and freeze-dried. The wholemeal flour of all samples were subjected to electrophoretic analysis.

Extraction of proteins were obtained according to Payne *et al*. (1980). Glutenins were selectively extracted following the sequential procedure of Singh *et al.* (1991). The 1-D electrophoresis based on procedure of Payne *et al*. (1980) was performed on sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE).

The results obtained show different electrophoretic protein patterns between field trial samples and those incubated in laboratory. The analysis of the shrivelled field samples appeared almost similar to the uninoculated control. The samples incubated on water agar plates showed no significant changes respect to the uninoculated samples until the fifth day of incubation. The sixth day of incubation loss of colour

intensity and smearing of all protein bands was observed.

These preliminary results suggest the possibility that the destruction of proteins in durum wheat kernels may be conditioned by various factors and/or activation (or expression) of different proteolytic enzymes.

Key words: *Stagonospora nodorum*, SDS-PAGE, Durum wheat, Proteins

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MOLECULAR ANALYSIS AND HISTOPATHOLOGY OF THE INTERACTION SUGARCANE-*PUCCINIA MELANOCEPHALA,* THE CAUSAL AGENT OF SUGARCANE COMMON RUST DISEASE

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Sugarcane (*Saccharum* spp.) common rust disease caused by the fungus *Puccinia melanocephala* H. Syd & P. Syd. is one of the major diseases of sugarcane, distributed along sugarcane growing areas worldwide. Compatible and incompatible sugarcane - *P. melanocephala* interactions were compared by histopathology and gene expression studies. Two sugarcane genotypes were used: the susceptible variety B4362 and its resistant mutant IBP8518. Plants from both genotypes were inoculated with *P. melanocephala* spore suspensions (4-5x10^5uredospores/ml) in controlled conditions (80-90 % relative humidity, 25˚C). Fungal structures and plant cell death were visualized with lactophenol-trypan blue staining. It was demonstrated that urediniospore germination, germinative tube elongation and stomata penetration of *P. melanocephla* on leaf surface of the resistant mutant IBP8518 is similar than in the susceptible B4362. Hypersensitive response in the resistant mutant is subsequent to the fungal penetration, like in gene-for-gene recognition.

Suppression subtractive hybridization (SSH) technology (Diatchenko *et al*., 1996) was used to capture and enrich rare transcripts expressed in sugarcane leaves inoculated whit *Puccinia melanocephala*. Transcription accumulation variations of genes were studied by RT-PCR and QRT-PCR. Database comparisons of ESTs revealed that, of a subset of 96 non redundant sequences induced by the fungus in limbo, 76% possessed putative identities indicative of involvement in signaling events, regulation of gene expression, defense and metabolism, while 24 % were of unknown functions.

Two members of the NAC transcription factors family were found to be differentially expressed in the resistant mutant at early time points of infection. Probably, they are involved in the regulation of PR genes and in the transcriptional activation of hypersensitive response in an analogue function as previously reported for NAC transcription factors by Oh *et al.* (2005), Kaneda *et al*. (2009) and Delessert *et al*. (2005). This result provides evidence on the roll of NAC transcription factors in the complex regulatory network of sugarcane defense against this pathogen.

Key words: NAC transcripts, Mutant, Up-regulated, Defence

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UNRAVELLING THE PHASES OF INFECTION AND GENE EXPRESSION DURING THE INTERACTION BETWEEN *PYRENOCHAETA LYCOPERSICI* AND TOMATO

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Corky root rot (CRR) is an economically devastating disease of tomato (*Solanum lycopersicum*) and other crop plants, caused by the soil-borne filamentous fungus *Pyrenochaeta lycopersici*. Data to progress in the understanding of the molecular knowledge of the infection process of this fungus are still lacking. At CRA-PAV we are currently investigating the mechanisms behind disease susceptibility and resistance against CRR using different molecular methods in order to identify fungal genes candidates to pathogen virulence. A previous experiment of fungus-plant interaction transcriptomic analysis by cDNA-AFLP allowed the identification of hypothetical fungal ESTs (Aragona and Infantino, 2008).

In the present work, a differential expression analysis by real-time PCR on tomato roots artificially infected with *P. lycopersici* at six different time post-infection events was set up for some of the previously identified ESTs. Our goal is to detect regulated fungal transcripts related to the development of the disease, therefore differentially expressed during the different time points of the infection, compared to vegetative mycelium. The last step will be the identification of the corresponding genes and their putative function. At present, we are characterizing a *P. lycopersici* EST having a high similarity with a fungal b-glucanase transcript by RACE analysis.

The attention towards this gene comes out from its possible role as virulence factor, such as other plant cell wall degrading enzymes known in literature. Semiquantitative RT-PCR revealed that this transcript is constitutively expressed in the fungus, but its expression differs at the different post-infection times and in the vegetative mycelium. Finally, a real-time PCR has been used for relative quantification of *P. lycopersici* biomass in relation with plant biomass, to find a correlation between expression of these fungal transcripts and the progress of *P. lycopersici* during the time course of root infection.

From the plant side, a transcriptomic analysis strategy for the search of tomato genes involved in CRR-resistance by cDNA microarray is in progress. The gene expression profiling, related to a defined time-course of the infection event, will be studied using three tomato cultivars: Corbarino, Moneymaker, both CRR-susceptible, and Mogeor, CRR-resistant. This work is being carried out in collaboration with the University of Modena and Reggio Emilia.

Key words: Corky root rot, Virulence factors, Real-time PCR

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STUDY OF COMBINED ABILITY EFFECTS TO YIELD TRAITS IN UPLAND COTTON UNDER *VERTICILLIUM* CONDITIONS

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Cotton yield is affected by several factors during the growing season. A soil borne fungus, *Verticillium dahliae* Kleb. can cause substantial yield loss in cotton (*Gossypium hirsutum* L.). Annual yield losses caused by *Verticillium* wilt exceeded 1.5 million bales worldwide (Bell, 1992). No absolute resistance to this disease is known in upland cotton up to now (Cano-Rios and Davis, 1981), so the use of resistant cultivars is considered the most practical and effective mean of control. The aim of this work was to evaluate upland cotton cultivars and their crosses through a breeding program of yield under *Verticillium* conditions. Five genotypes and their possible crosses without reciprocal were used and were simultaneously selected for yield traits and resistance. Seed cotton yield, boll weight, number of bolls per plant, seed index and fiber percentage were measured during two crop seasons each year at two different sites in plots with soil naturally infested with *Verticillium dahliae*. General Combined Aptitude (GCA) and Specific Combined Aptitude (SCA) were analysed using the Griffing model (Griffing, 1956). The studies of effects of GCA showed that the tolerant cultivars **'**Acala Prema**'** and **'**Acala Germain 510**'** were the most suitable to improve tolerance, boll weight and seed index, and **'**Deltapine Acala 90**'** was the best cultivar to improve seed cotton yield and tolerance to *Verticillium* wilt. The studies of effects of SCA indicated that the crosses between susceptible cultivar **'**Mª del Mar**'** with tolerant cultivars such as **'**Deltapine Acala 90**'** were the most advisable to unify seed cotton yield, fiber percentage and tolerance to *Verticillium* wilt, with **'**Acala Prema**'** to improve seed cotton yield and boll weight, and with **'**Acala Germain 510**'** to improve boll weight.

Key words: *Verticillium dahliae*, Disease, Cotton breeding, Yield traits, Griffing diallel

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PATHOGENICITY OF SOME FUNGI ISOLATED FROM ASH CANKERS ON *FRAXINUS EXCELSIOR*

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Since the early 1990's, decline of *Fraxinus excelsior* L. in Europe has attracted the attention of forest pathologist (Kowalski, 2006; Cech, 2007; Kirisits *et al*., 2008; Bakys *et al*., 2009; Kowalski and Holdenrieder, 2009). The disease results in dieback and mortality of affected trees and is considered to be the most important threat to ash forests in its distribution area (Kowalski and Holdenrieder, 2009). Several fungal species have been isolated from symptomatic tissues of diseased trees. Although the role of these fungi in the decline is still unclear, there is some experimental evidence that one of them, *Chalara fraxinea* T. Kowalski, could be the causal agent.

In the present study, *F. excelsior* nursery seedlings and *Fraxinus ornus* L. plantations were surveyed for occurrence of ash dieback symptoms. Shoot and stem lesions and cankers were sampled and fungal isolation were made. *C. fraxinea* was not found among the isolates. Two *Phoma herbarum* Sacc. and one *Podospora* sp. isolates representing three most frequently isolated fungal morphotypes were used in inoculation experiment on 3-year-old *F. excelsior* seedlings. Two Finnish *C. fraxinea* isolates were used as reference.

The inoculation experiment was set up in December 2009 in a growth chamber, and the tree seedlings watered periodically. The bark of the seedlings was inoculated 50 cm above soil level. For each isolate ten replicates were used. Agar plugs with mycelium were placed into the wounds with forceps and sealed with Parafilm™. For controls, sterile agar pieces were used.

The pathogenicity tests were scored after six weeks. All seedlings were cut at soil level, and transferred to a laboratory. The bark surface was disinfected with 70% ethanol, and the outer bark around the inoculation point removed with a sterile scalpel. Necroses in the inner bark were measured and recorded.

Only the two *C. fraxinea* isolates caused lesions that were significantly different from the controls ($p<0.05$) indicating that *P. herbarum* and *Podospora* sp. acted as saprotrophs.

Key words: *Chalara fraxinea*, *Phoma herbarum*, *Podospora* sp., Ash dieback, Turkey

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RAISED AND DEEP PITTED POTATO LESION INDUCING *STREPTOMYCES* FROM IRAN

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Among the *Streptomyces* species a few cause diseases on some plants. The most important plant pathogenic *Streptomyces* species are those which induce scab diseases on potato tubers (1). Three phytotoxins, including thaxtomin, concanamycin and FD-981 together with *nec1* gene are the main pathogenicity factors of these species (2, 3 and 4). Potato scab disease is one of the most important diseases in potato growing area in Iran. Potato tubers showing raised, netted, shallow and deep pitted lesion symptoms were collected from many potato fields and *Streptomyces* strains were isolated. The potato pathogenic strains were very heterogeneous based on their induced symptoms type and phenotypic features. They were pathogenic on potato, parsnip, horse radish, carrot and other tested plants and were identified as *S. scabies, S. acidiscabies, S. caviscabies* and *Streptomyces* sp. Most of the strains had a linear plasmid determined by pulsed field gel electrophoresis. Polymerase chain reaction revealed that all tested *streptomyces* strains carry the sequences related to *nec1* and thaxtomin biosynthetic genes. *Streptomyces* strains which induce potato raised and netted scab disease produced thaxtomin, determined by thin layer chromatography, but no pitted lesion inducing strains produce this phytotoxin. The last strains which did not produced thaxtomin also did not hybridize to thaxtomin biosynthesis gene probes. Deep pitted inducing representative strains produced disease inducing toxins other than thaxtomin.

Key words: *Streptomyces scabies*, Potato scab disease, Thaxtomin

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SUSCEPTIBILITY OF OLIVE TREE CULTIVARS TOWARDS *PSEUDOMONAS SAVASTANOI* PV. *SAVASTANOI*

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Olive knot disease, caused by *Pseudomonas savastanoi* pv. *savastanoi* (*Ps. savastanoi*) is considered nowadays among the potentially serious diseases of olive tree in the Mediterranean area. In Tunisia, this disease is frequently observed in the North and Central areas of the country, where there is abundance of hail and frost causing wounds to stems (Ouzari *et al.,* 2008). Indeed, the most effective method of disease control is the selection of resistant or tolerant cultivars. In particular, olive cultivars tolerant to the disease and resistant/tolerant to frost damage should be considered in environments characterized by late spring frost.

In this research, we were interested to study the susceptibility of olive trees cultivars towards *Ps. savastanoi.*

Strains used to evaluate susceptibility were Aw9 : (Aouedna, 2006, isolated from *Chemlali*), Ivia 1628-3: (Spain, Valencia, 1996, isolated from *Cornicabra*).

The identity of the pathogen was confirmed by PCR tests. The primers used in these tests were specific to the *iaaL* gene (Penyalver *et al.,* 2000) of the bacterium and directed the amplification of a 454 bp fragment.

The criteria used to estimate plant susceptibility and tolerance were the pourcentage of galles plants, tumour weight, and number of tumours.

Preliminary results revealed that majority of cultivars (*Chemleli, Chetoui, Meski, Picholine, Zarraji, Arbequina, Koroneiki, Arbosana*) were susceptible to the disease. Interestingly the local cultivar *Oueslati* was found tolerant to the disease. Further studies on the factors involved in susceptibility of cultivars of olive trees are in progress in our laboratory.

Key words: Susceptibility, Olive cultivars, *Pseudomonas savastanoi* pv. *savastanoi*

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SCREENING OF EUCALYPTUS SPECIES FOR SENSITIVITY TO CROWN GALL DISEASE

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During summer 1999, galled *Eucalyptus occidentalis* seedlings were observed in a commercial forest nursery in western Algeria, a growing area specialized in raising woody perennials and conifers for reforestation. Over 60,000 plantlets were infected, exhibiting one or more galls located at the crown of the plants. The rate of infection exceeded 95% on *Eucalyptus camaldulensis*, *E. cornuta* and *E. occidentalis* and 2% on *E. gomphocephala*. The species *E. cladocalyx* was not affected. Bacteria isolated from galls were identified by molecular traits as pathogenic *Agrobacterium* species (Krimi *et al*., 2006). Even though the incidence of eucalyptus crown gall in Algeria has never been determined, the disease is of economic concern to local nurserymen who cannot sell infected plant material. Moreover no control measures have ever been practiced and no data regarding the sensitivity of eucalyptus species to crown gall are available. The identification of species with low sensitivity to the disease will surely benefit eucalyptus growers.

The five eucalyptus species *Eucalyptus camaldulensis*, *E. cladocalyx*, *E. cornuta*, *E. gomphocephala* and *E. occidentalis* were experimentally tested for their sensitivity to crown gall disease by inoculating a reference (C58) and a local (E14) *Agrobacterium* sp. strain. Galls were induced on all five species by both strains, even though a different level of sensitivity was recorded. *E. cornuta*, *E. gomphocephala* and *E. occidentalis* were the most sensitive species. In general, strain E14, isolated from *E. occidentalis*, induced higher number of tumours of larger size than C58. The only way to manage crown gall disease on eucalyptus plants in Algeria is the use of healthy plant material and the selection of genotypes resistant to crown gall. This work represents the first attempt to screen for eucalyptus species less sensitive to *Agrobacterium* infection.

Key words: *Agrobacterium* spp., Forest nursery, Tumour, Eucalyptus spp.

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A SURVEY FOR VIRULENCE AND EPIPHYTIC FITNESS OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* STRAINS ISOLATED FROM MANGO TREES

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Pseudomonas syringae pv. *syringae* (Pss) is a common inhabitant of a wide variety of plants, where it also lives as an epiphytic microorganism. This plant pathogen is the causal agent of bacterial apical necrosis of mango (Cazorla *et al*., 1998) and it has the ability to produce an arsenal of effectors which determine the virulence degree of Pss strains.

Pss strains isolated from mango and others plants showed the ability to produce lipodepsipeptidic toxins, as syringomycin or syringopeptin and mangotoxin, an antimetabolite toxin described by our research group. Mangotoxin is a virulence factor produced by 87.6% of Pss strains isolated from mango, and its production increases the incidence and severity of the necrotic symptoms (Arrebola *et al.*, 2007). Furthermore, competition experiments showed that survival ability of the wild-type strain was slightly, but significantly, higher than mangotoxin defective mutants, suggesting that mangotoxin production could improve epiphytic fitness (Arrebola *et al.*, 2009).

On the other hand, the majority of Pss isolated from mango contains indigenous plasmids, the 62-kb plasmids being the most generalized. These 62-kb indigenous plasmids contain homologous genes to *cop*ABCD and *rul*AB operon, and their presence correlates with copper and ultraviolet light resistance, and also, with epiphytic survival ability (Cazorla *et al*., 2002; 2008). Copper-resistance in such strains, was evaluated by determining the minimal inhibitory concentration (MIC) of copper sulphate, and UV-resistance by performing survival analysis of Pss cells exposed to doses of B+A UV-fractions. Both resistance factors were also assayed in experiments under field conditions. Molecular analysis by cross-hybridization with specific sequences of copper resistance operon *cop*ABCD and the UV-resistance determinant *rul*AB confirmed the presence of homologous genes on most of the 62-kb plasmids analyzed.

Virulence and epiphytic survival factors assessed in this study are relevant to understand the lifestyle of *P. syringae* pv *syringae* strains as epiphyte and pathogen.

Keywords: *Pseudomonas syringae*, Virulence factors, Mango, Apical necrosis

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Petria 20 (2), 67-633 (2010)

INFLUENCE OF GLUCOCORTICOID RECEPTOR EXPRESSION ON *CUCUMBER MOSAIC VIRUS* (CMV) INFECTION IN TOBACCO PLANTS

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Steroid hormones are regulators of developmental physiological processes in animal systems. The most familiar receptors for animal steroid hormones are nuclear receptors of steroid/thyroid receptor superfamily. These receptors are transcription factors that are present in the cytoplasm or the nucleus. Binding of their ligand induces nuclear translocation of the complex and/or transcriptional regulation of specific target genes. In plants as well as in animals, many steroid molecules have been identified as essential growth regulators. Steroids are now widely accepted plant-hormones where they regulate several processes. However, no plant orthologs of the well-characterized animal nuclear steroid receptors have been identified, yet.

Cucumber mosaic virus (CMV) is one of the most economically important plant viruses. It has a worldwide distribution and has the widest host range of any known plant virus. It is naturally transmitted by aphids and experimentally by sap.

In order to assess the effect of a nuclear glucocorticoid receptor (GR) expression on the viral infection, tobacco plants expressing a mammalian nuclear GR (Irdani *et al.*, 1998; 2003) were challenged with CMV.

Seeds from two independent GR+ transgenic plants (NTGR 2, NTGR 6) were sown on selective media (Kan). Seedlings were checked for GR expression on root and leaf tissues by RT-PCR analysis. Seedlings were then transferred to soil and grown in growth chamber. As control, plants transformed with the empty vector (GR-) were included in the experiments. T1 GR+ and GR- plus wild-type tobacco plants were mechanically inoculated with purified CMV particles. The plants were scored for CMV symptoms and semi-quantitative ELISA were periodically performed to assess CMV concentration in the newly emerging leaves.

The results obtained comparing transgenic (GR+ and GR-) and wild-type control plants under CMV infection will be presented.

Key words: Transgenic plants, Steroid hormones, Phytovirus

Acknowledgements

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EXPRESSION OF GENE CLUSTERS IN GRAPEVINE CULTIVARS AFFECTED BY BOIS NOIR AND SEVERAL VIRUSES

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Phytoplasma and viruses represent the most detrimental pathogens that affect worldwide *Vitis vinifera,* inducing symptoms and metabolic alterations that modify quantitatively and qualitatively crop production with important consequent management costs. In the aim to investigate the interaction of these pathogens with grapevine plants, different samples (with mixed or single infection), naturally affected by Stolbur phytoplasma (agent of Bois Noir disease), *Grapevine Virus A* and *B* (GVA and GVB), *Grapevine Fleck Virus* (GFkV), *Grapevine Fan leaf virus* (GFLV), *Grapevine Leaf Roll associated Virus 1*, *2* and *3* (GLRaV 1, 2 and 3) were used; these samples were compared with naturally healthy and recovered controls, to identify plant response to systemic pathogen infection.

Roche NimbleGen® microarray chips have been used to identify differentially expressed genes between healthy vs. infected and healthy vs. recovered samples from different cultivars. The chip architecture consists in 29,550 probes in quadruplicate, representative of the whole *Vitis* genome.

Attention was focused on different kind of infection rather than cultivar type, in order to investigate over different expression among different infections and to identify common gene pathways.

Preliminary results showed that expression levels of thousand genes were altered in infected plants, involving various metabolic pathways, confirming data reported in literature (Albertazzi *et al.*, 2009); introducing a cut-off to analyze gene modulation, we obtained 95 over-expressed (more than 2-fold) and 62 under-expressed (less than 0.5-fold) probes (53 and 50 of which were annotated). Main classes of these probes represented genes coding "molecule binding" (61% for over- and 46% for under expressed), "signal transduction and internal regulation" (20% and 19%) and "molecule modification" (8% and 10%) proteins. Coming in detail, among the "binding" gene class, 13 (24% of total) coded for extracellular region molecules and 9 (17% of total) for internal and intracellular binding processes while regarding the "signal transducer" gene class, 6 (11% of total) coded for receptor molecules. In under expressed genes only 2 (4% of total) belonged to external structure molecules

in “binding” class, while a little increase of receptor activity transcripts (7 genes and 14% of total) in “signal transducer” class was observed.

This is the first analysis of gene expression profiling in whole grapevine genome with phytoplasma and viruses interaction using Roche NimbleGen® microarray chips.

Key words: Interaction, Microarrays, Virus, Phytoplasma, Grapevine

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CHARACTERIZATION OF THE PHASEOLOTOXIN BIOSYNTHESIS CLUSTER FROM *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*

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The gammaproteobacteria *Pseudomonas syringae* is divided into more than 50 pathovars based on host range. A distinguishing characteristic of this species is that many strains produce phytotoxins lacking host specificity and that often have a role in virulence or in epiphytic fitness. Phaseolotoxin is a modified tripeptide that inhibits enzymes of the arginine and polyamine biosynthesis pathways, and whose production has been described in pathovars *phaseolicola* (Pph), *actinidiae* (Pac) and *syringae* (Psy), which are currently assigned to three different genomospecies (Bender *et al.*, 1999; Gardan *et al.*, 1999; Tourte and Manceau, 1995). The genes for the biosynthesis of phaseolotoxin have been described in Pph and Pac, and involve at least 23 tightly clustered genes (Pht cluster), designated *argK* and *phtA-phtV*, that are included in a putative 38 kb pathogenicity island (Pht-PAI) (Aguilera *et al.*, 2007; Genka *et al.*, 2006). The Pht-PAI contains diverse transposable elements, as well as four terminal integrases that might mediate its horizontal transfer between Pph and Pac. To contribute to the understanding of the evolutive history of the Pht-PAI, we undertook the characterization of the phaseolotoxin biosynthesis genes in Psy CFBP3388. By sequencing two overlapping cosmid clones from CFBP3388, we identified a 25 kb region homologous to the Pht cluster containing all the 23 genes involved in phaseolotoxin biosynthesis and in the same relative order than in Pph and Pac. This region, however, is not included in a pathogenicity island, is not bordered by integrases and is integrated in a different genomic region than that in Pph and Pac. Additionally, the Pht clusters of strains of Pph and Pac show around 99.8% identity, whereas the Pht cluster from CFBP3388 is only an overall 83% identical to that of Pph; however, the deduced products of the Pht cluster showed variable levels of identity to those of Pph, from a high of 94.5% for *argK* to a low 61.9% to *phtV*. The genome of CBFP3388 does not contain homologues of the four integrases bordering the Pht PAI in Pph and the Pht cluster from CFBP3388 is not associated to the mobile elements described in the Pht-PAI, although the right border is delimited by a copy of IS*Pssy*. Our results suggest that the Pht cluster from CFBP3388 might represent an ancestor of the Pht-PAI present in strains of Pph and Pac, and that its association to diverse mobile elements has promoted its recent interpathovar transfer.

Key words: Antimetabolite phytotoxins, Arginine biosynthesis, Ornithine carbamoyl transferase, *argK-tox* cluster

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SESSIONE 6

Mycotoxins

ORAL PRESENTATIONS

MEDITERRANEAN MYCOTOXIN NETWORK: ISM AND MYCORED INITIATIVES

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Reducing mycotoxin contamination in the food and feed chains is one of the major challenge to improve human and animal health as well as the economic sustainability of agricultural communities in Mediterranean area. Mycotoxins are responsible for a variety of toxic effects including the induction of cancer, and digestive, blood, kidney and nerve defects. One quarter of the world's food crops, including many basic foods, are affected by mycotoxin producing fungi (CAST 2003). In order to comply with the needs of EU and address global strategies for mycotoxin reduction (Logrieco and Visconti, 2004), a large collaborative project for a four year duration on "Novel integrated strategies for worldwide mycotoxin reduction in food and feed chains", MYCORED as acronym, has been recently approved within the European FP7 - "Food, Agriculture and Biotechnologies" Work Programmes (www.mycored.eu).

MYCORED aims at developing strategic solutions for reducing mycotoxin contamination in major crops, and thereby eleviating concern in economically important food and feed chains. The following toxins and commodities are especially considered in the project: aflatoxins, trichothecenes, zearalenone, fumonisins in wheat/maize food and feed chains; ochratoxin A in grape-wine and wheat chains; and aflatoxins in dried fruit chain. Novel methodologies, efficient handling procedures and informationdissemination and educational strategies are considered in a context of multidisciplinary integration of know-how and technology to reduce mycotoxins exposure worldwide. Five work-packages (WPs) are expected to develop novel solution-driven strategies to reduce both pre-and post-harvest contamination in feed and food chains. They involve: i) optimization of plant resistance and fungicide use; ii) biocontrol to reduce toxigenic fungi in cropping systems, iii) predictive modelling and optimisation of logistics; iv) novel post-harvest and storage practices, and v) application of new food processing technologies. Two horizontal WPs will develop enabling methodologies for i) advanced diagnostics and quantitative detection of toxigenic fungi, and ii) rapid and multi-toxin detection of mycotoxins and relevant biomarkers. The project will significantly build on the outcome of several European projects (through most coordinators/partners of FP5 and FP6) on mycotoxins by supporting, stimulating and facilitating education and cooperation with countries having major mycotoxin concerns related to (international) trade and food safety and

human health. The direct involvement of ICPC countries (Argentina, Egypt, Russia, South Africa) and international organizations (CIMMYT, IITA) together with strong scientific alliances with International Experts will strengthen the project through sharing experiences and resources from several past/ongoing mycotoxin projects in a global context.

An International Society for Mycotoxicology (ISM) (www.mycotox-society.org) has been also funded to promote global networking and to increase scientific knowledge concerning biology, chemistry and any sciences/disciplines related to mycotoxins and toxigenic fungi, through membership networking, scientific meetings, symposia, discussions, technical courses and publications. The Society aims also to promote research on mycotoxins and toxigenic fungi thereby leading to prevention and reduction in exposure to mycotoxins, enhanced food safety and a greater public awareness of this area.

In the Mediterranean contest a MYCORED international workshop supported by ISM and PMU and entitled "Mycotoxicological risks in Mediterranean countries: economic impact, prevention, management and control" will be held in Cairo, Egypt on 25-27 October, 2010 (http://www.mycoredinternationalworkshop.org/home.html). The workshop will be focused on the cooperation among Mediterranean Countries, with an overview on the current situation on the occurrence of mycotoxins and toxigenic fungi in Mediterranean Bacin. Prevention and control of mycotoxins in mediterranean food and feed chain, as well as mycotoxins of public and animal health significance in the mediterranean bacil will be other relevant topic to be discussed.

Acknowledgements

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EFFECT OF *PENICILLIUM EXPANSUM* STRAIN R82 LIQUID CULTURE ON POSTHARVEST PATHOGENS

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Blue mould, caused by *Penicillium expansum* Link, is a severe disease worldwide on pome fruits even in production areas where the most advanced storage technologies are available such as northern Itlay (Spadaro *et al*., 2004).

Currently the application of the synthetic fungicide, such as thiabendazole, is a primary method of postharvest fungal decay control, however non conventional means are needed because of the negative public perceptions about the use of chemicals and the development of fungicide resistant strains. In recent years, alternative strategies in postharvest disease management has emerged as promising (Mari *et al*., 2010). In an attempt to develop new approaches for controlling postharvest fungal pathogens in pome fruits, experiments were carried out to determine the efficacy of secondary metabolites produced by *Penicillium expansum* isolate R82.

Penicillium isolate "R82", thiabendazole (TBZ) sensitive, was grown in potato dextrose broth. The liquid culture (LC) was lyophilized, resuspended in distilled water (1:10, 1:100, 1:1000 v/v), sterilized by filtration (0.45 mm) and its influence on growth (dry weight mycelium DWM) and germination (length of germ tube) of *Penicillium expansum*, *Monilinia laxa*, *Botrytis cinerea* and *Colletotrichum acutatum* was evaluated. The LC reduced significantly the DWM of all pathogens tested, while increased the length of the germ tubes with respect to control, however an abnormality in mycelium growth was observed. In the conidia germination trial, a 10 fold-dilution of LC fully inhibited *B. cinerea* spore germination while reduced the germination of the other fungi tested.

In *in vivo* trials, Golden Delicious apples and Doyenne du Comice pears were wounded, treated with LC, diluted as mentioned above, inoculated with equal amount (20 ml) of *P. expansum* or *B. cinerea* conidia suspension (10^3 conidia/ml) and kept at 20°C for 10 days. On apple, lesion diameter and disease incidence were not reduced by LC treatments, except the fruit treated with LC, 1000 fold-diluted and inoculated with *B. cinerea*.

In addition, another trial was carried out on Golden D. apples and Doyenne du C. pears. Fruits were treated with the conidia of R82 isolate (10^3 conidia/ml), inoculated with two TBZ resistant isolates of *P. expansum* (P13 or CADRP28) and kept at 20°C for 10 days. In order to identify the isolate responsible of rot, malt extract agar plates amended or not with TBZ (400 mg/g) were inoculated with small pieces of rotted tissues. No fungal growth was observed on TBZ amended media, confirming that all lesions were produced only by the isolate R82, TBZ sensitive.

In conclusion, R82 produces secondary metabolites that affect fungal growth and seems very promising for the control of postharvest rots.

Key words: *Penicillium expansum*, Thiabendazole, Apple, Pear, *Botrytis cinerea*

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OCHRATOXIN, A CONTAMINATION OF TABLE GRAPES IN APULIA REGION, ITAY

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The European Union production of table grapevine is estimated 43.2% of the world global production of grapes and Italy dominates with 11.9 % of the quantities produced. This crop is a target of numerous pathogens that affect the quality and the quantity of grapes. Some Aspergilli, in particular the black Aspergilli aggregates, (*Aspergillus carbonarius*, *A. niger,* etc.) represent a real thread not only because they can lead to a significant loss of the product, but also for their ability to synthesize mycotoxins.

The main mycotoxin of concern in grapes is ochratoxin A (OTA) which is nephrotoxic, hepatotoxic, teratogenic and carcinogenic to animals and has been classified as a possible carcinogen to humans (IARC 1993). Among black Aspergilli, *A. carbonarius* is considered the most important as OTA producing isolates are observed more frequently (41–100%) compared to isolates belonging to *A. niger aggregate*. Moreover OTA production by *A. carbonarius* isolates is generally higher compared with *A. niger* isolates even if *A. niger* is usually isolated more frequently compared to *A. carbonarius*.

The presence of OTA has been largely investigated on wine grapes but only few studies have been done on table grapes. In particular, Guzev *et al.*, (2006) reported that a higher number of OTA-producing isolates was isolated from the surface of table grapes cv. Superior compared to vine grapes in Israel. In this work we present the results of OTA survey in Italian table grape varieties Red Globe and Crimson grown in Apulia region during 2009. OTA contamination has been correlated in these grape varieties with the activity of lipoxygenase, an enzyme involved in the oxidative unbalance that, in turn, influence OTA biosynthesis.

Key words: Table grapes, Ochratoxin A, *Aspergillus carbonarius*, *Aspergillus niger*

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A COMPARATIVE STUDY OF THE EFFECT OF CYANOTOXINS ON *RHIZOBIA* ISOLATED FROM MOROCCO AND THEIR SYMBIOTIC ASSOCIATION WITH *VICIA FABA*

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In Morocco as well as in many countries over the world, several aquatic lakes present the problem of eutrophication which becomes more accentuated by the intense proliferation of blue green algae «cyanobacteria» (Bouaïcha, 2002; Oudra *et al.,* 2008). During the eutrophication, these cyanobacteria can produce toxins (soluble substances). When the water containing the toxic cyanobacteria was used for irrigation, the cyanobacterial toxins could generate negative impact on agriculture (McElhiney *et al.,* 2001); both yield and quality of agricultural crops and causing significant economic losses.

On the roots of leguminous plants, nodules containing symbiotic bacteria (*rhizobia*) are developed. It is very important to evaluate the effects of cyanotoxins (MC-LR) on the *rhizobia* and their symbiotic association with leguminous plants.

The main objective of this study is to determine the effects of the cyanotoxins such as the microcystins-LR (MC-LR) on the *rhizobia*-*Vicia faba* symbiosis.

Experiments were conducted under laboratory controlled conditions; three concentrations of cyanotoxins (0.01µg/ml, 0.05µg/ml and 0.1µg/ml) are tested on the growth in YEM broth of some strains of *rhizobia* isolated from nodules of *Vicia faba* cultures in Morocco. The obtained results showed that the effect of cyanotoxins is different depending on the rhizobial strain. Indeed cyanotoxins induce a significant decreasing on the growth of many strains of *rhizobia* but some of them show a toxin tolerance.

Further tests were carried out on faba bean plant. The concentrations tested were: 2.224 µg/ml, 6.672 µg/ml, 15.568 µg/ml MC-LR. The obtained results showed the negative effect of cyanotoxins on the growth and physiology of *Vicia faba*.

Regarding the nodulation of bean seedlings inoculated with a strain of *rhizobia* was reduced with a different rate depending on the concentration of toxins. The number of nodules was reduced by half at the concentration of 2.224 µg/ml of MC-LR equivalent and was reduced by 2/3 at the concentration of 6.672 µg/ml MC-LR equivalent. At the concentration of 15.568 µg/ml equivalent MC-LR, the nodulation was completely inhibited. This confirms that cyanotoxins act negatively on the nodulation, and could also have a negative impact on the plant symbiotic process, and by consequence the *rhizobia*-*Vicia faba* symbiosis.

Keywords: *Rhizobia*, Legumes, *Vicia faba*, Symbiosis, Nodulation, Cyanotoxins, Microcystins (MC-LR)

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PATHOGENICITY AND POTENTIAL TOXIGENICITY OF SEED-BORNE *FUSARIUM* SPP. ON SOYBEAN AND PEA

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Fungi of the genus *Fusarium* are important mycotoxin producers and plant pathogens which are often found on seed (Summerell *et al*., 2003). Using selective media, the presence of *Fusarium* species was examined on samples of soybean and pea seed. Seed infection on soybean varied from 6% to 12% (mean 9.4%) while on pea it ranged from 3% to 17% (mean 9.6%). Forty-seven isolates were collected from soybean, and 13 species were identified - *F. sporotrichioides*, *F. equiseti*, *F. verticillioides*, *F. semitectum*, *F. pseudograminearum*, *F. sambucinum*, *F. chlamydosporum*, *F. crookwellense*, *F. oxysporum*, *F. poae*, *F. solani*, *F. proliferatum*, and *F. compactum*. Forty-eight isolates were collected from pea, with 11 species identified: *F. proliferatum*, *F. verticillioides*, *F. sporotrichioides*, *F. semitectum*, *F. scirpi*, *F. oxysporum*, *F. poae*, *F. compactum*, *F. equiseti*, *F. avenaceum*, and *F. culmorum*.

In germination tests on blotter papers inoculated with conidia, 33 out of 47 tested isolates significantly reduced the number of normal soybean seedlings compared to control, while only 6 out of 48 tested isolates significantly reduced the number of normal pea seedlings when compared to the control. When inoculated on plants grown on Hoagland's No. 2 nutrient media, nearly all *Fusarium* isolates caused necrosis of soybean and pea root. Despite root necrosis, none of the isolates significantly reduced shoot and root dry mass of inoculated pea. None of the isolates significantly reduced shoot dry mass of soybean plants, but five isolates (species: *F. sporotrichioides*, *F. pseudograminearum* and *F. equiseti*) significantly reduced root dry mass of inoculated plants.

The presence of *tri*5 gene, essential for trichothecene mycotoxins biosynthesis (Niessen *et al*., 2004), was analysed with PCR in 38 isolates from soybean and 13 isolates from pea belonging to species which are potential trichothecene producers. Positive PCR assays were observed in 15 isolates from soybean (species: *F. sporotrichioides*, *F. crookwellense*, *F. pseudograminearum*, *F. sambucinum*, *F. equiseti* and *F. chlamydosporum*) and four isolates from pea (*F. sporotrichioides*, *F. poae* and *F. culmorum*).

To test whether soybean and pea grain are conductive substrates for trichothecene production, autoclaved soybean, pea and barley grain, used for comparison, were inoculated with 8 isolates of *F. sporotrichioides* and the quantity

of T-2 toxin produced in 28 days was determined. Concentrations of T-2 toxin ranged between 69.4 µg/kg and 2595.5 µg/kg, and no significant differences were determined between toxin production on soybean and barley grain, neither did on pea and barley grains.

The presence of FUM1 gene, essential for fumonisin mycotoxins biosynthesis (Baird *et al*., 2008), was analysed with PCR for 7 isolates from soybean and 27 isolates from pea belonging to species which are potential fumonisin producers. Positive PCR assays were observed in all isolates from soybean (species *F. verticillioides* and *F. proliferatum*) and 24 isolates from pea (*F. verticillioides* and *F. proliferatum*).

Production of fumonisin B_1 after 28 days was analysed on autoclaved soybean, pea, and maize grains, used for comparison, inoculated with 8 isolates of *F. verticillioides*. The quantity of fumonisin B_1 produced on inoculated soybean and pea grain after 28 days was not significantly different from the non-inoculated control, but it was neither significant on inoculated and non-inoculated maize grain.

Key words: *Fusarium*, Soybean, Pea, Trichothecenes, Fumonisins

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PRELIMINARY DATA ON EXTRACT PHYTOTOXICITY OF *PHOMOPSIS FOENICULI* FROM BULGARIA

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Umbel browning and stem necrosis of fennel (*Foeniculum vulgare* var. *vulgare*) caused by *Diaporthe angelicae* (anamorph *Phomopsis foeniculi*) was found as a harmful disease of this crop in Bulgaria. A quick spread of the disease symptoms at the distal part of the infection site was observed suggesting the involvement of transposable phytotoxins in the pathogenesis.

A study was undertaken to reveal the main secondary metabolites produced *in vitro* by the fungus. The effect of culture filtrate was assayed on uprooted seedlings of fennel (host) and tomato (non-host) plants by absorption. The phytotoxicity of the fractions and of pure compounds was proven applying leaf puncture assay on detached tomato leaves. Preliminary chemical investigation aimed to isolation, purification and chemical characterization indicated that the fungus produces several secondary low molecular weight metabolites some of which with high phytotoxicity. Their chemical and biological characterization and studies on their mode of action is currently being investigated. The geranylhydroquinone named foeniculoxin, found as the main phytotoxic metabolite produced by Italian isolates (Evidente *et al*., 1994), was not synthesized by Bulgarian strain.

Furthermore, investigation also need to ascertain if this latter strain produces the galactan and branched mannan produced by the Italian strains (Corsaro *el al*., 1998).

Key words: *Phomopsis*, Secondary metabolites, *Foeniculum vulagare*, Chemical and biological characterization

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EVALUATION OF SUSCEPTIBILITY AND TOLERANCE PHENOTYPE IN *TRITICUM AESTIVUM* VARIETIES CONTAMINATED WITH TWO DON-PRODUCERS *FUSARIUM GRAMINEARUM* ISOLATES

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It has been assessed that one of the main virulence factor involved in the *Fusarium* head blight (FHB) disease of wheat leading to a severe reduction of grain yield and quality is the production of toxins, predominantly deoxynivalenol (DON) in *F. graminearum*. This toxin delays germination and growth of wheat plants (Champeil, *et al.*, 2004), inducing hydrogen peroxide (H_2O_2) production, inhibiting protein synthesis and stimulating cell death in *planta* (Desmond *et al.*, 2008).

Mycotoxigenic fungi contamination is a real issue, especially for cereal industry. Therefore, in order to reduce the diffusion of plant disease and health risks due to DON toxicity, there is a real need to develop analytical methods able to identify DON-producing fungal variety and to quantify mycotoxins at an early stage of fungal contamination and in order to accomplish this need we intend to study the interaction between *F. graminearum* and *T. aestivum* kernels.

In this work, the interaction between two *Triticum aestivum* varieties, Blasco (tolerant) and Sagittario (susceptible), inoculated with two *F. graminearum* strains (Fg126 and Fg8308), was studied. Two primer pairs (N1-2) designed by Konietzny *et al.* (2003), on the gene sequences belonging to the thricothecene gene cluster were used to assess the level of DON production ability of our *Fusarium* strains through PCR method. The same primers were used for developing a SYBR green Real Time-PCR assay for quantifying the DNA of *F. graminearum* strains in artificially contaminated soft wheat. The results obtained indicate a different ability of the two strains in growing on the hosts and, particularly, a higher rate of growth of both strains on the susceptible variety *vs.* tolerant one. It is known that, among the broad range of defence responses activated in *planta* when *Fusarium* invasion occurs, the generation of reactive oxygen species (ROS), such as H_2O_2, is one of the earliest events. The activities of three antioxidant enzymes (catalase, superoxide dismutase

and glutathione peroxidase) correlated to ROS and of one more enzyme related to the defensive response (lipoxygenase), were monitored to give some explanation on the different behaviour of the two wheat varieties in front of *F. graminearum* contamination.

Moreover, the expression of different genes activated in the interaction by a relative RT-PCR approach was analysed. In the pathogen, these genes encode for the transcription factor Fg*ap1* active in the cell defence against oxidative stress, *ePG* a polygalacturonase involved in cell degradation and *tri6*, one of the thricotecenes biosynthesis regulator. In *T. aestivum,* the expression analysis of a glucosyl transferase (*gt*) and of the pathogenesis-related protein PR1 (*PR1*) were carried out. The first gene can be related to a biochemical mechanism of resistance to DON with the ability to convert DON in a less toxic glucosylated form (Lemmens *et al*., 2005).

Finally, quantitative detection by HPLC of DON, 3GDON, 3-ADON and 15-ADON produced from *Fusarium* species present on samples, was described. In addition to DON, some *F. graminearum* strains may also produce modified forms of DON called 3-acetyl DON (3-ADON) and 15-acetyl DON (15-ADON).

In conclusion, as far as fungal diseases are wide diffused, the control of contaminated matrices it's a priority. Thus, it's very important to deepen studies of plant-pathogen interactions, in order to develop control strategies (i.e. quantitative, specie-specific methods) to be applied in diagnostics (i.e. advanced analytical method for mycotoxin detection).

Key words: *Triticum aestivum*, *Fusarium graminearum*, Deoxynivalenol (DON).

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PETRIA 20 (2), 67-633 (2010)

VARIATION IN SEQUENCE AND LOCATION OF THE FUMONISIN MYCOTOXIN BIOSYNTHETIC GENE CLUSTER IN *FUSARIUM*

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Multiple *Fusarium* species in the *Gibberella fujikuroi* species complex (GFSC) and rare strains of *F. oxysporum* can produce fumonisins, a family of mycotoxins associated with multiple health disorders in humans and animals. In *Fusarium*, the ability to produce fumonisins is governed by a 17-gene fumonisin biosynthetic gene (*FUM*) cluster. Here, we examined the cluster in *F. oxysporum* strain O-1890 and nine other species (e.g. *F. proliferatum* and *F. verticillioides*) selected to represent a wide range of the genetic diversity within the GFSC. Flanking-gene analysis revealed that the *FUM* cluster can be located in one of four genetic environments. Comparison of the genetic environments with a housekeeping gene-based species phylogeny revealed that *FUM* cluster location is correlated with the phylogenetic relationships of species; the cluster is in the same genetic environment in more closely related species and different environments in more distantly related species. Additional analyses revealed that sequence polymorphism in the *FUM* cluster is not correlated with phylogenetic relationships of some species. However, cluster polymorphism is associated with production of different classes of fumonisins in some species. As a result, closely related species can have markedly different *FUM* gene sequences and can produce different classes of fumonisins. The data indicate that the *FUM* cluster has moved within the *Fusarium* genome during evolution of the GFSC and further that sequence polymorphism was sometimes maintained during the movement such that clusters with markedly different sequences moved to the same genetic environment.

Key words: Fumonisins, Gene cluster, *Gibberella fujikuroi* species complex

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SESSIONE 6

Mycotoxins

POSTERS

MODELLING, PREDICTING AND MAPPING THE EMERGENCE OF AFLATOXINS IN CEREALS IN THE EU DUE TO CLIMATE CHANGE

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The impact of climate change has been identified as an emerging issue for food and feed safety (Miraglia *et al.*, 2009). By its mandate to identify emerging risks in food and feed sectors, EFSA's Emerging Risks Unit has identified changing patterns in mycotoxin occurrence in cereals, maize, wheat and rice, due to climate change, as a potential area of concern. A project has recently begun with the following main aims: 1) to identify and screen all factors influencing the growth of *Aspergillus flavus* and *A. parasiticus*, and aflatoxin production, in maize, wheat and rice plants; 2) to identify data on climate change and generate climate change scenarios; 3) to develop predictive models for *A. flavus*, *A. parasiticus* and aflatoxins production in maize, wheat and rice; 4) to run predictive models with meteorological data obtained by climate change scenarios; 5) to draw maps describing scenarios of fungal and aflatoxin contamination in the selected crops in the pre-harvest stage in the EU; 6) to evaluate the possible increase of future risk for EU populations related to aflatoxin contamination in cereals.

The project activities are organised into three work packages devoted to: i) the inventory of available literature, ii) the selection of climate change scenarios, and iii) the modelling and mapping activity. The project officially started in December 2009 and will be completed in September 2011.

Key words: *Aspergillus flavus*, *Aspergillus parasiticus*, Ecology, Maize, Growth stage

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PETRIA 20 (2), 67-633 (2010)

FUMONISIN OCCURRENCE IN MAIZE KERNELS IN RELATION TO WATER LOSS AND EUROPEAN CORN BORER ATTACK

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Several studies considered the factors able to influence fumonisin (FUM) contamination in maize; meteorological conditions are defined as the key factor (Battilani *et al.*, 2003), but a relevant role is also attributed to the cropping system, the hybrid, and the pest borer attacks to ears. The dynamic of water activity during maize ripening has been also suggested as a possible genotype-related relevant factor (Battilani *et al.*, 2007).

The aim of this research was to study the dynamic of water in kernels in some maize commercial hybrids and the relationships between this factor and FUM occurrence in kernels; the severity of European corn borer (*Ostrinia nubilalis* Hübner, ECB) attacks on ears was also considered.

These parameters were studied in north Italy, in 2007 and 2008 on ten maize hybrids belonging to FAO class 500-700 (medium-late season).

ECB severity varied significantly between years and maize growing areas, with a major role of the year. Significant differences were noticed between hybrids, not related to the season length.

Differences were noticed among hybrids, also regarding available water (a_w) and humidity (H), mainly in those belonging to FAO class 500. Mean H was significantly different among fields and followed a similar behaviour if compared with a_w.

Significant differences were noticed among hybrids regarding FB_1 and FB_2 content.

FUM content in kernels was significantly and positively correlated to ECB attack, and negatively with a_w and H. The probability of FUM contamination in kernel above the legal limit of 4000 µg/kg was well described by the binary logistic regression. as function of a_w and H.; these are good predictors of FUM contamination.

The role of ECB attack in enhancing FUM contamination is surely confirmed; nevertheless, high contamination levels were detected also with low ECB attacks, so as low contaminations were associated to severe ECB attacks.

Based on these results, it is stressed that any tool able to limit ECB attack is a good preventive actions for FUM contamination, but they do not guarantee the absence.

Late season hybrids are considered as more prone to FUM contamination, but this is not totally confirmed by this research; a_w is important, but its trend can be very similar in hybrids belonging to different FAO classes. The behaviour of water loss suggests that the relevance of harvest time could be different in diverse hybrids; "slow dry down" hybrids seem more prone to FUM accumulation, probably due to the longer lasting of ecological conditions favourable for *F. verticillioides* activity.

Key words: *Fusarium verticillioides*, Fumonisins, Maize, Water activity, Humidity, Hybrids, *Ostrinia nubilalis*

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PETRIA 20 (2), 67-633 (2010)

ECOLOGY OF ONE AFLATOXIGENIC STRAIN OF *ASPERGILLUS. FLAVUS* ISOLATED FROM MAIZE IN ITALY

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The main objective of this study was to define the range of temperature and a_w conducive for growth and aflatoxin B_1 (AFB_1) production by one *A. flavus* strain isolated from maize in Northern Italy taking into account the role of maize ripening stage. The optimal range of temperature for *A. flavus* was found to be 19-35°C (Northolt and van Egmond, 1981), with 28°C being optimum for aflatoxin production (Scott *et al.,*1970; Sanchis and Magan, 2004). Regarding water activity (a_w), this fungus is able to grow and produce toxins down to 0.73 and 0.85 a_w, respectively.

One strain of *A. flavus* (MPVP A 2092) isolated from maize in North Italy was used for *in vitro* experiments. The strain, stored in the fungal collection of the Institute of Entomology and Plant Pathology of the Università Cattolica del Sacro Cuore of Piacenza, was previously found positive for AFs production (Giorni *et al.,* 2007) and its identification was confirmed by CSIRO (Australia). This strain was chosen because it was in a cluster of strains able to grow well and produce high amounts of AFB_1 in *in vitro* experiments (Giorni et al, 2007).

Nine different temperatures (5-45 °C, step 5°C) and 8 a_w levels (0.77, 0.80, 0.83, 0.85, 0.90, 0.93, 0.95 and 0.99 a_w) obtained with the addition of glycerol or salt were used.

To verify the role of maize growth stages on fungal infection, a minimal medium obtained from milling maize ears harvested at different days after pollination (DAP) was prepared. DAP times between 3 and 52, in 7 day steps were considered.

A*spergillus flavus* was not able to grow in extreme conditions; no mycelium was observed at 5 and 10 °C and at 0.77 and 0.80 a_w, even when incubation was 60 days. Fungal growth was initiated at 15°C (0.99 a_w) and at 0.83 a_w (25°C) after 20 and 10 days incubation, respectively. This differed from studies with strains from other parts of the world which were able to grow down to 0.73 a_w (Trucksess *et al.,* 1988; Sanchis and Magan, 2004).

Regarding AFB_1, there was a narrower temperature range for production, between 15 and 30°C, with significantly higher amounts at 20-25°C. This was in contrast with other studies where 28°C was optimum for AFB_1 production (Scott *et al.,* 1970; Sanchis and Magan, 2004). The amount of AFB_1 produced increased

with water stress (decreasing a_w), with the addition of glycerol, while it was always significantly lower and minimum at 0.90 with addition of salt.
Fungal growth was slightly influenced by growth stages of maize kernels tested. The highest growth rate was obtained at 52 DAP and it decreased in media prepared with younger ears, although not always significantly. Fungal growth was maximum at 35°C, followed by 30 and 25°C. Interestingly, no aflatoxins were found in all the conditions studied on different maize ripening stage matrices.

Key words: *Aspergillus flavus*, Ecology, Maize, Growth stage

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Petria 20 (2), 67-633 (2010)

FUSARIUM LANGSETHIAE IN ITALY: GEOGRAPHICAL DISTRIBUTION, PATHOGENICITY AND TOXIN PRODUCTION

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Occurrence of *Fusarium langsethiae* Torp & Niremberg on many cereal crops has recently caused great concern in Europe due to the production by this species of type A trichotecenes (T-2 and HT-2 toxins) harmful to humans and cattle (Edwards *et al.*, 2009). In Italy, *F. langsethiae* has been recently isolated from durum and common wheat kernels cultivated in central and southern Italy (Infantino *et al.*, 2007). In this work, the incidence of *F. langsethiae* was monitored on durum wheat kernels of cv. Simeto, susceptible to Fusarium Head Blight, cultivated in 16 Italian regions representative of different environmental conditions. A total of 31 and 37 samples were analyzed in 2007 and 2008, respectively. The analyses were performed on 200 seeds for each sample by the "deep freezing" blotter test. In 2007, samples positive for *F. langsethiae* infection were 0%, 41.2% and 23% in North, Centre and South Italy, respectively. In 2008, samples positive for *F. langsethiae* infection were 0%, 20% and 50% in North, Centre and South Italy, respectively. *F. langsethiae* incidence ranged from 0 to 6% in 2007, and from 0 to 7% in 2008. Artificial inoculations of cv. Simeto at three growing stages (GS60, GS75, and GS83) were performed in the field in order to assess the pathogenicity of three *F. langsethiae* isolates on durum wheat. On artificially infected kernel samples, T-2/HT-2 values were measured using an ELISA kit, while *F. langsethiae* DNA quantity was measured by Real Time PCR with specific primers. No symptoms were observed on inoculated heads. No statistical differences on *F. langsethiae* incidence on kernels were recorded among isolates, while significantly higher ($P < 0.05$) incidence values were observed when plants were inoculated at late growth stages (GS75 and GS83). T-2/HT-2 values measured on artificially infected kernels ranged from 19 to 125 ppb. Correlations between *F. langsethiae* incidence and toxin levels were not significant, while significant positive correlations were obtained for *F. langsethiae* DNA quantity and T-2/HT-2 levels. The present data are of interest because they represent the first large scale monitoring of *F. langsethiae* in Italy. The obtained results seem to confirm the scarce or null pathogenicity of this species on wheat, as already observed in other countries. The low levels of T-2/HT-2 contamination on artificially inoculated wheat seem to indicate a possible low risk of contamination of wheat by these toxins, as compared to other cereals cultivated in Northern Europe, like oat. Nevertheless, long-term monitoring

of *F. langsethiae* and more data on toxin production in cultivated wheat in Italy are still needed, while waiting for the setting of T-2/HT-2 limits on cereal products by the European legislation.

Key words: Wheat, Fungi, Monitoring, Mycotoxins, Human health

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CHEMOTYPE VARIABILITY OF *FUSARIUM GRAMINEARUM* SPECIES COMPLEX ISOLATED FROM CEREALS IN ITALY

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Fusarium graminearum (teleomorph, *G. zeae*) is a worldwide pathogen of cereals. In the last decade, studies based on phylogenetic analyses led to consider this species as a complex, the *F. graminearum* species complex (FGC), composed by at least 14 species (O'Donnell *et al.*, 2004; Starkey *et al*., 2007; Yli-Mattila *et al*., 2009). This species produces several mycotoxins, mainly trichothecenes, which are tricyclic sesquiterpenes strongly associated with chronic and fatal toxicoses of humans and animals. Several studies have shown that many strains of *F. graminearum* can produce multiple trichothecene analogues, in particular deoxynivalenol (DON) and nivalenol (NIV) and their acetylated derivatives, 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON). Therefore, as a result of loss of gene function, a wide chemotype diversity occurs within this species since there are strains that differ in their chemical profiles. Essentially, three main chemotypes have been described. Strains with the NIV chemotype produce NIV and 4-acetyl-NIV; strains with the 3-ADON chemotype produce DON and 3-ADON; and strains with 15-ADON chemotype produce DON and 15-ADON. Moreover, strains reported to produce both DON and NIV, were described as unknown chemotypes. Distinguish between different chemotypes is important because differences in the mycotoxin pattern of each strain can result in marked differences in toxicity and biological activity. Moreover, the taxonomic re-evaluation of *F. graminearum* increases the importance to clarify relationship between chemotypes and the species of the FGC at both chemical and molecular levels.

A set of 64 strains isolated from cereals in Italy belonging to the FGC were analyzed for identifying their chemical and molecular chemotypes (Desjardins, 2008) and their phylogenetic traits. The strains were obtained from the Culture Collection of ISPA-CNR, Bari (www.ispa.cnr.it/Collection). The phylogenetic analyses were based on the sequence analyses of translation elongation factor, ß-tubulin, and Histone 3 genes. Molecular chemotypes were investigated by multiplex PCR assays

to determine the potential of strains to produce DON-like trichothecenes versus NIV-like trichothecenes and 3-ADON versus 15-ADON. In the first assay, primer sequences were based on sequence variation in the *Tri5* and *Tri7* genes to distinguish potential DON versus NIV production as described by Quarta *et al*. (2005; 2006). In the second assay, primer sequences were based on sequence variation in the *Tri3* gene to distinguish potential 3-ADON versus 15-ADON production (Quarta *et al*., 2006). Finally, the mycotoxin profile for each strain was analyzed by using a high performance liquid chromatography method.

The analyses revealed that all strains of the FGC belonged to *F. graminearum sensu stricto* with the exception of two strains identified as *F. cortadaeriae*. The most occurring chemotype was 15-ADON. Chemotypes DON and NIV also occurred but at a low level, while only a single strain proved to produce 3-ADON. These data, showing that the Italian structure of the FGC is homogenous and that a wide chemotype variability can occur also within a single species of the FGC, can provide insight with respect to disease management, quarantine regulations and plant breeding strategies to better understanding the ecology, epidemiology, and population dynamics of FGC.

Key words: *Fusarium graminearum* species complex, Chemotype, Deoxynivalenol, Nivalenol

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Petria 20 (2), 67-633 (2010)

DEVELOPMENT OF METHODS FOR THE PREDICTION OF MYCOTOXINS CONTAMINATION

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Methods for rapid and early assessment of mycotoxins contamination are highly desirable to prevent the introduction of contaminated lots of grain into the food chain, since the extraction and analysis of samples are time-consuming processes not suitable for routine testing at the time of grain delivery to drying and storage services. In the last years our team, in collaboration with several other research groups, has focused on the development and evaluation of innovative methods for the early assessment of contamination. The proposed methods include: (i) Aereobiological sampling of fungal spores during the maize harvest with a cyclone-type air sampler followed by molecular or immunological analysis (Torelli *et al.*, 2010); (ii) Comparative image analysis with near infrared illumination (Torelli *et al.*, 2009); (iii) Fourier transform near infrared spectroscopy (FT-NIR) (Gaspardo, *personal comunication*); (iv) Prediction model based on agronomic data with a neural network approach (Torelli personal comunication); (v) Nanopore based biosensing with aptamer probes; (vi) Discrimination of mycotoxins contaminated maize by electronic nose (Gobbi *et al.*, 2005). Aflatoxins, fumonisins, deoxynivalenol and zearalenone were determined by ELISA and HPLC on hundred of samples collected during the last five years and the analytical measures were compared with the predictions estimated using the predictive methods. In addition to prediction methods, the lab also carries investigations on the efficient removal of mycotoxins in feeds by detoxification into non-toxic metabolites via microbial transformation (Benedetti *et al.*, 2006).

Key words: Fungi, Maize, Fumonisin, Electronic nose

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AFLATOXIN-PRODUCING *ASPERGILLUS* SPP. AND AFLATOXIN LEVELS IN SOME FOOD COMMODITIES IN ALGERIA

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The aim of this study was to analyze populations of *Aspergillus* section *Flavi* and to evaluate aflatoxins (AFs) contaminated wheat, wheat products (flour, semolina and bran), peanut and dry fruits (hazel nuts, nut, cashew nuts, almonds, grapes dry and prunes) commercialized in Algeria. A total of 180 samples (108 of wheat and 72 samples of peanut and dry fruits) were analyzed. The isolates were identified according to morphological, chemotypes (aflatoxins and cyclopiazonic production) and molecular (ITS1-5.8S-ITS2 sequencing) characters. The capacity for producing AFs was determined for 455 isolates on coconut agar medium (CAM). Aflatoxins were detected and quantified by HPLC using post-column derivatisation bromine in a Kobra cell.

The results revealed the abundance of *Aspergillus* spp. (24.5 to 100%) dominated by *A. flavus* and *A. niger*. The HPLC analysis showed that 72% of isolates were aflatoxigenic. The amounts of AFs range from 0.02 to 1994.63 µg/g of medium. *Aspergillus flavus* was the main aflatoxigenic species. AFB1was detected in 56.6% (n = 53) of the wheat samples, with contamination levels ranging from 0.13 to 37.4 µg/kg. Aflatoxins were detected in 100% (n = 20) of the peanut and dry fruits samples, with contamination levels ranging from 0.16 to 13.46 µg/kg. Because of their carcinogenic, mutagenic and teratogenic effects, the presence of aflatoxins may give rise to high risks to human health.

Key words: Aflatoxins, Wheat, Peanut, Dry fruits, *Aspergillus* section *Flavi*, Algeria

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PETRIA 20 (2), 67-633 (2010)

EVALUATION OF CULTIVAR SUSCEPTIBILITY AND STORAGE PERIODS TOWARDS AFLATOXIN B1 CONTAMINATION ON PISTACHIO NUTS

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Aflatoxins (AFs) are potent sources of health risks to both human and animals (Sweeney and Dobson, 1998). Among them, Aflatoxin B1 (AFB1) is the most hazardous toxic and the most frequent in various food commodities including pistachio nuts (Var *et al.,* 2007; Hussein & Brasel, 2001).

In this survey, the effect of the storage period on AFB1 accumulation on pistachio nuts was investigated. A total of 49 samples collected during the crop year of 2005 from the most cultivated pistachio cultivars in Tunisia were rapidly screened by enzyme-linked immunosorbent assay (ELISA) combined with an immunoaffinity step.

The obtained results showed that the contamination of pistachio nuts has occurred clearly after two years of storage for all the tested cultivars. In this study, the cultivar Mateur was found the most susceptible cultivar to contamination by AFB1. After 4 years of storage, the average contamination levels in nut samples were ranged from 2,7 ± 0,8 to 12,7 ± 3,1 µg/kg for AFB1 according to the cultivars. These levels exceeded the maximum permitted limit of 2 µg/kg set by the European Commission in nuts.

Key words: Aflatoxin B1, ELISA, Pistachio nuts, Storage, Contamination

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Petria 20 (2), 67-633 (2010)

RECENT ADVANCES ON THE MYCOTOXIN RISK IN FIG IN THE MEDITERRANEAN AREA

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Fig plant is a common and natural component of the flora in the Mediterranean area, where this cultivation is economically very important. Several reports have shown that fig fruits can be contaminated by several different kind of highly dangerous mycotoxins and their related toxigenic fungi. In particular, the occurrence in dried figs of aflatoxins, potent carcinogens produced by *Aspergillus flavus* and *A. parasiticus* and related to liver cell cancer in humans, has been recently determined at a high incidence (Iamanaka *et al*., 2007). Moreover, also ochratoxin A, a nephrotoxic and carcinogenic mycotoxin, has been often detected in dried figs and its occurrence has been related to the contamination of the fig fruits by black Aspergilli (Karbancioglu and Heperkan, 2008). Since both types of mycotoxins, aflatoxins and ochratoxin, can have carcinogenic effects on human and animal, their co-occurrence on dried fig fruits is a reason of higher concern due to the possibility of synergistic or additives effects. However, more recently, a further concern is related also to the occurrence on dried figs of fumonisin B_1 (FB_1; Karbancioglu and Heperkan, 2009), a mycotoxin related to esophageal cancer in human and several other diseases in animals, and produced by several *Fusarium* species among which the most important are *F. proliferatum* and *F. verticillioides*. Some reports on the occurrence of *Fusarium* species on figs have shown that species belonging to this genus can occur at high incidence since the first stages of fruit development (Michailides *et al*., 1990; Heperkan, 2006) and therefore a high risk of accumulation of *Fusarium* toxins on plant can occur. However, lack of a correct identification led to several misidentification of the toxigenic *Fusarium* species occurring on fig fruits. We recently identified in Apulia, southern Italy, *F. ramigenum* (with *F. proliferatum* present at much lower extent) as the main agent of fig endosepsis a worldwide disease of fig fruit. The identity of *F. ramigenum* strains was confirmed by sequencing a portion of the translation elongation factor gene and sequence identity was then confirmed using the GenBank BLASTn search. Moreover, by using flanking-gene analysis, we revealed that the fumonisin biosynthetic 17-gene cluster, that governs the ability to produce fumonisins, can be located in the *F. ramigenum* genome (Proctor *et al.*, 2010). Therefore, the chemical analyses by using high pressure liquid chromatography of *in vitro* fungal cultures on rice kernel grown in the dark for 4 weeks, confirmed the genetic studies and, for the first time, showed that most of strains of *F. ramigenum* can produce FB_1 (up to 1010 mg/kg). These data show that, beside aflatoxins and ochratoxin A, the concern for the mycotoxin contamination of fig must be also due to the contamination of fumonisin producing species of *Fusarium* genus, among which *F. ramigenum* is a most important species.

This is important since, on the contrary of cereals, there are no legal regulations regarding FB_1 in dried figs or fruits. Therefore, the reported determination of FB_1 at high incidences and concentrations in dried figs and the common occurrence on fig fruits of fumonisin-producing *Fusarium* species must be considered as a novel unexpected hazard.

Key words: Aflatoxins, Ochratoxin A, Fumonisins*, Fusarium ramigenum*

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PETRIA 20 (2), 67-633 (2010)

EXAMINATION OF SPECIES OF *ASPERGILLUS* SECTION *NIGRI* FOR FUMONISIN PRODUCTION AND PRESENCE OF THE FUMONISIN BIOSYNTHETIC GENE *FUM8*

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Fumonisins are mycotoxins associated with cancer and several other serious diseases in humans and animals. Production of the mycotoxins had been reported for over two decades in *Fusarium* species (Desjardins, 2006), but has been reported only recently in strains of *Aspergillus niger* (Frisvad *et al*., 2007). In addition, a homologue of the fumonisin biosynthetic gene (*FUM*) cluster, originally identified in *Fusarium verticillioides*, has been identified in the genome sequence of *A. niger* (Pel *et al*., 2007). Here, we examined seven species in *Aspergillus* Section Nigri that occur on grape for fumonisin production and presence of the *fum8* gene, which served as a marker for the *FUM* cluster. Fumonisin B_2 (FB_2) production was detected in nine of 32 *A. niger* strains examined, but not in any strains of *A. brasiliensis, A. carbonarius, A. foetidus, A. japonicus, A. tubingensis*, and *A. uvarum* that were examined. In addition, PCR and Southern blot analyses provided evidence for the presence of *fum8* in 11 *A. niger* strains but not in strains of the other species examined. These findings indicate that discontinuous distribution of fumonisin production in grape isolates of *A. niger* likely results from absence in some isolates of at least part of the *FUM* cluster. The results also confirm the taxonomic complexity of *A. niger* from grape and provide a possible explanation for previously observed variability in FB_2 contamination of grapes and wine.

Key words: Fumonisin gene cluster, *Aspergillus niger*, Grape, Section Nigri

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SESSIONE 7

Control strategies

ORAL PRESENTATIONS

EXPLOITING MICROBIAL INTERACTIONS FOR PLANT DISEASE CONTROL – A *TRICHODERMA* SUCCESS STORY

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Researchers in plant disease biocontrol have generally accepted the dogma that the best place to find a bioactive strain is from the same ecological niche as the target pathogen. Hence, sclerotial baits have been used to selectively isolate microbes from soil with mycoparasitic activity and isolations have been made from flower or fruit surfaces for microbes required to control flower or fruit infecting pathogens, respectively. The rationale being that these microbes will possess the same environmental tolerances as the pathogen and be better able to elicit a biocontrol effect. However, there is the opposing view, less widely held, that microbes should be selected from foreign environments since the pathogen will never have been exposed to these microbes and is more likely to be sensitive to their bioactivity. We have developed an approach that encompasses both of these concepts whereby biocontrol agents are selected based on their 'match' with key biological and environmental attributes exhibited by the pathogen e.g. nutritional characteristics, environmental tolerances but the search is not limited to specific ecological niches. For example, we have included soil microbes in screens against foliar pathogens and microbes isolated from pine trees in screens against onion. This strategy has been highly successful with bioactive microbes identified for numerous target pathogens on a wider range of crops. Work conducted by the research group with *Trichoderma* biocontrol agents provides an excellent example of how microbes can be selected for biological characteristics that best match the biocontrol blueprint (Card *et al*., 2009) This has provided a rapid and cost-effective means of identifying biocontrol agents with commercial potential.

Trichoderma species are regarded primarily as common soil saprophytes and so it is not surprising that there are numerous examples of their use as biocontrol agents against soil-borne diseases. However, reports in the literature and evidence from our research show that they can also provide biocontrol of foliar, flower, fruit and woody trunk diseases. This raises the question of what key ecological factors influence the success of *Trichoderma* as a wide ranging biocontrol agent. To answer this, we have conducted numerous studies investigating the influence of both abiotic (temperature, pH, moisture, nutrition etc) and biotic factors on the biocontrol performance of a number of *Trichoderma* biocontrol agents (McLean *et al*., 2005). From these extensive glasshouse and field ecology studies, we were able to determine the biocontrol inoculum threshold for *Trichoderma* spp. (10^5-10^6 cfu/g substrate), identify pH and N status as significant influencing factors on biocontrol performance and prioritise the key biological attributes (competitive saprophytic ability, tolerance

to abiotic stress) required by *Trichoderma* biocontrol agents to provide effective and consistent biocontrol.

This information has been used to develop more effective screening and selection programmes which has resulted in enhanced field performance. This programme of activity has resulted in the successful commercialisation of four *Trichoderma* biocontrol products for use in NZ agricultural and horticultural systems (Tenet® for control of onion white rot, Lettucemate™ for control of Sclerotinia lettuce drop (Rabeendran *et al*., 2006), Sentinel® for control of Botrytis grey mould of grapevine and Arborguard™ for use in forest nurseries).

Key words: Biocontrol, *Trichoderma*, bioactivity, ecological constraints

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IMPACT OF CERTAIN FUNGAL FILTRATES, AND SOIL AMENDMENTS IN COMPARISON OXAMYL ON *MELOIDOGYNE INCOGNITA* INFECTING SUNFLOWER.

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Sunflower, *Helianthus annus* L. is one of the most important oil crops cultivated in Egypt. Root-knot nematode, *Meloidogyne incognita* has been recorded to attack numerous economically important crops i.e. sunflower causing detrimental effects on plants and crops yield.

A plastic bag experiment was conducted to evaluate the influence of certain fungal filtrates i.e. *Trichoderma harzianum* or *T. viride* (60, 80 and 100% at 25ml / plant each), three animal wastes i.e. camel, cow and horse manures (at 10 g /plant), and plant dried leaf powders i.e. thorne apple, marigold and adhatoda (at 2.5, 5 and 10g / plant), in comparison with oxamyl (at 6ml /plant) separately for controlling *Meloidogyne incognita* on sunflower cv. Euroflor. Thirty six out of sixty three black plastic bags containing 1800g steam-sterilized sandy loam soil (1:1)(v:v) separately received the tested dose of each organic amendment. Subsequently, bags were watered and left one weak for decomposition.

Sixty 15 day-old sunflower seedlings that were grown each in a plastic bag were separately inoculated with 2000 second stage juveniles of *M. incognita* . Oxamyl and the fungal filtrates were separately introduced to sunflower seedling at the time of nematode inoculation. Three plastic bags with one seedling each were not inoculated with nematodes (controls). Each treatment was replicated three times. Plastic bags were arranged in a randomized complete block design on a greenhouse bench where temperature was kept at 31± 3C°. Forty five days after nematode inoculation, plants were up-rooted. Data for length and fresh weight of shoot and root, and shoot dry weight were recorded. Infected plant roots were examined for number of galls and nematode developmental stages, females and egg masses after staining with lactic acid-fuchsine. Nematode parameters were determined and recorded. Nitrogen (N), Phosphorus (P) and Potassium (K) and chlorophyll content of shoots were also determined and recorded.

All tested materials remarkably improved plant growth and significantly reduced nematode criteria. As the filtrate concentrations of T. *harzianum* or *T. viride* raised from 60 up to 100%, the percentage increase of plant growth parameters increased as well. A similar dose response trend was observed with the plant powders as well. These results are in accordance with those reported by Windham *et al*. (1989), Mostafa (1992), and Siddiqui *et al*. (2002). Among the tested components,

marigold powder at 5g /seedling accomplished the best growth of whole plant fresh weight and shoot dry weight (166.5 &167.85%) followed by thorne apple (5g), and *T. harzianum* at 100%, whereas, the lowest values were recorded by camel treatment (28.5 &25.0%) comparing to nematode alone, respectively. With respect to nematode parameters, marigold (5g) significantly achieved the highest reduction percentage for final nematode population (74.0%) followed by thorne apple (5g) (70.0%), cow manure (10g) (68.6%) oxamyl (67.6%) and then *T. harzianum* 100% (60.0%), respectively. These findings are also in congruence with those reported by Akhtar and Mohamaud (1997) and Elsherif *et al*. (2006). Oxamyl surpassed all tested materials in suppressing nematode criteria, i.e. root galls (72.5%), eggmasses numbers (72.68%), followed by thorne apple (5g) (72.2 & 69.5%), cow manure (69.99 & 66.61%), *T. harzianum* 100% (69.41 & 71.67%) and then marigold (5g) (65.44 & 64.74%), respectively. Moreover, N, P and K concentration in leave of sunflower were positively enhanced by all tested components whereas the opposite trend was noticed in the case of total chlorophyll content comparing to nematode alone.

Key wordes: Control, Fungal filtrates of *Trichoderma harzianum*, *T. viride, Meloidogyne incognita*, Oxamyl, Soil amendments, Sunflower

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EFFECTS OF BIOFERTILIZER AND MINERAL POTASSIUM ON THE BIOCHEMICAL COMPOUNDS OF TOMATO CV. RIO GRANDE INOCULATED WITH *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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The main goal of this study was to evaluate the effects of the biofertilizer (Halex) and mineral potassium on growth and biochemical characters of tomato cv. Rio Grande plants infected with the root-knot nematode *Meloidogyne incognit*a and *Fusarium oxysporum* f. sp. *lycopersici*.

The root-knot nematode population, obtained from infected eggplants in El-Hamama region, was identified as *M. incognita* on the basis of the perineal patterns morphology and esterase phenotype of adult females (Adam, 2006). Afterwards, a *M. incognita* pure culture was obtained, from a single egg mass, and multiplied on a susceptible tomato. The biofertilizer (Halex) was first tested on agar media and the pathogenicity of the fungus *F. oxysporum f.sp. lycopersici*) and *M. incognita* was evaluated on tomato seedlings. In order to know the effects of the Halex and mineral potassium on tomato cv. Rio Grande plants infected with *M. incognit*a and *F. oxysporum* f. sp. *lycopersici*, a pot experiment was conducted with the following treatments: tomato without fertilizer or fungus or nematodes (control); Halex; Halex + potassium; Halex + *Fusarium*; Halex + *M. incognita*; potassium; potassium + *Fusarium*; potassium + *M. incognita*; *Fusarium* alone; *M. incognita* alone; *Fusarium* + *M. incognita*; Halex + *Fusarium* + *M. incognita*; and potassium+ *Fusarium* + *M. incognita*.

The analysis of the plant biochemical compounds revealed that in all the treatments, with fertilization, the phenolic compound increased and the highest value was recorded in the treatment Halex + potassium. The content of lignin in tomato roots (Ride, 1975) increased in the Halex treated plants infected with *M. incognita* (0.812 mg/g of root), compared to plants infected with *M. incognita* alone (0.594 mg/g of root), and in plants treated with *Fusarium* + potassium, compared to plants with fungus alone (0.193 mg/g of root), and decreased in nematode infected plants + potassium. The activity of the peroxidase increased in the Halex treated plants and decreased in all fertilized infected plant treatments.

The activity of the polyphenol oxidase increased in Halex + potassium (127.15 units/g of plant), compared to unfertilized plants (108.9 units/g of plant), and *Fusarium* alone treatments.

The chlorophyll A and B content also increased in all the fertilized treatments and the highest value was detected in the Halex treatment (340.66 µg/g of leave), compared to unfertilized plants (248.79 µg/g of leave) (Moran, 1982).

Nitrogen and potassium analysis in the plant showed that: nitrogen increased in the plants inoculated with *Fusarium* + *M. incognita* (8.411%), compared to non-inoculated plants (6.002%); and in Halex treatment (80.085%), compared to unfertilized plants (6.206%); decreased in plants fertilized with potassium (2%) and in plants inoculated with *Fusarium* or *M. incognita* alone. The percentage of potassium decreased in all treatments with *Fusarium* or *M. incognita*. There was no effect of the fertilization on the potassium concentration in plant tissue.

Key words: Biofertilization, *Fusarium,* Halex, *Meloidogyne*, Potassium, Tomato

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AGRI-TERRA: COLLOIDAL INGREDIENT SYNERGY AND ENVIRONMENTAL RESPONSIBILITY

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Agri-Terra, manufactured by Cal-Agri Products, LLC of Los Angeles, CA, is a material composed of relatively inert, environmentally benign materials. The efficacy of this product results from ingredient synergy and colloidal action in the soil solution. The nematology laboratory in the Agriculture Center of Louisiana State University has been involved in the formulation and evaluation of this new nematicide since 2000. Over this period, Agri-Terra has proven to be a safe and efficacious material for the management of most economically important plant parasitic nematode species found in Louisiana. In field trials in which Agri-Terra has been employed as an "*at-planting, in-furrow*" spray treatment at the rate of 10GPA of a 1% solution, significant increases in yields were observed in five consecutive years with cotton and in two of three years with soybean (McGawley, 2007a; 2007b).

In a multi-year field trial with sugarcane the first ratoon crop produced a significant increase in the sugar content per ton of sugarcane. In 2006 and 2007 Agri-Terra was evaluated for control of nematode species associated with golf course turf. Eleven of 14 golf course sites treated with Agri-Terra showed improved turf quality and nematode management within seven weeks of application (McGawley *et al*., 2008). To date, this material has been tested on 12 economically important commodities (cotton, soybean, sugarcane, rice, tomato, bell pepper, cucumber, lettuce, mustard green, cabbage, endive and strawberry). Significant growth responses have been documented on seven (cotton, soybean, sugarcane, rice, tomato, bell pepper and cucumber) of the 12 crops; significant yield increases have been documented on six (cotton, sugarcane, tomato, bell pepper, cucumber and strawberry) and significant nematode control has been demonstrated on all of the crops. The most dramatic crop responses have been observed with cotton where yield increases in fields severely infested with reniform nematode (*Rotylenchulus reniformis*), the most serious pathogen of cotton, have averaged 62% over five years.

In America, Nematologists in California, Florida, North Carolina, Minnesota and Idaho have also evaluated the efficacy of Agri-Terra. Outside of America, trials have been conducted in Spain, China and Morocco. Data from almost all of these trials is in agreement with results from research in Louisiana.

Key words: Nematicide, Colloid, Reniform nematode

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ISOLATION AND CHARACTERISATION OF *TRICHODERMA* ISOLATES FROM THE RHIZOSPHERE OF NURSERY PLANTS

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Soil-borne diseases are important limiting factors in the production of woody, ornamental and officinal plants within nurseries. Environmental conditions such as high moisture and nutrient supply as well as agronomic techniques based on mono-cropping often promote the occurrence and spread of soil-borne fungal pathogens. The potential for control of such pathogens with fungal antagonists such as *Trichoderma* spp. has elicited considerable research interest in the last two decades stimulated by concern over the environmental impact of fungicides in soil and groundwater. The success of biological control relies on efficient adaptation of a given biocontrol agent to the local environmental conditions in which it is supposed to work. Thus, the selection of antagonistic micro-organisms should take into account efficacy towards the target pathogen along with the conditions under which the biocontrol agent should perform. In the framework of an Italian national project focused on the development of low-impact control strategies for major soil-borne diseases in nurseries, a recent investigation has commenced on the composition and characteristics of *Trichoderma* species from the rhizosphere of the nursery plants, *Olea europea* (olive), *Quercus ilex* (holm oak) and *Lavandula officinalis* (lavender).

The present study was carried out at a nursery located in the Province of Viterbo (42°26'45.98"N; 12°05'56.70"E). *Trichoderma* populations associated with the rhizosphere were isolated into culture colonies using the soil dilution method. Morphological identification was made from cultures grown on PDA at ~21°C. For molecular identification, DNA was extracted from mycelium by the methods of Lee and Taylor (1990). Ribosomal ITS fragments were amplified with primers ITS1 and ITS4 (White *et al*., 1990). The *Trichoderma* population was similar among the rhizosphere of the tested plants. Prevalent species were *T. asperellum, T. hamatum* and *T. harzianum* from olive tree, *T. asperellum, T. hamatum* and *T. virens* from holm oak and *T. asperellum* and *T. harzianum* from lavender. The antagonism of a *T. harzianum* strain obtained from olive was tested in dual culture against the pathogens *Sclerotinia sclerotiorum, Rhizoctonia solani, Verticillium dahliae, Phytophthora cinnamomi,* and *Phytophthora nicotianae* and through the production of volatile and non-volatile inhibitors. Antagonism occurred through hyphal contact and lysis. Volatile metabolites of *T. harzianum* caused a general inhibition of growth of the test fungi and oomycetes.

These results will complement further studies on the characteristics of the other species isolated and their combination in soil system tests will open up the possibility

of designing future strategies for the use of local antagonistic micro-organisms in nurseries through enrichment of locally developed green compost.

Key words: Biological control, Nursery, Soil-borne fungal pathogens, *Trichoderma* isolates

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BIOLOGICAL CONTROL OF MAIN OLIVE TREE PATHOGENS USING RHIZOBACTERIA AND ACTINOMYCETES

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Rhizobacteria and Actinomycetes are very important microorganisms due to their ability to enhance soil fertility and to have antagonistic activity against a wide range of plant root-pathogens.

In the present research, isolates of 40 rhizobacteria were obtained from olive tree rhizosphere in two Tunisian regions, Tunis and Nabeul, and were screened for *in vitro* antagonism to main olive tree pathogens: *Phoma* sp., *Botryosphaeria* sp., *Fusarium* sp., *Rhizoctonia bataticola* and *Rhizoctonia solani*. Out of these putative biocontrol isolates, ten were found to be strongly antagonistic to the pathogens and inhibited their growth in culture plates; the three most active strains that also exhibited *in vivo* biological control activities against the pathogens were selected for molecular analysis.

Key words: Antagonism, Screening, Root pathogens, PGPR, ADNr 16S

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EFFICACY OF *BACILLUS* SPP.IN BIOCONTROL OF *AGROBACTERIUM TUMEFACIENS*, IN PLANT GROWTH PROMOTION AND OTHER BENEFICIAL ACTIVITIES

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A. tumefaciens is a soil borne plant pathogen bacterium causing crown gall disease and affecting many species of dicotyledonous from almost 100 different families such as woody and herbaceous plants (De Cleene and Delay, 1976). The pathogen represents a serious problem for agriculture all over the world. It reduces the marketability of nursery stock and represents a quarantine pathogen in some European countries. In Tunisia, *A. tumefaciens* is considered the main bacterial disease of stone fruit rootstocks, against which only prophylactic control measures are available. Regarding to the drawbacks of the universal biological control agents *A. radibacter* (K84 and K1026) (Penyalver and López, 1999), it still has no solution to control this disease. Therefore, this study is aimed in searching of new antagonistic bacteria to control the crown gall disease caused by *A. tumefaciens*.

A total of 162 bacteria were isolated on LB medium, collected from different Tunisian biotopes (forest, oasis, sebkha, etc.). *In vitro* essays (double layer method and agar well diffusion assay) revealed 14 effective isolates against *A. tumefaciens* C58 strain.

Six antagonists, selected according to their efficacy *in vitro* were identified as several strains of *Bacillus* spp. and investigated for their effectiveness in biological control *in vivo* performed on *Lycopersicon esculentum*. Compared to the control, three *Bacillus* strains (*B. amylolequifaciens* JS7, *B. subtilis* GO20a and *Bacillus* sp. ZO4) reduced remarkably gall formation induced by *A. tumefaciens* C58 strain. But their impact on galls' weight was more important than on galls' number.

Other than biocontrol against *A. tumefaciens* C58 strain, some of *Bacillus* spp. strains have other beneficial activities. Firstly, some of them were effective *in vitro* against different other plant pathogens (*Pythium aphanidermatum, Rhizoctonia bataticola, Fusarium solani, Pectobacterium carotovorum, Xanthomonas juglandis* and *X. campestris*). Secondly, they were able to promote root elongation in seedlings of *L. esculentum*. Finally, an interesting protease and chitinase activities were identified in some of supernatants strains cultures.

The preliminary characterization of the antibacterial compounds showed that the antibacterial activity of *Bacillus* spp. strains has a proteinaceous nature. Different temperature and pH treatments of bacterial supernatants showed an optimal antibacterial activity at 60°C and pH 7.

Key words: *Agrobacterium tumefaciens*, *Bacillus* spp., Biocontrol, Root elongation, Proteinase, Chitinase

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SUDDEN OAK DEATH IN CALIFORNIA: LINKING BIOLOGY AND DISEASE MANAGEMENT

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Sudden oak death (SOD) was first described in California in the mid 1990s and its causal agent, *Phytophthora ramorum*, was discovered less than 10 years ago (Rizzo *et al*., 2002; Garbelotto and Rizzo, 2008). The biology of the pathogen and its epidemiology are briefly discussed with an emphasis on how they are directly contributing to formulate viable and effective management and regulatory prescriptions. A strong educational effort is currently under way to help local communities implement these management options, and to compare projected disease impacts on coastal oak woodlands in the presence and in the absence of active disease management. Strong evidence indicates the causal agent is exotic and that it was introduced multiple times in North America through the sale of infected ornamental plants. The SOD epidemic thus provides one of the best examples of how forest health is at risk because of collateral effects of economic activities often totally unrelated to forestry and forest uses. It also provides a vivid example of how modern technologies can be used to improve our understanding of the epidemiology of the disease and of how basic and applied research are inextricably intertwined and both needed to formulate practical disease management guidelines (Rizzo *et al*., 2005). The efficacy of chemical and silvicultural treatments, sanitation practices and the search for resistance in hosts will all be discussed. A key in the success of any disease management lies in the understanding of the epidemiology of the disease: in the case of *P. ramorum* in California, infection is mostly linked to sporulation supported by California bay laurel leaves and to warm and rainy spells (Garbelotto *et al*., 2003). Through controlled experiments we show that: 1) bay infection can be prevented by copper-hydroxide treatments (Garbelotto *et al*., 2008), 2) oak and tanoak infection can be lowered by preventive phosphonate treatments (Garbelotto *et al.*, 2007), 3) selective removal of bay laurels will reduce both inoculum potential and oak infection, 4) composting will eliminate all inoculum from bay leaves (Swain *et al.*, 2006). We also show that wounding will increase the infection probability of oaks one order of magnitude, but that such increase disappears four months after wounding. We are currently looking for natural resistance in coast live oaks, tanoaks and bays, but all evidence so far suggests that only multilocus resistance may be present and at very low frequencies. Instead, for all three species there is a strong environmental component that determines susceptibility (Anacker *et al.*, 2008; Dodd *et al.*, 2004): we are currently investigating the possibility of utilizing such environmental variability by targeting those trees that are responsible for the over-summering of the pathogen. By eliminating such trees, an

attempt is made to break the cycles of the SOD epidemic and to lower overall levels of infection. Scaling of management is based on newly attained information on dispersal of the pathogen by using population genetics approaches.

Key words: Exotic pathogen, *Phytophthora ramorum*, Chemical control

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PETRIA 20 (2), 67-633 (2010)

IN VITRO ANTIFUNGAL ACTIVITY OF *ALOE VERA* GEL (*ALOE BARBADEBSIS* MILLER)

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Human beings, animals and plants are susceptible to infections especially those caused by fungi so much so that antifungal compounds are now becoming increasingly important in the arsenal of anti-infectives. However, this has resulted in increased occurrence of multidrug resistance of microorganisms.

Azoles and other fungicides are not always the most effective treatments. Thus, there is need for research on new antifungal products from plants or microbes (Dulger et *al*, 2006). These products pose less adverse effects on human health and the environment. Also, herbs are considered a good pool of biologically active compounds and new molecules.

Aloe vera Linne or *Aloe barbadenbsis* Miller is a succulent plant that belongs to the liliaceal family. It contains mainly water and over than 75 nutrients and 200 active ingredients. The main active ingredient of the extract of *Aloe vera* is aloin.

Many scientific studies have demonstrated that *Aloe vera* has antimicrobial (Alemdar *et al.,* 2009), antifungal and antiviral activity.

This study investigates the antifungal activity of *Aloe vera* gel against *Alternaria alternata, Botrytis cinerea* and *Curvularia lunata* (pathogens of plants), *Penicillium expansum* (an opportunistic contaminant) and *Trichoderma viride* (an antagonist agent) by determining the minimum concentration of *Aloe vera* gel that inhibits mycelial growth of the test fungi.

The concentrations of the plant extract ranged from 0 to 1000 µl/ml. Fungal plugs, 5 mm in diameter, were placed in Petri dishes with a potato–dextrose–agar (PDA) culture medium, and treated with various concentrations of the *Aloe vera* gel (pulp of *Aloe vera* leaves). The cultures were incubated at 24±2 °C and the radial growth of mycelia measured daily for 7 days. The experiment was conducted under a totally random design with four replications.The activity of *Aloe vera* extract was compared to some test chemicals: azoxystrobin, carbendazim associated with flutriafol (fungicides used for phytosanitary treatment of vegetable crops in the fields) tested at registered rates. A phytochemical analysis of *Aloe vera* gel was also made.

The results showed a total inhibitory effect of the pulp of *A. vera* leaves on *Alternaria alternata*, *Botrytis cinerea*, *Curvularia lunata* and *Penicillium expansum* (at 1000, 100, 170 and 330 µl/ml, respectively). Thus, *Altenaria alternata* was the most resistant strain while *Botrytis cinerea* was the most sensitive. In contrast, *Trichoderma viride* was considered insensitive because no inhibition occurred and, in fact, the *Trichoderma* was slightly stimulated compared to the control.

For registered rates, azoxystrobin was effective at 100% against *Botrytis cinerea* while carbendazim associated with flutriafol were active against all pathogenic species tested except *Trichoderma viride.*

The phytochemical analysis revealed the presence of four major chemical groups (alkaloids, saponins, flavonoides and tannins) and aloin whose amount was determined by HPLC.

Keywords: *Aloe vera* gel, *Aloe barbadensis* Miller, Aloin, Antifungal activity, Pathogenic fungi

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CULTURE FILTRATE OF ACTIVE ACTINOBACTERIA AGAINST *PECTOBACTERIUM CAROTOVORUM* SUBSP. *CAROTOVORUM* INDUCES DEFENSE REACTION IN TOBACCO CELL SUSPENSIONS

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Priming of plant defense reactions by plant-associated bacteria was initially demonstrated using *Pseudomonas* spp. and other Gram-negative bacteria (Conrath *et al.*, 2001). In our knowledge, fewe studies report the elicitation of defense reactions by Gram-positive bacteria (Kloepper *et al*., 2004; Conn *et al*., 2008). In the present work, we studied the ability of an actinobacterium (*Streptomyces* sp. strain OE7) to protect potatoes against *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) and to induce defense responses in tobacco cell suspensions. The *Streptomyces* sp. strain OE7 inhibits the growth of *Pcc* in liquid culture medium. Cytosolic calcium variations, production of reactive oxygen species (ROS) and programmed cell death induction (PCD) were further evaluated in tobacco BY2 cells treated with the OE7 filtrate. When applied to aequorin-expressing BY2 cells, OE7 metabolite mixture induced an increase in $[Ca^{2+}]_{cyt}$. This variation was maintained throughout the experiment without returning to resting values. After 30 min of OE7 filtrate application, we recorded an increase in luminol-mediated chemiluminescence caused by H_2O_2 release into the culture medium. Oxidative bursts reached their maximum around 3 h, then, H_2O_2 levels decreased to control levels after 5 hours. H_2O_2 production was blocked by the NADPH oxidase inhibitor DPI and Ca^{2+} chelator BAPTA, suggesting that plasma membrane NADPH oxidase was involved in H_2O_2 production and that Ca^{2+} influx was an upstream event to the oxidative bursts induced by OE7 metabolite mixture. After 24 h of pretreatment, OE7 filtrate further induces a cell death, which extent could be decreased by cycloheximide and actinomycin D, inhibitors of translation and traduction. This active cell death could thus be considered as a PCD and a defense response. This PCD, reduced by BAPTA and Tiron is thus dependant on Ca^{2+} influx and oxidative bursts. Finally, when potato slices were treated with OE7 filtrate and inoculated with *Pcc* 48 h after the treatment, a reduction in rotted tissue was observed respect to the untreated slices. As a whole, our data indicate that *Streptomyces* sp. strain OE7 filtrate induces an early defence response in tobacco cells and protects potato slices against *Pcc*.

Key words: Actinobacteria, *Pectobacterium carotovorum* subsp. *carotovorum,* Priming, Defense reactions

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Petria 20 (2), 67-633 (2010)

EVALUATION OF CERTAIN PLANT EXTRACTS AGAINST EARLY BLIGHT OF TOMATO PLANTS UNDER GREENHOUSE AND FIELD CONDITIONS

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The antimicrobial activity of six plant extracts, *Ocimum basilicum, Azadirachta indica*, *Eucalyptus chamadulensis*, *Datura stramonium*, *Nerium oleander* and *Allium sativum*, were tested in controlling *Alternaria solani in vitro* and *in vivo*.

In vitro study the leaf extracts of *Datura stramonium, Azadirachta indica* and *Allium sativum* at 5% concentration caused highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2% respectively), while *Ocimum basilicum* at 1 and 5% and *Nerium oleander* at 5% caused the lowest inhibition of mycelia growth of the pathogen (Vijayan, 1989). In greenhouse experiments the highest reduction of diseases severity was achieved by fungicides (Ridomil–plus at 2 g/l) 82.8% followed by the extracts of *Allium sativum* at 5% and *Datura stramonium* at 1 and 5% concentration (Abdel-Sayed, 2006; Abada *et al*., 2008). All treatments, plants extracts and fungicides (Ridomil–plus), significantly reduced the early blight disease as well as increased the yield of tomato compared to infected control under field condition.

The maximum reduction of diseases severity was achieved by fungicide 74.2% followed by *Allium sativum* at 5% and the minimum reduction was obtained when tomato plant was treated with *Ocimum basilicum* at 1 and 5% (46.1 and 45.2% respectively). *Datura stramonium* and *Allium sativum* at 1 and at 5% increased the fruit yield 85.7, 76.2 and 66.7% compared to infected control.

Key words: *Alternaria solani*, Tomato, Early Blight, Datura, Garlic, Neem

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FORECASTING AND MANAGEMENT OF OKRA YELLOW VEIN MOSAIC VIRUS THROUGH ITS VECTOR CONTROL IN FAISALABAD (PAKISTAN)

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Okra yellow vein mosaic virus (OYVMV) is the most serious viral disease of okra (*Abelmoschus esculentus* L. Moench). The virus induces homogenous interwoven network of yellow veins enclosing islands of green tissues within the leaf. The disease causes 20-50% reduction in okra yield and is a severe threat to its production (Pullaiah *et al*., 1998). In Pakistan the annual losses estimated due to this disease are 20-30 % but during epidemics these may be up to 90% (Safdar *et al*., 2005). Disease always comes in epidemic when environmental conditions are suitable for its white fly vector (Safdar *et al*., 2005).

Forecasting is a helpful tool to predict the OYVMV and its whitefly vector (*Bemisia tabaci*). For this purpose, seven varieties of okra were subjected to different environmental conditions and biochemical control methods to evaluate their response against OYVMV in order to forecast this disease . The severity of OYVMV exhibited significant correlation with temperature, relative humidity and net radiation. All these environmental parameters influenced differently the disease severity. Severity of OYVMV disease was recorded to be highest at 37-41^{0}C, 29-35^{0}C, 44-56% relative humidity and 9.5 to 3 Mj/m^2/day net radiation. Similarly, white fly population was also found to be influenced by temperature and rainfall, whereas all other environmental factors exhibited non-significant effect on white fly population. A prominent increase was observed in white fly population at 35-41^{0}C and 6-7mm rainfall.

Evaluation of neem (*Azadirachta indica*) extracts, effective microbes (EM) and Imidacloprid with respect to their efficacy in controlling white fly and OYVMV of okra showed 33, 53, and 66% whitefly control and 9, 12 and 17% disease reduction, respectively in comparison with untreated control. Imidacloprid gave better results against whitefly and OYVMV disease as compared to biocontrol (EM) and neem extract, so it might be recommended for use, cosidering with environmental issues.

Key words: Epidemiology, Okra yellow vein mosaic virus, White fly, Control

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CLONING AND EXPRESSION OF THE IMMUNODOMINANT MEMBRANE PROTEIN (IMP) OF CANDIDATUS *PHYTOPLASMA AURANTIFOLIA*

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Phytoplasmas are among bacterial plant pathogens which cannot be cultured in known media and cause much yield losses in different plants around the world. In plant they are mainly limited to phloem tissue and they make diverse symptoms such as yellowing, dwarfing and one specific type of symptoms known as witches' broom. They are naturally transmitted by different type of insects of the order of *Hemiptera* such as leafhoppers, planthoppers and psyllids. They are able to make infection in their specific insect vector, and in most cases need both hosts for dispersal in nature (Weintraub and Beanland, 2006)).

The witches' broom disease of lime (WBDL), caused by 'Candidatus Phytoplasma aurantifolias' is the most devastating disease of acidian lime in southern part of Iran as it destroys thousands of trees yearly throughout these regions (Bove *et al.,* 2000). The disease has been previously established in southern countries of Persian Gulf, such as Oman and UAE as well, and has become unique limiting factor for gardeners who are dealing with this crop (Chung *et al.,* 2006). Traditional methods such as eradication of infected trees and insect vector control have shown limited effect on this case. Therefore, alternative approaches, like as antibody mediated resistance, could be considered (Safarnejad *et al.,* 2009, Le Gall *et al.,* 1998).

Final aim of present study is to obtain considerable resistance against causal disease by targeting of Immunodominant membrane protein (IMP) of pathogen by means of specific recombinant antibody fragments. The IMP is a important protein which is presented in surface of phytoplasma membrane and has key role in making infection at both host plant, acidian lime, and insect vector, *Hishimonus phycitis.*

The gene encoding IMP protein of 'Ca. Phytoplasma aurantifolia' was obtained from total DNA extracted from infected plants. For this aim, specific primers containing suitable restriction site and complementary binding regions to IMP was designed by appropriate program. The considering region encoding fragment of IMP was isolated by PCR amplification followed by insertion into pZ57R/T cloning vector. Intact clone containing right sequence was selected after digestion, PCR amplification and subsequent sequencing analysis. Next, IMP encoding region having right sequence was recovered and sub-cloned into pET28a bacterial expression vector. Large scale expression of recombinant protein was performed in BL21-de3 strain

of *E. coli* and purification was carried out through Immobolized metal ione affinity chromatoghraphy (IMAC) in column containing Ni-NTA agarose beads. Successful expression and purification steps were confirmed by SDS-PAGE followed by western blotting analysis. Now, we are trying to obtain specific recombinant monoclonal antibodies from naïve libraries containing single chain variable fragments (scFv) and single domain heavy chain variable fragments (VHH). Beyond this, since IMP protein has important role in pathogenicity of phytoplasma in plant and insect, then obtained protein can be exploited for determination of pathogen-host interactions studies.

Key words: WBDL, ' Ca. Phytoplasma aurantifolia', Immunodominant membrane protein

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INDUCTION OF RESISTANCE IN CHICKPEA (*CICER ARIETINUM*) AGAINST *ASCOCHYTA RABIEI* BY THE APPLICATION OF CHEMICALS AND PLANT EXTRACTS

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Owing to scarcity of new fungicides in the market and environmental problems, the researchers are now emphasizing other alternatives such as genetic potential of plants to resistance against pathogens and the use of biotic as well as abiotic agents for the development of induce or acquired resistance. Induced resistance is a well-known phenomenon for the management of plant diseases that was first reported by (Ray, 1901) in rust diseases. Up till now various chemicals salicylic acid, isonicotinic acid (INA), benzothiadiazole (BTH), ß-aminobutyric acid (BABA), $NaClO_3$, $HgCl_2$, paraquat, polyacrylic acid, SiO_2, Messenger (Harpin protine), Phoenix (Potassium phosphate) etc., have been used as inducer of resistance against fungi, bacteria and viruses (Schneider *et al.,* 1996; Kuc, 2001; Percival *et al*., 2009). The extracts of various plants have also been explored as natural resistance inducers like *Azadirachta indica* against Alternaria leaf spot of sesame (Guleria and Kumar, 2006), *Datura metel* against *Rhizoctonia solani*, *Xanthomonas oryzae* pv. *oryzae* against *Alternaria solani* (Kagale *et al.*, 2004; Latha *et al*., 2009).

We investigate the role of resistance inducing substances (chemicals and plant extracts) in three chickpea cultivars C-44, Pb-91, Bitter-98 in a field experiments against Ascochyta blight disease. These cultivars were selected on the basis of better yield potential shown in the experiments. Aqueous solution of Salicylic acid at 0.5, 1.0 and 1.5 mM, Bion® at 0.4, 0.8 and 1.2 mM, KOH at 25, 50 and 75mM, were applied whereas the plant extracts of *Azadirachta indica*, *Datura metel* and *Allium sativum* were applied at 5, 10 and 15%. The data regarding the reduction in disease was recorded with different intervals from 4 day to 14 days after the induction and inoculation with the pathogen.

The overall results revealed that significant disease reduction (79%) was provided by Bion® in the cultivar C-44 at 1.2 mM dose rate as compared to salicylic acid, whereas the least was showed by KOH. Among the plant extracts the maximum disease reduction (46%) against the disease was observed by the application of *Azadirachta indica* leaf extract whilst the extracts of *Datura metel* and *Allium sativum* did not prove effective in reducing the disease. The present findings suggest that enhancement of resistance before infection of chickpea plants could be an innovative control method for ascochyta blight of chickpea.

Key words: Induced resistance, Chemicals, Plant extracts, Reduction

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AN INTEGRATED MULTIVARIATE APPROACH TO NET BLOTCH OF BARLEY: VIRULENCE QUANTIFICATION, PATHOTYPING AND A BREEDING STRATEGY FOR DISEASE RESISTANCE

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Net blotch is a major foliar disease that is widely distributed in most of barley-growing regions of the world (Steffenson and Webster, 1992; El Yousfi and Ezzahiri, 2001). Knowledge of pathotype diversity and virulence in local populations of *Pyrenophora teres* is a prerequisite to screen for durable resistance to net blotch.

The current study aimed at quantifying the virulence level of moroccan isolates, identifying pathotypes, and selecting resistant genotypes. We developed a method to quantify virulence of *P. teres* isolates based on a conversion of infection responses into frequencies for use in correspondence analysis (Greenacre and Hastie, 1987). Coordinates of the first axis of this analysis had a virulence spectrum as a biological meaning and ranked isolates from virulent to avirulent. Mixed model analysis was also devised for quantifying virulence. Coordinates of the first dimension of correspondence analysis were linearly correlated to BLUPs (Best Linear Unbiased Predictors) of mixed model. GGE model (Yan et al., 2000) coupled with cluster analysis differentiated *P. teres* isolates into ten and nine pathotypes for net- and spot-forms, respectively. Populations of these two forms were not s imilar in terms of classes of virulence. For *P. teres* f. *maculata*, avirulent , moderately virulent and highly virulent isolates represented one-third of the population, whereas 90% of *P. teres* f. *teres* population was composed of avirulent to moderately avirulent isolates.

Barley differential sets were subsequently reduced to two new sets that simplified pathotyping through a key code that is based on resistance or susceptible reactions. Dendrograms of cluster analysis based on GGE analysis depicted genotype's reaction stability across all isolates, and using only resistant cultivars as sources of resistance from this analysis to control net blotch disease would not control all pathotypes. Therefore, we propose an alternative breeding strategy to control net blotch effectively.

Key words: *Pyrenophora teres*, Correspondence analysis, GGE model, Mixed model, Pathotypes, Quantifying virulence

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REACTION OF TWO MAIZE POPULATIONS TO S_1 LINE RECURRENT SELECTION UNDER LEAF BLIGHT STRESS

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Recurrent selection is a cyclic breeding technique that has become popular in comprehensive maize improvement strategies. Evaluation of S_1 lines *per se* is a common method of recurrent selection for improving quantitatively inherited traits in maize (Hallauer, 1992). To evaluate the efficacy of S_1 line recurrent selection for yield and yield related traits in maize under southern corn leaf blight (SCLB) stress, two cycles of S_1 line recurrent selection were conducted in maize populations Azam and Sarhad White (SW). A selection intensity of 20% was used in each selection cycle. During spring 2007 (March-June), the base populations from the third cycle of S_1 line recurrent selection were sown in the field and manual self pollination was carried out for S_1 line production. During the following season (July-October), the 196 S_1 lines (each for Azam and SW) along with the original populations as check were evaluated for resistance to SCLB under artificial inoculation, maturity traits and yield components. The same process was repeated during 2008.

Highly significant differences ($P < 0.01$) were observed among the S_1 lines of both populations for all the traits studied. High heritability estimates were observed for most of the traits except anthesis-silking interval and prolificacy. Observed response was higher than expected, for most traits in both populations (Pixely 2006; Pratt and Gordon, 2006). The two cycles of S_1 line recurrent selection significantly decreased the days to mid-anthesis and mid-silking in both populations. Anthesis-silking interval was significantly reduced in Azam (-26.4%). Ear height, ear length, kernel rows ear^{-1}, 1000 kernel weight and grain yield increased significantly by 5.9%, 5.1%, 1.5%, 5.6% and 8.6% for Azam while 1.7% (non-significant), 7.1%, 6.7%, 2.6% and 13.4% for SW population, respectively. There was a declining trend in SCLB severity for both Azam (-9.8%) and SW (-9.1%) population as desired. This increase in SCLB resistance can be credited as the main cause of yield improvement (Carson, 2006; Rahman et al, 2005).

These results demonstrated that S_1 line recurrent selection effectively improved SCLB resistance and grain yield in Azam and Sarhad White maize populations.

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COMPARISON BETWEEN METHODS OF POTATO EVALUATION FOR RESISTANCE TO *ALTERNARIA SOLANI* EARLY BLIGHT BY GREENHOUSE TEST AND *IN VITRO* ASSAY

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Potato (*Solanum tuberosum*) early blight, caused by *Alternaria solani*, is one of the most destructive fungal foliar diseases, particularly in hot climates where potato are produced under irrigation. The use of resistant varieties is the most important and environmentally safe method to control this disease thus it is very important to be able to use a reliable and rapid method for detection of resistance sources.

During this study, effects of a culture filtrate of *A. solani* on potato by an *in vitro* assay and greenhouse tests were compared to select resistance genotypes to the early blight. Virus free plantlets of potato and a virulent isolate of the pathogen were obtained from the National plant gene-bank of Iran –Seed and Plant Improvement Institute. First, the potato plantlets were multiplied *in vitro* on a MS medium and then divided into two groups: one inoculated with the culture filtrate of *A. solani* and the other transferred to pots for adaptation to greenhouse conditions. *In vitro* leaflets received a 1000-µl droplet of the *A. solani* culture filtrate; plants in the greenhouse were inoculated by spraying to run off with a suspension of 10^5 conidia/ml of *A. solani*. The experimental design was factorial on basis of completely randomized design (CRD) with two factors, three replications and five potato genotypes. During the *in vitro* assay, symptoms appear 1-2 days after inoculation while in the greenhouse test symptoms appear 3 days after inoculation. The AUDPC was calculated by daily evaluation of symptoms and analyzed using Duncan test (a= %0.01).

Significant differences among potato genotypes was observed in both methods ($p<0.01$) and the results were similar. It has been concluded that the *in vitro* assay can be used as a rapid and reliable evaluation method for potato resistance against *A. solani* early blight.

Key words: *Alternaria solani*, *Solanum tuberosum*, Resistant, AUDPC

Acknowledgements

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SUNFLOWER BREEDING MATERIAL TESTING TO *DIAPORTHE HELIANTHI*

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Phomopsis helianthi Munt.-Cvet. *et al.* (teleomorph *Diaporthe helinathi* Munt.-Cvet. *et al.*), is one of the most important sunflower pathogen in Europe, and has a great influence on grain and oil yield. It is first recorded on 1981 in Yugoslavia (described by Mihaljčević *et al.*, 1982) and from than is expanded all over the world and become one of the most prevalent diseases of cultivated sunflower. In favorable environmental conditions for disease development, it could cause significant decrease of grain yield (10-50%) and oil content (Laville, 1986; Duvnjak *et al*, 2006).

Growing resistant hybrids is the most effective measure for disease control. However, there are no completely resistant genotypes and the main challenge to the breeders represents searching for source of resistance and introducing it to genotypes with valuable agronomic traits. In sunflower breeding aimed on disease tolerance, artificial infection in controlled (laboratory) or uncontrolled (field) conditions is essential.

The aim of investigation was to estimate tolerance of 12 genotype created in the frame of the Agricultural Institutes Osijek's sunflower breeding program (2 cytoplasmatic male sterile (cms), A lines; 3 male fertile (mf), B lines; 2 restorers of fertility (rf), R lines; and 5 single cross, SC hybrids), on pathogen *D/P. helianthi* by artificial infection method in field. Investigation was conducted during four consecutive years (2006 - 2009) at the experimental field of the Agricultural Institute Osijek (Croatia). Plants were inoculated in full button stage (R2, according to Schnieter and Miller, 1981). Circular sector of mycelia discs cut from the periphery of the colonies growing in laboratory (Petri dishes with PDA, 12/12 light regime, 25°C) was lay-down on leaf stalk intercept (2-3 cm long) from one of mid-stem leaves. Infection spot was covered with a piece of wet cotton and aluminum foil to prevent mycelia dryness and create favorable micro-climatic conditions for pathogen development. Susceptibility estimation (material tolerance) was performed by measuring length of lesions over 3 measurements during three weeks after infection, each 7 days. Analysis of variance (ANOVA) and LSD test were processed by Statistical Analysis System for Windows software (SAS Institute, 2003).

Results of investigation show significant statistical differences among investigated years. The highest susceptibility genotypes were shown in 2007, and the lowest in 2009. Statistically significant differences in susceptibility on pathogen were established among tested genotypes. In average, A-lines were shown the

highest susceptibility on pathogen (3.42 cm), while SC hybrids were shown the lowest susceptibility (2.54 cm). The most susceptible genotype in investigation was A-line L-G/04 (3.75 cm), while the most resistant genotype was SC hybrid L-G/04A x L-190B (1.96 cm). In general, these results show that testing of susceptibility of SC hybrid parental components on *D/P. helianthi* contribute to advance in sunflower breeding program.

Key words: Breeding material, Sunflower, Artificial infection, *Diaporthe/Phomopsis* helianthi, Tolerance

Acknowledgements

This study was carried out within the projects of The Agricultural Institute Osijek '*Diaporthe/ Phomopsis* spp. and *Sclerotinia sclerotiorum* on soybean and sunflower', and "Stability of sunflower genotypes on important agronomic traits and oil quality" financed by the Ministry of Science, Education and Sports of the Republic of Croatia.

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SUSCEPTIBILITY OF OLIVE TREE HYBRIDS TO LEAF SPOT (*FUSICLADIUM OLEAGINUM*)

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The mitosporic fungus *Fusicladium oleaginum* is an obligate biotroph of olive (*Olea eurpea*) causing leaf spot disease (Benitez *et al*., 2005). This disease causes important yield losses of olives especially in the Mediterranean region (Trapero & Blanco, 2001). We have studied the susceptibility to the disease of 40 olive hybrids in an orchard located at Oued Souil (Nabeul), Tunisia. We have also examined the possible roles of polyphenol composition and density of trichomes in resistance to olive leaf spot.

Evaluation of the incidence of the disease in orchards (apparent infestation) and laboratory tests (latent infestation and leaf inoculation) showed variability of resistance to the disease among the collection of hybrids. Preliminary results of susceptibility parameters indicate that trichomes on olive leaves are one of the factors involved in the resistance. Qualitative phenol analysis revealed no correlation with the hybrids' response to pathogen. Further studies on leaf content of polyphenols and cuticle thickness are in progress in our laboratory.

Key words: *Olea europea*, Susceptibility, Polyphenols, Trichomes, Leaf cuticle

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SESSIONE 7

Control strategies

POSTERS

Petria 20 (2), 67-633 (2010)

ANTIOXIDANT DEFENCE IN THE *FUSARIUM VERTICILLIOIDES*-MAIZE PATHOSYSTEM

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About one quarter of the world's food crops are contaminated by mycotoxins (Magan *et al*., 2004). Fungal contamination harms grain yield and quality, reduces production from livestock fed with contaminated cereals, and causes mycotoxin-related human health problems (Charmley *et al*., 1994). Maize (*Zea mays* L.) is susceptible to pre- and post-harvest contamination by toxigenic fungi (Ominski *et al*., 1994). These include *Fusarium* spp. which produce several mycotoxins (Logrieco *et al*., 2007) including fumonisins. Efficient control of fumonisin contamination is lacking, and it will rely on further knowledge of infection processes and plant responses. Oxidative burst is an early plant response to pathogen infection, characterized by elevated production of reactive oxygen species (ROS) that are potentially toxic to cells. Antioxidant systems control ROS levels. Oxidative burst is a response to mycotoxins in some plant-fungus systems (Paciolla *et al*., 2008).

We have studied the detoxifying enzymes ascorbate peroxidase (APX), catalase (CAT), total peroxidase (POD) and superoxide dismutase (SOD) in plants of both a susceptible and a resistant maize hybrid inoculated *F. verticillioides* wild type or a mutant strain defective in fumonisin production. Detoxifying systems in the resistant hybrid indicate a plant mechanism to counteract pathogen toxicity. The APX and SOD activities were greater in resistant plants than in susceptible plants. Elevated APX activity (high affinity for H_2O_2) in the resistant hybrid was correlated with high SOD activity, which scavenges toxic anion superoxide to form H_2O_2. In the susceptible plants, however, activity of CAT and POD, which also control cellular H_2O_2 levels, did not have defence roles against *F. verticillioides*. The resistant hybrid resisted pathogen colonization and mycotoxin accumulation. After wild-type or mutant fungal attack, the resistant hybrid enzyme activity was the same as in uninoculated plants.

This work provides information on the *F. verticillioides*-maize pathosystem. Similar enzyme activity analyses could be applied in other maize/fungus associations to increase the rate of host resistance selection.

Key words: Antioxidant systems, Resistance selection

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EFFECTS OF CROP ROTATION ON WHEAT TAKE-ALL EPIDEMICS IN MAZANADARAN PROVINCE, IRAN

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Take-all, caused by *Gaeumannomyces graminis* var. *tritici*, is one of the most important root and foot rot diseases of wheat in many areas of the world (Walker, 1975), and it is an important disease in the Northern parts of Iran. Crop rotation is the best cultural practice for minimizing the yield losses caused by the disease (Colbach *et al*., 1997; Gilligan and Brassett, 1990). In a five year experiment the effects of wheat after wheat, oilseed rape or barley on take-all epidemics were evaluated. These are the only autumn or winter crops that are widely grown in Mazandaran Province. There were significant differences in take-all incidence, when wheat was followed by the other crops. The incidence and severity of the disease were greater in plots where wheat followed wheat. Wheat after oilseed rape showed reduced take-all incidence and severity compared to the other crops. Thus, oilseed rape is likely to be the best candidate as a rotation crop for reducing take-all risk in Mazandaran Province.

Key Words: Barley, *Gaeumannomyces*, Oilseed rape, Take-all, Wheat

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USE OF *BRASSICA JUNCEA* GREEN MANURE FOR THE CONTROL OF CROWN AND ROOT ROT OF WHEAT IN ITALY

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Crown and root-rot (CRR) is a disease of complex aetiology in which several fungal species are involved, among which *F. graminearum*, *F. culmorum* and *Microdochium nivale* play an important role. The disease is well known in Italy, and its economical importance is growing in the last years (Santori and Infantino, 2009). Typical symptoms of the disease are browning and rotting of the crowns and roots, often causing whitenings of the heads containing shrivelled kernels of no commercial value. Apart of seed dressing, control of the disease is problematic, due to the difficult application of chemicals to the soil and to the scarce availability of resistant varieties. Crop rotation could be effective, but its use is sometimes limited by the non profitability of available break crops.

Biofumigation is the use of *Brassica* green manure for the control of many soil-borne pathogens, namely fungi and nematodes (Matthiessen and Kirkegaard, 2006). The principle of its action is the release of isothiocyanates into the soil upon hydrolization of the glucosinolates present in many *Brassica* spp. by the activity of a mirosinase, after mechanical breakage of the tissues. However, any eventual impact of biofumigation on non-target soil organisms other than those involved in CRR has to be verified. In fact it is known that some fumigation practices could affect the soil microbial communities, reducing soil biodiversity and fertility (Mocali *et al*., 2008). Aims of the present work are: i) to verify the efficacy of *Brassica juncea* green manure in the control of CRR of wheat; ii) to monitor soil microbial activity and both genetic and functional changes in the soil microbial communities after biofumigation. To these aims, half of a field with a known high CRR incidence was sown with *B. juncea* var. Scala at the CRA-PAV experimental farm at Monterotondo (Rome, Italy). After chopping and incorporation of 60 days-old plants into the soil, durum wheat var. Liberdur was sown. The disease progression is being studied by checking the incidence of the most important fungal pathogens present in the soil and by analyzing the incidence and severity of the disease on wheat at different growth stage until harvest. Preliminary mycological analyses showed *M. nivale* as the species most frequently isolated from wheat seedlings. Treatment with *B. juncea* reduced incidence of *M. nivale* of 83.0 % as compared to the check. The microbial activity is being monitored by means of biochemical soil indicators, such as C-biomass

and microbial respiration, whereas the genetic and functional diversity were be analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) and Community Level Physiological Profile (BIOLOG), respectively. Preliminary results showed a significant decrease of microbial activity, suggesting a widespread impact of the treatment on non-target soil microbial communities. The final results will increase our knowledge for the safe use of biofumigation for the control of soilborne diseases of extensive crops as wheat.

Key words: *Brassica juncea, Fusarium,* Plant diseases, Biofumigation, Biodiversity

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ANTAGONISM OF MELON SOIL-BORNE FUNGAL PATHOGENS BY FUNGI ISOLATED FROM COMPOST

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The soil-borne plant pathogens *Fusarium oxysporum* f. sp. *melonis* and *Monosporascus cannonballus* are among the most damaging fungal pathogens of melon grown under greenhouse conditions (Chilosi *et al*., 2008). Chemical methods used to control such pathogens are either not very efficient or cause negative effects on environmental and human health. Within a sustainable disease management strategy for horticultural crops, the use of compost is particularly useful due to its general effectiveness in decreasing soil-borne fungal diseases (Bonanoni *et al.*, 2007).

The aim of this study was to isolate antagonistic fungi from compost made with green and municipal waste and evaluate their *in vitro* effects on *F. oxysporum* f. sp. *melonis* (FOM) and *M. cannonballus* (MC). In addition, greenhouse experiments were carried out to determine the effect of composts on the incidence of these diseases.

Fungi were evaluated on the basis of their antagonism *in vitro*. The antifungal activity of each compost fungal isolate against FOM and MC was studied using the dual culture technique. Morphological identification was made from cultures grown on PDA. Prevalent antagonistic fungal species belonged to the genera *Trichoderma*, *Penicillium* and *Aspergillus*. For molecular identification, DNA was extracted from mycelium by the methods of Lee and Taylor (1990). Ribosomal ITS fragments were amplified with primers ITS1 and ITS4 (White *et al*., 1990). Dual culture experiments showed that all test fungi significantly inhibited mycelial growth of FOM and MC compared to the untreated control. Inhibition of FOM varied from 25% for *Penicillium* spp. to 60% for *Trichoderma* spp.; inhibition of MC varied from 40% for *Penicillium* spp. to 100% for *Trichoderma* spp. Experiments under greenhouse conditions showed that compost amendment was capable of reducing the severity of collapse caused by MC on melon.

The present results show that compost amendments can play an important role in reducing economic losses from soil-borne diseases of melon, especially under greenhouse conditions.

Key words Antagonistic fungi, Compost, *Monosporascus cannonballus, Fusarium oxysporum* f.sp. *melonis*

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CONTROL OF SOIL-BORNE PATHOGENS BY MICROORGANISMS SELECTED FROM COMPOST

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Suppression of soil-borne plant diseases with composts has been widely studied. Composts have been found to be suppressive against several soil-borne pathogens in various cropping systems (Noble and Coventry, 2005). However, an increase of some diseases due to compost usage has also been demonstrated, since compost is a product that varies considerably in chemical, physical and biotic composition, and, consequently, also in ability to suppress soil-borne diseases (Termorshuizen *et al.,* 2006; Pugliese *et al.*, 2008). Carisse *et al.,* (2003) isolated microorganisms from composts and tested them for control of damping-off of cucumber caused by *Pythium ultimum*. Microorganisms showed different levels of disease control when assessed one by one.

The objective of the present work was to isolate microorganisms from a suppressive compost and to test them for their activity against soil-borne pathogens. A compost originated from green wastes, organic domestic wastes and urban sludges that showed a good suppressive activity in previous trials was used as source of microorganisms. Serial diluted suspensions of compost samples were plated on five different media: selective for *Fusarium* sp., selective for *Trichoderma* sp., selective for oomycetes, potato dextrose agar (PDA) for isolation of fungi, lysogeny broth (LB) for isolation of bacteria. In total, 101 colonies were isolated from plates and tested under laboratory conditions on tomato seedlings growing on perlite medium in Petri plates infected with *Fusarium oxysporum* f. sp. *radicis-lycopersici* and compared to a commercial antagonist (*Streptomyces griserovidis*, Mycostop, Bioplanet). Among them, 28 showed a significant disease reduction and were assessed under greenhouse condition on three pathosystems: *Fusarium oxysporum* f. sp. *basilici* on basil, *Phytophthora nicotianae* on tomato and *Rhizoctonia solani* on bean.

Fusarium spp. selected from compost generally showed a good disease control against *Fusarium* wilts, while only bacteria significantly controlled *P. nicotianae* on tomato under greenhouse conditions. None of the microorganisms was able to control the three soil-borne pathogens together, in particular *Rhizoctonia solani*. Results confirmed the good suppressive activity of the compost under study against soil-borne pathogens.

The selection of antagonists from compost is a promising strategy for the development of new biological control agents against soil-borne pathogens.

Key words: Suppressiveness, Composting, Wastes, *Fusarium*, *Trichoderma*

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IN VITRO AND *IN VIVO* ANTAGONISTIC ACTIVITY OF *BACILLUS* SPP. FROM ORGANIC COMPOST AGAINST *FUSARIUM OXYSPORUM* F.SP. *MELONIS*

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In central Italy, the causal agent of melon vascular wilts *Fusarium oxysporium* f. sp. *melonis* (FOM) is the main fungal pathogen of melon (*Cucumis melo* L.), causing a crucial problem and economic losses (Chilosi *et al.,* 2008). Recently, the adopted methodology for suppression of soil-borne pathogens, such as *Fusarium oxysporum*, *Pythium* and *Phytophthora* species, is using the organic amendment, which is particularly important not only to improve plant growth but also to make the plant less susceptible to pathogen infection; this suppression relates to both physiochemical and microbiological features of the substrates (McKellar and Nelson, 2003; Mazzola, 2002).

The aim of this study was to investigate the *in situ*, *in vitro* and *in vivo* inhibition and suppression activities of antagonistic bacteria from commercial compost (ECOS) towards FOM. The bacteria isolated and identified from the compost were generally found belonging to the aerobic spore forming bacterial group (*Bacillus* spp.).

In situ assay was usually designed to detect the suppression effect of the matured compost, in which autoclaved and non-autoclaved compost was placed onto the center of the fungi seeded plate's surface. *In situ* results had indicated considerable decrease in fungal growth in plates containing non-autoclaved compost compared to the autoclaved one.

Moreover, the suppressive effect of the compost against the fungi under test was examined using two *in vitro* assays: the pouring method and the dual culture-plate method on PDA (Mila Santos *et al*., 2008). The pouring method was more efficient to find the bacterial strains present in the compost that have an important role in suppressing the growth of tested fungi by producing clear inhibition zones. The spore forming bacteria were able to produce diffusible antifungal compounds, neither HCN nor siderophores. Based on the results obtained in the *in vitro* assays, four *Bacillus* spp. were selected to be tested for their suppressive effect in further bioassays *in vivo*.

The findings reported here demonstrate that the use of microbial communities to induce a disease suppressiveness of soil could be a potential tool for the management of soil borne pathogens.

Key words: Melon, Soil borne pathogen, Spore forming bacteria, Suppressiveness, Antagonism

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EFFECT OF ESSENTIAL OILS ON THE POPULATION DENSITY OF *FUSARIUM OXYSPORUM* F. SP. *PISI* IN SOIL AND CONTROL OF FUSARIUM WILT

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Wilt of peas (*Pisum sativum* L.), caused by *Fusarium oxysporum* Schlecht. f. sp. *pisi* (van Hall) Snyd. & Hans. (Fop), is a serious economic threat to pea production. Several methods have been used to evaluated pea sedlings for resistance to Fop. Biologically based and environmentally safe alternatives, such as biological control agents, natural plant products, and cultural methods, are being investigated for possible use as components in integrated management programs (Bowers and Locke, 2000).

In this study the antifugal effect of potassium phophite (Kalex), known to increas crop resistance to diseases, and 4 different plant extracts [*Cinnamomum zeylanicum* L. (Cinnamon), *Thymus serpyllum* L. (White Thyme), *Cymbopogom nardus* L. (Lemongrass), *Origanum vulgare* L. (Oregan)], reported as the most active on *Fusarium oxysporum* (Barrera-Necha *et al*., 2009; Pawar and Thaker, 2006; Daouk *et al*., 1995), have been evaluated on 2 isolates of *F.oxysporum* f. sp. *pisi*, CBS127.73 (Fop1) and R2B F871 (Fop2), isolated from *Pisum sativum* L. in UK and USA respectively. Soil artificially inoculated with Fop1 and Fop2 was treated with a different concentrations of oil emulsions and potassium phosphite (1, 5, and 10%). The survival of Fop1 and Fop2 was evaluated at different times after treatments (0, 1, 3, 7, 14, and 21 days) by dilution plate technique as CFU/cm^3 of pot soil (Bowers and Locke, 2000). The effect of treatments on seedlings was assessed on the base of percentage of seed gemination and symptoms developing.

Significant differences between Fop1 and Fop2 have been observed ($P<0.05$). After 21 days, all plant extracts, but not potassium phosphite, were the most effective to inhibit the growth of the Fop1 isolate at each concentration ($P<0.05$). Treatments with 5 and 10% acqueous emulsions of oregan and 10% acqueous emulsions of lemongrass significantly reduced the population density of Fop2 isolate ($P<0.05$).

The possible use of oil extracts in the management and control of disease caused by *F. oxysporum* f. sp. *pisi* is discussed.

Key words: *Pisum sativum*, *Fusarium oxysporum* f. sp. *pisi*, Wilt, Plant Extracts

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EVALUATION OF PLANT-GROWTH PROMOTING RHIZOBACTERIA FOR GROWTH PROMOTION AND BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *RADICIS-LYCOPERSICI* ON TOMATO

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Fusarium oxysporum f. sp. *radicis-lycopersici* (*Forl*) is the causal agent of tomato foot and root rot (TFRR), one of the most important diseases of tomato worldwide (Jones *et al.*, 1991). The relatively poor efficacy of chemical control and the lack of resistance in some commercially important tomato cultivars have focused attention on the feasibility of biological control of the pathogen (Baysal *et al*., 2008)

In this study, laboratory experiments were conducted to evaluate four *Bacillus* strains of plant-growth promoting rhizobacteria (PGPR) and their mixtures, as biological control agents against *Forl*. The influence of the PGPR strains on tomato growth was also estimated.

The rhizobacteria used for the study were *Bacillus amyloliquefaciens* IN937a, *Bacillus pumilus* SE34 and the commercial products of *B. subtilis*, GB03 (Companion®) and FZB24 (FZB24® li.). PGPR strains were applied as a soil drench just after seeding and a second application was repeated 20 days later with bacterial suspensions (Anith *et al.*, 2004). Tomato roots were inoculated with *Forl* when the seedlings were 4 weeks old.

Disease severity measurements 15 days after plant inoculation with the pathogen showed that treatment with IN937a+GB03 resulted in the highest biocontrol protection (63%), followed by GB03, FZB24+GB03 and SE34+GB03, which resulted in 50, 43 and 37% protection, respectively, compared to the untreated control. Treatment with IN937a+GB03 demonstrated significantly lower levels of disease than any individual PGPR strain, indicating either additive or synergistic effect on disease reduction achieved by mixing PGPR strains. Treatments with IN937a and SE34 did not reduce the disease incidence significantly compared with the positive control.

For growth promotion assays, several plant growth characteristics namely shoot height, shoot fresh and dry weight were measured. In general, all PGPR applied singly or in combinations caused positive growth responses among all the parameters measured under laboratory conditions compared to the nonbacterized controls.

Specifically, shoot height, shoot fresh and dry weight were promoted by all bacterial applications by 18-57%, 22-55% and 38-63%, respectively, compared to the untreated control. Among them, plants treated with strain SE34 showed the most significant increases in the three plant-growth parameters tested.

Key words: Biological control, Tomato foot and root rot, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, PGPR

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ANTIOXIDANT DEFENCE IN *FUSARIUM VERTICILLIOIDES*/MAIZE PATHOSYSTEM

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Maize (*Zea mays* L.) is a plant matrix susceptible to fungal contamination both pre- and post-harvest. The main genera found are *Fusarium*, *Aspergillus* and *Penicillium* (Ominski *et al*., 1994). *Fusarium* species are important colonizers of maize because of their ability to produce several mycotoxins such as fumonisins, moniliformin, trichothecenes, beauvericin and fusaric acid (Logrieco *et al*., 2007). Efficient control strategies for fumonisin contamination are still lacking and their development relies on a deeper knowledge of the infection process and of the plant response. It is known that an early plant response to pathogen infection consists in an oxidative burst characterized by high production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion that are potentially toxic for the cells. It has been reported that the higher ROS level occurring in some plant-fungal interactions may be correlated with the type of mycotoxin produced (Paciolla *et al*., 2008). Antioxidant systems, such as detoxifying enzymes and low-molecular weight antioxidants, control the level of ROS. In this work we have studied the detoxifying enzymes ascorbate peroxidase (APX), catalase (CAT), total peroxidase (POD) and superoxide dismutase (SOD) during the defensive strategy activated by susceptible or resistant maize hybrid plants five days after treatment with a *F. verticillioides* wild type or with a mutant strain defective in fumonisin production.

The two tested maize cultivars show a difference in antioxidant defence enzymes. In resistant plants the APX and SOD enzymatic activities were higher than in susceptible ones. In this respect the high activity of APX, enzyme with a high affinity for hydrogen peroxide, shown by resistant maize hybrid respect to the susceptible, was correlated to the high SOD activity, enzyme considered an efficient scavenger of toxic anion superoxide to form hydrogen peroxide. On the other hand, in susceptible plant, the higher activity of CAT and POD, other enzymes controlling H_2O_2 cellular level, seems not to have an important role in the defence response activated by plant against *Fusarium verticillioides*.

The fungal-resistant maize cultivar shows a higher constitutive capacity to resist pathogen colonization and mycotoxin accumulation than the susceptible cultivar. The potentiated detoxifying systems occurring in maize resistant hybrid

respect to the susceptible one suggest the presence of a strategy developed by plant useful to counteract the toxicity of pathogen. This work provides novel information on the *F. verticillioides*-maize pathosystem and also will allow, by the extension of the analysis of enzymatic activities in other maize cultivars considered resistant to pathogens different by *F. verticillioides*, to evaluate if these antioxidative enzymes could be considered useful hallmarks for fast plant resistance selection programmes.

Key words: Antioxidant systems, *Fusarium verticillioides*, Maize, Mycotoxins, Resistance

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BIOLOGICAL CONTROL OF *COLLETOTRICHUM ACUTATUM* BY *TRICHODERMA* IN STRAWBERRY FIELDS

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Anthracnose is one of the major fungal diseases of strawberry (Howard *et al*., 1992). In 1998, *Colletotrichum acutatum* J.H. Simmonds was first reported in Huelva (De los Santos and Romero, 1999), the most important area of strawberry (*Fragaria* x *ananassa* Duch.) production in Europe. Considerable yield loss can be inflicted by the pathogen under the appropriate environmental and cultural conditions. Chemical, physical, and biological alternatives to methyl bromide (MB) have been evaluated in strawberry fruit production (Duniway, 2002; Moser *et al*., 2008; Porras *et al*., 2007).

The objective of this research was to evaluate the use of *Trichoderma* spp. for biocontrol of anthracnose. Large scale field experiments were conducted in a strawberry crop located in Moguer (Huelva, SW Spain), from October to May for three consecutive growing seasons, to evaluate the use of *Trichoderma* biocontrol of anthracnose (*Colletotrichum acutatum*). *Trichoderma harzianum* and *T. viride* (Tusal®) were applied via drip irrigation and dip, by addition to the soil 7-days before planting (10^8 conidia/m^2), and by dipping strawberry roots in a suspension of *Trichoderma* (10^6 conidia/ml) immediately prior to planting. A randomised complete block design with four replications was used. Each plot was 12.5 x 3.3 m and had three raised beds. Plants from the nursery were examined to detect latent infections of *C. acutatum*.

The highest percentage of anthracnose infected transplants was detected in the second year (16% of plants from the nursery). Crown infections were initiated in the nursery but were not apparent until after plants were set in production fields. The fungus continued to grow in infected plants, which later died suddenly following warm weather in the autumn and the following spring. *Trichoderma* applications significantly reduced anthracnose disease incidence and plant mortality by 69.3% relative to the untreated control. Solarization alone did not reduce anthracnose disease incidence. Nevertheless, the combination of solarization and *Trichoderma* reduced disease relative to the untreated control, although this was not significantly different to the *Trichoderma* alone treatment.

This work contributes to the development and optimization of *Trichoderma* biocontrol as an alternative to traditional chemicals for control of *C. acutatum* in strawberry production.

Key words: Biological control, *Fragaria* x *ananassa,* Anthracnose, *Colletotrichum acutatum, Trichoderma harzianum, Trichoderma viride*

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SUPPRESSING *RHIZOCTONIA SOLANI* ROOT ROT OF POINSETTIA WITH COMPOST

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Root and stem rot fungi occur throughout the nursery causing huge losses at any stage of plants development. *Rhizoctonia solani* Khün is one of the most cosmopolite plant pathogens, causing root, stem and foliar diseases of many important herbaceous and woody ornamentals, among which Poinsettia (*Euphorbia pulcherrima* Wild. ex Klotzsch) (Chase 1998). Synthetic chemicals and fumigants have been widely used to prevent, control or eradicate this soilborne plant pathogen. Although effective, their continued applications may cause ecological and management consequences such as water and soil pollution. The study of alternative methods for plant disease control in agriculture and nursery is required (Krause *et al*., 2001).

The objective of the present work is to assess the efficacy of a compost mix for the suppression of Rhizoctonia rot of Poinsettia. Compost was made by organic wastes, which included organic wholesale vegetable market and wood wastes at a 7:1 ratio. Young Poinsettia plants were transplanted in pots containing 0, 20, and 40% of the compost mix and inoculated with *Rhizoctonia solani* AG4 (Phillips, 1991). Compost suppressive ability was evaluated in a growth chamber assay. The percentage of dead plants and the Area Under Disease Progress Curve (AUDPC) (based on disease severity repeated assessments) were used to evaluate the effectiveness of compost treatments against Rhizoctonia rot as suggested by Termorshuizen *et al*. (2007).

Compost was effective in controlling Rhizoctonia rot of Poinsettia only at a percentage of the 20% reducing plant mortality of about 26% and reaching a suppressivness of about the 25%. However, an increase of the disease at the highest concentration of compost (40%) has been observed. Data reported in literature for other pathosystems showed that chemical characteristic of the product could strongly influence its disease suppressive properties (Hoitink and Fahy, 1986; Hoitink *et al*., 1996).

Key words: *Euphorbia pulcherrima*, Root Rot, Biological Control

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BIOCONTROL OF DAMPING-OFF CAUSED BY *SCLEROTIUM ROLFSII* AND *FUSARIUM OXYSPORUM*

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Two isolates of *Trichoderma harzianum* selected for their ability to control *Sclerotium rolfsii in vitro* (Davet and Roure, 1986; Khattabi *et al.*, 2001and 2004; Henis and Papavizas, 1983) were tested for their ability to control the damping-off of tomato and some leguminous plants caused by *Fusarium oxysporum* or *Sclerotium* rolfsii in natural soil. A similar test was carried out in soil amended with manure.

The antagonists reduced the damping-off caused separately by *Sclerotium rolfsii* or *Fusarium oxysporum*. The highest effect was observed when *Trichoderma harzianum* was added to the amended soil. Manure in combination with *Trichoderma harzianum* reduced the percentage of plant mortality. It seemed that manure favored the development of each isolate of *Trichoderma harzianum* and enhanced their antagonistic effect on *Sclerotium rolfsii.* The combined use of *Trichoderma harzianum* and manure offered a valuable option to contribute to the biological control of damping-off caused by *Sclerotium rolfsii.*

Key words: *Trichoderma harzianum,* Antagonism, Combination, Manure, *Sclerotium rolfsii*

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CHANGES IN GROWTH, PEROXIDASE ACTIVITY AND PHENOLIC COMPOUNDS CONTENT OF ONIONS TREATED WITH *TRICHODERMA ASPERELLUM*

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In Mexico, onion is the third vegetable of greater consumption. Onions suffer from root diseases caused by species of *Sclerotium*, namely white rot (*Sclerotium cepivorum*) and southern blight (*Sclerotium rolfsii*). Chemical control methods against these diseases are used, but they can cause environmental problems; the use of biological control techniques have also been proposed for onion production, i.e. the application of *Trichoderma* spp, antagonist and parasitic fungi, that inhibited growth and sclerotial production on *Sclerotium* species and under experimental conditions have given good results (Punja, 1985). Beside the biocontrol effects, the treatment with *Trichoderma* spp. could be also effective in promoting growth and yield of various crops, and inducing resistance against pathogens (Vinale *et al.*, 2008).

Ruíz-Rosales *et al.* (2007) evaluated one isolate of *Trichoderma asperellum* Tc74 with antagonistic activity against *S. rolfsii*. However, the biochemical changes induced in onions by *T. asperellum* Tc74 treatment and their correlation with the lower infection by *S. rolfsii*, where not analysed. Therefore, the objective of this investigation was to know the changes on growth, peroxidase activity and phenolic compounds content of onions treated with *T. asperellum.*

Onion seeds of three varieties: white (Crystal white), red (Red satan) and violet (Mata hari) were germinated in pots with substrate previously inoculated for 20 days with *T. asperellum* Tc74 (4.08×10^7 spores kg^{-1} substrate). Plants were treated again with *T. asperellum* applying 1×10^7 spores kg^{-1} substrate after four and eight weeks of growth. Controls were plants growth with non-inoculated substrate with *T. asperellum*. Seed germination percentage was evaluated daily and germination velocity was defined as the relation of the number of seeds germinated in the germination time.

After eight weeks, leaves, roots, and bulbs were weighted by an analytical balance. Eight week old bulb samples were used to evaluate peroxidase activity following the method describe by Jetiyanon (2007) and phenolic compounds content using the Folin-Ciocalteau reagent according to Shohael *et al*. (2005). Phenolic compounds content was expressed as mg equivalent of galic acid (EGA) g^{-1} of dry weight (DW).

The seed germination percentage of the three onion varieties ranged from 90

to 94 % at 8 days. The germination velocity of red onion was of 49 seeds day^{-1} in the non-inoculated substrate and it was reduced to 40 seeds day^{-1} on substrate treated with *T. asperellum*; in violet onion, germination velocity was similar in the seeds germinated with substrate non-inoculated and inoculated with *T. asperellum* (47 and 46 seeds day^{-1}, respectively); the germination velocity of white onion was 46 seeds day^{-1} in the non-inoculated substrate and it was enhanced up to 55 seeds day^{-1} on substrate inoculated with *T. asperellum*.

Eight weeks after treatment with *T. asperellum* the leave weight was increased 1.9, 2.5, and 2.0 times in the varieties white, red, and violet, respectively. Root weight was improved 8.0, 4.5, and 8.0 times with the treatment of the varieties white, red and violet. Bulb weight also was enhanced 2.0, 1.8, and 1.0 times in the onions white, red, and violet, respectively.

Peroxidase activity of bulbs was increased with the inoculation 4.0, 2.3, and 3.7 times in the varieties white, red, and violet, respectively.

Phenolic compound content was similar (2 mg EGA g^{-1} DW) in the bulb of the white variety grown with substrate inoculated and non-inoculated. However, phenolic compound content were higher in bulb of the red and violet varieties developed with substrate inoculated (11, 9.5 mg EGA g^{-1} DW, for each one), than with substrate non-inoculated (8.5, 6.0 mg EGA g^{-1} DW, respectively).

In conclusion, treatment of three onion varieties with substrate inoculated with *T. asperellum* promotes plant growth and increases the peroxidase activity and phenolic compound content that could be related at the induced defense response.

Key words: *Allium cepa*, Onion, *Trichoderma*, Resistance

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BIOACTIVITY OF GLUCOSINOLATE-DERIVED ISOTHIOCYANATES AGAINST *SCLEROTINIA SCLEROTIORUM* (LIB.) de BARY

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Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic homotallic pathogen and causes *Sclerotinia* stem and root rot (syn. white mold) of tomato and other vegetable crops. The fungus has a large host range of more than 400 species and a worldwide distribution including important crops and numerous weeds (Boland and Hall, 1994). The pathogen can cause serious losses in yield and quality on a number of important field and vegetable crops in Turkey (Kurt and Erkılıç, 1997) and in many countries (Huang *et al*., 2006).

Diseases caused by *S. sclerotiorum* have been traditionally difficult to control with reasonable management strategies. Due to the lack of adequate levels of host resistance, foliar and soil fumigation treatments against ascospore and mycelial infections have been major control methods for *Sclerotinia* diseases (Bardin and Huang, 2001).

Brassica crops such as broccoli, cabbage, cauliflower, kale, turnip, radish, canola, rapeseed and various mustards, produce significant levels of potentially antimicrobial secondary compounds called glucosinolates (GSLs). The products of this reaction, particularly isothiocyanates (ITCs) have a broad range of biocidal characteristics including insecticidal, nematicidal, fungicidal, antibacterial and phytotoxic effects as part of a process known as biofumigation (Sarwar *et al*., 1998).

Thus, the objectives of this research were to determine *in vitro* toxicity of individual pure compounds of aliphatic and aromatic ITCs on different fungal growth parameters which include mycelial growth, sclerotial viability and carpogenic germination of *S. sclerotiorum*.

Pure compounds were methyl, allyl, butyl, ethyl, benzyl, phenyl-ethyl and phenyl ITCs. Virulence tests using susceptible tomato seedlings showed that isolate Ss31 of *S. sclerotiorum* used had the highest disease severity by 100%.

All ITC_S, tested at five different concentration, were found to inhibit the growth of *S. sclerotiorum* in a dose-dependent manner. Aliphatic ITCs were more active than aromatic ITCs in inhibition of mycelial growth at the vapour phase. Aliphatic allyl ITC was the most fungitoxic compound among all tested ITC, showing the lowest EC_{50} value ($0.007 \mu l L^{-1}$) for mycelial growth at the vapour phase. In the experiment performed by incorporating ITCs into the PDA medium, aromatic ITCs were more effective inhibiting radial growth of mycelium than aliphatic ones.

In the antifungal effects of ITC compounds on the sclerotial viability, at a

concentration of 30 µlL^{-1} at volatile phase, sclerotial viability of *S. sclerotiorum* was significantly ($P<0.05$) more affected by the benzyl ITC than methyl ITC, phenyl ITC, 2-PE ITC and ethyl ITC. Volatile ITC compounds were extremely effective in the inhibition of sclerotial viability of *S. sclerotiorum*.

The apothecial growth of *S. sclerotiorum* in relation to the concentration of ITCs in the vapour phase was inhibited in levels ranging from 30 to 77.5%.

Butyl and benzyl ITCs are recommended for soil treatment before sowing or planting to avoid phytotoxic effects. The results of the present study provide evidence that some ITCs may provide good opportunities to control *S. sclerotiorum* by decreasing the sclerotial viability and apothecial production in the soil.

Key words: *Sclerotinia sclerotiorum*, Isothiocyanates, Bioactivity

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PRELIMINARY RESULTS ON THE *IN VITRO* INHIBITION OF GROWTH AND SPORANGIA PRODUCTION OF *PHYTOPHTHORA NICOTIANAE* BY BIOLOGICAL TREATMENTS

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Phytophthora nicotianae Breda de Haan (= *P. parasitica* Dastur) is one of the most widespread and destructive soilborne plant pathogens associated with 301 host species (Erwin and Ribeiro, 1996). It causes root rots, stem necroses, and crown decline, as well as fruit and foliar blights on many agronomic and horticultural plants in seed beds, nurseries, fields, and landscape plantings. Pathogen propagules spread through soil particles and infected plants. Strategies for disease control focus on inoculum reduction in soil. Biological control may be a viable strategy to manage soilborne pathogens. Examples of the effectiveness of formulated plant extracts are already available in the literature for a number of soil-inhabiting microbial groups such as *Fusarium* spp., *Verticillium* spp. and *Phytophthora* spp. (Bowers and Locke, 1998; 2000; 2004). If natural plant products can reduce populations of soilborne plant pathogens and control disease development, then these plant extracts have potential as environmentally safe alternatives and as components in integrated pest management programs.

The objective of the present research was to evaluate the effectiveness of essential oils of oregano, thyme, and rosemary, as well as chicken manure and potassium phosphite, in reducing *P. nicotianae* growth and sporangia production in *in vitro* dose response studies. Two isolates of the pathogen were used. Essential oils significantly reduced pathogen growth if applied at the highest concentrations ($P < 0.05$). Chicken manure, although effective in mycelial growth inhibition, lead to an increase in the number of sporangia produced. Phosphite showed the highest efficacy in the inhibition of both mycelial development and sporangia production. The role of those biological substances as potential alternatives in the management of *P. nicotianae* is discussed.

Key words: *Phytophthora* control, Phosphite, Essential oils, Chicken manure

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EFFECT OF ESSENTIAL OILS ON GROWTH OF PHYTOPATHOGENIC FUNGI

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More than 1300 plants are known to be potential sources of antimicrobial compounds but only some have been studied scientifically (Wilkins and Board, 1989; Paster *et al*., 1990).

The effect of clove (*Eugenia caryophyllus*), rosemary (*Rosmarinus officinalis*), cinnamon leaf (*Cinnamomum verum*), sage (*Salvia officinalis*), scots pine (*Pinus sylvestris*), neroli (*Citrus aurantium ssp. amara*), peppermint (*Mentha piperita*), aniseed (*Pimpinella anisum*), caraway (*Carum carvi*), lavander (*Lavandula angustifolia ssp. angustifolia*) and common thyme (*Thymus vulgaris*) oils (Pranarom International) on growth of *Fusarium graminearum*, *F. verticillioides*, *F. subglutinans*, *F. oxysporum*, *F. avenaceum*, *Diaporthe helianthi*, *Diaporthe phaseolorum var. caulivora*, *Phomopsis longicolla*, *Phomopsis viticola*, *Helminthosporium sativum*, *Colletotrichum coccodes* and *Thanatephorus cucumeris* were evaluated. The essential oils were analysed by the Pranarom laboratory using GC-MC. The experiment was performed according to the method of Saikia *et al*. (2001). A 5-mm diameter sterilized filter paper disc was placed at the centre of a Petri dish with PDA and inoculated with 5 µl of essential oil. Four discs (5 mm diameter) of mycelial plugs were equidistantly placed on each Petri dish. Petri dishes were kept in an incubator at 22ºC and 12/12 h light/dark regime. The inhibition zones around the filter paper discs were measured after 8 days.

The results of the effect of essential oils showed thal all oils except scots pine and neroli had antifungal activity against some or all test fungi. Greatest inhibition was exhibited by common thyme followed by aniseed oil, cinnamon leaf oil and clove oil. Common thyme and aniseed oils had a statistically significant negative impact on mycelium growth of all the test fungi except *T. cucumeris* and *F. graminearum*, respectively. Clove oil and cinnamon leaf oil had a statistically significant negative impact on the growth of all test fungi except *T. cucumeris* and *F. graminearum*. When compared to the control, scots pine, neroli and sage oils stimulated mycelium growth of *D. helianthi* while scots pine, sage, peppermint and lavander oils stimulated mycelium growth of *H. sativum*. Full details will be given in the presentation.

Key words: Essential oil, Antifungal activity

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PRELIMINARY ASSAYS FOR INTEGRATED PEST MANAGEMENT IN LEBANON

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Peach and apple orchards are usually repeatedly treated with pesticides to control pests and pathogens (Agrios, 2005). Evaluation of control strategies often shows that treatments can be avoided without increasing pest damage or yield loss (Aluja *et al.,* 2009). Integrated Pest Management (IPM) procedures aim to apply treatments only when the infection/infestation risk is high and only use pesticides with favourable eco-toxicological profiles (Ehler, 2006). In Europe IPM is widely used, but in developing countries superfluous treatments with pesticides banned in Europe are often used on many crops (Morse & Buhler, 1997).

This study monitored pests and pathogens of apple and peach in two Lebanese regions, and compared an IPM schedules with the treatment strategies applied by growers.

Assays were carried out in an apple orchard (West Bekaa) and a peach orchard (Marjayoun). Untreated plots (approx. 1 ha) were used to monitor disease symptoms each week, and sample insects (pheromone traps). Analogous plots were treated using Italian IPM procedures, and treatment schedules were compared with grower practices.

In West Bekaa, apart from sporadic appearance of powdery mildew on young shoots, no disease symptoms were detected on the untreated trees: no fungicide treatment was applied in the IPM plot, while the growers applied numerous treatments against powdery mildew and apple scab. In Marjayoun, sporadic shot hole symptoms were detected early in the season in the untreated plot. Powdery mildew, on young shoots and on fruits, particularly on nectarine, was controlled in the IPM plot, using sulphur and IBS fungicides.

The main pests of peach were *Anarsia lineatella, Ceratitis capitata* and thrips (*Frankliniella* spp.). On apple, *Cydia molesta*, *Orgyia antiqua*, *Archyps podanus*, *Pandemis cerasana* were not frequent, while *Synanthedon myopaeformis,* causing root and trunk damage, was detected throughout the season.

Information collected during the weekly surveys indicated that, apart from powdery mildew on peach in Marjayoun, the main losses were caused by insects. The use of different traps is crucial to quantify pest populations in orchards and define treatment strategies. IPM procedures in Lebanon are hampered by lack of availability

of pesticides with favourable eco-toxicological profiles or as alternatives to avoid the selection of resistant strains/populations.

Keywords: Apple, Peach, Treatment strategy

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SUPPRESSIVE EFFECT OF WASTE VEGETABLE BIOMASS TREATED BY TECHNOLOGICAL PROCESS OF "STEAM-EXPLOSION WOOD" AGAINST SOIL-BORNE PLANT PATHOGENS

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A preliminary study has demonstrated disease suppression of waste vegetable biomass obtained by the technological process of "Steam-Explosion Wood" (SEW) (Sharma and De Corato, 2009). Among biomasses processing, the SEW has been the object of great interest since the 1990's for the production of biocombustibles of 2nd generation from energy crops (Zimbardi *et al*., 2007). The SEW is a thermo-mechano-chemical processing: in a steam reactor in which moisture penetrates lignocellulosic structures by diffusion, it condenses under high pressure, thereby wetting the material. The moisture hydrolyses the acetyl groups of hemicellulose fractions, forming furfurals and organic acids (Tanahashi, 1990). Steam-exploded biomass of *Miscanthus* × *giganteus*, a herbaceous vigorous perennial species that grows to 0.8–2 m (rarely 4 m) tall (Sharma *et al*., 2004), is a good and inexpensive renewable resource that could be useful in crop protection as an alternative to the use of chemical fungicides and fumigants. It was used in this study to assess its suppressive effect against soil-borne plant pathogens that infect horticultural crops and cause a great economical impact on the Mediterranean Italian areas.

Disease suppression was tested *in vivo* in five plant/fungus pathosystems: tomato/*Phytophthora nicotianae*, cucumber/*Pythium ultimum*, lettuce/*Fusarium oxysporum* f. sp. *lactucae*, melon/*F. oxysporum* f. sp. *melonis* and bean/*Rhizoctonia solani*. Two plots of potting mixes were made, adding compost and biomass separately to peat substrate at a concentration range of 10, 20 and 30% of potting mix. Seven days before transplanting, the potting mixes and peat control were artificially infected with the fungi. They were mixed into substrates and incubated for 7 days in plastic bags at 20–25°C. For each pathosystem, different disease levels were related to that of peat control, in order to compare the suppression levels among the trials at the end of each experiment. The percentage of disease suppression was calculated as: Ds = [(Np – Nm) / Np] × 100 (Np=average of number of diseased plants in peat control, Nm=average of number of diseased plants in the potting mixes containing compost/ biomass infected with fungi and in peat without pathogens).

Statistical analysis of cucumber/*P. ultimum* pathosystem showed a significant

suppression of the biomass with respect to compost at all the amounts tested. Also in bean/*R. solani* the biomass addition showed a significant disease suppression at all the amounts tested. In case of tomato/*P. nicotianae*, the biomass addition showed a significant disease suppression when it was added at 20 and 30% dose, but it did not show any statistical difference at 10% dose. In case of lettuce/*F. oxysporum* f. sp. *lactucae*, the biomass addition showed no significant difference in disease suppression at all the doses tested. Finally, in case of melon/*F. oxysporum* f. sp. *melonis*, the biomass was not suppressive at all the amounts tested. In conclusion, the addition of *M.* × *giganteus* biomass treated by SEW processing increased the disease suppressiveness level of peat substrate in the pathosystems cucumber/*P. ultimum*, bean/*R. solani* and tomato/*P. nicotianae*, compared to commercial compost used in these trials (Pugliese *et al*., 2007). Disease suppressiveness was, most likely, related to furfurals and organic acids produced during the processing.

Key words: Disease suppressiveness, *Miscanthus* × *giganteus*, Soil-borne plant pathogen, Steam-exploded biomass

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THE GENETIC AND MOLECULAR ROLE OF AIF PROTEINS (APOPTOSIS INDUCING FACTOR) IN THE ACTIVATION OF THE PLANT INNATE IMMUNE SYSTEM

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Hypersensitive response (HR) is a form of plant Programmed Cell Death (PCD) and is defined as a rapid, defence associated death of plant cells in contact with the pathogen. The pathogen elicits a host HR, it fails to multiply to high population levels and causes no disease symptoms. This phenomenon happens within a few hours after the contact with the pathogen and it only occurs directly at the infection sites of resistant plants (Chisholm *et al.,* 2006; Jones and Dangl, 2006). To understand the HR more clearly, we are looking for similar mechanisms between HR in plants and the well characterised PCD in mammalian, termed as apoptosis. PCD is an active process that involves signalling pathways controlling cellular reduction. The PCD pathways in plants are less well characterised. In plants PCD normally occurs during development or after abiotic and biotic challenges, such as wounding, or attacks by pathogens and pests (Hofius *et al.,* 2007).

It is well established that in mammalian cells AIF is an important protein in mediating PCD.The mammalian AIF protein is a phylogenetically old, 57 kDa flavoprotein, which shares similarity to bacterial, fungus and plant oxidoreductases. This protein contains mitochondrial and nucleus signal sequences and is typically localised in the mitochondrial intermembrane space. But after apoptogenic stimuli, the protein translocates to the nucleus and induces peripheral chromatin condensation and DNA fragmentation (Cande *et al*., 2002; Penninger and Kroemer, 2003). In *A. thaliana* five different putative Apoptosis Inducing Factor like proteins were identified and all five genes are expressed in the plant. One aim of this study was to identify these plant homologues apoptosis inducing factor like proteins and to investigate if these proteins are involved in HR and disease resistance. T-DNA knock-out mutants At-AIF-2, At-AIF-3 and At-AIF-5 were characterized in *Arabidopsis* and the mutants were tested for whether they are compromised in HR and disease resistance against several pathogens: *Hyaloperonospora arabidopsis*, *Pseudomonas syringae* pv. *tomato*, *Verticillium dahliae, Fusarium oxysporum f.sp. raphani* and *Alternaria brassicicola.* Characterisation of these lines and the results of the virulence and HR assays will be presented.

Key words: Plant immune system, Hypersensitive response, Programmed cell death, *Arabidopsis thaliana*

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IN VITRO AND IN VIVO ANTIFUNGAL ACTIVITY OF TEA TREE (*MELALEUCA ALTERNIFOLIA*) AND THYME (*THYMUS VULGARIS*) ESSENTIAL OILS AGAINST SOME PATHOGENIC SEEDBORNE FUNGI

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In recent years interest has grown in developing alternative measures to chemicals for crop protection, including the use of plant extracts (Tinivella *et al*., 2007). Many studies have been mainly focused on the pharmacological actions of essential oils derived from aromatic and medicinal plants due to their antimicrobial and antioxidant properties. Among them, tea tree (*Melaleuca alternifolia* Cheel) and thyme (*Thymus vulgaris* L.) oils have been reported to possess antifungal activity (Terzi *et al*., 2007; Pina-Vaz *et al*., 2004; Carson *et al*., 2006) and to be the most interesting in agriculture due to their effectiveness, low cost and availability. Seed borne diseases represent a critical problem for successful production, especially in organic farming systems, where less efficient plant protection agents are available for managing plant diseases. The aim of this study is to test the efficacy of those two essential oils for seed treatments against some important pathogenic seed borne fungi, e.g.: *Fusarium graminearum*, *F. culmorum*, *Drechslera avenae, Alternaria radicina*, *A. dauci*, *Ascochyta rabiei*, *Colletotrichum lindemuthianum*.

Here we present the results of the antifungal activity of the two oils, through i*n vitro* and *in vivo* assays. The mycelial growth was evaluated on solid PDA medium amended with TTO and TO up to 1% v/v. Results confirm that both the oils have a clear reducing effect on fungal growth, as already reported in literature, with TO to be the most potent agent against all the fungi. The efficacious concentration, that causes no mycelial growth, was determined for each couple pathogen/oil to obtain the minimum effective concentration that produce the desired effect on the whole group of pathogens: 1% for TTO and 0.25% for TO.

To perform *in vivo* analysis, the two essential oils were applied as liquid seed treatments on naturally infected seeds of a durum wheat cultivar. Stocks of seeds were treated by immersion in solutions prepared with sterile distilled water at different concentrations of the oils. Seeds treated with sterile distilled water and untreated seeds were used as control. Seeds were analysed using blotter test method, to determine seed infection after treatment and evaluate treatment efficacy against the pathogens. Moreover, in order to evaluate essential oils phytotoxicity, germination tests were performed on durum wheat seeds by dipping the seeds for 30 min in a solution with different oil concentrations.

The results showed that tea tree oil had a good activity against the fungi with

a very low toxicity even at the maximum concentration investigated (2.5%), as it did not inhibit seed germination; on the contrary, thyme oil is very effective against the fungi present on the seeds even at very low concentration (0.1%), but is also very toxic because it inhibits the seeds germination for more than 50% if applied at concentration of 0.3 %. The identification of the best solution concentration of the thyme oil, which combines the highest antifungal activity and the lowest toxic effect, is in progress.

Key words: Essential oils, Antifungal activity, Seed, Seed treatment

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SENSITIVITY OF *PODOSPHAERA XANTHII* TO TRIFLOXYSTROBIN IN APULIA

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QoI fungicides inhibit mitochondrial respiration by binding to the Qo site (Quinone outside) of the cytochrome b, which is part of the cytochrome bc1 complex (complex III) located within the mitochondrial membrane of fungi and other eukaryotes (Sauter *et al.*, 1999). A few years after their introduction in 1998, resistance to QoI fungicides has been observed in many phytopathogenic fungi (Sierotzki *et al.*, 2000), including *Podosphaera xanthii,* the causal agent of powdery mildew in cucurbits (Fernandez-Ortuno *et al*., 2006).

Powdery mildew populations were sampled in cucurbits growing areas of Apulia (South Italy) during 2002-2007 growing seasons, and 64 isolates of *P. xanthii* from 32 fields were obtained and assayed for their sensitivity to trifloxystrobin (Flint®, Bayer CropScience), selected as representative of QoIs fungicides. The fungicide was suspended in sterile water together with 1 mg ml^{-1} salicylhydroxamic acid (SHAM) to inhibit the alternative respiration, to final concentrations of 0, 0.1, 1, 10, 50, 125, 250, 375 μg ml^{-1}. Zucchini cotyledons of cv. Diamant 1 were dipped for 1 min in above fungicide-SHAM solutions, dried on paper towels, cut in 1 cm^2 portions and put (5 per plate) on agarized medium in Petri dishes. Leaf tissues were inoculated at a single point with about 20 conidia of *P. xanthii*, and maintained at 25°C under a 12-hour photoperiod. Ten days after inoculation, infections were estimated as a percentage of infected leaf surface, using the following empirical scale: 0 = no infection, 1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = >76% infected surface. The effective concentration inhibiting the pathogen at 50% (EC50) and the minimum inhibitory concentration (MIC) were assessed. Results pointed out a high variability in response to trifloxystrobin in *P. xanthii* isolates even from the same field. EC_{50} ranged from <0.1 μg ml^{-1} to >375 μg ml^{-1}, MIC values ranged from 10 μg ml^{-1} to >375 μg ml^{-1}. About half of the isolates had EC50 >375 μg ml^{-1} trifloxystrobin, a concentration three times greater than the field dose (25 μg ml^{-1}), with no differences ascribable to host species or geographical origin. The other 50% of isolates was sensitive to trifloxystrobin, showing EC^{50} values ranging from <0.1 to 50 μg ml^{-1}, and MIC values ranging between 10 and 125 μg ml^{-1}. This group included all isolates collected in 2002 and 2003, close to the time of introduction of QoI fungicides in Italy.

The results indicate that in Apulia, resistance to QoIs is very common, as a result of a strong selection pressure exerted by repeated sprays with these fungicides on the pathogen's populations. Apulian growers, therefore, will be forced to face this challenge in managing cucumber powdery mildew, and they should adopt a greater stringency of anti-resistance strategies.

Key words: Fungicide resistance, QoI fungicides

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BIOCONTROL OF CUCURBITS POWDERY MILDEW BY RHIZOBACTERIA INDUCING SYSTEMIC RESISTANCE

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The Cucurbitaceae is a major family for economically important species, particularly those with edible fruits. The major cultivated types include cucumber, melon, watermelon, squash and pumpkin. Melon (*Cucumis melo*) is one of the most important horticultural crops in Spain with a production of more than 1 million tons in 2006 and 337 millions € in profits.

Powdery mildew is a devastating disease of cucurbits especially on melon, causing important economical losses all over the world (Pérez-García *et al*., 2009). Fungicide applications and the use of resistant cultivars are the main means of control. Unfortunately, the limited availability of commercially acceptable resistant cultivars, the increasing problem of fungicide resistance and public concerns about the hazardous effects of chemicals on the environment (Fernández-Ortuño *et al*., 2008), have led growers to explore environmentally friendly alternatives or complements to chemicals for the management of cucurbit powdery mildew such the use of biological control agents (Romero *et al*., 2007).

Considering the ectoparasitic life style of powdery mildews, it has been often assumed that they could be efficiently targeted by mycoparasites or antibiotic-producing microorganisms, however, their use generally require a high relative humidity for optimal disease-suppressive activity, conditions fairly achieved in greenhouses but not in open field plantations. In Spain melon crops are mainly grow in open fields. For this reason, an interesting approach to overcoming this environmental restriction could be the use of rhizobacterial strains able to promote the induction of systemic resistance in the plant. In a previous work we have selected several rhizobacterial strains, two *Pseudomonas fluorescens* strains (UMAF6031 and UMAF8402), two *Bacillus subtilis* (UMAF6639 and UMAF6614) and one *Bacillus cereus* strain (UMAF8564), able to elicit protection in melon against cucurbit powdery mildew, achieving disease reduction values ranging from 43 to 52% (García-Gutiérrez *et al*., 2009).

The objective of the present study is to unravel the defence mechanisms underlying the induction of systemic resistance promoted by these rhizobacteria as well as to identify the signal transduction pathways that regulate this enhanced powdery mildew resistance in melon plants (Romero *et al*., 2008). For this purpose we have selected several plant defence marker genes such as *PR-1, PR-5, LOX, POX, ETR, CTR, PAL1, PAL2*, and acidic and basic β-1,3-glucanase and chitinase genes, for studies of gene expression by qPCR. Furthermore, we have carried out

ISR assays against other melon pathogens such as *P. syringae* pv. *lachrymans* and *Botrytis cinerea*, and on other cucurbit crops such as cucumber and zucchini against powdery mildew, in other to explore the range of disease and hosts more suitable for these rhizobacteria. Moreover, we are testing these bacteria on *Arabidopsis* against powdery mildew in order to take advantage of the tools developed in this plant to study signal transduction pathways.

Key words: Powdery mildew, ISR, PGPR, Cucurbits

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MULTIPLE RESISTANCE TO CARBOXIMIDE, QoI AND OTHER FUNGICIDE GROUPS IN *BOTRYTIS CINEREA* FROM KIWIFRUIT

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Botrytis cinerea, the causal agent of gray mold, is one of the major fungal diseases of kiwifruit resulting in severe postharvest losses (Michailides and Elmer, 2000). The control of the disease is mainly based on the pre-harvest application of fungicides. Fungicides used against the pathogen include the "old" botryticides iprodione, carbendazim, cyprodinil, pyrimethanil, fludioxonil, fenhexamid and the newly introduced fungicides boscalid and pyraclostrobin. However, *B. cinerea* is a classical 'high-risk' pathogen and development of resistance to several classes of fungicides has been frequently reported worldwide (Leroux, 2007). Recently, the presence of field pathogen strains with reduced sensitivity to the newly introduced botryticides boscalid and pyraclostrobin has been reported (Stammler *et al.*, 2008; Jiang *et al.*, 2009).

During the 2008 and 2009 kiwifruit storage periods, unusually high levels of post harvest fruit rot were observed in the kiwifruits growing area of Thessaloniki. Therefore, the current study was initiated to investigate the sensitivity profile of *B. cinerea* to older fungicides and to determine the EC_{50} values to the newer botryticides boscalid and pyraclostrobin used against the pathogen.

Forty three *B. cinerea* isolates were obtained from fruits obtained from pyraclostrobin and boscalid treated orchards, while 33 isolates were obtained from orchards never treated with boscalid and pyraclostrobin. Sensitivity measurements to pyraclostrobin were based on the inhibition of spore germination while for boscalid a microtiter method was used. The measurement of resistance frequency to carbendazim, iprodione, fludioxonil, cyprodinil and fenhexamid was based on the inhibition of mycelial growth at fungicide discriminatory concentrations.

The test isolates were divided in two groups according to their sensitivity to boscalid and pyraclostrobin. Forty three isolates showed EC_{50} values greater than 50 mg l^{-1} and 16 mg l^{-1} to boscalid and pyraclostrobin, respectively, while 33 were sensitive to both fungicides. All 43 resistant isolates originated exclusively from boscalid and pyraclostrobin mixture treated orchards during the three previous years. In addition, determination of the sensitivity profile to the remaining fungicides revealed several profiles, including simultaneous resistance to chemically unrelated fungicides. None of the tested isolates was resistant to fludioxonil and fenhexamid.

The results of this study constitute the first report of *B. cinerea* field isolates resistant to both boscalid and pyraclostrobin, and strongly suggest that we are about to face a major problem concerning the control of this very important pathogen. Avoiding failures of disease control due to fungicide resistance development is a main task and additional management strategies should be implemented.

Key words: Boscalid, Chemical control, Gray mold, Pyraclostrobin

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EFFICACY AND MECHANISM OF ACTION OF YEAST BIOCONTROL AGENTS FOR THE CONTROL OF POSTHARVEST DISEASES OF STONE FRUIT

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Monilinia laxa, one of the main postharvest pathogens of stone fruit, could also be controlled by the application of antagonistic microorganisms (Spadaro and Gullino, 2004). While different filamentous fungi, yeast and bacteria have been selected and studied against *Monilinia* spp. on peaches and nectarines (Ippolito *et al.*, 2000; Karabulut *et al.,* 2004), only a very few of them is commercially available. Three isolates of *Pseudozyma fusiformata*, *Metschnikowia* sp., and *Aureobasidium pullulans* showed a high biocontrol efficacy against *M. laxa* on peaches (Zhang *et al.*, 2010). By co-culturing *in vitro M. laxa* in the presence of the three antagonists, neither the inactivated cells nor the culture filtrate of the three isolates had any significant effect on spore germination or germ tube elongation, permitting to exclude the production of secreted toxic metabolites. The antagonistic activity of *A. pullulans* PL5 and *P. fusiformata* AP6 was dependent on the cell concentration. *Metschnikowia* sp. AP47 significantly inhibited the spore germination at the three concentrations tested (106, 107, and 108 cells/mL). The efficacy of the three strains was tested on peaches stored at three different temperatures, and their effectiveness was higher at 1°C than at 8°C or 20°C. In trials carried out in semi-commercial conditions with peaches inoculated by spraying 105 spores/mL of *M. laxa* and stored for 21 days at 1°C and 96 % RH, a cell concentration effect on the control of brown rot incidence was observed. In such experiment, AP6 and PL5 showed no significant differences in the efficacy when applied at 1×108 cells/mL or at 1×107 cells/mL, indicating that they could be used at a lower concentration in potential biofungicide formulations. Finally, in an experiment in semi-commercial conditions on fruits not inoculated with the pathogen with 21 days storage at 1°C and 96 % RH, the evaluation of postharvest quality parameters, including firmness, total soluble solids, ascorbic acid content, and titratable acidity, showed that no one of the three screened antagonists impaired peach quality, when applied before storage. The interactions between the antagonist *A. pullulans* and some postharvest pathogens were studied *in vitro* and/or *in vivo* in order to highlight its possible modes of action. *A. pullulans*, when cocultured *in vitro* with *M. laxa* or *B. cinerea* showed beta-1,3-glucanase, exo-chitinase, and endo-chitinase activities.

Key words: *Aureobasidium pullulans,* beta-1,3-glucanase, Chitinase, *Metschnikowia* sp., *Monilinia laxa*, Peach, *Pseudozyma fusiformata*

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NEW FORECASTING MODELS AS EFFECTIVE TOOLS FOR RATIONAL DISEASE CONTROL STRATEGIES IN VINEYARDS

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Different approaches in formulating epidemiological models in plant pathology are available, and the resulting systems are characterized by different levels of accuracy and robustness (Rossi *et al.*, 2009). Many models have been elaborated starting from field observations to define quantitative relationships between epidemics and their influencing weather factors: all these models are empirical and suffer from the typical problems of this approach. Nowadays many mechanistic models exist for both field and orchard crops (Rossi *et al.*, 2009): these models (also referred to as explanatory, theoretical, or fundamental) explain epidemic behaviour on the basis of what is known about how the system works in relation to the influencing variables.

A research project funded by the Emilia-Romagna Region of Italy aimed to extend and improve the application of epidemiological models in crop protection (Caffi *et al*., 2008). In particular, two mechanistic models were used in real time to schedule fungicide applications against primary infections of grapevine downy (Rossi *et al.*, 2008) and powdery mildews (Caffi and Rossi. 2009). A disease warning system based on these models and on short-term weather forecasts was developed and its use was evaluated in experimental vineyards over a 3-year period in Northern Italy (Caffi *et al.*, 2009). For each vineyard, the number of fungicide spray applications and the disease intensity were compared for treatments following the recommendations of the warning system with treatments following a standard grower's fungicide schedule and with an unsprayed control.

The real time warning system allowed reductions in fungicide applications over the 3 years averaging 60% for downy mildew and 40% for powdery mildew, while maintaining regular production.

Keywords: Grapevine, Downy mildew, Powdery mildew, Disease control, Warning system, Mechanistic modelling

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VARIATION OF QoI RESISTANCE FREQUENCY IN *PLASMOPARA VITICOLA* OOSPORES

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Plasmopara viticola (Berk. et Curt.) Berl. and De Toni is the causal agent of grapevine downy mildew, one of the most relevant diseases in vineyard. Since many infection cycles can occur in the same growing season, numerous fungicide treatments are carried out to prevent severe epidemics (Gisi and Sierotzki, 2008). Repeated applications of fungicides belonging to the same resistance class exert a selection pressure which, in the QoI case, generates distinct populations. QoI resistance in *P. viticola* is due to a single nucleotide polymorphism (SNP), in which alanine replaces glycine at the amino acid codon 143 in the target cytochrome bc1 component of complex III (Sierotzki *et al.,* 2000). Specific assays were developed for the detection of resistant individuals and of the mutation rates in *P. viticola* oospore populations: a biological assay, based on the germination rate of the oospores at a discriminatory dose of azoxystrobin (10 mg/l), and an allele-specific real-time PCR assay for the detection of the point mutation responsible for QoI resistance (Toffolatti *et al.,* 2006). *P. viticola* oospores are differentiated by sexual reproduction at the end of grapevine growing season, overwinter in soil and germinate in the following season, providing the inoculum for primary infections. Since QoI resistance is inherited maternally (Blum and Gisi, 2008) studies on the oospores collected at the end of the growing season reflect the result of the fungicide selection pressure during the past season, and indicate the potential resistance frequency of the primary inoculum for the following season. Aim of the study was to follow the changes in QoI resistance rates during consecutive seasons in vineyards where QoI treatments were applied with different strategies (solo, in mixture), in order to investigate the effect of different treatment strategies, or where QoIs were withdrawn, to investigate if resistance can be reversed. Samplings were carried out in October from 2004 to 2007 in 33 vineyards located mainly in southern Italy (Puglia) but also in northern Italy (Lombardia, Veneto, Piemonte and Emilia Romagna). Among these vineyards, seven were monitored for at least two consecutive years following the interruption of QoIs.

The results showed a significant increase in the average resistance rates in samples collected from vineyards repeatedly treated with QoI fungicides, especially as solo formulations, but also a separation of the samples in two subpopulations: one characterized by low (about 15 %) and the other by high (about 80 %) rates of resistance. To a high selection pressure, in fact, does not always correspond to high resistance levels, a phenomenon explained by the migration of sensitive strains from surrounding vineyards and sexual recombination. Reduced frequencies of resistance

were observed where QoIs were applied in mixture with fungicides belonging to different resistance groups. Finally, a decreasing trend from initial high resistance levels towards values similar to those recorded in untreated vineyards followed the interruption of QoI sprays for at least two years. A reduction in the resistance values can be, therefore, obtained by an appropriate resistance management.

Keywords: Downy mildew, Grapevine, Resistance monitoring

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PHYLLOSPHERE GRAPEVINE YEAST *AUREOBASIDIUM PULLULANS* REDUCES *ASPERGILLUS CARBONARIUS* (SOUR ROT) INCIDENCE AND OCHRATOXIN A IN WINE PRODUCING VINEYARDS IN RHODES, GREECE

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Berry rot, known as sour rot or Aspergillus rot, is mainly caused by black aspergilli which are very common in vineyards. Surveys conducted in different countries have revealed that *Aspergillus carbonarius*, and *A. niger* aggregate, are the predominant aspergilli in vineyards (Battilani *et al.,* 2006, Tjamos *et al.,* 2006). It is known that *A. carbonarius* and *A. niger* are ochratoxin A (OTA) producers (Battilani *et al.,* 2001, Tjamos *et al.,* 2004). Ochratoxin A is a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive properties. Grapes and derived products such as raisins, grape juices and wines have been reported as potentially contaminated with OTA. Several studies have shown that *A. carbonarius* and occasionally *A. niger* are the OTA sources in wine grapes; therefore, our efforts have been mainly focused on developing biocontrol methods for restricting *A. carbonarius* contamination of wine-producing grapes. For this purpose, phyllosphere yeasts were isolated from leaves of vine canes and evaluated in a detached berry assay for their ability to suppress *A. carbonarius* growth. Seventeen of the 21 yeast isolates significantly reduced *A. carbonarius* growth, i.e. sour rot infection compared to untreated controls in laboratory tests. The most effective yeast isolate *Aureobasidium pullulans*, isolate Y-1, was field tested on two varieties of red grape, Grenache Rouge and Agiorgitiko located on the Island of Rhodes. It was demonstrated that *A. pullulans* Y-1 was as effective as the commercial fungicide fludioxonil+cyprodinil, in reducing sour rot infection, *A. carbonarius* presence on berries at harvest and OTA contamination in must (Dimakopoulou *et al.*, 2008).

Keywords: Biological control, Black aspergilli, Ochratoxin A

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POST-HARVEST TREATMENT WITH OZONE TO CONTROL DECAY OF TABLE GRAPES BY MICRO-ORGANISMS

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Ozone is a strong naturally-occurring oxidizing agent (Suslow, 2004). Its ability to kill micro-organisms by oxidation of their cell membranes makes it an ideal post-harvest treatment option (Bourbos and Barbopoulou, 2005). Trials were carried out to assess whether ozone can reduce decay of table grapes. Table grapes cv. Italia and cv. Red Globe were packaged in plastic boxes covered or uncovered with plastic bags in the presence or absence of a sulfur dioxide fumigant.

Table grapes were stored in chambers at 1°C and submitted to ozone exposure for a proper duration; the same packages, not exposed to ozone treatment, were used as control. The number of colony forming units (cfu) of fungi, yeasts and bacteria on the berries surface was evaluated by using a selective medium, and the incidence of natural decay was assessed by applying McKinney index after 3 days' storage. Results show a significant rot reduction in grapes treated with ozone and stored without plastic bags and without sulfur dioxide fumigant for both periods of ozone exposure; the number of cfu of fungi, yeasts and bacteria was dramatically decreased after ozone treatment. Ozone treatment was more effective in cv. Red Globe than in cv. Italia. The application of the appropriate dose for a sufficient period of time can effectively reduce post-harvest losses and allow a longer shelf-life and storage duration.

Key words: Ozone, Table grape, Post harvest

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Petria 20 (2), 67-633 (2010)

ANTAGONISTIC ACTINOMYCETES FROM MOROCCAN SOIL TO CONTROL THE GRAPEVINE GRAY MOLD

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Biological control is an alternative to pesticides for protection against crop diseases (Compant *et al*., 2005). A key to progress in the protection of grapevine against *Botrytis cinerea* using biological control is to select *in vitro* the best agent to be applied in the field. Among bacteria, the actinomycetes are important producers of bioactive compounds and constitute a potential as biocontrol agents. The Moroccan actinomycete microflora has poorly been explored to search new means of biocontrol suggesting that a careful exploration of new habitats might be useful (Ouhdouch *et al*., 2001). For this, one hundred and forty-two different actinomycete strains were isolated from *Vitis vinifera* L. rhizosphere from four Moroccan sites (Loqman *et al*., 2009). They were tested against five phytopathogenic fungi (*Pythium ultimum*, *Fusarium oxyysporum* f. sp. *albedinis*, *Sclerotium rolfsii*, *Verticillium dahliae* and *B. cinerea* to evaluate their antifungal effects. Results showed that 24 isolates had an *in vitro* inhibitory effect toward at least 4 of the indicator fungi, but only 9 inhibited all these phytopathogens. Microscopic observation of *B. cinerea* mycelium from the zone of contact between the fungus and actinomycete showed a growth disruption of fungal mycelium. These nine isolates were subsequently evaluated individually using *in vitro* grapevine plantlets for their ability to protect against plant gray mold. We found that pre-inoculation of plantlets with these isolates allow them to withstand against *B. cinerea*. When plants (inoculated only with actinomycetes isolates) were sampled several weeks later, and their tissues were macerated in buffer and cultured on Potato Dextrose Agar (PDA) medium, the isolates were able to grow on the medium demonstrating that actinomycetes establish sufficient endophytic populations in plant (unshown data). This characteristic may allow them to produce significant amounts of antibiotic compounds which may induce plant defence mechanisms.

However, the inhibitory effect of these isolates on the growth of soil-borne fungal pathogens and disease development is probably derived from more than one mechanism. Although the exact mechanisms by which these actinomycetes isolates operate to reduce disease incidence is not elucidated, one possibility is that these

biocontrol agents could exert a direct inhibitory effect on hyphal growth and structure of fungal pathogens (Loqman *et al*., 2009). The taxonomic status of these strains was established using a polyphasic approach; seven of these strains were shown to belong to the genus *Streptomyces* and two to the genus *Micromonospora.* Further studies are necessary to evaluate the effect of these potential biocontrol agents in greenhouse and field conditions, as well as to purify and characterize the secondary metabolites produced by these actinomycetes.

Key words: Actinomycetes, Screening, Identification, *Vitis vinifera*, Biocontrol, *B. cinerea*, Grey mold

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Petria 20 (2), 67-633 (2010)

BIOCONTROL OF DATE PALM PATHOGEN, *FUSARIUM OXYSPORUM* F. SP. *ALBEDINIS,* USING THREE BACTERIA ISOLATED FROM SOIL

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The bayoud, caused by *Fusarium oxysporum* f. sp. *albedinis* (*Foa*), is the most destructive disease of date palm in Morocco and Algeria. It has been reported that more than 2/3 of the Moroccan date palm groves have been destroyed during the last century (Fernandez *et al.*, 1995). No strategy has been yet established allowing the reduction of the impact of bayoud disease. The use of *Foa* antagonists could constitute a promising strategy to control this disease. Several studies have been published on the biological control of plant diseases (Silva *et al.*, 2004; Zhang *et al.*, 2008). Concerning this study, three bacteria species were used; two species isolated from Tunisian soil, *Bacillus amyloliquefaciens* and *Burkholderia cepacia* and one, not yet identified, isolated from a solid waste compost of olive. The bacteria were examined for their potential role to control *Foa* and to protect the date palm seedlings against bayoud disease. Bacterial and fungal suspensions were injected into roots of six month-old date palm seedlings. After one month, the results showed a reduction in disease symptoms based on the extend of necrosis which reflected the colonization of the host plant by the pathogen. In addition, it was found that new phenolic compounds, known to play a crucial role in resistance of date palm to *Foa* (El Hadrami, 2002) were accumulated.

The effects of the antifungal compounds released by the antagonists into the culture media on *Foa* were also studied. The results revealed that the three strains of bacteria exhibited distinct antifungal activities against *Foa*. Cytological alterations expressed by cell ballooning and lyses, and brown pigmentation of the mycelium have been detected into *Foa* mycelium grown with antagonists.

Keywords: Bayoud, necrosis, Phenolic compounds, Antifungal compounds

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Petria 20 (2), 67-633 (2010)

EFFICACY OF UREA, BORAX AND *TRICHODERMA* TREATMENTS AGAINST *HETEROBASIDION* SPORE INFECTIONS OF STUMPS OF *ABIES NORDMANNIANA* SSP. *BORNMÜLLERIANA*

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Heterobasidion annosum sensu lato has caused significant losses in managed coniferous forests throughout Europe and Asia Minor (Woodward *et al*. 1998; Annesi *et al.,* 2005; Doğmuş-Lehtijärvi *et al.,* 2008). As the main route of infection in managed forests is freshly cut stump surfaces, from where the fungus spreads to nearby trees via root contacts, the control efforts have focused on stump treatments (Nicolotti *et al*., 1999; Pratt, 2000).

In this study, stumps of freshly-cut *Abies nordmanniana* ssp. *bornmülleriana* Mattf. (mean diameter 20.6 ± 3.9 STD) in Bolu province in Turkey were treated manually with either borax powder (0.01 g/cm^2), 30% aqueous urea solution (0.1 ml/cm^2), or a non-commercial *Trichoderma harzianum* Rifai (isolated from an A. *nordmanniana* ssp. *bornmülleriana* stump) spore suspension (10^4 spores/ml, 0.1 ml/cm^2). Each stump and treatment combination was repeated 20 times (totally 80 stumps including untreated controls). The treatments were performed in October 2007, whereafter the stumps remained exposed to natural infection by *Heterobasidion* spores. The stumps were sampled six months later; from each stump the top 1 cm was discarded and a 2 cm thick disc was cut and put into a nylon bag. After one week's incubation at 24°C, stump area colonized by *Heterobasidion* was determined from the discs under stereomicroscope using a transparent film with a cm^2 grid. The efficacy of each treatment was calculated by comparing the proportion of the total disc area colonized by *H. abietinum* with that of the control.

The frequency of colonized stumps was 25, 50 and 70% in the urea, borax and *Trichoderma* treatments, respectively, while it was 85% in the control. The mean proportion of the stump area colonized by *Heterobasidion* in urea (0.4±0.2%, SE), borax (2.3±1.4) and *Trichoderma* (6.1±2.0) treatments was significantly lower than that in the controls (12.5±2.8%; $p<0.01$). The urea and borax treatments showed the highest mean efficacies, 96.8 and 81.6%, respectively, contrasting the relatively low efficacy of the *Trichoderma* treatment, 51.2%. The results indicate that urea treatment could be used successfully against spore infection of ssp. *bornmülleriana* stumps by *Heterobasidion* in Turkish forests.

Key words: Biological control, Chemical control, Root and butt rot, Stump treatment

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Petria 20 (2), 67-633 (2010)

BAYOUD DISEASE: EFFECTIVENESS OF PLANT EXTRACTS FOR CONTROLLING *FUSARIUM OXYSPORUM* F. SP. *ALBEDINIS*

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Fusarium oxysporum (Schlecht.) f. sp. *albedinis* (Killian & Maire) W.L.Gordon (*Foa*) causes Bayoud disease, a principal cause of death of date palms (*Phoenix dactylifera* L.) in North Africa (Hassni *et al.,* 2007). This disease has an important environmental impact, due to the loss of plants as carbon sinks and the increase of the incidence of desertification, and economical consequences, linked to lost production and difficulty of vegetable cultivation in areas without palm shadow. For these reasons control measures are required. Several control approaches, mainly based on chemical treatments, have been applied in the past, but none gave effective disease eradication or decreased *Foa* inoculum for long periods. New methods of disease control are required.

The aim of this research was to examine effects of potassium phosphite and essential oils from cinnamon, lemongrass, oregano, and thyme on *Foa* isolates collected in Algeria. The survival of two isolates (*Foa*13 and *Foa*28) has been analysed in soil treated with 1, 5, and 10% aqueous emulsions of oils and potassium phosphite. The population density of *Foa* was determinated at 0, 1, 3, 7, 14, and 21 days after inoculation in soil (Bowers and Locke, 2000). Significant differences between the two isolates have been observed. After 21 days of inoculation all treatements showed a significant inhibition ($P < 0.05$) of growth of isolate *Foa*28 except for aqueous emulsions of phosphite at 5 and 10% and of lemongrass at 5%. Oils from thyme at all concentrations, oregano at 5 and 10% and lemongrass at 10% were the most effective treatments against. Potassium phophite did not reduce the population density of *Foa*13 isolate in the trials. These *in vitro* results confirm the potential of essential oils for control of Bayoud disease. Preliminary *in vivo* results on date palm seedlings are reported.

Key words: *Phoenix dactylifera*, Date palm, Bayoud, Essential oils

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PETRIA 20 (2), 67-633 (2010)

PSEUDOMONAS CHLORORAPHIS SUBSP. *AUREOFACIENS* STRAIN M71 CONTROLS *SEIRIDIUM CARDINALE* INFECTIONS ON CYPRESS

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Bark canker of cypress (*Cypressus sempervirens*) is a disease caused by the fungus *Seiridium cardinale*. Under favourable environmental conditions, the disease may kill the trees within a few months. Cypress is largely used as an ornamental plant both in urban and rural areas and it is appreciated for the quality of its timber, for its tolerance to drought and poor soils and for its usefulness in agriculture. In Tuscany, cypress characterizes the most beautiful landscapes known all over the world. In this region at the end of the last century, the mean incidence of diseased trees was estimated to be around 25%, but reached 75% in the Florence district where severe damage to landscape and economic losses were recorded (Graniti, 1998). Control of cypress canker has been based on sanitation, breeding for resistance and chemical prevention. Benzimidazole compounds have been shown to be the most effective, preventing the onset of new infections in nurseries (Panconesi and Raddi, 1986). Recent EC measures revoked the use of these chemicals due to their toxicity and heavy environmental impact. Alternative methods to control cypress canker are strongly needed and the exploitation of natural antagonists may be a valid option.

Bacterial strains belonging to *Pseudomonas chlororaphis* species are known to be powerful antagonists effective against several soil-borne pathogens of herbaceous plants (Weller, 2007). Strain M71 of *Pseudomonas chlororaphis* subsp. *aureofaciens* was tested for its antagonistic activity against *S. cardinale*. *In vitro* tests showed that the bacterium totally inhibited the radial growth of the fungus. Moreover, the culture filtrate of strain M71 inhibited the germination of conidia of *S. cardinale*. Two antibiotic compounds, extracted from the culture filtrate, were purified and identified by spectroscopic and chromatographic methods as phenazine-1-carboxylic acid (PCA) and 2-hydroxyphenazine (2-OH P). The two phenazines were tested for their activity against *S. cardinale* but only PCA showed a strong inhibitory activity against the fungus.

Five-year old plants of *C. sempervirens*, grafted on *C. sempervirens* seedling rootstocks were used for *in vivo* trials. Three different trials were carried out in order

to determine: i) the effectiveness of *P. chlororaphis* subsp. *aureofaciens* strain M71 in preventing infection by mycelial inoculum of *S. cardinale*; ii) the effectiveness of *P. chlororaphis* subsp. *aureofaciens* strain M71 in preventing infection by conidial inoculum of *S. cardinale*; iii) the effectiveness of a phenazine deficient mutant of *P. chlororaphis* subsp. *aureofaciens* strain M71b in preventing infection by mycelial inoculum of *S. cardinale*. The phenazine producer strain M71 completely prevented disease induction both by mycelium and spore inoculum, while the phenazine deficient mutant only slightly reduced the canker size. These results strongly support the hypothesis that phenazines are involved in the biocontrol of cypress canker. A further experiment carried out under controlled conditions showed that *P. chlororaphis* subsp. *aureofaciens* strain M71 has an interesting epiphytic fitness since it was able to establish itself on the head of cypress plants.

Key words: Biocontrol, Cypress canker, Epiphytism, Phenazine

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PETRIA 20 (2), 67-633 (2010)

EFFICACY OF BIOFUNGICIDE AQ10 AND POLYMER NU - FILM IN CONTROLLING POWDERY MILDEW

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Powdery mildew on oak, is caused by the fungus *Microsphaera alphitoides* Griff. *et* Maubl. *M. alphitoides* did not occur epidemically in Central or North-Western Europe before 1907, but was introduced from North America and now occurs as a pathogen on several tree genera in forests, parklands and nurseries throughout Europe. The pathogen can retard the growth of young plants and kill tree seedlings. Recurring attacks of *M. alphitoides* in combination with other pathogens or herbivory by mammals and insects can cause older oaks to die.

Greatest damage occurs on young oak, that in cases of strong attacks requires to use of chemical protection – via treatment by fungicides (Rajkovic, 2009). Because of the desire to reduce the negative consequences of applying chemicals, biological control is becoming increasingly important.

Attempts have been made to use *Ampelomyces quisqualis* isolates as biological control agents of powdery mildews infecting various crops (Hofstein *et al*., 1996). An *Ampelomyces* isolate has been commercialized by Ecogen, Inc. (USA) under the trade name AQ10 biofungicide for use against powdery mildew infections of many crops (Bélanger & Labbé, 2002; Kiss, 2003). However, some trials have shown that *Ampelomyces* isolates are less efficient biocontrol agents of powdery mildews than a number of other fungal antagonists (Verhaar *et al*., 1999; Bélanger & Labbé, 2002; Kiss, 2003; Szentivanyi, 2003).

AQ-10 biofungicide contains fungal spores of *Ampelomyces quisqualis* and is reported to control powdery mildew by parasitizing and killing the fungal organisms that cause the disease. AQ-10 is not selective for specific strains of powdery mildew. The efficacy of biofungicide AQ10 has been reported to increase with the addition of polymers during application.

The aim of this work was to verify the effect of the biofungicide AQ10 individually and in combination with the polymer Nu-film 17 in controlling powdery mildews on oak. Preliminary tests were performed by using standard OEPP methods (1999) on oak seedlings infected by *M. alphitoides*. Treatments were carried out in four replicates over the period June - September 2009.

Infection and phytotoxity were determined on 11/07/2009 using the EPPO methods (1999 and 1997, respectively). Data processing and standard statistical methods (intensity of infection by Towsend-Heuberger formula and efficiency after the Abbott formula, analysis of variance and Ducan test) were applied.

When AQ10 was used alone, in doses 30 g/ha, percentage of infection was 15.35%, while efficacy was 48.05%. When the medium and highest doses were applied (50g/ha and 70 g/ha) efficacy was 75.80% and 79.19%, respectively. On the untreated variant percentage of infection was 29.55%. When AQ10 was applied in combination with polymer Nu-film 17 all variations in all combinations showed a satisfactory efficacy (84.26% - 92.89%) and a low percentage of infection (4.65% - 2.10%). Fungicide Sulfur SC efficiency was 84.43%.

Key words: Biofungicide, Efficacy, Powdery mildew, Oak

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PETRIA 20 (2), 67-633 (2010)

OCCURRENCE OF *ZETIASPLOZNA UNICOLOR* AS AN ENDOPHYTE OF MYRTLE (*MYRTUS COMMUNIS*) IN ITALY

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In the frame of studies concerning biodiversity, fungal endophytes are increasingly exploited as a source of biologically active compounds. In fact, a number of endophytic species have proved to be able to produce secondary metabolites originally extracted from their host plants. An investigation concerning fungal endophytes of myrtle (*Myrtus communis*) was undertaken throughout 2009 in Campania and Basilicata regions in southern Italy. Isolations were made from subcortical tissues of secondary branches of wild plants, and a small collection of 35 fungal strains was obtained. Two isolates were found to produce appendaged conidia, which are typical of *Pestalotia* and related genera (Guba, 1961; NagRaj, 1993). Observations of culture morphology and conidial structures led us to ascribe these isolates to *Zetiasplozna unicolor* (Berk. & M.A. Curtis) Nag Raj. This species has been mentioned as a pathogen of myrtle, but previous reports from Italy have depicted its occurrence as quite sporadic, or limited to artificial plantations. Attempts to induce leaf-spot symptoms on myrtle cuttings in hydroponic culture inoculated with conidial suspensions of both isolates failed, and recovery of the fungus from the treated leaves was unsuccessful at the end of the experiment. Combined with considerations by other authors, the available data indicate that *Z. unicolor* is a weak and/or occasional pathogen of myrtle in Italy, and consistent with an hypothesis that it could rather establish a compatible interaction as an endophyte in natural conditions.

Key words: Fungal endophytes, *Pestalotia* spp., Leaf-spot

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ANTIBACTERIAL ACTIVITIES OF NATURAL EXTRACTS AND THEIR POTENTIAL FOR THE CONTROL OF PATHOGENS OF KIWIFRUIT AND TOMATO

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Kiwifruit and tomato are affected by several bacterial diseases, e.g.: kiwifruit by *Pseudomonas syringae* pv. *syringae* (Pss) and *P. viridiflava* (Pv); tomato by *P. s.* pv. *tomato* (Pst) (Balestra *et al*., 1998; Balestra and Varvaro, 1999). Control of plant bacterial pathogens is limited to spraying cupric salts and to cultural practices. Recent restrictions on copper use for agriculture and horticulture in Europe made necessary new control strategies. The potential of some natural extracts to control different pathogens has already been established (Burt, 1994; Bloor, 1995; Iacobellis *et al.*, 2005). Our aim was to determine *in vitro* the antibacterial activity of natural extracts from some plants sampled in Italy: *Allium sativum, Ficus carica, Laurus nobilis, Lavandula spica, Mentha piperita, Pelargonium zonale, Salvia officinalis, Punica granatum, Olea europea* and *Vitis vinifera*; some essential oils: *Argania spinosa, Azadirachta indica, Boswellia sacra, Cymbopogon nardus, Leptospermum scoparium, Malaleuca alternifolia*; and one animal extract, lysozyme, against a collection of Pss, Pv and Pst strains; moreover, some extracts from plants sampled in New Zealand (Waikato): *Alectryon excelsus, Aristotelia serrata, Arthropodium cirratum, Cordyline australis, Corynocarpus laevigatus, Griselinia littoralis, Hebe pauciramosa, Olearia paniculata, Phormium tenax, Pittosporum crassifolium, Pittosporum eugenioides, Podocarpus totara, Pseudopanax gilliesii, Pseudopanax laetus* and *Pseudopanax lessonii,* were tested against a collection of Pss and Pv strains. The tests were carried out by spotting four drops (30 µl each) of ethanolic extracts (10 mg/l) onto plates of nutrient agar seeded with the test bacteria (100 µl, 10^5 cfu/ml). The zones of inhibitions were recorded after incubation at 27±2°C for 48-72 h.

None of the New Zealand native plant extracts showed antibacterial activities against the bacteria tested, while *L. spica, A. sativum, L. nobilis, P. granatum* extracts and lysozyme showed antibacterial activity against all the strains tested of Pss, Pst and Pv.

Key words: *Actinidia*, Antimicrobial, Bacterial speck, Phytopathogenic bacteria, Vegetal extracts

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ANTI-NEMATODE ACTIVITY OF ALCOHOLIC EXTRACTS AND OIL OF SOME MEDICINAL PLANTS AGAINST THE ROOT- KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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The root-knot nematode *Meloidogyne incognita*, an economically important nematode species, is recognized as one of the causes of serious yield losses on a wide range of crops. In order to manage and control this nematode, several control measures have been used by chemicals nematicides , resistant cultivars, crop rotation and biological control. Chemical control is really expensive and are harmful to the environment and human health (Tsay *et al*., 2004). Therefore, there is a need to find alternatives to highly toxic and polluting chemicals such as plant extracts, root exudates, plant volatiles with nematicidal activity (Adegbite and Adesiyan, 2005).

The aim of this work was to evaluate the nematicidal properties of alcoholic extracts and seed oils from four medicinal native plants (castor bean, *Ricinus communis* L., chinaberry, *Melia azedarach* L., and rapeseed, *Brassica napus* L.) against *M. incognita*.

Leaves of each plant were macerated with 70% ethanol (300 ml) three times, 48h each time, at room temperature (Cristobal-Alejo, 2006). Seed oils were obtained by Soxhlet extraction with petroleum ether solvent (Hosseininejad, 2004). The efficacy of alcoholic extracts and seed oils at different concentrations (0, 50, 100, 200, 300, 400, 500 and 1000 µl) against *M. incognita* was studied in vitro conditions, at 26±2°C, using 100 second-stage juveniles (J2) and 100 eggs.

Immobility of J2 was recorded after 24, 48, 72 h of exposure and hatching after 7 days. The results of J2 mobility and egg hatching was effected significantly ($p \leq 0.05$), on the alcoholic extracts and seed oils with significant differences ($p \leq 0.05$) between alcoholic extract or seed oil, concentration and time of exposure. The analysis of J2 mobility revealed that all treatments had nematicidal effect being the seed oil the most effective with values of 76.33% for chinaberry, 71.33% for castor bean and 53.66% for rapeseed after 72 h in the 1000 µl concentration. Concerning the effect of alcoholic extracts on the mobility of *M. incognita* J2, the values obtained, in 1000 µl concentration, after 72 h of exposure, were 68.66% for chinaberry, 61.33% for castor bean and 47% for rapeseed. Hatching, in 1000 µl concentration after 72h of exposure, was affected in all treatment being the highest value 25.67% for castor bean alcoholic extract followed by rapeseed alcoholic extract (21.67%), chinaberry alcoholic extract (20%), rapeseed oil (20%), castor bean oil (16%), and chinaberry oil (13.67%). These results clearly indicated that extracts and seed oil of these plants had an effect on *M.*

incognita activity and may have potential to be used as biological control agents to control root-knot nematodes.

Keywords: Alcoholic extracts, Biological control, *Meloidogyne incognita*, Seed oil

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NEMATICIDAL ACTIVITY OF ESSENTIAL OIL AGAINST ROOT-KNOT NEMATODES

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Root–Knot nematodes constitute a serious danger on vegetable crops in the world (Kiewinick and Sikora, 2006) and in Algeria (Lamberti *et al*., 1975; Sellami, 1999). Available nematicides are too costly for use on most crops and they are dangerous to the public health.

During the last years, various plants have been reported to have nematicidal or nematostatic proprieties (Gommers, 1981; Chitwood, 2202). So, investigations were carried out to assess the effect of essential oils from leaves of *Thymus fontaneisi*, *Origanum floribundum*, *Mentha spictata*, *Mentha peligium* (Lamiaceae) and *Artemisia herba-alba* (Asteraceae) at different concentrations (125, 250, 500, 1000 ug/l) on the mortality of juveniles after 24, 48 and 72 hours of exposure, and hatching of eggs of *Meloidogyne incognita* maintained in the same solutions during twelve days.

The efficacy of these oils was compared with a control solution: Ethanol+0,3% of tween 20 and Ethoprophos. DL50s were calculated for these effects.

Results indicated that all essential oils increased the mortality rate and decreased hatching eggs rates. However, these effects depended on the nature of essentials oils, the exposure period and concentration. Recently, Oka *et al.* (2000) reported the efficacity on juvenile mobility and hatching of *Meloidogyne javanica* at lower concentrations (250 at 500 ul/l) of essential oils of *Origanum syriacum* and *Origanum vulgare* Finally, further investigations are required to ascertain the use of plant oil extracts as alternative methods to nematode management.

Key words: Larval mortality, Egg hatch suppression, Plant extracts, *Meloidogyne incognita*

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ANTAGONISTIC ACTIVITY OF *TRICHODERMA HARZIANUM* AND *T. VIRENS* AGAINST *HETERODERA SCHACHTII*

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Sugar beet cyst forming nematode (*Heterodera schachtii*) is a widespread pathogen of the sugar beet all over the world and one of the most important pathogens of the sugar beet in Iran. The antagonistic fungi of nematodes consists of a great variety of organisms which include the nematode- trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs, parasites of nematodes cyst and fungi which produce toxic metabolites to nematodes. Nematicidal fungi included *Verticillium chlamydosporum, V. lecani, Hirsutella rhossiliensis*, *Trichoderma harzianum, and T. virens* (Westphal and Becker, 2001).

For biological control of *Heterodera schachtii*, 10 isolates of *Trichoderma* related to two species *T. harzianum* and *T. virens* were examined in laboratory and green house on eggs and cysts for two years.

These included 5 isolates from the soils of sugar beet fields of Mashhad (north-east of Iran) and 5 isolates obtained from the collection of biocontrol fungi (Iran).

The cyst forming nematode (*H. schachtii*) were extracted from the soil of sugar beet fields and identified by the key of Mulvey and Golden (1983). The nematode population was prepared on sugar beet in autoclaved soil in green house. Parasitism of isolates of *Trichoderma* on eggs and cysts was studied in laboratory and greenhouse.

For the green house experiments, the obtained cysts were mixed at the rate of 400 eggs and J2/100 g soil with autoclaved and non autoclaved soil inoculated separately by 10 isolates of two *Trichoderma* species at the rat of 10^7spor/g soil. They were maintained in green house condition and irrigated normally. Experiments were carried out in autoclaved and non autoclaved soils (field soils) separately with 12 treatments and 3 replications including non infested control (using Ragbi nematicide in field soils experiment), control without nematode and *Trichoderma*, infested control and treated with isolates of *Trichoderma* using Randomized Complete Design in green house.

The plants were harvested 70 days later and the final population of nematodes in soil of pots were determined.The fresh and dry root weight and the fresh and dry leaves weight were also noted.

Results obtained from the laboratory assay showed that isolates of *Trichoderma* parasitized 60% eggs in average. Among them, two isolates *T. harzianum* Bi and *T. virens* VM_1 with 76.18% and 72.55% parasitism respectively showed to be more efficient comparing with the control.

In green house, analysis of variance for the biocontrol potential of isolates, final population of nematode, fresh and dry root weight, fresh and dry leaves weight inoculated with isolates of *Trichoderma* was carried out. The results revealed a significant differences ($P<0.05$) between treatments and control according to Duncan's Multiple Range Test. *T. harzianum* Bi and *T. virens* VM_1 decreased population of nematodes, increased yield in autoclaved and field soils. In autoclaved soils two isolates *T. harzianum* Bi and *T. virens* VM_1 decreased population of nematodes by %76.68 and %72.65 respectively comparing with the control. The Ragbi nematicide, *T. harzianum* Bi and *T. virens* VM_1 decreased population of nematodes by %81.65, %75.15 and %72.85 respectively comparing with the control in field soils experiments.

Meyer *at al*. (2001) reported that *T. virens* G1-3 was decreased population of *Meloidogyne incognita*. Also, the population decline of *M. arenaria* in the soil by using *T. harzianum* T-12 and *T. koningii* T-8 were determined (Windham *et al.,* 1989). Rao *et al.* (1998) reported similar results about controlling *M. incognita* by *T. harzianum* and *T. lignorum*. In this research, we considered that two isolates *T. harzianum* Bi and *T. virens* VM_1 were caused to increase yield and to decrease population of *H. schachtii*.

Key words: Biological control, *Heterodera schachtii*, *Trichoderma*

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EVALUATION OF SELECTED WHITE BEAN ACCESSIONS FROM NPGBI COLLECTION FOR RESISTANCE TO *FUSARIUM* SPP.

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Fusarium wilt and root rot are worldwide important diseases of common bean (*Phaseolus vulgaris*) present in many bean growing regions, that produces severe yield losses. *Fusarium* wilt is caused by *Fusarium oxysporum* Schlechtend. Fr. f. sp. *phaseoli* Kendrick and Snyder (*Fop*), while *Fusarium* root rot by *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyder & Hansen (*Fsp*). These diseases are reported to be severe in white bean cultivars, which are grown in Central Iran.

Since cultural methods have limited effectiveness in controlling these diseases and chemical methods are not environmentally safe, it is necessary to obtain bean cultivars with high level of resistance. In this research, 47 accessions of white bean landrace collection of National Plant Gene-Bank of Iran were evaluated for resistance against two *Fop* isolates and one *Fsp* isolate obtained from infected white bean plants, collected during 2008-2009 in research farms of National Plant Gene-Bank of Iran in Karaj (Tehran province) and Khomein (Markazi province). White bean accessions were evaluated under greenhouse conditions by artificial inoculation of plants with the contaminated soil method. Disease severity was scored every two days from 10 to 22 days after the inoculation. Area under disease progress curve (AUDPC) was calculated for every accession. Mean comparison analysis showed that none of the accessions examined in this study was completely resistant but considerable variation and significant differences were observed in AUDPC. Ten accessions with partial resistance can be used in breeding programs to develop moderately resistant bean cultivars against *Fusarium* wilt and root rot.

Key words: *Fusarium oxysporum, Fusarium solani, Phaseolus vulgaris,* AUDPC, Resistant

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BREEDING FOR STEM RUST RESISTANCE IN ICARDA'S DURUM WHEAT BREEDING PROGRAM FOR THE MEDITERRANEAN REGION

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Stem rust disease of durum wheat caused by the fungus *Puccinia graminis* is a global biotic stress. In 1999, a new virulent race of stem rust emerged in Uganda called UG99 (TTKS) which infects most current resistant wheat varieties by overcoming the most common resistance genes. UG99 has already spread to East Africa and to some countries in the Middle East and could spread throughout the world. Growing susceptible varieties can insure the spread of UG99 with devastating impacts on economies and food supplies. So, wheat breeders select for resistant varieties by exposing them to disease.

Durum wheat breeding program at ICARDA is working since many years ago to improve resistance to stem rust. Every year thousands of segregation populations and advanced lines are screened under artificial infection in the Middle East and North Africa region. Selection to UG99 resistance was initiated recently where during the last 3 years numbers of lines and populations were screened under artificial infection condition at Debre Zeit station in Ethiopia: 664 in 2007, 1527 in 2008, and 2040 in 2009. The results indicated that percentage of resistance was for the last 3 years: 7.5% (2007), 32% (2008), and 49% (2009).

Most of the resistant lines carry also good agronomic traits and high yielding potential. Accordingly, three varieties were released in Ethiopia during last years (Aghrass, Gedifla/Gerrou, Maamouri). Additionally, among other sources, ICARDA durum wheat breeding program is using these varieties as source for upgrading stem rust (UG99) resistance.

Key words: Durum wheat, *Puccinia graminis,* Stem Rust, UG99

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RESISTANCE OF SPRING BARLEY VARIETIES TO RAMULARIA LEAF SPOT IN THE CZECH REPUBLIC

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Ramularia collo-cygni (RCC) is barley fungal pathogen of increasing importance in Europe, New Zealand and South America (Sutton and Waller, 1988; Sheridan, 1996; Sachs, 2006). Spring barley varieties were evaluated for the resistance to Ramularia leaf spot disease caused by RCC.

A set of 150 varieties of spring barley was tested in field trials at three locations in the Czech Republic (Luzany, Stupice, Kromeriz) in 2009. Each plot was sown in three replications. The evaluation was done using a 1-9 scoring scale, where 1 = maximum infection and 9 = no disease symptoms. The plots were treated with herbicides only. The occurrence of the pathogen RCC was sporadic at the end of June, while the scoring was performed at all locations at the stages of milk-waxy and waxy maturity. At Luzany, if RCC infection symptoms were scored at milk-waxy maturity, the differences among varieties varied from 3.0 to 5.0; an average difference in the disease expression among earliness categories at heading was only 0.5 (3.6 to 4.1). At waxy maturity, all varieties exhibited maximum RCC symptoms (1.0), i.e. drying leaf blades, large spots on leaf sheath, peduncle as well as awns. At Stupice, at scoring RCC infection symptoms at milk-waxy maturity, the differences ranged between 1.9 and 4.7 with an average infection for all varieties 3.1. The highest disease severity was observed in the variety Xanadu (1.9) and conversely the lowest severity in the varieties Barke (4.1), Isotta (4.5) and Carvilla (4.7). All examined varieties scored at waxy maturity manifested maximum RCC infection symptoms (1.0) similar to those at Luzany. At Kromeriz, scores of the 1-9 scale varied from 3 to 1. The lowest disease severity was found for the variety Isotta (4).

The experiment involved varieties whose resistance to *Blumeria graminis* is based on the gene *mlo*. However, in 2009 with heavy RCC infection, when almost no differences were observed among varieties, no correlation between RCC infection and *mlo* gene presence was confirmed. In this year, no variety resistant to RCC was found in the set of spring barley varieties across the three locations. Lower severity of disease symptoms was recorded in later varieties.

Key words: *Ramularia collo-cygni,* Disease evaluation, Visible symptoms

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SEARCH FOR SOURCE OF RESISTANCE TO *PYRENOPHORA GRAMINEA* IN *HORDEUM VULGARE*

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Barley leaf streak is an important disease in Algeria (Abdouche, 2000). The use of resistant varieties of *Hordeum vulgare* L., is important for controlling the disease (Skou *et al.*, 1994). Several heterogeneities have been reported among barley varieties for disease resistance (Demarly and Sibi, 1996). Developing new disease-resistant varieties is the method of choice (Benbelkacem *et al.*, 1999). Specific crosses were made among Algerian barley genotypes. Forty eight genotypes were selected and characterized in terms of agronomic traits and resistance to *Pyrenophora graminea*. We focused on three virulent strains of the pathogen which were artificially inoculated to plants. A diallel was achieved by a satisfactory number of crosses among genotypes and the F1 seed-hybrids were obtained. Analysis of variance, general parameters and specific parameters of the combination of each type of crosses were used to obtain the investigation results which were thoroughly analyzed and discussed. The F2 was obtained by self-pollination of F1. The F2 was very heterogeneous and resulted in obtaining a homozygote line. This goal was obtained by choosing hybrids, obtaining the next generation and looking for homogeneous traits of interest. A one phenotype may correspond to different genotypes (Jestin, 1992). From The F1 to F6, we studied the impact of disease on agronomic traits. We studied the hybrid vigor (heterosis) of F1 and F2: comparison of F1 and the corresponding average parent heterosis (H), at significant level of crosses, and the impact of disease on some agronomic characters will be presented.

Key words Barley, Genotype, Hybridization, Inoculation, Resistance, Leaf streak

Acknowledgments

Our study aims to improve the Algerian barley genotypes for resistance to leaf streak.

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IN VIVO AND *IN VITRO* EFFECTS OF N AND K ELEMENTS ON DURUM WHEAT RESISTANCE TO SEPTORIA LEAF BLOTCH

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Septoria leaf blotch caused by *Mycosphaerella graminicola* (Fuckel) Schröter ex Cohn (anamorph: *Septoria tritici* Roberge ex Desmaz.) is considered as the most important foliar disease of wheat commonly encountered in all northern areas (Sayoud et *al.*, 1999).

Crop fertilization can influence the susceptibility to *M. graminicola.* The level of infection reflects the nitrogen content of the soil (Tompkins *et al.,* 1993). Furthermore, when the soil is deficient in potassium, the severity of the disease is more marked than when it has an adequate content of this element (Shipton et *al.,* 1971).

In Algeria, few studies were carried out to study the influence of fertilizers on the sensitivity of wheat to Septoria leaf blotch. Thus, the objective of this work consists in studying the effects of different modalities of nitrogen and potassium fertilizers on the reaction of durum wheat to Septoria.

Four modalities of fertilization (M1: without fertilization; M2: 2 q/ha of Bioctyl before sowing; M3: 3 q ha^{-1} of Bioctyl before sowing + 1 q ha^{-1} of Urea at tillage stage + 1 q ha^{-1} at beginning heading stage; M4: M3 + 5 l ha^{-1} of Agripotash at heading stage) were used in order to test the effect of N and K supply on Septoria leaf blotch development on a susceptible durum wheat cultivar Vitron.

Tow trials were carried out: the first one was conducted under field conditions by adopting a randomized complete block design with four replications, where seedlings were artificially inoculated with conidia suspension of *M. graminicola,* adjusted to 10^6 spores per ml; the second trial was carried out *in vitro* conditions by using the detached leaf method (Arraiano et *al.*, 2001; Véchet and Vojackova, 2005), where detached leaf (flag leaf) taken at the beginning heading-flowering stage were also inoculated with a inoculum concentration of 10^6 conidia ml^{-1}.

The results indicate that a potassium supply before sowing seems to decrease the intensity of disease. The nitrogen supplies given at the tillering and at the beginning heading stages promote the extension of the disease to the last leaf causing considerable yield losses. A foliar supply of potassium at the heading stage can remedy to this situation. *In vitro* results confirm those of field trial concerning the effect of N and K and some results reported by Benmouhamed *et al.* (2001). The obtained results will be used in future experiments including the use of pesticides to improve the grain production.

Key words: Durum wheat, *Septoria tritici* leaf blotch, Fertilization, Nitrogen, Potassium

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TRANSFORMATION OF PLUM VARIETIES WITH PPV-DERIVED RNAI CONSTRUCTS

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Plum pox virus (PPV), the causal agent of sharka disease, is one of the most important pathogens affecting stone fruits, causing significant economic losses. Most stone fruit varieties are highly susceptible to the virus and until now breeding programs have not produced resistant and/or commercially acceptable plants. The goal of this research was to obtain *Prunus* varieties resistant to sharka by a biotechnological approach.

PPV-derived gene constructs based on the hairpin RNAi technology were developed (Di Nicola-Negri *et al.*, 2005). Distinct PPV genome regions were cloned and arranged to form an intron-spliced RNA with a hairpin structure. One sequence starts at the 5' end of PPV genome and includes a part of the P1 gene, the other partially cover the HC-Pro gene that encodes the viral suppressor of RNA silencing. The constructs were tested in *Nicotiana benthamiana* where they induced high resistance to a wide range of PPV isolates (Di Nicola-Negri and V. Ilardi,. 2006) and PPV resistance was kept also under biotic and abiotic stress (Di Nicola-Negri *et al*., in this volume).

Based on these results three of the above constructs (h-UTR/P1, h-P1/HCpro and h-HCpro) were introduced into Stanley and Tardicotes plum varieties. Hypocotyl slices from mature seeds were transformed by *Agrobacterium tumefaciens* system. Kanamicyn selection was adopted to screen transgenic shoots. Different sensitiveness to the antibiotic selection was shown by the two plum varieties. Two Stanley clones transformed with h-UTR/P1 and ten Tardicotes clones (three with h-UTR/P1, three with h-P1/HCpro and four with h-HCpro) were obtained after antibiotic selection. PCR analysis, with specific primers, showed that for each cv/construct couple at least two clones amplified all constructs sequences: 35S promoter, PPV derived sequences, DAG intron, terminator and *NPTII* gene. Moreover, in those clones *A. tumefaciens vir* gene was never detected.

PPV resistance evaluation of these clones will be the next step of the project.

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Indice per autori/*Workshop Index*

Abbasi M. 101
Abbasi Moghadam A. 507-590
Abdelmohsin M. E. 190
Abed F. 116-575
Abnaouf A. A. 190
Abo-Elyousr K. A. M. 394
Abou-Jawdah Y. 173-268
Abtali Y. 108
Adam M.A. 481
Aday A. G. 241-573
Adolfi A. 381
Afschari F. 403
Agoritsis S. 95
Aguado A. 415
Al Ghaithi A.G. 332
Al Jabri A.H. 332
Al Mazrouai S.S. 332
Ahmad S. 497
Ahmed Y. 125
Alavi S.V. 182-243
Alawani A.M. 481
Albanese G. 303-307
Albanese P. 568
Aleandri MP. 485-520
Aleem E. 358-
Alfaro-Fernández A. 143-154-156-158 160-260-262-275-346-364-366
Alkadri D. 105
Almabrok A.S. 481
Al-Hilali S.A. 305
Ali O.O. 137
Ali Söğüt M. 149
AAl-Momani A.M. 247
Al-Sadi A.M. 305
Al-Said F.A. 305
Al-Turaihi E. 186
Al-Yahyai R.A. 305
Amin A. 137
Amrirmijani A.R. 317
Andolfi A. 445
Anfoka G. 271
Angelini E. 180
Annesi T. 127-135
Antonelli M. 524
Antoniou P. 95-566
Aparicio-Bello J. A. 537
Arnold D. 299
Aragona M. 413
Arous S. 237
Arrebola E. 424
Assante G. 401
Attar N. 269
Aureli G. 409-447-459
Avramidis N. 4 66
Ayoubi F. 439
Babay-ahri A. 322
Babini A.R. 358
Bacha H. 468
Bagheri A. 392
Balali G.R. 324
Balažic L. 178
Balestra G. M. 147-582
Barakate M. 494
Barari H. 405-517
Barba M. 168-176-307-518
Barbarossa L. 356
Bardaji L. 430
Bardas G. 558
Barka EA. 569
Barrau C. 532
Baschieri T. 1 97
Bassimba D.D.M. 143
Battilani P. 453-455-457-566
Baz M. 494
Belaskri A. 423
Belisario A. 112-122-201-289-328
Bellaedi M.G. 266
Belocchi A. 459
Bencheikh M. 387
Ben Dhiab A. 511
Benedetti A. 518
Bensassi F. 468

Bentata F. 319
Berraf A. 120
Bertazzon N. 180
Bertolini P. 4 37
Bessedik F. 1 16
Bhatnagar D. 435
Bianchi G.L. 180
Borgo M. 180
Borodynko N. 151-264-372
Boscia D. 354
Bouazza F. 492
Boubaker A. 154-366-488
Boubourakas I.N. 397
Boudabous A. 487
Boughrarou N. 596
Boukef S. 93
Boureghda H. 315
Bouri M. 488
Bouroubi N. 315
Bouteau F.. 494
Bouznad Z 120-199-407-596-598
Bragaloni M. 195
Bravi R. 258
Bravo-Luna. L. 537
Brown J.K. 221
Brunner P.C. 93
Budziszewska M. 227-368-370
Bugiani R. 562
Burruano S. 239
Busman M. 450
Cacciola O.S. 251-326-334-461
Caffi T. 562
Campanella V. 118
Candresse T. 237
Capote N. 415
Capretti P. 139-209-211-283
Carella A. 581
Carrión V. 424
Caruso A. 168-277
Castagnone-Sereno P. 289
Cavicchi L. 266
Cazorla F.M. 348-424
Cebrian M.C. 143-154-156-158-160
260-262-275-346-364-366
Ceccarelli B. 116-131-209-526-575
Cennamo G. 164-166
Čepin U. 178
Chandel V. 309
Chattaoui M. 487-511
Chatzipavlidis I. 391
Chilosi G. 485-520-524
Christophorou R. 231
Cimmino A. 381
Cinelli T. 350-381
Cirvilleri G. 145
Clément C. 569
Codina J.C. 424
Coelho M.A. 120
Conigliaro G. 239
Córdoba-Sellés M.C. 154-156-158-160
260-262-275-346-364-366
Correia V.G. 285
Cosic J. 109-188-509-544
Cvjetković B. 330-443
D'Amico L. 127-135
D'Avino V. 164
D'Egidio M.G. 447
D'Onghia A.M. 125-291-439-524
Dallai D. 97
Damadi S.M. 101
Damiano C. 600
Danti R. 577
Davino A.S. 162-168
Davino M. 168
De Amicis F. 180
De Corato U. 548
De Felice S. 459
De Leonardis S. 515-530
De Mendoza A.H. 260
De Simone D. 135
De Vicente A. 348-424-556
Deadman M.L. 332
Debbi A. 199
Debener T. 301
Della Rocca G. 577

Devran Z. 149
Dianpeng Z. 560
Di Giambattista G. 552
Di Nicola-Negri E. 395-426-600
Di Stefano C. 129-207-542
Diamandis S. 129
Dibari C. 139
Dihazi A. 571
Dimakopoulou M. 95-566
Djeghmoum C. 315
Doğmuş-Lehtijärvi H.T. 241-573
Döken M. T. 383
Dolezal A.L. 389
Durreshahwar 505
Duvnjak T. 509
Edraki V. 141
Edwan H. 271
Ehwaeti M.E. 481
Ekzayez A. 269
El-Ahmed A. 297
El- Aissami A. 319
El Bassir H. 511
El Hadrami I. 571
El-Hendawy H. 344
El-Khalifeh M. 592
El Khalloufi F. 441
El Sadi A.M. 332
El-Sherif A.G. 479
El Shimy H. 125
El Yousfi B. 503
Elamri N. A. 299
Erincik Ö 383
Esmaiili A.R. 111-385
Essakhi S. 568
Evidente A. 381-445-577
Fabbri A.A. 389-439-447
Fadavi Khalajloo G. 342
Fadil S. 223
Faedda R. 251
Faggioli F. 307-428
Falahati Rastegar M. 362
Falloon R. E. 89-293
Fanelli C. 389-439-447
Faretra F. 295-554
Fasseas K. 397
Fatmi M. 145
Fattouh F. 358
Faure C. 237
Fazli M. 392
Feducci M. 139
Feki O. K. 571
Felici B. 518
Fellas P. 231
Ferrándiz J.C. 260
Ferrarini A. 428
Ferraro V. 239
Ferretti L. 303
Firrao G. 464
Flamini L. 133
Font M.I. 143-154-156-158-160-260 262-275-346-366
Fontana A. 303
Formenti S. 455
Foroutan A. 107-108-404-405-517
Franceschini S 116-129-133-207-526 534-542-575-
Frías J. 415
Gallelli A. 215
Galli M. 112
Garbellotto M. 490
García-Gutiérrez L. 556
Garibaldi A. 114-249-522-560
Gatta S. 447
Gauthier N. 91
Gentile A. 600
Georgiadou M. 95
Ghazanfar M.U. 501
Gil V. 411
Giorni P. 457
Giovanardi D. 97
Gobbi E. 464
Griessinger D. 184
Gruden K. 178
Guardo M. 168-277
Gülden Aday A. 417
Gullino M.L. 114-249-522-560

Güneş U. 540
Gutiérrez-Aguirre I. 178
Gutiérrez-Barranquero J.A. 348
Haegi A, 201
Haidar A. 268
Haj Ahmad F. 271
Hajarian N. 403
Hajjeh H. 295
Hajlaoui M. R. 203-468
Hallan V. 309
Hantula J. 283
Hanusova N. 594
Haq Q.M.I. 223
Harrat W. 598
Hashemi M. 590
Hasiów-Jaroszewska B. 151-154-264 372-
Hassikou R. 492
Henni J. 387
Herrera-Vásquez J.A. 154-156-160-275-364-366-
Hill R.A. 477
Höfte M. 411
Hussien A. 291
Iazzoni A. 426
Ibijbijen J. 319
Ibrahim M. 358
Iftikaar Y. 497
Ilardi V. 395-426-600
Infantino A. 201-413-459-518
Ioannou N. 231-338
Iori A . 409
Ippolito A. 118-125-205-334
Iqbal Z. 497
Irdani T. 426
Ismail I. 269
Ismail Amona F.A. 479
Ivic D. 443
Jafarpour B. 153-273-362
Jaiti F. 571
Jamjari A. 494
Jaoua S. 571
Jebbouj R. 503
Jeffries C. 217
Jimenez E. 411
Jones J.D.G. 550
Jordá C. 143-154-158-160-260-262-346 364
Juárez M. 160
Jurkovic D. 109-188-544
Jyothsna P. 223
Kalai L. 203
Kalantar E. 324
Kaliterna J. 330
Kamasa J. 257
Kanetis L. 338
Karadeniz M. 417
Karaoglanidis G. 5 28-558
Karnavas G. 95
Katis N.I. 221
Katooli N. 584
Kfoury L. 546
Keshavarz Tohid V. 141-320
Khattabi N. 536
Khelafi H 116
Khodakaramian G. 392-419
Khodaparast S.A. 287-317-340
Khoshkalam N. 322
Koltai H. 397
Korhonen K. 283
Kountouri S.D. 550
Koutita O. 558
Krawczyk K. 257
Krimi Z. 423
Krizmanić M. 509
Kumar Y. 309
Kumari Safaa G. 269
Kurt Ş. 540
Kyriakopoulou P.E. 397
L'Aurora A. 409
Labhilili M. 319
Laguardia C. 554
Lahrouni M. 441
Lamichhane J. R. 147
Lanzoni C. 219
Latinovic J. 124-253

Lehtijärvi A. 241-417-573
Le Romancer M. 237
Ligorio A. M. 334
Linde M. 301
Liović I. 509
Lister R.A. 293
Logrieco A. 435-461-470-472-515-530
Lo Piccolo S. 239
Loqman S. 215-569
Loreti S. 215
Lotfi M. 317
Lucero G. 542
Luchi N. 139-209-211-283
Ludersdorfer T. 550
Luigi M. 307
Luongo L 112-122-201-289-328
Luque J. 381
Maakaroun R. 546
Magan N. 457
Magnano di San Lio G. 326-334
Magro P. 520
Mahdikhani Moghadam E. 255-340-584
588
Mahdjoubi K. 586
Malathi V.G. 223
Mammella M. A. 251-326
Mancini V. 211
Mangllii A. 225
Manoussopoulos I.N. 331
Marais A. 237
Marchi G. 350
Mari M 437
Maric P. 594
Markakis E. 566
Marković M. 579
Marletta T. 277
Marocco A. 515-530
Martelli G.P. 354
Martignoni D. 520
Martin F. 326
Marzachì C. 352
Mascia T. 360
Masi M. 381-577
Matmoura A. 466
Matusinsky P. 594
Mazza G. 135
McDonald B.A. 93
McGawley E.C. 483
McLean K. L. 477
McMullen M. 184
Meca G. 461
Mehdikhani Mogadam E. 213-342
Meimoun P. 494
Mekkeliche L 596
Melck D. 445
Mentzen W. 3 89
Merz U. 293
Miazzi M. 295-554
Miceli C. 118
Miglietta F. 453
Mijić A. 509
Miličević T. 330-443
Minafra A. 354
Minakirova V. 594
Minassian V: 320
Minguzzi S. 219
Miraglia M. 453
Mirkarimi H. R. 507
Mitic D. 579
Mnari-Hattab M. 91-203
Mocali S. 518
Mohammady N. 344
Mokaram Hesar A. 213-255
Molina L.M. 143-346
Molino Lova M. 546
Mondello V. 239
Montes-Belmont R. 537
Monticelli S. 600
Montuschi C. 197
Moosavi Jorf S.A. 320
Morelli M. 354
Moretti A. 450-453-461-470-472
Moriondo M. 139
Mosalayee M.K. 141
Mosca S. 251
Mostafa M. 385

Motta A. 381
Motta E. 127-135-201
Mougou I. 421
Mozafari J. 507
Msallem M. 421-487-511
Mugnai L. 381
Mukhtar S. K. 190
Mulè G. 472-515-530
Mulholland V. 217
Murillo J. 348-430
Mustafa S. 344
Myresiotis C. 528
Nabhan S. 301
Nachit M. 297-592
Nakada N. 483
Nappo A. G. 266
Nardi S. 112
NasrEsfahani M. 111
Neema C. 387
Neophytou G. 231-338
Nicoletti R. 581
Nigro F. 205
Nipoti P. 105-133
Nobili C. 447
Nocentini A. 139
Novak A. 188
Obrepalska-Steplowska A. 227-368-370
Olady M. 517
Oloriz M.I. 411
Orzali L. 552
Oskay F. 241-417-573
Oudra B. 441
Oufdou K. 441
Ouhdouch Y. 569
Pacifico D. 352
Paciolla C. 515-530
Padasht F. 317
Paffetti D. 283
Pakbaz S. 362
Palmano S. 217-352
Pancaldi D. 105
Pane A. 326
Panjehkeh N. 324
Pantelides I.S. 377-391
Papadopoulou-Mourkidou E. 528
Papayiannis L.C. 221-231
Paplomatas E.J. 95-377-391
Parrella G. 164-166-266
Pasquini G. 428
Pastorelli R. 426
Payne G.A. 389
Pellegrin F. 91
Peraica M. 443
Pérez-García A. 3 48-424-556
Perlerou C. 129
Perrone C. 445
Petruzzella A.L. 461
Petter F. 184
Pfohl-Leszkowicz A. 466
Phillips A.J.L. 120-281-285
Picciau L. 352
Pietri A. 457-566
Pisi A. 105
Pittas L. 338
Pizzichini L. 133
Pizzuolo P. 542
Poggi Pollini C. 219
Pompe-Novak M. 178
Porras M. 532
Portal O. 411
Pospieszny H. 151-257-264-368-370
372
Postic J. 109-544
Proctor R.H. 450-472
Prodi A. 105-116-133
Pucci N. 201
Pugliese M. 114-522
Punelli F. 428
Punelli M. 389
Puopolo G 577
Quattrucci A. 582
Ragnese F. 133
Rahman H. 505
Raio A. 423-577
Rajkovic S. 579
Ram R. 309

Ramponi C. 455
Rana T. 309
Rastegar M.F. 273
Ratti C. 219-358
Ravlic M. 544
Ravnikar M. 178
Reda R. 520
Reggiani N. 219
Resnick N. 397
Reverberi M. 389-439-447
Rezaee S. 403
Rezgui S. 93
Rhouma A. 421-468-487-488-511
Riba A. 466
Riccioni L. 122-195-201-552
Ricelli A. 439-447
Rimondi S. 197
Ritieni A. 472
Roccotelli A. 334
Rodeva R. 445
Rohani H. 342
Rojas l. 411
Romero F. 415-532
Roselló J.A. 364
Roschetti A. 307
Rossetti A. 582
Rossi V. 455-562
Rossini E. 112-133
Rouhani H. 588
Rouissi W. 437
Roy A.S. 184-235
Rubies Autonell C. 219-358
Rymelska N 151
Sabaou N. 466
Sabokkhiz M. A. 153-273-362
Saccone C. 291
Saddler G.S. 217
Safarnejad M. R. 499
Sahi S. 501
Salandri L. 395
Salari M. 324
Sallam N.M.A. 496
Salomoni D. 105
Samri S E. 494
Samsatly J. 268
Sanchez A. 411
Sanjuan S. 260
Santomauro A. 554
Santori A, 289-459-518
Santos B. 415
Santos J.M. 120-285
Sanzani S.M. 205
Saponari M. 303
Scagnelli S. 336
Scala V. 409-447-459
Schena L. 205-251-326-334
Schimio R. 303
Sciarroni R. 303
Scirè M. 127
Scotton M. 112
Scuderi G. 145
Sebaaly C. 524
Sellami S. 586
Senape V. 258
Sepúlveda-Jiménez G. 537
Sergeeva V. 99-251
Serghini M. A. 571
Serratore G. 258
Setti B. 387
Shahriyari F. 499
Shahzad M. 497
Shamay Irani S. 5 90
Shams-Bakhsh M. 499
Shamsi R. 297
Sharifnabi B. 385
Sharma N. 548
Shukla R. 223
Sidero F. 258
Sigillo L. 258
Singh V.B. 223
Smith J.A. 101
Sobh H. 268
Soltani I. 154-366
Somma S. 461
Sorrentino G. 168-277
Soylu E.M 540

Spadaro D. 249-560
Spigno P. 166
Spina V. 258
Stea G. 450-472
Steckler R.M. 483
Stefani E. 97
Stemberkova L. 594
Stewart A. 477
Stoyanova Z. 445
Striglis I.A. 391
Suffert M. 184
Surico G. 350-381
Susca A. 450-472
Tabaković-Tosic M. 579
Tagliavento V. 520
Taheri S. 507
Taibi K. 319
Takamatsu S. 287
Taktak W. 571
Tanha Maafi Z. 213
Taouil H. 319
Teymuri P. 182-243
Tiberini A. 162-176-225-360
Tizioualou G. 407
Tizzani L. 201
Tiwari N. 223
Tjamos E.C. 83-95-566
Tjamos S. E. 95-377-391-566
Tsitsigiannis D.I. 95-550
Toffolatti S.L. 564
Tomao A. 575
Tomassoli L. 162-176-225-360
Tonti S. 105-133
Torelli E. 464
Torés J.A. 424
Torres V. 275
Torta l. 239
Tran D. 494
Trapella R. 197
Trzmiel K. 229
Tsaltas D. 231-338
Tuğba Doğmuş-Lehtijärvi H. 417
Tzima A. 377
Tziros G. 129
Uva P. 389-428
Vaezi S. 590-Valente M.T. 413
Valentini F. 145
Valizadeh M. 322
Van der Fels Klerkx I. 453
Van Der Lee T. 450
Van Hove F. 450
Vanneste J.L. 582
Vannini A. 116-129-131-133-207-209
328-485-526-534-542-575
Varvaro L. 147-524
Varzakas T.H. 95
Veloukas T. 558
Venturini G. 401
Vercesi A. 336-401-546-564
Vettraino A. M. 116-129-131-133-207
209-328-485-526-534-542-575
Vicario S. 291
Vicchi V. 162
Villaescusa F.J. 260
Visconti A. 435
Vitale S. 201-289-328
Vivian A. 299
Voludakis A. E. 397
Vrandecic K. 109-188-509-544
Vryzas Z. 528
Vucinic Z. 124-253
Vuono G. 129-207
Waalwijk C. 450
Wajnberg E. 289
Wakil W. 501
Wallace A. R. 293
Wieczorek P. 227-370
Wołczańska A. 581
Woloshuk C. 389
Wydra K. 301
Yahyaoui A. 93-297
Yaich M. 145
Yanniotis S. 95
Yasari E. 108-405
Yaseen T. 125-291-439-568
Yildiz A. 383

Zafari D.	392	Zammouri S.	91
Zaidi A. A.	309	Zandi Z.A.	137
Zakynthinos G.	95	Zanón M.J.	346
Zamanizadeh H.R.	182-243	Zwolińska A.	257