10th INTERNATIONAL VERTICILLIUM SYMPOSIUM
16-20 NOVEMBER, 2009
CORFU ISLAND, HELLAS

PROGRAM
ABSTRACTS OF PLENARY, KEYNOTE, ORAL AND POSTER PRESENTATIONS
LIST OF PARTICIPANTS

FRONT COVER PICTURE

A TYPICAL SCENERY OF THE AEGEAN ISLAND ASTYPALAIA DURING SUMMER

Combination of land and sea with the messenger Ancient Greek God Hermes

Symbolic meaning: The ancient Greek God Hermes is carrying the message concerning the solution of controlling Verticillium over the seas and cultivated lands around the world.....

Picture and comments by Eris Tjamos

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CORFU HOLIDAY PALACE HOTEL
CORFU ISLAND, HELLAS

Dear colleagues

The International Verticillium Steering Committee and the Local Organizing Committee of the 10th International Verticillium Symposium are pleased to have fulfilled the organization of the symposium.

Over 80 scientists from 14 countries will attend the symposium.

Ninety-one plenary, keynotes, oral and poster presentations will be given.

You will enjoy staying in one of the most beautiful, picturesque islands of the Mediterranean Sea. You will hear a lot about the history of the people and admire the civilization of the country you are visiting particularly for those coming for the first time in Greece. You will visit the ancient and medieval sites and places, where Greeks lived for thousands of years. You will be also experienced the hospitality of the modern Greeks and enjoy food and drinks. Beyond attending the symposium you will be in a very friendly and creative environment for holding fruitful scientific discussions and creating new accountancies and links leading to future research cooperation.

For the International Verticillium Steering Committee and the Local Organizers

Eris Tjamos

The organizers acknowledge with thanks organizational and financial support by

Department of Plant Pathology, Agricultural University of Athens, Greece
Hellenic Society of Phytiatry

Alfa Agricultural Supplies S.A
Plastika Kritis
Syngenta
Geotechnical Chamber of Greece

FINAL SCIENTIFIC PROGRAM

MONDAY NOVEMBER 16

0730 – 09.00 LATE REGISTRATION

09.00 WELCOME OPENING ADDRESS

INTERNATIONAL VERTICILLIUM STEERING COMMITTEE AND LOCAL ORGANIZING COMMITTEE

PLENARY SESSION 1

MOLECULAR ASPECTS

CHAIRPERSONS: NONDAS PAPLOMATAS - JANE ROBB

09.30 KRISHNA SUBBARAO-USA
Global Migration of *Verticillium dahliae* - Fact or Fiction page 23
University of California Davis, Davis, CA, USA

10.10 STEVE KLOSTERMAN-USA
***Verticillium* Comparative Genomics** page 24
Steve Klosterman[1], Krishna V. Subbarao, Katherine F. Dobinson[3], Paola Veronese[4], Bart P. H. J. Thomma[5], Maria D. Garcia-Pedrajas[6], Amy Anchieta1, Zehua Chen[7], Dez Barbara[8], Ronnie de Jonge[5], Partha Santhanam[5], Karunakaran Maruthachalam[2], Zahi Atallah[2], Stefan Amyotte[3], Patrik Inderbitzin[2], Zahi Paz[9], David I. Heiman[7], Sarah Young[7], Qiandong Zeng[7], Reinhard Engels[7], Michael Koehrsen[7], James Galagan[7], Bruce Birren[7], Christina Cuomo[7], Seogchan Kang[10], Scott E. Gold[9] and Li-Jun Ma[7]
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10.50-11.20 COFFEE BREAK AND POSTER VIEWING

11.20 KATHERINE DOBINSON-CANADA
Form and function: Molecular analysis of *Verticillium* morphogenesis page 25
Agriculture & Agri-Food Canada, Canada

12.00 BART THOMMA-THE NETHERLANDS
The molecular genetics of the interaction of *Verticillium* with the plant hosts *Arabidopsis* and tomato page 26
Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

12.40-1300 GENERAL DISCUSSION

13.00-14.30 LUNCH

ORAL PRESENTATIONS

TAXONOMY AND GENETICS
MOLECULAR BIOLOGY AND GENOMICS

CHAIRPERSONS: KRISHNA SUBBARAO - BART THOMMA

14.30 DEZ BARBARA-UK-KEYNOTE PRESENTATION

Analyses of mating type genes in *Verticillium* species page 27

Dez Barbara[8], Patrik Inderbitzin[2], Emily Clewes, C Grant, Steve Klosterman[1], Krishna V. Subbarao[2], Katherine F. Dobinson[3], Paola Veronese[4], Bart P. H. J. Thomma[5], Maria D. Garcia-Pedrajas[6], Amy Anchieta1, Zehua Chen[7], , Ronnie de Jonge[5], Partha Santhanam[5], Karunakaran Maruthachalam[2], Zahi Atallah[2], Stefan Amyotte[3], Zahi Paz[9], David I. Heiman[7], Sarah Young[7], Qiandong Zeng[7], Reinhard Engels[7], Michael Koehrsen[7], James Galagan[7], Bruce Birren[7], Christina Cuomo[7], Seogchan Kang[10], Scott E. Gold[9] and Li-Jun Ma[7]

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15.00 MARIA DEL MAR JIMÉNEZ-GASCO

New insights on the phylogenetic relationships of vegetative compatibility groups in *Verticillium dahliae* page 28

Maria del Mar Jiménez-Gasco[1], M. Berbegal[2], G.M. Malcolm[1], J. Armengol[2] and R.M. Jiménez-Díaz[3]

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15.20 MONICA BERBEGAL

Development and application of new molecular markers for the analysis of genetic diversity in *Verticillium dahliae* populations page 29

Monica Berbegal[l], C. Garzón[2], A. Ortega[3], J. Armengol[4], R.M. Jiménez Díaz[5] and M.M. Jiménez-Gasco[6]

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15.40 SIBEL DERVIS

***Verticillium dahliae* on olives in Turkey: Distribution, vegetative compatibility and molecular Characterization and Virulence of Selected Isolates** page 30

S.Dervis[1], L.Erten[2], J. Mercado-Blanco[3], A.Valverde-Corredor [3]and E. Pérez-Artés [3]

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Bornova, Izmir, Turkey [3]Instituto de Agricultura Sostenible, CSIC. Alameda del Obispo s/n. 14080 Córdoba, Spain

16.00 JANE DEBODE

Quantitative detection of multiple *Verticillium* species in soil using real-time PCR page 31

J.Debode[1], K.Van Poucke[1], S.C.Franca[2], M. Höfte[2], M. Maes[1] and K. Heungens[1]

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16.20 JANE ROBB–CANADA-KEYNOTE PRESENTATION

Proteomic analysis of defence gene expression in a model tomato-*Verticillium* pathosystem page 32

Jane Robb[1], Barbara Lee[1], Alex Kurosky[2] and Ross N. Nazar[1]

[1]Molecular and Cellular Biology, University of Guelph, ON, Canada and Department of Biochemistry and Molecular Biology, [2]University of Texas Medical Branch, Galveston, TX, USA

16.50 CHRISTIANE GATZ-GÖTTINGEN RESEARCH CONSORTIUM

Analysis of *Verticillium longisporum*-induced gene expression in *Arabidopsis thaliana* page 33

Christiane Gatz, Elke Gerhard Braus, Elke Diederichsen, Wolfgang Dröge-Laser, Volker Lipka, Ivo Feußner, Petr Karlovsky, Andrea Polle, Thomas Teichmann and Andreas von Tiedemann

Georg-August-University Goettingen, Germany

17.20 DIMITRIS TSITSIGIANNIS

Involvement of genes related to Programmed Cell Death in Arabidopsis-*Verticillium dahliae* interaction page 34

S.D. Kountouri[1], M.X. Panagiotopoulou[1], X. Koutelieri[1], J.D.G. Jones[2] and D.I. Tsitsigiannis[1]

[1] Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece [2]The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, United Kingdom

17.40-18.10 COFFEE BREAK AND POSTER VIEWING

18.10 SUSANNA A. BRAUS-STROMEYER

Chorismate synthase and colonization of xylem vessels of B. napus by the phytopathogenic fungus *Verticillium longisporum* page 35

Susanna A. Braus-Stromeyer, Seema Singh and Gerhard H. Braus

Institute of Microbiology and Genetics, Goettingen, Germany

18.30 ABDELBASSET EL HADRAMI

Molecular and biochemical analysis of the defences and counter-defences in potato x *Verticillium dahliae* pathosystem page 36

A. El Hadrami, L.R. Adam and F. Daayf

University of Manitoba, Department of Plant Science, 222 Agriculture Building, Winnipeg, Manitoba, R3T 2N2, Canada

18.50 IAKOVOS PANTELIDES

Changes in ethylene perception of Arabidopsis plants lead to differential defence responses against *Verticillium dahliae* page 37

I. S. Pantelides, S. E. Tjamos and E. J. Paplomatas

Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

19.10 STEVEN KLOSTERMAN

Lettuce genes differentially expressed in a lettuce- *Verticillium dahliae* interaction page 38

Steven J. Klosterman[1], Amy Anchieta[1], Karunakaran Maruthachalam[2], Ryan J. Hayes[1] and Krishna V. Subbarao[2]

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19.30 PARTHASARATHY SANTHANAM

Identification of the *Verticillium dahliae* secretome by sequence characteristics page 39

Parthasarathy Santhanam, Ronnie de Jonge and Bart Thomma

Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

19.50 ERMIS YANES PAZ

Transcriptome analysis on Verticillium-infected tomato to identify genes involved in host defence page 40

Ermis Yanes-Paz[1], Sajid Rehman[1], Yuling BAI[2], Ramón Santos Bermudez[3], Orlando Borrás-Hidalgo[4]and Bart P.H.J. Thomma[1]

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20.30: COCKTAIL GET TOGETHER PARTY

TUESDAY NOVEMBER 17

ORAL PRESENTATIONS

HOST-PATHOGEN INTERACTIONS

CHAIRPERSONS: KATHERINE DOBINSON - ANDREAS VON TIEDEMANN

08.30 NONDAS PAPLOMATAS-GREECE- KEYNOTE PRESENTATION

Molecular and genetic determinants of the *Verticillium dahliae* - plant host interaction page 41

Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

09.00 JULIE PASCHE

Evaluation of genetic resistance to *Verticillium dahliae* in potato using in vitro cultivation and quantitative PCR page 42

Julie S. Pasche[1], Asunta Thompson[2], Ipsita Mallik[1] and Neil C. Gudmestad[1]

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09.20 ELKE DIEDERICHSEN

Characterization and genetics of different disease parameters caused by *Verticillium longisporum* in *Brassica* and *Arabidopsis* page 43

E. Diederichsen[1], S. Konietzki[1], D. Socquet-Juglard[1], P. Karlovsky[2] and E. Häffner[1]

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[2]*Georg-August-Universität Göttingen, Institut für Pflanzenpathologie, Grisebachstr. 6, D-37077 Göttingen, Germany*

09.40 MICHAEL REUSCHE

Verticillium longisporum* induces new xylem vessel formation in leaves during an infection of *Arabidopsis thaliana* and *Brassica napus page 44

M. Reusche, V. Lipka and T. Teichmann

Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Abteilung Zellbiologie der Pflanze, Georg-August- Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany

10.00 ALIKI TZIMA

The Sucrose Non Fermenting Protein kinase (SNF1) gene is involved in virulence and expression of genes involved in cell wall degrading machinery of *Verticillium dahliae* page 45

Aliki Tzima[1], E. J. Paplomatas[1], P. Rauyaree[2*] and S. Kang[2]

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1020-10.50 COFFEE BREAK AND POSTER VIEWING

10.50 PAOLA VERONESE -USA-KEYNOTE PRESENTATION

How phytopathogenic *Verticillium* spp. manipulate host defence-related secondary metabolism page 46

Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA.

11.20 EVA HÄFFNER

QTL mapping of *Verticillium* resistance traits in *Arabidopsis thaliana* page 47

Eva Häffner and Elke Diederichsen

Freie Universität Berlin, Institut für Biologie – Angewandte Genetik, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany

11.40 ARNE WEIBERG

The role of the phytohormone salicylic acid in defence of *Brassica napus* to *Verticillium longisporum* page 48

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[2] *Department of Botany, University of Pune, India*

[3] *Plant Breeding Unit, Department of Crop Sciences, Georg-August University of Goettingen, Germany*

12.00 MANOLIS MARKAKIS
Quantification of defoliating and non defoliating pathotypes of *Verticillium dahliae* in Greek olive cultivars infested by a microsclerotia inoculum page 49
E. Markakis, S.E. Tjamos., P., Antoniou, E.J. Paplomatas, and E.C. Tjamos
Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

12.20 JESUS MERCADO-BLANCO
A close look at the olive-*Verticillium dahliae*-*Pseudomonas fluorescens* in planta interaction page 50
Jesús Mercado-Blanco[1], Carmen Navarro-Raya[1], Antonio Valverde-Corredor[1], Stefan G. Amyotte[2], Katherine F. Dobinson[2,3] and Pilar Prieto[1]
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12.40 ALIKI TZIMA
Roles of the G protein β subunit and the catalytic Protein kinase A signalling genes in virulence and physiology of *Verticillium dahliae* page 51
Aliki Tzima[1], E. J. Paplomatas[1], D. I. Tsitsigiannis[1] and S. Kang[2]
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13.00 PHOTOGRAPHY OF THE GROUP
13.15-15.00 LUNCH

POSTER SESSION
15.00-16.00 POSTER VIEWING

16.00-18.20 SHORT POSTER PRESENTATIONS AND POSTER DISCUSSION

MODERATORS: PETR KARLOVSKY - ABDELBASSET EL HADRAMI

TAXONOMY AND GENETICS
MOLECULAR BIOLOGY AND GENOMICS

16.00 MAPIA RATAJ-GURANOWSKA
New case of the identity between vegetative compatibility groups VCG 1 and VCG 2B page 52
Rataj-Guranowska M. and Łukaszewska-Skrzypniak N.
Institute of Plant Protection-National Research Institute, Poznan, Poland

16.05 CARMEN NAVARRO-RAYA
Identification and characterization of an MFS-transporter gene from *Verticillium dahliae* in the interaction with olive page 53
Carmen Navarro-Raya[1] , Enriqueta Moyano[2] , Joselin Benítez-Alfonso[2] , Katherine F. Dobinson[3,4], Juan Muñoz-Blanco[2] and Jesús Mercado-Blanco[1]

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16.10 KATHERINE DOBINSON

Transposable elements of *Verticillium dahliae* page 54

S.G. Amyotte[1] K.F. Dobinson[1,2], P. Veronese,[3] S.J. Klosterman[4] , K.V. Subbarao[4,5], S.E. Gold[6] S. Kang[7] and L.-J. Ma[8]

Department of Biology, University of Western Ontario1, London ON, Canada; Southern Crop Protection and Food Research Centre, Agriculture and Agri-food Canada, London, ON, Canada[2]; Department of Plant Pathology, NC State University, Raleigh NC, USA[3]; USDA-ARS, Salinas CA, USA[4]; Department of Plant Pathology, University of California, Davis CA, USA[5]; University of Georgia, Athens GA, USA[6]; Department of Plant Pathology, Pennsylvania State University, University Park PA, USA[7]; Broad Institute of MIT and Harvard, Cambridge MA, USA[8].

16.15 KATHERINE DOBINSON

Autophagy and resting structure development in *Verticillium dahliae* page 55

Sylvie M. van Twest[2], Sandra J Grant[1] , Jessica Cucollo[2] and Katherine F. Dobinson[1,2]

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16.20 IOANNIS PAPAIOANNOU

Searching for heterokaryon incompatibility genes in *Verticillium* page 56

Ioannis A. Papaioannou and Milton A. Typas

Department of Genetics & Biotechnology, Faculty of Biology, University of Athens, Panepistimiopolis, Athens 15701, Greece

16.25 STEFAN SEEFELDER

Wilting disease in the Hallertauer hop growing region -Molecular characterization of various *Verticillium* strains page 57

S.Seefelder[1], E.Seigner[1], E.Niedermeier[1], S.Radišek[2], and B.Javornik[3]

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16.30 HAIQUAN XU

Identification and characterisation *Verticillium longisporum* gene encoding a transcription factor similar to repressor of polysaccharide hydrolases AceI of *Trichoderma reesei* page 58

Haiquan Xu, Malte Beinhoff, Arne Weiberg and Petr Karlovsky

Institute of Plant Pathology and Plant Protection, Göttingen University, Grisebachstrasse 6, 37077 Göttingen, Germany

16.35 PETR KARLOVSKY

Characterisation of potential pathogenicity-related gene *Vl_6.2* of *Verticillium longisporum* page 59

Haiquan Xu, Arne Weiberg and Petr Karlovsky

Institute of Plant Pathology and Plant Protection, Göttingen University, Grisebachstrasse 6, 37077 Göttingen, Germany

16.40 MALTE BEINHOFF
Detection and functional analysis of a polyketide synthase gene in *Verticillium longisporum* page 60
Malte Beinhoff, Hanno Wolf, Wolfgang Hiegl, Arne Weiberg and Petr Karlovsky
Institute of Plant Pathology and Plant Protection, Göttingen University, Grisebachstrasse 6, 37077 Göttingen, Germany

HOST PATHOGEN INTERACTIONS

16.45 MALTE BEINHOFF
Characterization of Nep-like proteins (NLPs) of *Verticillium longisporum* according to their relevance for pathogenicity in *Brassica* napus page 61
Malte Beinhoff, Arne Weiberg, Haiquan Xu and Petr Karlovsky
Institute of Plant Pathology and Plant Protection, Göttingen University, Grisebachstrasse 6, 37077 Göttingen, Germany

16.50 ARNE WEIBERG
A cDNA-AFLP transcriptome database of Brassica napus in response to *Verticillium longisporum* infection page 62
A. Weiberg, Karlovsky P. and von Tiedemann A.
Division of Plant Pathology and Crop Protection, Georg-August University of Goettingen, Germany

16.55 ALIKI TZIMA
Insights into the role of the Necrosis and Ethylene inducing Protein (VdNEP) gene in virulence of *Verticillium dahliae* page 63
Aliki Tzima[1], E. J. Paplomatas[1], D. I. Tsitsigiannis[1], M. Tsagouris[1], and S. Kang[2]
[1]Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece
[2]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA

17.00 IAKOVOS PANTELIDES
Ethylene perception via Never ripe and LeETR4 affects the resistance of tomato plants to vascular infection by *Verticillium dahliae* page 64
I.S. Pantelides, S.E. Tjamos and E.J. Paplomatas
Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

17.05-17.30 COFFEE BREAK

17.30 ANJALI RALHAN
Verticillium longisporum* induced gene expression in *Arabidopsis thaliana page 65
Anjali Ralhan, Hella Tappe and Christiane Gatz
Albrecht-von-Haller-Institute for Plant Sciences, George-August-University Goettigen, Germany

17.35 JANE ROBB
Defense gene responses in a plant endophyte interaction page 66
H.O. Shittu, C.D.M. Castroverde, R.N. Nazar and E.J. Robb
Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

17.40 ABDELBASSET EL HADRAMI

In vitro elicitation of pathogenicity-related genes in *Verticillium dahliae* using potato root extracts page 67

Ahmed F. El-Bebany, Maria A. Henriquez, Mohamed A. Badawi, Lorne R. Adam, Abdelbasset El Hadrami and Fouad Daayf

University of Manitoba, Department of Plant Science, 222 Agriculture Building, Winnipeg, Manitoba, R3T 2N2, Canada

17.45 ABDELBASSET EL HADRAMI

Changes in *Verticillium dahliae's* pathogenic abilities after serial passages on original or alternative hosts page 68

Hassna A. Alkher[1], Abdelbasset El Hadrami[1], Khalid Y. Rashid[2], Lorne R. Adam[1], and Fouad Daayf[1]

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17.50 ABDELBASSET EL HADRAMI

On *Verticillium dahliae* plasticity and flexibility: a case study of cross-pathogenicity between potatoes and sunflowers page 69

Hassna A. Alkher[1], Abdelbasset El Hadrami[1], Khalid Y. Rashid[2], Lorne R. Adam[1], and Fouad Daayf[1]

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PATHOLOGY, PHYSIOLOGY AND BIOCHEMISTRY

17.55 JAVIER LÓPEZ-ESCUDERO

Improvement of inoculation methods for finding resistance of olive to Verticillium wilt caused by *Verticillium dahliae* page 70

C. Trapero, L. Rallo, M.A. Blanco-López and F.J. López-Escudero

Departamento de Agronomía, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Celestino Mutis, 14071, Córdoba, Spain

18.00 JAVIER LÓPEZ-ESCUDERO

Physiological differences expressed by susceptible and resistant olive cultivars inoculated with *Verticillum dahliae* page 71

F.Birem, E.Alcántara, M.A. Blanco-López and F.J. López-Escudero

Departamento de Agronomía, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Celestino Mutis, 14071, Córdoba, Spain

18.05 THOMAS TEICHMANN

Cytokinin treatments ameliorate symptom development during *Verticillium* infection of *Arabidopsis* page 72

M. Reusche, V. Lipka and T. Teichmann

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18.10 ABDELBASSET EL HADRAMI

Production and scavenging of ROS during potato-*Verticillium dahliae* interactions: *Strboh* genes expression and role of secondary metabolites page 73
M. Badawi, L. R. Adam, A. F. El-Bebany, A. El Hadrami and F. Daayf
University of Manitoba, Department of Plant Science, 222 Agriculture Building Winnipeg, MB, R3T 2N2 Canada.

18.15-18.45 GENERAL DISCUSSION

WEDNESDAY 18 NOVEMBER

FULL DAY EXCURSION OF SCIENTIFIC AND ARCHAEOLOGICAL INTEREST TO THE GREEK MAINLAND (IGOUMENITSA, IOANNINA, METEORA-KALAMBAKA)

THURSDAY NOVEMBER 19

PLENARY SESSION 2

DISEASE MANAGEMENT AND CONTROL ASPECTS

CHAIRPERSONS: MATTEO CIRULLI - RAFAEL M. JIMENEZ-DIAZ

09.00 ERIS TJAMOS - GREECE
Current and emerging problems of Verticillium wilts: Research challenges, policies and practical applications page 74
Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

09.40 RAFAEL M. JIMENEZ-DIAZ - SPAIN
Verticillium wilt in olives in southern Spain, past and present: How a minor problem became the major threat for an olive industry? page 75
College of Agriculture and Forestry, University of Córdoba, and Institute for Sustainable Agriculture, CSIC; Alameda del Obispo s/n, P.O.Box 4084, 14080 Córdoba, Spain

10.10 ABRAHAM GAMLIEL - ISRAEL
Managing Verticillium: past experience, current challenges and future prospects (advances) page 76
Institute of Agricultural Engineering, ARO, The Volcani Center, Bet Dagan 50250, Israel

10.50-11.20 COFFEE BREAK

11.20 GABRIELE BERG - AUSTRIA
Biological control of Verticillium Diseases: Possibilities, problems and praxis page 77
Gabriele Berg and Henry Müller
Graz University of Technology, Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria

12.00-12.15 GENERAL DISCUSSION

12.15 – 13.00 LUNCH

13.00 AFTERNOON ROUND TRIP VISIT TO THE MOST INTERESTING SITES OF CORFU CITY AND THE ISLAND

EVENING : SYMPOSIUM DINNER

FRIDAY NOVEMBER 20

ORAL PRESENTATIONS

EPIDEMIOLOGY, DISEASE MODELING AND FORECASTING DISEASE MANAGEMENT

CHAIRPERSONS: ERIS TJAMOS – JELLE HIEMSTRA

08.30 MATTEO CIRULLI -ITALY-KEYNOTE PRESENTATION
Complete control of Verticillium wilt of olive is obtained using resistant rootstocks page 78

Matteo Cirulli[1], Giovanni Bubici[1] and Salvatore Frisullo[2]
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EPIDEMIOLOGY, DISEASE MODELING AND FORECASTING

09.00 JUAN A. NAVAS-CORTÉS
The influence of agronomic factors on prevalence and distribution of *Verticillium dahliae* vegetative compatibility groups and pathotypes infecting olive in Andalusia, southern Spain page 79

J.A.Navas-Cortés[1], C. Olivares[2], J.L.Trapero-Casas[1], B.B. Landa[1], M.M. Jiménez-Gasco[3] and R.M.Jiménez-Díaz, [1,2]
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09.20 JAVIER LÓPEZ-ESCUDERO
Distribution of Verticillium dahliae through watering systems in irrigated olive orchards in Andalucia page 80

F.J. López-Escudero, S. García-Cabello and M.A. Blanco-López
Departamento de Agronomia, Universidad de Córdoba, Campus Univ. Rabanales, Edificio Celestino Mutis, 14071, Córdoba, Spain

09.40 JUAN A. NAVAS-CORTÉS
High resolution thermal Remote Sensing imagery for Verticillium wilt detection in olive page 81

C.Lucena[1], J.A.J.Berni[2], M.Montes-Borrego[1], J.L.Trapero-Casas[1], B.B.Landa[1], P.Zarco-Tejada[2] and J.A.Navas-Cortés[1]
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10.00 BRANKA JAVORNIK

Studies of *Verticillium* resistance in hop page 82

Branka Javornik[1], Stanislav Mandelc[1], Sebastjan Radišek[2], Jernej Jakše[1], Andreja Čerenak[2] and Zlatko Satović[3]
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10.20-10.50 COFFEE BREAK AND POSTER VIEWING

BIOLOGICAL CONTROL

10.50 GABRIELE BERG

***Stenotrophomonas* – new insights into a potent *Verticillium* antagonist** page 83

Gabriele Berg[1], Massimiliano Cardinale[1], Christoph Schmidt[1], Muhammedali Alavi[1], Henry Müller[1]and José Luis Martínez[2]
[1]TU Graz, Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria [2]Centro Nacional de Biotecnología (CSIC), Darwin 3, Madrid, Spain

11.10 SOTIRIS TJAMOS

Mode of action of a non-pathogenic *Fusarium oxysporum* strain against *Verticillium dahliae* page 84

I. Pantelides, S.E. Tjamos, I. Striglis, I. Chatzipavlidis and E.J. Paplomatas
[1]Department of Plant Pathology, [2]Laboratory of General and Agricultural Microbiology, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece

11.30 RAFAEL M. JIMÉNEZ-DÍAZ

Control of Verticillium wilt of olive caused by defoliating *Verticillium dahliae* with the Trichoderma-based Bioten® formulation page 85

R.M. Jiménez-Díaz[1,2], J.L. Trapero-Casas[2], J.Boned[3], B.B. Landa[2] and J.A. Navas-Cortés[2]
[1]College of Agriculture and Forestry, University of Córdoba, Campus Rabanales, 14071 Córdoba; [2]Institute for Sustainable Agriculture, CSIC; Alameda del Obispo s/n, P.O.Box 4084, 14080 Córdoba, Spain; [3]ISAGRO ESPAÑA, S. L., Maldonado 63, E-C, 2º I, 28006 Madrid, Spain

11.50 ABDELBASSET EL HADRAMI1

Study of some commonalities between the action mechanisms of selected green manures used to control potato Verticillium wilt page 86

Abdelbasset El Hadrami[1], Lorne R. Adam[1], Fouad Daayf[1] and Mario Tenuta[2]
[1]Department of Plant Science, 222, Agriculture Building; [2]Department of Soil Science, University of Manitoba, Winnipeg, R3T 2N2, Manitoba, Canada

12.00 FRANCO NIGRO

Microbial antagonists and compost-based growing media affect the growth of olive plantlets and the inoculum density of *Verticillium dahliae* microsclerotia page 87

T. Yaseen[1], A.M. D'Onghia[1], A. Ippolito[2], and F. Nigro[2]
[1]Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM)/Mediterranean Agronomic Institute, Via Ceglie 9, 70010 Valenzano (BA), Italy; [2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari - Via Amendola 165/A, I - 70126 Bari, Italy

DISEASE MANAGEMENT

CHAIRPERSONS: ABRAHAM GAMLIEL –BRANKA JAVORNIK

12.30 POLYMNIA ANTONIOU
Verticillium wilt management in outdoor field crops: The watermelon case in Greece page 88
Polymnia Antoniou, Sotiris Tjamos and E. C. Tjamos
Department of Plant Pathology, Faculty of Crop Science, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

12.30 LEAH TSROR
Attempts to control Verticillium wilt on olive in Israel page 89
L. Tsror (Lahkim)[1], A. Dag[2], M. Hazanovsky[1], I. Zipori[2], N. Bar-On[3], N. Priel [3], Y. Tugendhaft[3] and S. Lavee[4]
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12.50 TOM GULYA
A new strain of *Verticillium* threatens U.S. sunflower production: distribution and sources of resistance page 90
T. J. Gulya[1] and S. A. Radi[2]
[1] Sunflower Research Unit, USDA- Agricultural Research Service, Fargo, ND 58105, U.S.A.;
[2]Dow AgroSciences, Sunflower Research, Breckenridge, MN 56520, USA

13.10 JELLE HIEMSTRA
Verticillium resistance in maple page 91
J.A. Hiemstra
Nursery Stock section, Applied Plant Research PPO, Wageningen University.P.O. Box 85, 2160 AB Lisse, The Netherlands

13.30 ABRAHAM GAMLIEL
Predicting the efficacy of soil fumigation in controlling Verticillium wilt - from research to a farmer decision-making tool page 92
A. Gamliel[1], S.Triky-Dotan[1], M.Austerweil[1], B.Steiner[1], Y. Peretz-Alon[2], J.Katan[3] and R. Volcan[4]
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[3]Hebrew University, Faculty of Agriculture, Rehovot, 76100 Israel;
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14.10-15.10 LUNCH

15.10 POSTER PRESENTATION AND DISCUSSION

MODERATORS: JOSE LOPEZ ESCUDERO-SIBEL DERVIS

EPIDEMIOLOGY, DISEASE MODELING AND FORECASTING

TAXONOMY AND GENETICS

Bornova, Izmir, Turkey; [3]Ege (Aegean) University, Department of Plant Protection, Faculty of Agriculture, 35100 Bornova, İzmir, Turkey

15.45 ELEFTHERIOS LIGOXIGAKIS
Vegetative compatibility groups of *Verticillium dahliae* isolates obtained from cultivated and weed plants in Crete page 100
D.J. Vakalounakis and E.K. Ligoxigakis
National Agricultural Reaserch Foundation (N.A.G.RE.R.), Plant Protection Institute GR 71003 Heraklio, Crete, Greece

15.50 ELEFTHERIOS LIGOXIGAKIS
Determination of pathotypes of *Verticillium dahliae* and new hosts of *V. dahliae* race-2, and spread of the pathogen in Crete page 101
E.K. Ligoxigakis
National Agricultural Reaserch Foundation (N.A.G.RE.R.), Plant Protection Institure GR 71003 Crete, Greece

15.55 TUAN VAN TRAN
Fungal adhesion to plants – the first step of early infection and systemic colonization of Brassica napus by *Verticillium longisporum* page 102
Tuan van Tran, Susanna A. Braus-Stromeyer and Gerhard H. Braus
Institute of Microbiology and Genetics, Goettingen, Germany

16.00-16.20 COFFEE BREAK AND POSTER VIEWING

DISEASE MANAGEMENT

MODERATORS: LEAH TSOR - FRANCO NIGRO

16.20 SORAYA FRANÇA
Potential of lignin incorporation in soil to control Verticillium wilt of cauliflower page 103
S.C. França[1], S.Van Beneden[1], K.Spiessens[2], L. De Rooster[2], S. Pollet[3], D. Callens[3] and M. Höfte, [1]
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16.25 JELLE HIEMSTRA
Non-chemical control of *Verticillium* and nematodes in tree nursery soils page 104
G.W. Korthals[1], J.H. Visser[1], B.J. van der Sluis[2], A.P. Smits[2] and J.A. Hiemstra[2]
Applied Plant Research PPO, Wageningen University, The Netherlands [1]Section Arable Farming, Multifunctional Agriculture and Field Production of Vegetables, P.O. Box 430, 8200 AK Lelystad, The Netherlands.[2]Nursery Stock section, P.O. Box 85, 2160 AB Lisse, The Netherlands

16.30 LEAH TSROR
A Knowledge-based System to Predict Plot infestation with *Verticillium dahliae* in Potato page 105
E. Goldstein[1,2], Y. Cohen[1], A. Hetzroni[1], L. Tsror[3], U. Zig [4] and I. Lensky [2]

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16.35 SEBASTJAN RADISEK

Verticillium wilt on hops in Slovenia: phytosanitary control and disease management page 106

Sebastjan Radišek[1], Uroš Kolenc[2], Jolanda Persolja[3], Andreja Čerenak[4], Vlasta Knapič[5], Ema Pavlič-Nikolić[6] and Branka Javornik[7]

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16.40 MASSIMO FERRARA

Effects of Acibenzolar-S-methyl on resistance induction and stem colonization by *Verticillium dahliae* in nursery produced olive planting stocks page 107

M. Ferrara[1], I. Pentimone[1], M. Mammella[2], A. Ippolito[1]and F. Nigro[1]

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BIOLOGICAL CONTROL

16.45 KATJA DROFENIGG

Cultivar – specific *Pseudomonas* communities in the rhizosphere of olives page 108

Katja Drofenigg[1], Sergio Aranda [2], Blanca B. Landa[2], Gabriele Berg[1]and Henry Müller[1]

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16.50 KATJA DROFENIGG

Molecular analysis of endophytic communities of wild olive from different site of the Mediterranean region page 109

Katja Drofenigg[1], Henry Müller[1], Christian Berg[2] and Gabriele Berg[1]

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16.55 ELISABETTA SCHILIRÓ

Unraveling the olive roots colonization process by Verticillium wilt effective biocontrol *Pseudomonas* spp strains page 110

Elisabetta Schiliró[1], Pilar Prieto[2], Raquel Valderrama[3], Mercedes Maldonado-González[1], Juan B. Barroso-Albarracín[3] and Jesús Mercado-Blanco[1]

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17.00 JAVIER LÓPEZ-ESCUDERO

An outbreak of Verticillium wilt in Hedgerow olive orchards in Andalucía (Southern Spain) page 111

F.J. López-Escudero, L.F. Roca, A.Trapero and M.A. Blanco-López

Grupo de Patología Agroforestal, Departamento de Agronomia, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071-Córdoba, Spain

17.05 FRANCO NIGRO

Effect of cured compost and biocontrol agents on the viability of *Verticillium dahliae* microsclerotia in the rhizosphere of nursery-grown olive plants page 112

G. Lima[1], D. Vitullo[1], F. De Curtis[1], M. Ferrara[2] and F. Nigro[2]

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17.10 FRANCO NIGRO

Effect of Guanito on olive plantlets growth and on the inoculum density of *Verticillium dahliae* microsclerotia page 113

M. D'Onghia[1], T. Yaseen[1], A. Ippolito[2] and F. Nigro[2]

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17.15 CLOSING REMARKS

17.30

ELECTION OF NEW INTERNATIONAL VERTICILLIUM STEERING COMMITTEE

COUNTRY AND DATE FOR HOLDING THE 11th INTERNATIONAL VERTICILLIUM SYMPOSIUM

GLOBAL MIGRATION OF *VERTICILLIUM DAHLIAE* – FACT OR FICTION?

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The migration of fungal phytopathogens in infected, infested or contaminated plant matter, such as seed or vegetative material, is cause for concern and regulation in various parts of the world. Reports on the international transfer of *Verticillium dahliae* in plant material abound in the literature. These studies relied on the direct recovery of the pathogen from planting material (Omer et al. 2000; du Toit et al. 2005), or on the indirect association by similarity of genetic or phenotypic markers (Dobinson et al. 1998; Korolev et al. 2001; Nigro et al. 2005; Jimenez-Diaz et al. 2006; Radisek et al. 2006). The variety of hosts described in these studies and their wide geographic distribution highlight the perennial and chronic nature of the disease and the incessant quest to understand the significance of gene flow in Verticillium wilt outbreaks, especially that *V. dahliae* is commonly transferred across natural and international boundaries by human activity. Because of the nature of the molecular or biological markers previously used, the ability to retrace the evolutionary and migratory history of genotypes remained limited. A number of the studies published on the population biology of *V. dahliae* have utilized either dominant markers or phenotypes, both of which are not amenable to evolutionary analyses relying on the coalescent or Bayesian theories. To alleviate such impediments, we developed and utilized a set of 22 unlinked simple sequence repeat (SSR or microsatellite) markers, which offered a set of robust co-dominant markers that may be used to retrace the evolutionary and migratory histories of the fungus. These markers were employed on a collection of strains of *V. dahliae* isolated from lettuce and other vegetable and small fruit plants in coastal California (western USA), spinach seed from Northern Europe, the US Pacific Northwest and South America, tomato and lettuce seed from the San Joaquin Valley of central California, and ornamental plants from Wisconsin (North Central USA). The virulence phenotype of a number of these strains was also determined in replicated greenhouse trials. The genotypic diversity obtained from the SSR haplotypes was elevated. Conversely, higher frequencies of those with same genotype were recovered from all spinach seed populations and from the tomato population. Both races 1 and 2 were also found in all populations, with the exception of the tomato population, which was solely comprised of race 2 strains. The first major finding involves the lack of correlation between the virulence phenotype and genotype, which suggests that *V. dahliae* undergoes common recombination. Additionally, the lack of significant differentiation between the spinach seed populations and the other populations (except tomato) indicates that the genotypes of the pathogen are re-sampled in various regions, because of their introduction in infested seed. Strains isolated from lettuce plants in coastal California were assigned to spinach populations, and similarly many of the strains from spinach seed from various countries were assigned to other spinach sources. However, no strains were assigned to the lettuce seed population, including those from coastal California lettuce plants. Similarly, using sequences of the IGS rDNA, strains from spinach seed were successfully placed in each of the three clades obtained from the Bayesian phylogenetic analysis of the IGS rDNA sequences, indicating that spinach populations were not differentiated from populations collected in coastal California. Based on data acquired from the genome sequence of strain VdLs17, and fine mapping using 2603 AFLP makers, the length of linkage in *V. dahliae* is estimated to be ~1,000 nucleotides, similar to the sexual human genome. The findings of this study support previous evidence of intra and international migration of *V. dahliae* and provide credence to suggestions that the fungus undergoes frequent recombination, and is not solely clonal.

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VERTICILLIUM COMPARATIVE GENOMICS

STEVEN J. KLOSTERMAN[1], KRISHNA V. SUBBARAO[2], KATHERINE F. DOBINSON[3], PAOLA VERONESE[4], BART P. H. J. THOMMA[5], MARIA D. GARCIA-PEDRAJAS[6], AMY ANCHIETA[1], ZEHUA CHEN[7], DEZ BARBARA[8], RONNIE DE JONGE[5], PARTHA SANTHANAM[5], KARUNAKARAN MARUTHACHALAM[2], ZAHI ATALLAH[2], STEFAN AMYOTTE[3], PATRIK INDERBITZIN[2], ZAHI PAZ[9], DAVID I. HEIMAN[7], SARAH YOUNG[7], QIANDONG ZENG[7], REINHARD ENGELS[7], MICHAEL KOEHRSEN[7], JAMES GALAGAN[7], BRUCE BIRREN[7], CHRISTINA CUOMO[7], SEOGCHAN KANG[10], SCOTT E. GOLD[9]AND LI-JUN MA[7]

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Verticillium dahliae, the cause of Verticillium wilt on economically important crops and ornamentals, exhibits extraordinary genetic plasticity, capable of colonizing a broad range of plant hosts in diverse ecological niches. One factor that has hindered progress in developing new control strategies for Verticillium wilt is our limited understanding of the biology and ecology of *V. dahliae* and related pathogens. We have employed a genomic approach to compare *V. dahliae* to a related species, *V. albo-atrum*, that produces distinctive survival structures and exhibits host range differences. A 7.5X assembly of the 33.8 Mb genome of a lettuce isolate of *V. dahliae*, in addition to a 4X assembly of an alfalfa isolate of *V. albo-atrum* are now publicly available via the Broad Institute. About 38,000 EST reads from three cDNA libraries of *V. dahliae* were generated, and the genomes of both species have been annotated. Through comparative analyses, we have identified four regions on two chromosomes that are specific to *V. dahliae*. Each of these spans ~ 300 kb and are enriched in repetitive DNA. The expression of *V. dahliae*-specific genes encoded in these regions was confirmed by the presence of corresponding ESTs. We are currently examining these regions and conducting genome-wide analyses of predicted protein sets from both fungi to gain insight into diversity, pathogenicity, and other aspects of *Verticillium* spp. biology.

FORM AND FUNCTION: MOLECULAR ANALYSIS OF *VERTICILLIUM* MORPHOGENESIS

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Verticillium dahliae and *V. albo-atrum* are characterized by distinct phases of growth during disease progression and resting structure development. Our research has been directed toward elucidating the molecular mechanisms that direct these morphogenetic changes in *V. dahliae* and *V. albo-atrum*. In the pre-VGS (*Verticillium* genome sequence) era we used expressed sequence tag (EST) and small-scale microarray analyses to identify genes expressed differentially in *V. dahliae* during i) dimorphic growth in a simulated xylem fluid medium, and ii) *in vitro* growth under conditions that favour development of microsclerotia (MCS), the long-lived, melanized structures that form in the very late stages of the *V. dahliae* life cycle and are well able to survive adverse field and laboratory conditions. Since then, our studies have focused primarily on functional characterization of several genes identified in the above analyses, including: orthologous class II hydrophobin genes in *V. dahliae* and *V. albo-atrum*, a gene with high similarity to the yeast autophagy gene *ATG8*, and more recently, a gene of unknown function for which expression is highly upregulated during growth under microsclerotial development-inducing conditions. An efficient *Agrobacterium tumefaciens*-mediated gene replacement system has been exploited for disruption of these genes, and comparative in-depth expression analyses are being carried out to evaluate the temporal and spatial changes ingene and gene product expression that occur during resting structure development and dimorphic growth in both *V. dahliae* and *V. albo-atrum*. Results to date suggest a link between MCS formation and dimorphic growth, and further have begun to reveal molecular differences in the developmental pathways that govern resting structure formation in *V. dahliae* and *V. albo-atrum*.

THE MOLECULAR GENETICS OF THE INTERACTION OF *VERTICILLIUM* WITH THE PLANT HOSTS ARABIDOPSIS AND TOMATO

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So far, a locus responsible for resistance against *Verticillium* has been cloned only from tomato [1]. This *Ve* locus governs resistance against race 1 strains of *V. dahliae* and *V. albo-atrum*, and contains two closely linked inversely oriented genes, *Ve1* and *Ve2*. We recently investigated the role of *Ve1* and *Ve2* in *Verticillium* resistance of tomato. Surprisingly, we found that *Ve1*, but not *Ve2*, mediates *Verticillium* resistance in tomato [2]. Using VIGS, we determined the role of a number of known disease resistance signaling components in *Verticillium* resistance. We found that the receptor-like kinase SERK3 (BAK1) is required for Ve1-mediated resistance, providing the first example of race-specific disease resistance for which SERK3/BAK1 is required [2]. Through cDNA-AFLP followed by VIGS analysis of candidate genes we are trying to identify novel components that are required for Ve1 mediated-resistance in tomato.

In addition to tomato, we also use Arabidopsis as a host for *Verticillium dahliae*. We recently discovered a role for gene silencing in *Verticillium* defense [3]. Furthermore, mutants displaying enhanced *Verticillium* resistance have been isolated and characterized.

Finally, we are interested to characterize the secretome of *V. dahliae*, potential virulence factors that are secreted *in planta*, amounting to over 780 proteins. The secretome contains seven lysin motif (LysM) effectors that are homologous to the recently identified *C. fulvum* effector protein Ecp6 [4,5]. The role of LysM effectors in pathogen virulence will be addressed.

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ANALYSES OF MATING TYPE GENES IN *VERTICILLIUM* SPECIES

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Sexual mating has not been recorded in any of the plant pathogenic *Verticillium* species but as ascomycetes they are presumably derived from species which were once sexual. Two idiomorphic genes with DNA binding motifs determine mating type in many fungi. Such genes determining mating type have been reported in other asexual fungi and we have looked for them in *Verticillium*.

A MAT1-2-1 gene (with an HMG-box motif) has been reported from Japanese and other isolates of *V. dahliae* (Usami et al, 2009, Phys. Mol. Pl Path. *73*, 133) but this group have not reported finding a MAT1-1-1 gene (which should carry an alpha-box motif). Building both on the work of Usami et al. and the sequence coming from the *Verticillium* genome sequencing project we here report the presence of both mating type idiomorphs in isolates of *V. dahliae*. We have also found a MAT1-2-1 gene homologue in Non-Lucerne isolates of *V. albo-atrum* whilst Lucerne isolates of *V. albo-atrum* carry the MAT1-1-1 idiomorph. Within the two idiomorphs there are sequence variants and preliminary data suggests this variation may correlate with other properties, probably as an indicator of clonal lineages rather than causally related.

Long-spored isolates from cruciferous hosts (*V. longisporum*) are interspecific amphihaploid hybrids and as expected these carry two distinct copies of the mating type locus. However these have sequences distinct from either of the haploid species. The significance of this in relation to the origins of these isolates will be discussed.

NEW INSIGHTS ON THE PHYLOGENETIC RELATIONSHIPS AMONG VEGETATIVE COMPATIBILITY GROUPS IN *VERTICILLIUM DAHLIAE*

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The analysis of vegetative compatibility groups (VCGs) has been widely used over the past 20 years to assess genetic diversity in *Verticillium dahliae* population infecting numerous crops of interest. The main reasons for that use have been the availability of a technically simple methodological procedure, and the assumption that VCGs represent genetically homogeneous groups of individuals. The more recent application of molecular markers to the study of *V. dahliae* populations has raised the concern that VCGs may be genetically more diverse than previously thought, and that some subgroups within a VCG may not be closely related to each other. The objective of this study was to determine the phylogenetic relationships that might exist among VCGs and their subgroups. For this purpose, we analyzed sequences of the intergenic spacer region of the ribosomal DNA (IGS) of 56 *V. dahliae* isolates representing the main VCGs and subgroups (VCG 1A, VCG 1B, VCG 2A, VCG 2B, VCG 4A, VCG 4B, and VCG 6) from different geographic origins and hosts. Alignment of IGS sequences revealed a very complex structure with numerous large indels with varying copy numbers between isolates. Maximum parsimony (MP) analysis indicated that certain subgroups (e.g., VCG 1A and VCG 1B) are indeed closely related and placed in the same clade; however, other subgroups (e.g., VCG 4A) are related more closely to members of a different VCG (e.g., VCG 2B) than to subgroups of the same VCG (VCG 4B). Furthermore, MP analysis based on IGS and other anonymous polymorphic sequences showed that VCG 2B is polyphyletic and comprises at least three distinct phylogenetic lineages. These results raise questions concerning the significance of VCG groups and subgroups, the need for reassessment of VCGs in *V. dahliae*, and the adequacy of using current VCG analysis for assessing genetic diversity in *V. dahliae* populations.

DEVELOPMENT AND APPLICATION OF NEW MOLECULAR MARKERS FOR THE ANALYSIS OF GENETIC DIVERSITY IN *VERTICILLIUM DAHLIAE* POPULATIONS

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Twelve novel polymorphic markers, five microsatellites and seven polymorphic sequences, were developed for analysis of genetic diversity in populations of the fungal soilborne plant pathogen *Verticillium dahliae*. The new markers were identified from a genomic library highly enriched for microsatellites. Screening of polymorphic loci was done using 25 *V. dahliae* isolates of diverse geographic origin, host source and vegetative compatibility group (VCG). Three different methods were used to score allele sizes: polyacrylamide gel electrophoresis (PAGE), sequencing of PCR-amplified loci, and fluorescent capillary electrophoresis. The newly developed markers were used to assess the genetic structure of two *V. dahliae* populations obtained from artichoke (30 isolates) and potato (20 isolates) plants collected from fields located in a region of intensive vegetable production in eastern-central Spain, where both crops are grown in rotation. The resolution of these two populations using the new markers was compared with that provided by previously reported PCR-based markers and VCGs.

Sequence analysis of the alleles proved to be the most informative technique evaluated for scoring microsatellite data. When comparing the new markers with the combination of PCR-based markers and VCG markers, gene and genotypic diversity values for the *V. dahliae* populations studied were similar. The relatively high genetic differentiation observed among the two populations and the high genotypic diversity suggest a divergence between populations of the pathogen from artichoke and potato. This could be a result of evolution of *V. dahliae* from the original, resident population towards adaptation on the two host crops. Results indicate that the new markers have high potential for resolving population structure within *V. dahliae* and may contribute to a better understanding of the population biology of the fungus and disease epidemiology.

VERTICILLIUM DAHLIAE ON OLIVES IN TURKEY: DISTRIBUTION, VEGETATIVE COMPATIBILITY AND MOLECULAR CHARACTERIZATION AND VIRULENCE OF SELECTED ISOLATES

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This study reports observations made in a comprehensive survey of the Verticillium wilt of olive in 14 provinces of Turkey over 6 years. Vegetative compatibility groups (VCGs) and PCR-based moanalyses were used to assess the distribution of the defoliating (D) and nondefoliating (ND) pathotypes of *Verticillium dahliae* in surveyed areas. Pathogen prevalence was 35.0% of all olive orchards surveyed and incidence of the disease was 3.1%. VCG1A was the predominant (29.3%) VCG and was able to infect major cultivars (Memecik, Ayvalik, Gemlik, Domat, Yamalak Kabasi, Kilis Yaglik, Uslu, Halhali, Manzanilla, Nizip Yaglik and Maras) grown in Turkey. The other VCGs 2A and 4B were of minor relevant (5.8%). Disease incidence for VCG1A was greater (6.9-1.1%) than the VCG2A and VCG4B in 10 provinces (Manisa, Aydin, Kahramanmaras, Izmir, Mugla, Kilis, Denizli, Gaziantep, Mardin and Balikesir) but lower (0.2-0.5%) in three provinces (Hatay, Osmaniye and Bursa) being not found in Canakkale. Bioassays (19) revealed that a continuum of virulence within VCG1A isolates (13) and that virulence was also related to susceptibility of olive genotypes (Manzanilla, Ayvalik and Gemlik) tested. PCR-based analyses (115) were carried out to assess molecular pathotyping of representative isolates and to qualify D and ND pathotypes. Specific PCR markers for D and ND pathotypes corresponded with VCG1A and VCG2A/4B isolates, respectively. Remarkably, the *V. dahliae* VCG1A/D pathotype population infecting olive in Turkey was molecularly different form that one previously identified in Spain. This study is also the first record of both D and ND pathotypes from olives in the Mediterranean and D pathotype from olives in the southeastern Anatolia regions of Turkey

QUANTITATIVE DETECTION OF MULTIPLE *VERTICILLIUM* SPECIES IN SOIL USING REAL-TIME PCR

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Soils can simultaneously contain different microsclerotia-forming *Verticillium* species such as *V. dahliae*, *V. longisporum*, and *V. tricorpus*. *V. dahliae* infects a wide range of mainly non-cruciferous hosts, *V. longisporum* infects almost exclusively crucifers, and *V. tricorpus* is suspected to be a beneficial biocontrol species. As even low numbers of microsclerotia of *V. dahliae* and *V. longisporum* can lead to high levels of disease, and no reliable curative measures exist to control these pathogens, accurate pre-planting assessment of their inoculum potential in soil is important. Simultaneous detection of *V. tricorpus* may further help in the evaluation of the disease suppressiveness of the soil.

The most commonly used technique to quantify the microsclerotia of these closely related *Verticillium* species is wet sieving of the soil, followed by plating on semi-selective medium and microscopic analysis. A first limitation of this technique is that morphological differentiation between microsclerotia of different *Verticillium* species on medium is very labor-intensive and often difficult. A second limitation is that the standard technique is usually restricted to small soil samples (12.5 g), which limits the sensitivity of the assay. A third limitation is that the assay takes several weeks to complete.

In this study, a method is described based on centrifugation of the soil in 70% sucrose for scaling up the amount of soil from which the microsclerotia are extracted. In addition, real-time PCR assays are developed for specific detection and quantification of *V. tricorpus* and *V. longisporum*, using primers designed to the ribosomal DNA internal transcribed spacer 2 (rDNA ITS2) and the β-tubulin gene, respectively. The newly developed sampling method is combined with a previously described real-time PCR assay for *V. dahliae* and the new real-time PCR assays to simultaneously detect the three *Verticillium* species in soil. In addition, the method is validated by comparing it to the conventional method using various artificially and naturally infected field soils. The new protocol is faster (days), more reliable, and more sensitive. This protocol can therefore help in evaluating the risk for disease development and contribute to durable disease control strategies.

PROTEOMIC ANALYSES OF DEFENCE GENE EXPRESSION IN A MODEL TOMATO-*VERTICILLIUM* PATHOSYSTEM

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Relatively little is known about the molecular mechanisms utilized by plants to defend themselves against fungal vascular pathogens. Based on DNA microarray analyses, in past studies we used a model tomato-*Verticillium* pathosystem to compare the global expression of genes in compatible and incompatible interactions. While very significant patterns of mRNA changes were defined, how these are translated at the protein level remained unclear. In the present study, 2D gel electrophoresis and proteomic analyses were applied to evaluate the actual changes in protein levels. Whole cell protein was extracted simultaneously with RNA to permit parallel comparisons of both mRNA and proteins from the same samples. Proteins representing 13 of the most intense changes were identified using mass spectrometry. Three were products of commonly identified pathogenesis related genes, three were peroxidase related and two were osmotin related proteins, all products of genes commonly reported to be involved in genetic responses to stress or pathogens. The levels of induction relative to other cellular proteins were particularly striking, an observation which underlines the plant's heroic systemic response to a vascular disease such as *Verticillium* wilt.

Supported by the Natural Sciences and Engineering Research Council of Canada.

ANALYSIS OF *VERTICILLIUM LONGISPORUM*-INDUCED GENE EXPRESSION IN *ARABIDOPSIS THALIANA*

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The *Verticillium* research unit, which consists of eight partners of the Georg August University Göttingen and one partner of the Free University Berlin, has chosen to elucidate the interaction between *V. longisporum* and *Arabidopsis thaliana* and *Brassica napus* as a common experimental system to address the question how plants can sense and react to foreign organisms in the xylem. One fruitful aspect of this research group is the strong focus on one experimental system that is investigated using the different expertises of laboratories working in the fields of biochemistry, cell biology, molecular and applied genetics, plant physiology, plant pathology, and microbiology, and entomology. The project profits from the modern infrastructure (proteomics, transcriptomics, metabolomics, microscopy) available on campus. In detail, we address the question concerning the identity of the fungal PAMPs or elicitors that evoke plant responses to *Verticillium longisporum* in roots and in the xylem. Second, we analyze, which responses of the plant serve to accommodate the fungus and which responses serve defense mechanisms (see Abstracts by Diederichsen et al., Häffner et al., Reusche et al.). Third, we aim to elucidate the plant signal transduction chains that elicit the responses (see Abstracts by Ralhan et al., Teichmann et al., Weiberg et al.). Finally, the adaptation of the fungus to living in the xylem is explored (see Abstracts by Braus-Stromeier et al. and van Tran et al.). The talk will give a brief overview of the research approaches of the different groups. Finally, I will present data from our group that focus on the use of *Verticillium longisporum*-induced marker genes to elucidate the contribution of known and unknown singaling pathways to the gene expression pattern elicited by the plant

(See also Abstract by Ralhan et al.).

INVOLVEMENT OF GENES RELATED TO PROGRAMMED CELL DEATH IN *ARABIDOPSIS - VERTICILLIUM DAHLIAE* INTERACTION

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The plant hypersensitive response (HR), a form of programmed cell death with many common characteristics with the mammalian apoptosis, is associated with the rapid death of host cells triggered during the entrance of the pathogen in plant tissues. When the pathogen elicits a host HR, it fails to multiply to high population levels and causes no disease symptoms. Many recent studies indicate the existence of common biochemical pathways of programmed cell death/apoptosis between the plant, mammalian and microbial cells. A detailed bioinformatics analysis of the genome of *Arabidopsis* led to the identification of mammalian orthologous genes known to be involved in programmed cell death. In the present study, we investigated the role of mammalian AIF (Apoptosis Inducing Factor) and the frataxin orthologous genes known to play essential role in apoptosis. The mammalian AIF protein is a phylogenetically old, 57 kDa flavoprotein, which shares similarity to bacterial, fungus and plant oxidoreductases. The frataxin (FT) is involved in iron-sulfur cluster biosynthesis, controls the oxidative stress response, is essential for development and its defective expression causes Friedreich's Ataxia in humans. *A. thaliana* contains 5 different putative homologous AIF genes and 1 frataxin. T-DNA knock-out mutants in these genes were characterized in *Arabidopsis* and the mutants were tested for whether they are compromised in HR and disease resistance against different pathogens included *Verticillium dahliae*. Characterization of these lines and the results of the virulence and HR assays will be presented.

Another important protein required for the activation of hypersensitive response (HR) and disease resistance against *Cladosporium fulvum* and *Pseudomonas syringae* pv. *tomato* in tomato as well as Tobacco Mosaic Virus (TMV) and *Pseudomonas syringae* pv. *tabacci* in tobacco is the F-box protein ACF1. ACF1 is expressed in plant cells in the first 30 minutes after the recognition of the *Cladosporium fulvum* avirulence protein Avr9 by the tomato resistance protein Cf9. Inactivation of the F-box protein in tobacco using gene silencing methodologies leads to a signification reduction in the development of HR and a significant increase in plant sensitivity to several pathogens. In the present study, characterization of the *Arabidopsis* ACF1 homologues, *vfb* genes led to the conclusion that these proteins are also involved in activation of HR. The mutants were studied for the susceptibility and/or resistance to different pathogens such as *Verticillium dahliae*, *Hyaloperonospora arabidopsis* and *Pseudomonas syringae* pv. *tomato* DC3000.

CHORISMATE SYNTHASE AND COLONIZATION OF XYLEM VESSELS *OF B. NAPUS* BY THE PHYTOPATHOGENIC FUNGUS *VERTICILLIUM LONGISPORUM*

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Verticillium longisporum is a near diploid fungal pathogen, which enters its host *B. napus* through the roots and colonizes the xylem vessels. The xylem contains little nutrients including low concentrations of amino acids. We isolated the gene *VLARO2* encoding chorismate synthase by complementation of the corresponding yeast mutant strain. Chorismate synthase produces the first branch point intermediate of aromatic amino acid biosynthesis. Two isogenes are present and a novel RNA-mediated gene silencing method reduced gene expression by 80% and resulted in a bradytrophic mutant impaired in the expression of chorismate synthase. In contrast to the wild type, silencing resulted in increased expression of the cross-pathway regulatory gene *VLCPCA* (similar to *cpcA*/*GCN4)* during saprophytic life. The mutant fungus is still able to infect the host-plant *B. napus* and the model *A. thaliana* with reduced efficiency. *VLCPCA* expression is increased *in planta* in the mutant and the wild type fungus. We assume that xylem colonization requires induction of the cross-pathway control, presumably because the fungus has to overcome imbalanced amino acid supply in the xylem.

MOLECULAR AND BIOCHEMICAL ANALYSES OF DEFENSES AND COUNTER-DEFENSES IN THE POTATO X *VERTICILLIUM DAHLIAE* PATHOSYSTEM

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In our quest to provide alternative strategies for potato Verticilium wilt management, we pre-screened a set of bacteria and plant extracts for their efficacy in reducing disease severity under controlled and field conditions. In this preliminary screening, the most effective treatments acted through enhancement of plant defense responses. Under controlled conditions, the changes in the host response to *V. dahliae,* in presence or in absence of these treatments, included a variation in soluble phenolics. In particular, the flavonol glycoside rutin (querectin 3-*O*-rutinoside) was highly induced in response to the most effective treatments (up to 160 μM). These levels were revealed to be toxic to *V. dahliae in vitro*. Treatments that were ineffective still induced the same compound, but only to levels below the toxic threshold. Additional investigations led us to determine the ability of *V. dahliae* to cleave and use the sugar moiety of rutin (rutinoside: glycosyl-rhamnoside) as a carbon source and to further metabolize the remaining flavonol (quercetin) to by-products such as phloroglucinol and protocatechoylphloroglucinol carboxylic acid (2-PCPGCA). This ability allows *V. dahliae* overcome the toxicity of some of potato defense-related metabolites. Furthermore, it allows it to generate phloroglucinol derivatives that could enhance its prevalence in the rhizosphere. The 2-PCPGCA is also a source of protocatechoyl acid, one of the precursors for salicylic acid (SA), which could trigger SA-pathway *in planta*, thus interfering with the jasmonic acid (JA)-pathway, seemingly the main signaling pathway induced in potato against *V. dahliae*. This study illustrates a defense-counter defense race between potato and *V. dahliae*. It also shows that any biocontrol agent able to help the plant winning this race would be beneficial in controlling Verticillium wilt.

CHANGES IN ETHYLENE PERCEPTION OF *ARABIDOPSIS* PLANTS LEAD TO DIFFERENTIAL DEFENCE RESPONSES AGAINST *VERTICILLIUM DAHLIAE*

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The defence components of *Arabidopsis thaliana* plants against *Verticillium dahliae* were explored by the responses of mutant plants impaired in known pathogen response pathways. Pathogenicity tests of mutant plants revealed enhanced resistance in *etr1-1* [ethylene (ET) receptor mutant] plants, but not in salicylic acid-, jasmonic acid or other ET-deficient mutants, indicating a crucial role of *ETR1* in defence against *V. dahliae*. Quantitative Real-time PCR analysis revealed that the decrease in symptom severity shown in *etr1-1* plants was associated with significant reductions in the growth of the pathogen in the vascular tissues of the plants, suggesting that impaired perception of ET via *ETR1* results in increased disease resistance. Furthermore, the extent of *V. dahliae* growth within the vascular tissues of plants was determined to be positively correlated with disease severity. 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*, *GSTF12*, *GSTU16*, *CHI-1*, *CHI-2* 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.

IDENTIFICATION OF LETTUCE GENES DIFFERENTIALLY EXPRESSED IN A *VERTICILLIUM DAHLIAE*-LETTUCE INTERACTION BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION

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The method of suppression subtractive hybridization (SSH) was employed to identify lettuce (*Lactuca sativa*) genes that are differentially expressed in symptomatic leaves during a lettuce-*V. dahliae* interaction. A subset of cDNAs from the forward-(symptomatic) and reverse-subtracted (asymptomatic) SSH libraries were sequenced and subjected to homology searches using several databases. Genes identified as expressed only in symptomatic leaves included those with homology to pathogenesis-related (PR) protein genes such as *PR-5* (thaumatin-like protein), *PR-3* (endochitinase), *PR-1* (unknown function), *PR-12* (defensin), and a putative lettuce cysteine protease (*LsCP1*). In contrast, of those clones identified as differentially expressed in the asymptomatic leaf tissue, only cDNAs sharing homology with *PR-14* (lipid transfer protein) were identified. Increased expression of *PR-3* and *PR-5* in symptomatic leaves was confirmed by quantitative RTPCR, and *LsCP1* was only expressed in symptomatic leaves. *LsCP1* shares homology with plant cysteine proteases that are expressed in senescing leaves and may contribute to the leaf symptoms observed in this lettuce-*V. dahliae* interaction.

IDENTIFICATION OF THE *V. DAHLIAE* SECRETOME BY SEQUENCE CHARACTERISTICS

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Phytopathogenic fungi deliver effector proteins into the host extracellular space (apoplastic effectors) or into the host cell (cytoplasmic effectors) where they interact with their targets. Large effector repertoires have been identified from bacterial pathogens, and an intrinsic biological function has been uncovered for a growing number of these effectors. Besides bacterial pathogens, the number of identified (potential) effector molecules of filamentous fungal and oomycete plant pathogens is also growing. Based on the presence of the RxLR host targeting motif, it is currently predicted that the genomes of several oomycete plant pathogens encode hundreds of effectors. Nevertheless, the intrinsic biological function of only a few filamentous pathogen effector molecules has been identified so far. Sequence characteristics of fungal effectors are that they contain an N-terminal secretion domain, and many effectors are rich in cysteines. Identification and characterization of these unknown effectors will provide insight into the host pathogen interactions.

With the availability of the *Verticillium* (*V. dahliae* and *V. albo-atrum*) genome sequences we attempted to predict the secreted proteins (secretome). This prediction was based on the results from a number of programs that predict the presence of an N-terminal secretion domain and the subcellular localization. One fourth of the predicted secretome contains cell wall-degrading enzymes and one third are conserved hypothetical proteins. When compared with other sequenced fungal plant pathogens, the number of polysaccharide lyases is significantly higher in *V. dahliae.* Analysis also revealed the presence of four proteins with extracellular cysteine-rich EGF-like domain and eight necrosis-inducing proteins of the NLP-class.

Proteases and protease inhibitors were predicted based on the blast results from a number of web based programs such as MEROPS, Pfam SignalP, WolfPsort, Superfamily protein database. The prediction resulted in 199 and 178 proteases in *V. dahliae* and *V. albo-atrum*, respectively. One third of these proteases are secreted.

TRANSCRIPTOME ANALYSIS ON *VERTICILLIUM*-INFECTED TOMATO TO IDENTIFY GENES INVOLVED IN HOST DEFENSE

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Despite the economical importance of *Verticillium* spp., relatively little is known about the molecular basis of *Verticillium* pathogenicity and host resistance against this fungus. To identify key components involved in the interaction between *Verticillium* and tomato, comparative transcriptomics was performed on susceptible and resistant tomato lines of the cultivar MoneyMaker inoculated with a race 1 isolate of *Verticillium* using cDNA-AFLP. As a resistant line, Ve1 transgenic MoneyMaker was used (Fradin et al., 2009). A total number of 176 selective primer combinations were tested and 1434 differentially expressed transcript-derived fragments (DE-TDFs) were identified. As the DE-TDFs that are up-regulated specifically in resistant tomato upon *Verticillium* inoculation (302 DE-TDFs) may be involved in host resistance, these are functionally analyzed using virus-induced gene silencing (VIGS). The initial results of this analysis will be presented.

Fradin EF, Zhang Z, Juarez Ayala JC, Castroverde CCM, Nazar RN, Robb J, Liu C-M, and Thomma BPHJ (2009). Genetic dissection of Verticillium wilt resistance mediated 1 by tomato Ve1. Plant Physiology 150: 320-332.

MOLECULAR AND GENETIC DETERMINANTS OF THE *VERTICILLIUM DAHLIAE* - PLANT HOST INTERACTION

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The interaction of plant pathogenic fungi with the host-plants has been studied widely in recent years due to the development of dynamic molecular tools that have facilitated the identification and functional analysis of pathogenic determinants and host defense responces that govern these relationships. However, the vast majority of fungi studied so far are of airborne nature and only limited research has been devoted to soilborne pathogens that cause vascular wilt diseases mainly due to their distinct life-style. Among the genes targeted are those involved in signalling, in the early stages of plant recognition and in the degradation of plant cellwall by the pathogen. Fungal genes involved in signalling are heterotrimeric G proteins, adenylate cyclase, mitogen activated protein (MAP) kinases, cAMP dependent protein kinase A (cPKA). It has been reported in the literature 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. We have disrupted the β subunit of G protein and the catalytic protein kinase A signalling genes and demonstrated their important role in virulence and physiology of *V. dahliae*. Moreover, we disrupted the sucrose non-fermenting protein kinase (*SNF1*) gene and revealed his implication in virulence and expression of genes involved in cell wall degrading machinery of *V. dahliae*. Finally, we provided insights into the role of the necrosis and ethylene inducing protein (*VdNEP*) gene in virulence of *V. dahliae* by over expressing it in wild type strains. As far as the second factor (the host plant) of this interaction, we demonstrated through pathogenicity experiments on mutated *Arabidopsis thaliana* or tomato plants that ethylene plays an important role in the host defence responses to *V. dahlae* infection. Ethylene insensitive plants expressed reduced foliar symptoms and pathogen colonization of their vascular system. This reaction was dependent on the specific ethylene receptor that was implicated. We intend to continue our efforts in studying the interactions of the vascular wilt pathogen *V. dahliae* with the host plant in identifying and analysing molecular determinants of this interplay.

EVALUATION OF GENETIC RESISTANCE TO *VERTICILLIUM DAHLIAE* IN POTATO USING IN VITRO CULTIVATION AND QUANTITATIVE PCR

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Several factors have lead to the increased importance of Verticillium wilt including reduced tillage and shorter rotations between potato (*Solanum tuberosum* L.) crops, as well as an increase in use restrictions of the soil fumigant metam sodium. As a result, there has been a renewed emphasis in screening cultivars and germplasm for resistance to *Verticillium dahliae* Kleb. to enhance genetic resistance to the disease. This in turn represents an increase in the need for accurate and rapid assays to detect the pathogen in host tissue in order to define the exact nature of the host:parasite interaction. Several cultivars have been reported to exhibit resistance to Verticillium wilt, but no attempts were made to quantify the level of pathogen present to determine if the host response was one of true resistant or tolerance. While recent research comparing traditional plating methods of pathogen quantification to visual wilt symptoms appears to be accurate and reliable, these methodologies are time and labor intensive (Jansky, 2009 American Journal of Potato Research. DOI 10.1007/s12230-009-9107-x). A quantitative real-time PCR assay would drastically reduce the time and labor required to quantify the *S. tuberosum*:*V. dahliae* interaction.

Potato cultivars with purported resistance to *V. dahliae* and control cultivars were grown in a *V. dahliae* infested research plot. Real-time PCR primers and probe developed from the trypsin protease gene of *V. dahliae* (Dobinson et al., 2004. Current Genetics. 45:104-110) were compared to traditional plating methods for pathogen quantification in potato stem tissue with Pearson's correlation revealing a strong relationship ($r = -0.70$; $P < 0.0001$) between these assays. Real-time PCR successfully amplified the *V. dahliae* in all eight cultivars evaluated including numerous plants where no *V. dahliae* colonies formed on plates. Results from the quantitative real-time PCR assay also were supported by wilt evaluations taken in the field. The correlation between real-time PCR Ct values and percent wilt evaluated two weeks after stem sampling also was strong ($r = -0.75$; $P < 0.0001$). These results indicate that not only can this PCR assay be utilized to detect *V. dahliae* in potato stems grown under field conditions, but will also enable quantification of the pathogen in potato plants providing breeders with the ability to rapidly screen germplasm and to distinguish between genetic resistance and tolerance to the pathogen.

CHARACTERIZATION AND GENETICS OF DIFFERENT DISEASE PARAMETERS CAUSED BY *VERTICILLIUM LONGISPORUM* IN *BRASSICA* AND *ARABIDOPSIS*

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Verticillium infections on Brassicaceae are predominantly caused by *V. longisporum*. The disease has gained significant relevance for oilseed rape, *Brassica napus*. However, disease symptoms occur only very late in the field and are not very distinct, such as premature seed ripening, vascular discoloration or chlorosis of leaves and stems. Wilting, a major symptom in other crops is never observed in Brassicaceae. Greenhouse assays have been developed to identify resistant accessions using young plants, which are based on scoring of different symptoms such as stunting, leaf loss and chlorosis. More recently the quantitative determination of fungal biomass has been included as a disease parameter, in particular to study the systemic colonisation of the shoot by the fungus. This raises the question which parameter(s) are most meaningful to describe host resistance and pathogenesis.

In greenhouse assays we found considerable variation for the degree of stunting in different *Brassica* species and *Arabidopsis*. By determining the presence of the fungus in apical stem segments using either agar plate assays or qPCR, we could show that the time point of systemic colonisation or whether the fungus is able to colonise systemically at all varies with the host genotype. While the *Brassica* hosts were often delayed in their development due to infection, two ecotypes of *Arabidopsis* were both accelerated. To get a better understanding of this complex disease phenotype, we started a mapping project in *Brassica* and *Arabidopsis*. Segregating populations (F2/ F3) were studied for disease and developmental parameters. In *Arabidopsis* the systemic colonisation was the most reliable parameter to describe resistance, whereas stunting showed strong environmental implications. In *Brassica* the scoring of disease symptoms on young plants was reproducible in particular when extreme genotypes were compared. The systemic colonisation of the shoot apex seemed to be, however, also in *Brassica*, the most meaningful parameter. Several QTL could be identified in both species that controlled the systemic colonisation, while the inheritance of stunting was less clear in both species. In both species some overlapping QTL were found which were controlling developmental features and colonisation. Most parameters did show some correlations, however, the correlations were not too close, indicating that the studied traits are controlled to some extent by different genes.

VERTICILLIUM LONGISPORUM INDUCES NEW XYLEM VESSEL FORMATION IN LEAVES DURING AN INFECTION OF *ARABIDOPSIS THALIANA* AND *BRASSICA NAPUS*

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Verticillium longisporum is a soil-borne fungal pathogen which causes vascular disease in oilseed rape and other members of the family *Brassicaceae*. The fungus enters via the plant root system, invades the xylem vessels and then systemically colonizes the hypocotyl and shoot xylem elements. We use GFP and RFP-labeled *Verticillium* strains to study the infection process in *Arabidopsis thaliana*. As the fungus proliferates into the leaf vascular bundles, transdifferentiation of mesophyll cells to xylem elements was observed in *A. thaliana* and *Brassica napus*. We currently study the role of transcription factors involved in the *Verticillium* induced transdifferentiation.

THE *S*UCROSE *N*ON *F*ERMENTING PROTEIN KINASE (*SNF1*) GENE OF *VERTICILLIUM DAHLIAE* IS INVOLVED IN VIRULENCE AND EXPRESSION OF GENES INVOLVED IN PLANT CELL WALL DEGRADATION

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The sucrose non fermenting gene (*SNF1*) regulates the induction of catabolite-repressed genes, including cell wall degrading enzymes. To assess its role in pathogenicity of *V. dahliae, VdSNF1,* the SNF1 ortholog, was disrupted in three *V. dahliae* races; race 1 of tomato and the defoliating and non-defoliating strains of cotton. *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 significantly less virulent on tomato and eggplants. Representative mutants of tomato race 1 were further characterized for the expression of cell wall degrading enzymes (CWDEs) and growth on different carbon sources. Specific 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 wild type and ectopic strains. Tomato stem cross sections at the cotyledon level showed reduced xylem vessel colonization by an *EGFP* transformed race 1 mutant strain compared with the wild type strain that heavily colonized vascular bundles and adjacent parenchyma cells. Quantification of fungal biomass in plant tissues by Real-Time PCR, further confirmed reduced colonization ability of roots, stems and cotyledons by the *VdSNF1* mutant. The results of the present study suggest a role of *VdSNF1* in virulence, vascular colonization and production of hydrolytic enzymes of the soilborne fungus *V. dahliae*.

HOW PHYTOPATHOGENIC *VERTICILLIUM* SPP. MANIPULATE HOST-DEFENSE-RELATED SECONDARY METABOLISM

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Most of our understanding of plant defense against pathogens has come from studies with microorganisms causing foliar diseases. To contribute to the elucidation of host defense mechanisms against systemic invaders, we are using a comprehensive approach for the genetic dissection of plant responses to phytopathogenic species of the genus *Verticillium.* At the present, there is little knowledge of the deterministic molecular and genetic basis of plant resistance/tolerance to *Verticillium spp*. To address this knowledge gap, we are studying the interaction of *V. longisporum* (*Vl*) and *V. dahliae* (*Vd*) with the model plant *Arabidopsis thaliana.* When challenged with different isolates of these two fungal pathogens, *Arabidopsis* plants showed comparable levels of stunted growth and early flowering disease symptoms however, *Vl*-induced chlorosis was much more severe. We found that in *Arabidopsis*, like in other plant species, *Verticillium spp.* infection induced disease symptoms by interfering with the host developmental program and discovered, *via* genetic analysis of natural variation and forward genetics, four independent loci contributing to tolerance/basal resistance likely functioning as negative regulators of pathogen-induced early flowering and/or accelerated senescence-like syndrome. We have identified the gene associated to the locus *VERTICILLIUM HIGHLY SUSCEPTIBLE 3* (*VHS3*) and shown it encodes AtBRM, a member of the SNF2 family of chromatin remodeling proteins known to control shoot and flower development. Microarray experiments and metabolites analysis indicated that *Vl* but not *Vd* isolates were capable of reprogramming the tryptophan (Trp) metabolic pathway resulting in lack of accumulation of the Trp-derived defense compounds indolic glucosinolates (IGs). We are proposing *Vl* pathogenicity strategy involves recruiting the transcription factor *WRKY 70* as negative regulator of the IG biosynthesis.

QTL MAPPING OF *VERTICILLIUM* RESISTANCE TRAITS IN *ARABIDOPSIS THALIANA*

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Verticillium longisporum is one of the most challenging pathogens of cruciferous hosts in Northern temperate regions. By inducing premature ripening, it reduces yield to a considerable degree. We used the related model plant *Arabidopsis thaliana* to study host reactions towards *V. longisporum*, identify resistance traits and investigate their genetic basis. By crossing the *A. thaliana* ecotypes Burren (Bur) and Landsberg *erecta* (L*er*), which differ in their reactions towards *V. longisporum*, we established an F2/F3 mapping population in order to find QTL controlling resistance reactions. A linkage map of polymorphic SSR and InDel markers was constructed in the F2 generation. For resistance testing of F3-families, a semi-natural greenhouse testing system was developed. Three F3 greenhouse tests were performed in the season from late winter to late spring.

Several symptoms of *Verticillium* infection could be differentiated in the *A. thaliana* ecotypes Bur and L*er* and in the segregating population: Systemic colonisation of the shoot system, developmental acceleration, stunting, chlorosis and *Verticillium*-induced axillary branching. For systemic colonisation, four QTL could be detected in at least two of the three F3-tests. The locus with the largest effect, explaining 27% of the total variation, was located in the region of the *erecta* gene. Whereas four QTL could be found which controlled the duration of the developmental cycle, no QTL controlling developmental acceleration could be detected. Several QTL controlling stunting under *V. longisporum* pressure could be detected. However, this trait showed a strong dependence on environmental factors. Two QTL conferring stunting resistance were more significant in the winter test and were of L*er* origin, whereas three QTL on another chromosome had a larger effect in the late spring test and partly showed a heterozygous effect. For chlorosis, no QTL mapping was performed in the F2/F3 population since the parameter varied with the developmental stage of the plants and the F3-families were too heterogeneous in this respect. For *Verticillium*-induced axillary branching, two QTL of Bur origin could be identified. The study emphasizes the complexity of the *Verticillium* syndrome in *A. thaliana*. Different symptoms and corresponding resistance traits are controlled by different sets of QTL, which are overlapping only to a minor proportion. Our mapping approach supports the dissection of this complex disease phenotype by unravelling the genetic basis of the different parameters involved.

THE ROLE OF THE PHYTOHORMONE SALICYLIC ACID IN DEFENSE OF *BRASSICA NAPUS* TO *VERTICILLIUM LONGISPORUM*

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Salicylic acid (SA) is a phytohormone involved in the regulation of plant defense reactions against pathogens. The role of SA in the activation of systemic acquired resistance (SAR) has been verified at biochemical and genetic levels in a range of different plant systems. Deposition of callose into plant cell wall, hypersensitive reaction, and expression of phytoalexins are typical resistance mechanisms triggered by SA. These patterns of resistance are not obviously activated in *B. napus* after *V. longisporum* infection. Instead, stunting and premature flowering are typical disease symptoms in this pathosystem.

We confirmed a significant increase of SA levels in hypocotyl tissue of *B. napus* 14 days after inoculation with *V. longisporum*, while jasmonic acid, a second phytohormone controlling defense reactions against biotic stress, is not induced. Exogenous pretreatment of *B. napus* with SA induces SAR and lead to an enhanced resistance against *V. longisporum* as evident from significant increase of endogenous SA level in hypocotyl tissue 7 dpi and also up-regulation of PR-1, a marker gene for SA signaling pathway. Additionally to PR-1 further genes involved in pathogen defense like a polygalacturonase inhibiting peptide (Pgip5), a phenylalanine ammonium lyase (PAL2-2), and a thioglucoside glucohydrolase (TGG2) showed enhanced transcript abundances in *B. napus*. The eminent role of SA in plant defense against *V. longisporum* has been confirmed with inoculation experiments using NahG transgenic *B. napus*. These engineered plants hydroxylate SA enzymatically and exhibit increased susceptibility to *V. longisporum.*

Induction of SA may be part of the plant innate immune system activated in *B. napus* through *V. longisporum* infection. However, interestingly, this happens in a susceptible interaction which may indicate that the colonization by *V. longisporum* is only partially delayed by the SA response as this pathogen might have evolved strategies to overcome *Brassica* defense.

QUANTIFICATION OF DEFOLIATING AND NON DEFOLIATING PATHOTYPES OF *VERTICILLIUM DAHLIAE* IN GREEK OLIVE CULTIVARS INFESTED BY A MICROSCLEROTIA INOCULUM

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Verticillium wilt is the most serious olive disease in the Mediterranean countries and worldwide. The most effective control strategy is the use of resistant cultivars. However, limited information is available about the level and source of resistance in most of the olive cultivars and there are no published data using microsclerotia, the resting structures of *Verticillium dahliae*, as the infective inoculum. In the present study, we correlated symptomatology and the presence of the fungus along with the DNA relative amount of a defoliating (D) and a non-defoliating (ND) *V. dahliae* pathotype in the susceptible cv. Amfissis and the tolerant cvs Kalamon and Koroneiki, as quantified by the Real-Time qPCR technology. The viability of the pathogen in the plant tissues was confirmed by isolating the fungus on PDA plates, while symptom assessment proved the correlation between the DNA relative amount of *V. dahliae* in plant tissues and cultivar susceptibility. It was further demonstrated that the D and ND strains were present at a significantly higher level in cv. Amfissis than in cvs Kalamon and Koroneiki. It was finally observed that the relative amount of the pathogen in roots was lower than in stems and shoots and declined in plant tissues over time.

A CLOSE LOOK AT THE OLIVE-*VERTICILLIUM DAHLIAE*-*PSEUDOMONAS FLUORESCENS IN PLANTA* INTERACTION

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Verticillium wilt (*Verticillium dahliae* Kleb.) is the most serious biotic threat for olive (*Olea europaea* L.) cultivation worldwide. Efficient control of this disease should be accomplished by means of an integrated management strategy, with emphasis on before-planting measures. An interesting element of such a preventive strategy is the protection of pathogen-free planting material during plant propagation and/or at transplanting. This could be implemented by the use of biocontrol agents (BCAs). Previous studies have revealed that *Pseudomonas* spp. strains native to olive roots antagonize *V. dahliae in vitro*, and effectively suppress disease caused by the most aggressive (defoliating, [D]) pathotype. The objectives of this work were to: 1) monitor by confocal laser scanning microscopy (CLSM) the infection and colonization of the entire olive plant by an enhanced yellow fluorescent protein (EYFP)-tagged transformant of the *V. dahliae* D pathotype; 2) assess the biocontrol activity of an enhanced green fluorescent protein (EGFP)-tagged *P. fluorescens* PICF7 derivative in young olive plants against the D pathotype; and 3) analyse the interaction between the EYFP-tagged *V. dahliae* isolate and the *P. fluorescens* EGFP-tagged PICF7 strain on/in olive roots. An EYFP-tagged *V. dahliae* derivative (VDAT-36I) was obtained by *Agrobacterium tumefaciens*-mediated transformation. The colonization process of 'Arbequina' plantlets by VDAT36-I, and the *in planta* interaction with the endophytic strain PICF7 (EGFP-tagged) have been determined on/in olive tissues using a nongnotobiotic system, CLSM and vibratome-tissue sectioning. Isolate VDAT-36I quickly colonized olive root surface, successfully invaded root cortex and vascular tissues via macro- and micro-breakages, and progressed to the aerial parts of the plant through xylem vessel cells. Strain PICF7 used root hairs as preferred penetration site, establishing microcolonies in the intercellular spaces of the root cortex. Early and localized root surface and root endophytic colonization by *P. fluorescens* PICF7 is needed to impair full progress of verticillium wilt in olive.

CATALYTIC SUBUNIT OF PROTEIN KINASE A IN VIRULENCE AND PHYSIOLOGY OF *VERTICILLIUM DAHLIAE*

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G proteins transduce external signals to intracellular targets, regulating cellular and developmental processes, and also affect virulence in several plant pathogenic fungi. Mitogen-activated protein kinase (MAPK) and cAMP dependent signaling cascades have been implicated as downstream targets of G proteins in filamentous fungi. The major downstream protein implicated in the cAMP-dependent signaling pathway is the cAMP-dependent protein kinase A (PKA). To gain insight into the role of the cAMP-dependent signaling pathway in virulence and development of the soilborne, wilt causing fungus *Verticillium dahliae*, the G protein β subunit (*VdGb*) and the PKA (*VdPKAC1*) genes were individually disrupted in tomato race 1 strain of *V. dahliae* through gene replacement. Deletion mutants (70*ΔGb* and 70*ΔPKA*) showed reduced virulence, which was more drastic in the *Gb* mutants as they caused almost no visible symptoms. Moreover, disruption of the *Gb* or *VdPKAC1* gene caused induction of microsclerotia production, increase in germination and decrease in ethylene production compared to the wild type strain. Similar to virulence, the change in these phenotypes was more prounced in 70*ΔGb* than in 70*ΔPKA*. In addition, 70*ΔGb* mutants presented a vertical rather a radial growth pattern on agar media. Overexpression of *PKA* in 70*ΔGb* mutant restored the radial wild type growth, germination and conidiation. Mutants were unable to produce sclerotia, but were able to cause typical disease symptoms on tomato plants. The findings of the present work suggest interaction between *Gb* and *PKA* in regulating virulence, physiology and development in *V. dahliae*.

NEW CASE OF THE IDENTITY BETWEEN VEGETATIVE COMPATIBILITY GROUPS VCG 1 AND VCG 2B

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VCG 1 was absent in our studies of vegetative compatibility in *Verticillium dahliae* originated from several plants in countries in Europe. We took 11 Turkish isolates of this pathogen isolated from olives and cotton which have been determined in the past as members of VCG 1. However most of them were contaminated with yeasts to the extent which made the recovery of *nit* mutants impossible. Finally we were able to recover mutants from four olive isolates. Only one of them numbered olive 110 was self-compatible. We paired randomly several mutants from each of four isolates with sets of testers from USA and The Netherlands. After two weeks strong heterokaryons have been formed between one isolate: olive 267 and American testers of VCG 2: strong between VCG 2B and medium between VCG 2A tester, medium with Dutch NL II (corresponding to VCG 2). Very week heterokaryon was formed between olive 267 and American VCG 1 tester. Also week heterokaryons were formed between Dutch NL II testers and isolates 108 and 110. It seems that all these isolates assessed as VCG 1 members belong rather to VCG 2 (=NL II). In the past we recorded similar pattern of pairing between several European isolates and VCG 2 A, 2B (NL II) and VCG 1 testers.

Such results need careful reconsideration and comparative studies. In the past in similar situation VCG 3 was assessed to VCG 4.

IDENTIFICATION AND CHARACTERIZATION OF A MFS-TRANSPORTER GENE FROM *VERTICILLIUM DAHLIAE* IN THE INTERACTION WITH OLIVE (*OLEA EUROPAEA* L.)

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Verticillium wilt of olive (VO) is one of the most serious diseases affecting this woody crop worldwide, and may cause severe losses and plant death. Severity of attacks by *Verticillium dahliae* depends upon virulence of the pathogen isolates, which can be classified into defoliating (D) and nondefoliating (ND) pathotypes based on their ability to cause or not defoliation of green leaves from shoots and twigs, respectively. Investigations of VO have traditionally focused on studies of pathogenicity and resistance mechanisms, primarily from biochemical, epidemiological and physiological viewpoints. However, knowledge is scarce of the molecular basis, either for resistance in olive cultivars or pathogenicity/virulence of pathotypes of *V. dahliae*. In order to identify genes involved in the *V. dahliae*-olive interaction, two suppression subtractive hybridization libraries were obtained, potentially enriched in genes whose expression is induced (up regulated) or repressed (down regulated) during infection of olive by *V. dahliae*. Sequencing of some 1000 ESTs has enabled us to select several ESTs whose sequences showed high sequence similarity to genes from other fungal species. Here, we present the identification, characterization and disruption by transposon mutagenesis of a putative major facilitator superfamily (MFS) transporter gene of *V. dahliae*. DNA and protein sequence analyses have revealed high similarity with members of this gene family found in different fungi. Blast analysis revealed that the deduced protein sequence was 60% identical to that of *Cryptococcus neoformans*. Gene expression studies (quantitative real-time RT-PCR) indicated that the putative *V. dahliae* MFS-transporter gene is expressed under different growth conditions, and even induced in culture medium simulating the xylem fluid. Finally, targeted gene disruption has been accomplished by inserting the hygromycin-resistance gene cassette into several positions within the MFS coding region. Several disruption plasmids are available, and mutants are expected to be produced in a representative isolate of the *V. dahliae* D pathotype by *A. tumefaciens*-mediated transformation. Mutants will be valuable to study the role of this gene in the *V. dahliae*-olive interaction.

TRANSPOSABLE ELEMENTS IN *VERTICILLIUM DAHLIAE* AND *V. ALBO-ATRUM*

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Genome sequences have recently become available for the filamentous fungi *Verticillium dahliae* and *V. albo-atrum*. Since transposable elements have previously been implicated in fungal genome evolution, we set out to characterize such elements in the genome of *V. dahliae* isolate VdLs.17. Several DNA transposons and LINE-like retroelements were identified, as well as five long terminal repeat (LTR) retroelements, which will be the primary focus here. Two of the LTR elements were of the copia class, one of which (VdLTRE1) contains the DNA fingerprint sequence E18. The other three elements (VdLTREs2, 3 and 4) are closely related gypsy class elements, and highly similar to the *Fusarium oxysporum* and *Colletotrichum graminicola* elements Skippy and Cgret, respectively, with LTRE3 containing the *V. dahliae* retrotransposon sequence previously identified by Usami et al, 2005. Although there is evidence for RIP-like mutation of the LTR elements, full-length copies of the elements have been identified in VdLs.17, suggesting that some or all of them are active. Consistent with that possibility, ESTs have been obtained that correspond to each element. Variation of retroelement distribution will be described with respect to genome evolution and the potential use of these elements as diagnostic markers.

AUTOPHAGY AND RESTING STRUCTURE DEVELOPMENT IN *VERTICILLIUM DAHLIAE*

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Autophagy is a highly conserved, catabolic membrane trafficking response that is induced in eukaryotic cells by various stresses such as nutrient starvation, hypoxia, overcrowding, high temperatures, and accumulation of damaged/superfluous organelles and cytoplasmic components. In various filamentous fungi, augophagy has been shown to be required for nutrient recycling during starvation, and also to be involved in cellular differentiation and accompanying developmental processes such as germination, sporulation, and infection structure formation.

The development of melanized microsclerotia is essential for *V. dahliae* survival, and these resting structures, which retain viability in the soil in the absence of susceptible hosts, pose a formidable challenge to disease control efforts. In *V. dahliae* autophagy has previously been implicated in microsclerotia development. We have identified in our *V. dahliae* cDNA collections several sequences that correspond to autophagy genes, and one of these encodes a protein with high similarity to yeast ATG8, which has been specifically identified as a macroautophagy marker. We have hypothesized that this protein (which we have designated *VdATG8*) plays a role in development of *V. dahliae*, specifically in microsclerotia formation, and that autophagy may further be involved in the process of *in planta* colonization. We have therefore initiated functional characterization of the *VdATG8* gene during cell growth and development. We have generated gene disruption mutants and demonstrated that the resulting *vdatg8* strains are defective in conidiation, microslerotia formation, and yeast-like growth, specifically the transition from mycelial to yeast-like growth. For further analysis of the autophagic process in *V. dahliae*, we are carrying out additional studies to assess gene activity under autophagy-inducing and –inhibiting growth conditions, and to generate strains bearing reporter gene constructs so that gene expression and protein localization can be monitored directly during *in vitro* and *in planta* growth.

SEARCHING FOR HETEROKARYON INCOMPATIBILITY GENES IN *VERTICILLIUM*

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In filamentous fungi, anastomosis between hyphae from different strains often leads to the formation of heterokaryons and provides the means for the occurrence of mitotic genetic recombination (parasexual cycle). However, even more frequently, the genetically different hyphae either fail to anastomose or fuse but do not establish heterokaryotic growth, a phenomenon described as vegetative incompatibility. Despite its widespread occurrence in natural populations of fungi, the function of this incompatibility is still an open question; it probably aims at the limitation of horizontal spread of putatively deleterious extrachromosomal elements, or at the foundation of a basis for evolution of isolated groups within a species by limiting outbreeding. The genetics of heterokaryon incompatibility has long been studied in many fungal species, including the asexual phytopathogenic fungus *Verticillium*, but an investigation of this process at the molecular level has been mainly undertaken in only two model fungi, namely *Neurospora crassa* and *Podospora anserina*.

We show here that an extensive *in silico* search of the genome sequences of *V. dahliae* and *V. albo-atrum* allows the discovery of many putatively heterokaryon incompatibility-associated genes, including homologues of hyphal anastomosis, heterokaryon incompatibility, induced-during-incompatibility, HET domain containing, suppressor, modifier, programmed cell death and mating type genes. Based on comparative studies of all available heterologous sequences from other genomes of ascomycetes, appropriate primers were synthesized for the amplification of genes representative of the above functional classes from both *V. dahliae* genomic DNA (a corresponding genomic library was occasionally used) and cDNA (RT-PCR). Putative *cis*-regulatory elements, structures and domain organization were predicted *in silico*. Population studies of three *het*-like genes in a large collection of *Verticillium* strains provide clues to their participation in the control of heterokaryosis and the development of molecular tools for rapid classification of new isolates in vegetative compatibility groups. The construction of knock-out mutants for the above genes is currently underway.

WILTING DISEASE IN THE HALLERTAUER HOP GROWING REGION -MOLECULAR CHARACTERIZATION OF VARIOUS *VERTICILLIUM* STRAINS

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Hop wilt, caused by *Verticillium albo-atrum* Reinke & Berthold and *Verticillium dahliae* Klebahn is a very dangerous disease in hops. This soilborne pathogen enters the hop by root invasion and then colonizes the hop vascular system. Up to now no fungicides are effective to control this disease. Hop wilt was first reported in England in 1924 were 1933 the lethal form was discovered (Keyworth 1942). Up to now no lethal *Verticillium* strains have been identified in the Hallertau. In Slovenia since 1997 an outbreak of the lethal form was registered with *V. albo-atrum* as causal agent. During the next 6 years nearly 180 ha of Slovenian hop gardens have been harmed by this lethal form (Radišek et al. 2004). Since 2005, the *Verticillium* disease increased in some regions of the Hallertau. All varieties, even the tolerant ones were affected. What needs to be clarified is whether lethal *Vericillium* strains from England or Slovenia have already appeared in the Hallertau growing region, or whether new highly virulent races have developed here. In the summer of 2008, work commenced at more than 30 locations with collecting 20-30 cm bine sections from hop yards heavily infected with *Verticillium*. Sections were removed from 123 heavily diseased hop plants and from 28 phenotypically healthy ones in the immediate vicinity of the diseased plants. Cultures of the *Verticillium* fungus were investigated. *Verticillium albo-atrum* infection was confirmed microscopically in all 151 samples from severely damaged and also from phenotypically healthy hop plants. Not a single sample contained *Verticillium dahliae*. A qualitative *PCR*-test was performed in a number of samples to determine the *Verticillium* species directly from the infected bines. The primers disclosed by the European and Mediterranean Plant Protection Organisation (OEPP/EPPO Bulletin, 2007) for detecting *V. albo-atrum* were established. *Verticillium albo-atrum* was present in all samples collected from the Hallertau. For further molecular analyses *Verticillium* isolates from Slovenia, England and Poland were used as references using the method developed by Radišek as shown above. SCAR-markers as well as AFLP-markers showed no fragments detecting lethal *Verticillium* strains from Slovenia. The preliminary results obtained up to now allow the conclusion that no lethal forms of *Verticillium* have invaded the Hallertau from Slovenia. This leads to the assumption that the lethal forms observed in Germany are the result of genetic recombination of regional Verticillium strains. Further investigations are necessary to obtain more data on the genetic variability.

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RNAi-BASED GENE SILENCING OF A PUTATIVE TRANSCRIPTION FACTOR IN *VERTICILLIUM LONGISPORUM* AND ITS ROLE IN PATHOGENICITY

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Verticillium longisporium (VL) is a soil-borne pathogen of *Brassicaceae* infecting economical important crops like oil seed rape (*Brassica napus*). Due to its systemic infection chemical plant protection is insufficient for crop protection. The design of resistant varieties is on demand and the discovery of new gene targets being involved in VL pathogenicity will help us to develop improved resistance traits against VL.

In a cDNA-AFLP approach investigating the effect of xylem sap metabolites of *B. napus* on the transcriptome of VL we identified a regulated transcript *Vl12.1* showing homology to a zinc-finger transcription factor (ZnF_C2H2). We profiled the expression of *Vl_12.1* during infection of *B. napus* in root/hypocotyl tissue and found a reduced transcript accumulation in reference to *in vitro* grown mycelium in a xylem simulating artificial medium, indicating a suppression of this gene during the infection process.

pBLAST sequence analysis of *Vl_12.1* showed the highest homology to the *ace1* transcription factor in *Trichoderma reseii*. In this saprophytic fungus *ace1* acts as a regulated transcriptional suppressor of cellulase and xylanase genes.

We applied RNAi technology for gene silencing of this *ace1*-like gene in VL by expressing a gene-specific RNA-hairpin. Gene-silenced mutants did not show any visual difference in triggering typical infection symptoms in *B. napus*. Also testing for alterations in cellulase activities on cellulose-containing agar medium indicated no difference between the silenced mutants and the VL wild type. To further clarify role of the VL ace1-like gene in cellulase regulation as well as in pathogenicity to B. napus an over-expression of this gene is under construction.

CHARACTERISATION OF POTENTIAL PATHOGENICITY-RELATED GENE *VL_6.2* OF *VERTICILLIUM LONGISPORUM*

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Gene *Vl_6.2* of *Verticillium longisporium* was identified in a cDNA-AFLP screening as induced by metabolites from xylem sap of *Brassica napus*. The expression of *Vl_6.2 in planta* increased significant as compared to growth in vitro in xylem-simulating medium.

The full-lengt *Vl_6.2* gene was isolated from a genome library of V. longisporum. The gene has an ORF of 4540 nucleotides, two introns and translated cDNA is predicted to code for 1536 amino acids (aa). Sequencing cDNA confirmed the prediction. The protein contains four putative WSC carbohydrate binding domains.

The expression of *Vl_6.2* was suppressed to 70% of its activity by expressing anti-sense RNA in *Verticillium longisporium* as determined by real-time RT PCR. The effect of silencing on the infection of is currently being investigated.

SDS-containing medium inhibited the growth of strains with the silenced *Vl_6.2* gene to a larger extent than the wild type strain.

Because of the low efficiency of antisense strategy, we are generating strains with *Vl_6.2* silenced by RNAi strategy.

DETECTION AND FUNCTIONAL ANALYSIS OF A POLYKETIDE SYNTHASE GENE IN *VERTICILLIUM LONGISPORUM*

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The biosynthesis of polyketides in fungi is carried out by large multifunctional enzymes called polyketide synthases (PKS) which are encoded by a single gene. Polyketides from phytopathogenic fungi are known to often play a role during the host-pathogen interaction as phytotoxins, pathogenicity or virulence factors. Here we report our results on a polyketide synthease of *Verticillium longisporum*.

We detected a polyketide synthase gene (*Vl-pks1*) in *V. longisporum* isolate VL43 using degenerated primers based on fungal L-ketoacylsynthases (KS)-domain. The fragment shows a high homology to fungal PKS-genes of the WA-type, especially those involved in the biosynthesis of 1,8-dihydroxynaphthalene-(DHN)-melanin. For functional analysis of *Vl-pks1* the complete gene sequence was obtained by primer walking. Southern hybridisation was performed in order to determine the copy number of the gene in the genome of *V. longisporum*, which is known to be amphidiploid. In planta expression of the gene was investigated using root hypocotyl tissue of *V. longisporum* infected *Brassica* napus plants and compared to in vitro grown mycelium. Strongly enhanced transcript levels of *Vl-pks1* in planta motivated us to construct knock-down mutants by RNA-mediated gene silencing. Infection assays on *B. napus* plants were used to elucidate the role of *Vl-pks1* in the pathogenic lifecycle of *V. longisporum*.

CHARACTERIZATION OF NEP-LIKE PROTEINS (NLPS) OF *VERTICILLIUM LONGISPORUM* ACCORDING TO THEIR RELEVANCE FOR PATHOGENICITY IN *BRASSICA* NAPUS

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Recently, a new family of small phytotoxic peptides, called Necrosis and Etylen induceng Peptides (NEPs), was described. Since the first NEP were detected more than 10 years ago, many other NEP-like proteins (NLPs) were detected in various microbiological species. Most of them are plant pathogens wich have more than one copy of NLPs, although it is known that many of these copies are pseudogenes. Conserved sequence structures of NLPs are the GHRHDWE motif in the centre and cysteine residues at the N-terminus supposed to be relevant of peptide activity. In many dicotyle plants NLPs have the ability to trigger cell death. The cellular role of the peptides is not clear yet: NLPs may act as true elicitors by inducing plant immune system or necrotic leasons may result from their phytoxic effects.

In this study we identified and characterized genes for 6 NEPs in *Verticillium longisporum*. These VlNEP genes are homologous genes to VdNEP originating from *Verticillium dahliae*. Southern hybridisation was performed to determine to copy number of the genes in amphidiploid genome of *V. longisporum*. We analysed the expression of the gene in hypocotyl tissue of infected *Brassica napus* plants. For VlNEP type A, strongly enhanced transcript levels were detected in planta, which motivated us to construct knock-down mutants of *V. longisporum* by RNA-mediated gene silencing. Typical symptoms caused by *V. longisporum* such as stunting and early flowering has rarely been described for the effects of NEPs from other fungi. Infection assays on *Brassica napus* plants with silenced VlNEP gene type A showed a strong reduction in symptom severity.

A CDNA-AFLP TRANSCRIPTOME DATABASE OF *BRASSICA NAPUS* IN RESPONSE TO *VERTICILLIUM LONGISPORUM* INFECTION

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Brassica napus is under growing demand for biofuel production. Intensification of rapeseed cultivation increases threats of particularly host-adapted pathogens like *Vertcillium longisporum*. After root infection *V. longisporum* invades the vascular system of host plants, but in contrast to typical wilting symptoms, *V. longisporum* causes no wilting but stunting and premature flowering in *Brassica*. Under greenhouse conditions stunting is evident at 14 days after inoculation. *V. longisporum* remains restricted to the basal part of hypocotyl at this time, while massive mycelial colonization within the plant was detected at maturing stages of *Brassica*. We propose that systemic effects of *V. longisporum* infection induces disease symptoms in *B. napus* and molecular signalling plays the key role.

A cDNA-AFLP transcriptome database of *B. napus* is in progess with the primary goal to identify genes differentially expressed in response to *V. longisporum* infection. The RNA sample set up includes 3 different time points after inoculation (3, 21, 28 dpi) and distinct plant segments (root, hypocotyl, stem). To investigate local and systemic effects of infection on the *Brassica* transcriptome, cDNAs of samples are profiled showing significant high and low fungal biomass determined by quantitative real-time PCR *a priori*.

We consistently improve the transcriptome representation of our cDNA-AFLP database by amplifying additional cDNA fragments using new PCR primer combinations. Based on our preliminary results, we estimate a set of 35,000 individual cDNA signals covered by a total set of 512 PCR primer combinations. By using laser-assisted automatic DNA sequencer system a full quantitative analysis of cDNA signals is performed. For this purpose, we implement a PERL-based program to automatically process data for signal intensity recognition, normalization of signal intensities and identification of differentially expressed cDNAs. Accurate cDNA fragment sizing using DNA size markers facilitates the bidirectional linkage of our cDNA-AFLP database to public gene databases of *B. napus*. With information of cDNA fragment size, restriction sites and selective nucleotides in cDNA-AFLP PCR both the prediction of a corresponding gene to a cDNA fragment and expression data for particular gene sequence are available.

INSIGHTS INTO THE ROLE OF THE NECROSIS AND ETHYLENE INDUCING PROTEIN (*VDNEP*) GENE IN VIRULENCE OF *VERTICILLIUM DAHLIAE*

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VdNEP is a necrosis- and ethylene-inducing protein that was isolated from a *V. dahliae* cotton strain. In the present study we investigated the role of *VdNEP* in virulence of *V. dahliae*. Two overexpression vectors for *VdNEP* were constructed and transformed into *V. dahliae*; in the first vector, the ORF of *VdNEP* was cloned under the control of the *Aspergillus nidulans trpC* promoter (An-trpC prom) and the *Neurospora crassa* beta tubulin terminator (Nc-Bt term), while in the second vector *VdNEP* was cloned under the control of the strong *Magnaporthe grisea* ribosomal protein promoter (Mg – RP prom) and the *Neurospora crassa* beta tubulin terminator (Nc-Bt term). These overexpression vectors were introduced into three *V. dahliae* races; race 1 of tomato as well as the defoliating and non-defoliating strains of cotton. In pathogenicity assays on cotton plants, increased necrosis symptoms were observed in plants inoculated with *Mg – RP prom-VdNEP* transformants compared to the wild type strain. Increased virulence of the *Mg – RP - VdNEP* transformants is being further investigated on tomato and other hosts. Moreover, a TRV-expression vector of *VdNEP* was constructed and transient expression of *VdNEP* in tomato plants caused typical necrosis symptoms.

ETHYLENE PERCEPTION VIA *NEVER RIPE* AND *LEETR4* AFFECTS THE RESISTANCE OF TOMATO PLANTS TO VASCULAR INFECTION BY *VERTICILLIUM DAHLIAE*

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To investigate whether impaired perception of ethylene via the ethylene receptor ETR4 affects the resistance of tomato plants against *Verticillium dahliae*, a *Tobacco rattle virus* (TRV) based virus induced gene silencing (VIGS) system was employed, to knock down the *LeETR4* gene expression in tomato plants. The role of the ethylene receptor *Never ripe* (*Nr*) was assessed in pathogenicity tests of *Nr* mutant plants with the pathogen. *LeETR4*-silenced and mutant *Never ripe* plants were inoculated with *V. dahliae* and the disease symptoms were scored. The pathogenicity experiments revealed that the Verticillium disease severity in the *Nr* and *ETR4*-silenced plants was statistically reduced compared to wild-type and control plants, respectively. Quantification of *V. dahliae* by qPCR showed that the 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, suggesting that loss of function of the Nr receptor results in increased disease resistance. Fungal reduction was evident at each sampling day in the *Nr* plants compared to the wild-type plants, whereas the fungal quantification in the *ETR4* silenced and *TRV*-only inoculated plants showed similar levels of fungal biomass.

VERTICILLIUM LONGISPORUM-INDUCED GENE EXPRESSION IN *ARABIDOPSIS THALIANA*

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Verticillium longisporum is a soil-borne fungal pathogen causing vascular disease predominantly in oilseed rape but also in other members of the family *Brassicaceae*. The fungus enters the root, invades xylem elements, proliferation in the xylem vessels, where is produces conidia and microsclerotia. As a result of the infection, plants are stunted; show yellowing leaves and dicreased yield. The general aim of the study is to functionally analyse the Arabidopsis genes that are induced in the course of interaction with *Verticillium longisporum* and to explore the mechanisms of their transcriptional activation.

In order to identify, which genes are induced after infection, whole genome micro array analyses was done at 5 and 18 days post infection (dpi), respectively. The 18 dpi array yielded 22 genes (VliGs: *Verticillium* induced genes) that were reproducibly induced in petioles of infected plants and which can be used as reliable marker genes for the infection. According to their deduced amino acid sequence, they fall into six groups: carbohydrate modifying proteins, lipid binding proteins, peroxidases, aquaporin protein families, copper ion-binding proteins and calmodulin-like proteins. The 5 dpi array yielded putative regulatory genes like e.g. an AP2 domain containing transcription factor.

In addition, the increased expression of marker gene of known defense pathways (*PDF1.2* representing the jasmonic acid (JA)/ethylene (ET) pathway, and *PR1* representing the salicylic acid (SA) pathway) was detected between 7 and 15 dpi. To assess the roles of these pathways in defence against *Verticillium* mutants were tested for susceptibility. When comparing the leaf area and the fresh weight of mutants in JA and SA biosynthesis (*dde1*; *sid2*), we found that the biosynthesis of these hormones does not play a major role in the interaction. Surprisingly, *coi1* (*coronatine insensitive 1*) and *ein2-1* (ethylene insensitive 2) mutant plants showed reduced disease symptoms and reduced expression of selected VliGs, indicating the possible role of COI1 and EIN2 in regulating resistance/tolerance to *Verticillium.* Whether COI1 functions independently of JA or in combination with oxylipins possibly produced by the fungus remains to be elucidated.

DEFENCE GENE RESPONSES IN A PLANT-ENDOPHYTE INTERACTION

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Endophytes can colonize plants with minimal symptoms and substantial benefit to the host, including a defence against normally virulent pathogens. Craigella tomatoes (*Lycopersicon esculentum*) can be infected with a virulent wilt pathogen, Verticillium dahliae, race 1, or a non-host isolate, DVD - E6, resulting in susceptible or tolerant interactions, respectively. The present study sought to examine a plant's regulatory responses in susceptible, tolerant or mixed interactions, to identify adaptive changes which can lead to tolerance or even pathogen resistance. A specialized pathogen-specific microarray chip (TVR3) was developed and used, together with RT-PCR mRNA assays, to examine patterns of defence gene expression when plants first were infected with either pathogen or endophyte. The results demonstrate that the host clearly detects and responds genetically with either fungus but that the response differs significantly depending on the colonization profile. An apparent interplay between Dvd-E6 and the plant is able to program the plant's defence gene expression in a manner that significantly improves resistance against the very large pathogen. Supported by NSERC.

IN VITRO ELICITATION OF PATHOGENICITY-RELATED GENES IN *VERTICILLIUM DAHLIAE* USING POTATO ROOT EXTRACTS

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Using two highly- and weakly-aggressive isolates of *V. dahliae*, elicited *in vitro* with potato root extracts derived from a susceptible (Kennebec) or a moderately resistant cultivar (Ranger Russet), we investigated the differential accumulation of induced transcripts in this pathogen. A total of 573 differentially accumulated transcripts were detected in the two tested isolates in response to the elicitation using a technique that combines subtractive hybridization and AFLP, along with 16 primers' combinations representing *EcoRI/MseI* AFLP adaptors +A/T/C/G to ensure a full coverage of the subtractive hybridization products. The number of differentially expressed genes in response to the elicitation was slightly higher (5%) in the highly-aggressive isolate as compared to the weakly-aggressive one. The recovery of 185 selected transcripts from the SDS-PAGE followed by PCR re-amplification and sequencing revealed 41 matches with known sequences of assigned functions in *V. dahliae* pathogenicity (i.e., polygalacuronases) or with conserved hypothetical proteins from NCBI or MIT GenBanks. The remaining sequences were unique and did not match with any sequence currently available in these databases.

CHANGES IN *VERTICILLIUM DAHLIAE*'S PATHOGENIC ABILITIES AFTER SERIAL PASSAGES ON ORIGINAL OR ALTERNATIVE HOSTS

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Verticillium dahliae holds a high level of diversity and pathogenic variability. In order to investigate to what extent isolates recovered from one host can adapt to an alternative host over time, we inoculated and recovered weakly aggressive isolates from both potato and sunflower onto their susceptible original and alternative hosts in several successive generations. We developed a pathogenicity index to assess any gain or loss of pathogenicity (aggressiveness and virulence combined) by the isolates over the generations. Interestingly, just after four successive passages, potato weakly aggressive isolates were able to gain pathogenicity on potato and sunflower while those from sunflower lost their adaptability on potato cultivars, especially on the moderately resistant cultivar Ranger Russet. Knowing that changes in pathogenicity among fungi can be gradual in their appearance, relatively stable and genetic in nature or sudden and cyclic, our study revealed that *V. dahliae* can quickly adapt to host defenses and increase its pathogenicity on either the original or alternative hosts.

ON *VERTICILLIUM DAHLIAE* PLASTICITY AND FLEXIBILITY: A CASE STUDY OF CROSS-PATHOGENICITY BETWEEN POTATOES AND SUNFLOWERS

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Verticillium dahliae is known for its widespread around the world; its longevity and survivability in the soil and its ability to infect a wide host range including potatoes and sunflowers. This translates into plasticity and flexibility of the pathogen allowing it to infect, grow and develop in presence of its original or alternative hosts. However, that does not occur without a fitness cost and a variation in pathogenicity. *V. dahliae* cross-pathogenicity was already documented on several hosts including cotton, mint, lettuce, broccoli, cabbage, cauliflower, strawberry, artichoke, bell and chili pepper, eggplant, potato, tomato, and watermelon. In Manitoba (Canada), potato and sunflower growing areas are overlapping and may sometimes be considered in a crop rotation. In the present study, we examined *V. dahliae* cross-pathogenicity between potato and sunflower using a set of 10 isolates from each host on two potato cultivars (Kennebec, susceptible, and Ranger Russet, moderately resistant) and two sunflower hybrids (IS8048, susceptible, and 6946, moderately resistant). Our results indicated that potato isolates were equally aggressive on both hosts while isolates from sunflower were better adapted on sunflower than they were on potato, suggesting that rotations when sunflower follows potato should be avoided.

IMPROVEMENT OF INOCULATION METHODS FOR FINDING RESISTANCE OF OLIVE TO VERTICILLIUM WILT CAUSED BY *VERTICILLIUM DAHLIAE*

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The use of resistance is one of the most important mean of control of Verticillium wilt of olive (VWO), caused by *Verticillium dahliae*. Olive shows a wide source of genetic variability that can be explore for finding resistance. Nevertheless, developing effective inoculations methods that allow to differentiate resistant from susceptible reactions, to short incubation period of infections, and to reduce space and time for obtaining results, is necessary. The objective of this work was to develop rapid, reliable and effective methods for screening a great number of olive genotypes for resistance to VWO in young plants. Three methods (transplanting to a natural infested soil, bare-root dip and direct dipping of plant cultivation tray, in a conidial suspension) were tested. Moreover, three types of plant material of 'Picual' (9-month wild olive seedlings, and 9 and 2-month rooted olive cuttings), were assessed. After inoculations, plants were grown in controlled conditions, and disease severity weekly evaluated by assessing symptom severity, using a 0-4 scale. Root dip-inoculated wild olive seedlings and nine-months rooted olive cuttings showed similar onset and disease development, exhibiting symptoms from the 4^{th} week after inoculation, and reaching at 15 weeks after inoculation a final mean severity and percentage of dead plants, of 3.3 and 3.9, 68 and 90%, respectively. Disease incidence was the 100% in both treatments. On the contrary, two-month rooted olive cuttings root-dip inoculated showed no symptoms during recording period. Moreover, disease symptoms were not observed in any plant material inoculated by dipping of cultivation trays. Plants growing in infested soil have not showed wilt symptoms yet, and are currently being evaluated. Olive seedlings can be successfully infected by *V. dahliae*, and plants show consistent symptoms. Therefore, root dip inoculation of seedlings could be an effective and useful method for identifying resistance in olive. This method also saves time and space in comparison with standard inoculation methods of older plants, such as nine-moths rooted cuttings, commonly used up to now in routine inoculation experiments. Finally, additional trials currently conducted, have preliminarily demonstrated that seedlings age for infection success by the pathogen, using root dip or stem puncture inoculation, could be reduced to 5 weeks.

PHYSIOLOGICAL DIFFERENCES EXPRESSED BY SUSCEPTIBLE AND RESISTANT OLIVE CULTIVARS INOCULATED WITH *VERTICILLIUM DAHLIAE*

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The progress of several physiological parameters has been investigated in nine-months-old olive cuttings of 'Frantoio' (moderately resistant to Verticillium wilt of olive) and 'Picual' (highly susceptible) after being inoculated with a cotton defoliating isolate of *Verticillium dahliae* under controlled conditions. For each cultivar, the plants were inoculated by dipping their bare root system in a conidial suspension og the pathogen. The control plants were treated similarly without the pathogen. Disease progress was evaluated by assessing symptom severity (defoliation, wilt, chlorosis and necrosis) using a 0-4 scale. Susceptibility of 'Picual' was confirmed by the high values of final severity (3.95), area under the disease progress curve (66.7%) and percentage of dead plants (90%), with symptoms appreciable from the 4^{th} week after inoculation. In contrast, 'Frantoio' was moderately resistant, showing values of 1.1, 18.8% and 10% for the respective parameters. The chlorophyll content in leaves was estimated by a non destructive method (SPAD). Basal leaves had similar content in inoculated and control plants. However, the content in apical expanded leaves showed a light but significant reduction in inoculated respect to the control plants, effect that was similar for both cultivars and that appear from 8^{th} week after inoculation. Water consumption was lower in inoculated than in control plants after inoculation. However from the 3^{rd} to the 6^{th} week, in coincidence with the first phase of symptom development, the effect was similar for both cultivars, and could be related to the concentration of gums and tyloses in xylem vessels to avoid colonization by the pathogen. From the 9^{th} week after inoculation, the effect was more prevalent in 'Picual' and could be related to the high defoliation it suffered. This defoliation was associated with an increase in ethylene. Plant growth was reduced in the inoculated plants of both cultivars, but the reduction was much more important in 'Picual'. The fresh weight of inoculated plants of 'Picual' was 66.5% less than control, due to a lower growth of plant root, absence of new shoot growth and severe defoliation and desiccation. On the contrary, weight plant reduction in 'Frantoio' was only light (21.7%), and mainly due to differences in the production of new twigs that were 83.3% lower in inoculated plants than in control.

CYTOKININ TREATMENTS AMELIORATE SYMPTOM DEVELOPMENT DURING *VERTICILLIUM INFECTION* OF *ARABIDOPSIS*

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Verticillium longisporum is a soil-borne fungal pathogen which causes vascular disease in oilseed rape and other members of the family *Brassicaceae*. The fungus enters via the plant root system, invades the xylem vessels and then systemically colonizes the hypocotyl and shoot xylem elements. At late stages of infection coinciding with leave senescence, the fungus starts to grow out of the vascular tissue and switches from biotrophy to a saprophytic lifestyle. We observed that cytokinin-mediated inhibition of plant senescence compromises proliferation of the fungus. We conclude that cytokinin level modulation may be necessary for fungal lifecycle completion.

PRODUCTION AND SCAVENGING OF ROS DURING POTATO-*VERTICILLIUM DAHLIAE* INTERACTIONS: *STRBOH* GENES EXPRESSION AND ROLE OF SECONDARY METABOLITES

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Production of reactive oxygen species (ROS) is one of the earliest events following pathogen recognition in plants. The respiratory burst oxidase (*Rboh*) encodes the key enzymatic subunit of plant NADPH oxidases. *Rboh* represents the plant genes family homolog of the catalytic subunit of phagocyte NADPH oxidase ($gp91^{phox}$). Both are involved in superoxide formation. Our investigation intends to better understand the role of *rboh* genes in potato defense responses against *V. dahliae*. We followed the transcriptional regulation of *Strboh A, B, C, D* and *F* in potato roots and leaves from a susceptible and moderately resistant cultivars, after inoculation with either a highly or a weakly aggressive isolate of *V. dahliae*. Genes *Strboh A, B, C* and *D* were differentially up-regulated and their expression patterns were different between the leaves and the roots. In parallel, secondary metabolites accumulation accompanying these early events was analyzed using HPLC-DAD and –FLD. Spatiotemporal integration of both types of stress responses is being analyzed and correlated to the chronology of the interaction events.

CURRENT AND EMERGING PROBLEMS CAUSED BY VERTICILLIUM WILTS: RESEARCH CHALLENGES, POLICIES AND PRACTICAL APPLICATIONS

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Verticillium characteristics involve several features such as lack of host specificity within an extremely broad host range of annual and perennial hosts. Distinct epidemiological aspects involving mode of survival and dispersal of the pathogens characterized by an interrelationship among annual hosts, weeds and perennials, are also key factors for the incidence of Verticillium wilts. The complicated, extensive seed transmission currently includes lettuce, fennel and linseed and surprisingly enough olive fruits. These hosts are added in the current list of capsicum, aubergines, spinach, alfa-alfa and others. Furthermore, dissemination of *Verticillium dahliae* by infected dried leaves, as it has been demonstrated among others for cotton, safflower, olives and ash and most possibly for many more others, not being tested or reported in the literature, intensifies the impact of the disease.

We further focus on impressive selected cases to demonstrate the epidemiological complexity of the pathogen in Greece (Aetolia-olives and cotton), Spain (Andalucía-olives and cotton) and USA (Bakersfield case in California- olives and safflower/cotton) and implicate responsibilities of the private or the state sectors in properly consulting the farmers. Beyond the existing problems several alarming cases of first reports appear continuously in the literature. Among others *Actinidia chinensis*, in Chile (2003) or elsewhere in the world put in danger the international kiwifruit industry. Stevia, indigenous of Paraguay, a potential alternative for cotton or tobacco cultivation in Europe is also susceptible to *V. dahliae*. While cucurbitaceous hosts are occasionally resistant to races of *Fusarium oxysporum*, they are vulnerable to Verticillium wilt. In the mean time new reports of Verticillium wilt of melon (Tunisia) and of watermelon (Texas, USA) broaden the records of the disease. Current research efforts are concentrated on molecular and control aspects of Verticillium wilt in field crops (sunflower, oilseed rape, cotton and hops) or vegetables. These efforts are necessary to improve or enrich existing control measures involving phytosanitary means, soil fumigation, solarization, biofumigation and cultural means, resistant varieties and grafting vegetables on resistant rootstocks. As for resistant olive rootstocks, research groups in Italy and Greece have shown promising results. Laboratories in Europe have being working on biological control of Verticillium wilt regardless of the problems related with registration and the commercial application. Beyond commercialization biocontrol failures have being also linked to inappropriate selection of biocontrol agents or insufficient information for exploiting successful candidates. Chemical control in existing orchards remains a big challenge due mainly to the lack of chemicals with apoplastic movement. Interestingly enough globalization of agriculture or climatic changes indirectly affect dispersal of Verticillium wilt pathogens (e.g. olives in Greece). So I believe that the great initiative of the Universities of Florida and Nebraska in USA to establish Postgraduate Studies in Plant Medicine provides properly educated and trained scientists (Plant Doctors), necessary to cope with the complicated problems of pant diseases and pests in a real professional way. The endless list of factors and parameters includes finally the translation of our research to application. During the Quebec 13th IC of MPMI in July 2009 Prof. Emeritus Luis Sequeira expressed his concern over the fact that significant progress in our understanding of host-parasite interactions has not translated into new and effective means of controlling plant diseases. There is a sense of urgency, as some of our major crops are facing devastating diseases and we cannot provide solutions. The prospects of moving to a better understanding of Verticillium wilt and solve current practical problems through molecular studies on *Verticillium* spp. will be discussed.

VERTICILLIUM WILT OF OLIVES IN SOUTHERN SPAIN, PAST AND PRESENT: HOW A MINOR PROBLEM COULD BECOME THE MAJOR THREAT FOR THE OLIVE INDUSTRY?

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Approximately 65% of 2.5 million ha of olive cultivated in Spain are grown in Andalusia (southern Spain). In this region, Verticillium wilt (*Verticillium dahliae*) was first found mildly affecting commercial olive orchards in limited areas in early 1980s and it has been of minor concern for the olive industry until recently. Nowadays, severe attacks by the disease occur in that area making Verticillium wilt (VW) the main threat for the olive industry in Andalusia. The increase in distribution and importance of VW of olive in Andalusia occurred concomitant with changes in cropping practices aimed at increasing olive production, which include use of own-rooted planting stocks to establish high-tree-density, drip-irrigated orchards in newly cultivated soils or fertile soils previously cropped to *V. dahliae* hosts, such as cotton. Those changes in the management of the crop have led to traditional groves being replaced by intensively managed orchards, which may have played a role in the extent of VW in olive in Andalusia. A major factor in that increase is the widespread dissemination of the highly virulent defoliating (D) pathotype of vegetative compatibility group 1A, which nowadays dominates the population of the pathogen in Andalusia. The D pathotype was found infecting olive in 83.1 % of 65 arbitrarily-selected, wilt-affected orchards in the five more important olive-growing provinces of the region and accounted for 78% of 637 *V. dahliae* isolates from 433 trees in those orchards. Such predominance of the D pathotype has strong implications for the management of VW of olive in Andalusia as: (i) the threshold of inoculum density for disease with D isolates is much lower compared with that of nondefoliating isolates; (ii) fallen, infected olive leaves are efficient source of inoculum for spreading the pathogen within and among orchards and giving rise to secondary infections; and (iii) olive cultivars of commercial interest are highly to moderately susceptible to D *V. dahliae*. Results from research at different laboratories in Andalusia are of use for the integrated management of VW in olive through disease prediction, risk assessment, pathogen-free certification of planting stocks, and biological protection of healthy planting material. However, such research progress has failed to reach practical applications. On the contrary, a number of marketed but yet untested phytosanitary formulations are overwhelmingly being recommended to olive producers for control of the disease, which lack of efficacy contributes to discredit professional advice for integrated management of Verticillium wilt.

MANAGING VERTICILLIUM: PAST EXPERIENCE, CURRENT CHALLENGES AND FUTURE PROSPECT

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Verticillium dahliae affects a wide range of susceptible agricultural crops causing plant senescence, early dying syndrome and substantial economical damage. The broad spectrum of horticultural crops as well as weeds, together with the fungus ability to produce long-lived resting structures has made its widespread and difficulty to control. Soil fumigation with a combination of methyl bromide and chloropicrin was introduced in 1956 in California, has proven to be very effective inn controlling Verticillium wilt, and turn to be an integral part of cultivation of many crops resulting in higher yield. Since than up to date, soil fumigants still play a major role in Verticillium management in various annual and perennial crops. The phase-out of methyl bromide has directed the focus of management on alternatives chemical fumigants, on combination of chemicals and on nonchemical strategies. Grafting and the use of resistant or tolerant host cultivars together with cultural (e.g. crop rotation practices contribute to the reduction of the pathogen populations in soil. This approach draws much research and development attention as chemical alternatives diminish. Soil solarization is effective in controlling Verticillium in wide range of annual and perennial crops. The efficacy of this approach is further improved by combination with reduced dosage of fumigants and other control means. Organic amendments emerge during the last decade as a promising approach to the management of *Verticillium* in various crops. The incorporation of large quantities of organic matter, such as certain crop residues (crucifers) and organic wastes and their decomposition produce toxic volatile compounds which control various pathogens and generate suppressive conditions to reestablishment of pathogens. Future management of Verticillium will be direct to integrated approach which combines crop rotation with crop residues which can serve as organic amendments, effective pre-plant treatment (which also improves microbial balance in the plant-root interface), and optimal crop management.

BIOLOGICAL CONTROL OF *VERTICILLIUM* DISEASES: POSSIBILITIES, PROBLEMS AND PRAXIS

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Verticillium causes serious diseases connected with high yield losses in a broad variety of crops. Due to the specific ecological behaviour it is difficult to control the pathogen with chemical fungicides. In addition, negative impact on the environment was reported for protection strategies against soil-borne pathogens, e.g. by fumigants. On the other side, *Verticillium* is an interesting target for biological control, which presents an environmental friendly plant protection strategy using the natural antagonistic potential. On and in plants, including *Verticillium* host plants, there exist an enormous potential of antagonistic microorganisms, which are able to suppress the growth or kill the pathogen.

During the last years, interesting biocontrol studies showing efficient results in the suppression of *Verticillium* symptoms were reported. In these studies, a long list of phylogenetically diverse microorganisms, e.g. *Pseudomonas* spp., *Trichoderma* spp., *Talaromyces flavus*, were used. In contrast to these promising results, only a few products are on the market. Problems to translate these interesting approaches into praxis are scale-up, formulation and registration of Biological Control Agents. Furthermore, inconsistent effects under field conditions are another hurdle. Due to new technologies and results from molecular ecology it is possible, step by step, to solve these problems.

One example, which will be presented in detail, is RhizoStar®, a product on the basis of *Serratia plymuthica* HRO C-48. This endophytically living bacterium is not only able to suppress symptoms caused by *Verticillium* in diverse host plants but also to stimulate growth of plants. Quorum sensing is crucial for biocontrol activity of *S. plymuthica* HRO-C48. In the pathosystem *Verticillium dahliae*-oilseed rape the essential role of AHL-mediated signalling for disease suppression was demonstrated. HRO-C48 emits a broad spectrum of volatile organic compounds (VOCs), which are involved in antifungal activity and, interestingly, whose relative abundances are influenced by quorum sensing.

COMPLETE CONTROL OF VERTICILLIUM WILT OF OLIVE IS OBTAINED USING RESISTANT ROOTSTOCKS

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The potential of grafting susceptible cultivars on resistant rootstocks was evaluated for the control of Verticillium wilt in olive. Cultivars Frantoio (highly resistant clone), Coratina (partially resistant clone) and Leccino (highly resistant clone) were used as scions and rootstocks in all combinations. Two years old self-rooted plants were used for grafting, and one year later they were inoculated by root-dipping with the defoliating pathotypes of *Verticillium dahliae*. Plants were pot-grown and maintained in greenhouse. Disease external symptom severity was evaluated weekly until 90 days after inoculation, when vascular browning was assessed and *V. dahliae* re-isolation was attempted as well.

The reactions to Verticillium wilt of the three tested cultivars were confirmed in this research, and were not affected by grafting each of them on itself. Based on external symptoms, Frantoio rootstock protected partially Coratina and completely Leccino from Verticillium wilt, while Coratina and Leccino rootstocks did not provide any protection on the scions. These results were also confirmed by vascular browning reactions. On rootstocks of Leccino, and less markedly of Coratina, an increase of Verticillium wilt occurred in scions of Frantoio, compared with Frantoio scions grafted on Frantoio or non-grafted Frantoio plants. Such disease increase occurred for all the disease evaluations such as the severities of external symptoms, vascular browning and *V. dahliae* re-isolation.

Finally, in order to study *V. dahliae* colonization progress in the rootstock/scion combinations, quantitative estimations of xylem vessel plugging were carried out on transverse micro-sections (20 μm thick) of rootstocks and scions. The results will be discussed during the presentation of this paper.

THE INFLUENCE OF AGRONOMIC FACTORS ON PREVALENCE AND DISTRIBUTION OF *VERTICILLIUM DAHLIAE* VEGETATIVE COMPATIBILITY GROUPS AND PATHOTYPES INFECTING OLIVE IN ANDALUSIA, SOUTHERN SPAIN

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Verticillium wilt, caused by *Verticillium dahliae*, has become the main threat for the Andalusia olive industry. Severity of attacks by *V. dahliae* is associated with the spread of a highly virulent, defoliating (D) pathotype of vegetative compatibility group 1A (VCG1A). This study was conducted to determine the effect of factors associated to olive production on the prevalence of *V. dahliae* VCGs and pathotypes in the five most important olive-growing provinces in Andalusia. Genetic diversity in 637 *V. dahliae* isolates from 433 trees in 65 orchards was studied by VCG typing using *nit* mutants of the OARDC reference strains and local testers, as well as by PCR assays that differentiate the D and nondefoliating (ND) *V. dahliae* pathotypes and DNA sequence analysis of a 539/523-bp *V. dahliae*-specific PCR amplicon.

Four VCGs were identified: VCG1A (78% of isolates), VCG2A (20%), VCG2B (0.6%) and VCG4B (1.4%). VCG1A isolates were typed as D pathotype, while isolates of other VCGs were ND. Three sequences of the *V. dahliae*-specific amplicon were identified among the isolates that correlated to VCG (*seq1*/VCG2B; *seq2*/VCG2A, VCG4B; *seq4*/VCG1A). A single VCG prevailed among isolates within an orchard (77%). The association VCG1A/VCG2A occurred in 15% of the orchards, and 8% of them included some other VCG associations. VCG1A was the most prevalent genotype in all provinces, except Granada (south-east Andalusia). This genotype prevailed in orchards established with unrooted olive sticks at low to medium planting density in virgin soil, or in soil previously cropped to *V. dahliae* hosts, and irrigated with underground water. In contrast VCG2A (ND) was the most prevalent VCG in Granada but its prevalence was not influenced by crop age, cropping history or plant density. Geographic location, plant density, and crop age of olive orchards were used as predictors in discriminant analysis for classifying the occurrence of VCGs or pathotypes in olive orchards. A linear discriminant-function model was developed that can be of use to predict occurrence of *V. dahliae* VCGs or pathotypes in an orchard based on estimates of the selected agronomic features and geographic location of the orchard.

DISTRIBUTION OF *VERTICILLIUM DAHLIAE* THROUGH WATERING SYSTEMS IN IRRIGATED OLIVE ORCHARDS IN ANDALUCIA

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Dispersal of *Verticillium dahliae* microsclerotia (MS) by irrigation water has been always considered very probable, although only little direct experimental evidences were available. The aim of this research was determine the presence of *V. dahliae* MS in water or in soil or plant debris particles carried by water, in infrastructures of the main pumping station of an important Irrigation Community in Southern Spain, in an area occupied by olive orchards, in which pathogen causes Verticillium wilt of olive (VWO). Pathogen was present in all the engineering structures of the community involved in water transporting to irrigated plots. The study starts in Cordobilla reservoir, from which water is pumped to the community main channel. Analyses of samples collected from the soil pellet decanted in this channel showed mean ID of *V. dahliae* that reached 2.24 MS/gr of pellet. Pathogen ID also reached 1.28 Ms/gr in the pellet settled down inside the reception tank, where water is initially gathered in the station after being taken up from the main channel. During watering season, the pumping station continuously delivers water to plots. Farmers use in the watering pipe connection in plots different kind of filters, such as sand filters, commonly used in drip irrigated olive orchards that usually retain particles bigger than 120 μm. We have found the pathogen in the sand of filters of several olive orchards affected by VWO with a mean of 0.007 MS/gr of sampled sand. Moreover, fungus MS were also found in the pellet decanted is storing water ponds at these olive fields. Finally, for determining the amount of *V. dahliae* MS directly delivered in soil of plots through the drippers during irrigation, a similar situation was reproduced in the pumping station installations. The simulation consisted of inserting between the station water main a filtering system that allowed to recover particles suspended in water 35 to 120 μm in size, to explore large water volume. Monthly analyzes of mean water volumes of 20 m^3 allowed to detect the fungus in variable amounts that reached 0.041 and 0.94 MS/m^3 during November and April, respectively. Results demonstrated that *V. dahliae* is distributed through irrigation system, which can provide to the pathogen a long-distance spreading in wide cultivation areas. This fact has surely contributed to increase VWO disease incidence and severity in Andalucía.

HIGH RESOLUTION THERMAL REMOTE SENSING IMAGERY FOR VERTICILLIUM WILT DETECTION IN OLIVE

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Obtaining information of spatio-temporal patterns and spread of plant diseases in susceptible crops may provide valuable information for designing optimum strategies for disease management. This information may be particularly relevant for Verticillium wilt (VW) of olive in southern Spain, caused by *Verticillium dahliae.* This disease is the most limiting factor for this crop during the last decade due to its rapid an extensive spread and the occurrence of a highly virulent defoliating (D) pathotype of *V. dahliae*. Recent developments in optical sensor technology have the potential to enable direct detection of plant diseases under field conditions at pre-visual stages, even before the occurrence of severe symptoms.

Our study aimed at determining whether high resolution airborne thermal imagery can be used to detect early stages of *V. dahliae* infection and to monitor VW development in olive orchards. The study was conducted in two 7-ha commercial high planting density olive orchards located in Cordoba (10-yr old 'Picual') and Seville (3-yr old 'Arbequina') provinces. On each orchard, thermal remote sensing imagery was obtained in the 8-14 μm spectral region, using a camera installed onboard an unmanned aerial vehicle (UAV), yielding 25 cm spatial resolution. Remote sensing parameters and spatio-temporal VW disease incidence and severity was determined for each tree in early May and mid July to account for the different stages of disease development.

Results indicate that in both olive orchards VW affected trees showed a patched distribution, with the occurrence of several foci of variable size of infected trees across the orchard. VW disease tree severity was positively and spatially correlated with canopy temperature, showing differences up to 4ºK when compared with pure-crown temperature extracted from healthy trees. These results suggest the feasibility for VW detection when submeter thermal imagery is used due to the lower transpiration of diseased trees as compared with healthy trees.

Research supported by grants P08-AGR-03528 from CICE-Junta de Andalucía, Spain, and AGL2009-13105-C03 from Ministerio de Ciencia e Innovación, Spain.

STUDIES OF VERTICILLIUM RESISTANCE IN HOP

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In Slovenia, hop growing is an export oriented agricultural branch producing highly valued aromatic varieties, all bred by the hop institute in Žalec. Because of the outbreak of lethal wilt, the development of cultivars with resistance to *Verticillium albo-atrum* is currently the main focus in hop breeding since growing resistant/tolerant cultivars is the most effective means of preventing the disease. The use of marker-assisted selection (MAS) would greatly accelerate the development of wilt-resistant hop cultivars, so a research program has been set up to study gene(s) conferring resistance *to V. albo-atrum* and to develop resistance linked markers applicable in MAS.

A genomic approached has been applied for mapping and cloning gene(s) and we have so far developed various molecular markers, established a family segregating for Verticillium resistance and constructed a genetic map. Differential gene expression in response to inoculation of susceptible and a tolerant hop cultivars is also being studied, using cDNA-AFLP, and candidate genes will subsequently be examined. The research will continue with the development of markers linked to resistance gene(s) and isolation of genomic and cDNA clones with validation of their function.

In parallel, proteomics has been used to study the pathogenicity of *V.albo-atrum* and hop-Verticillium interactions. A 2-D electrophoresis reference map of mycelium proteins was first established and a comparative analysis of the proteome of mild and lethal pathotypes revealed differences which may in part explain the differences in their virulence. Hop xylem sap proteins and changes in the root proteome were analysed by inoculating a susceptible and a tolerant hop cultivar. Two induced plant defence proteins were identified in xylem sap, the function of which is being examined and changes in the root proteome detected by DIGE showed differences in the response to inoculation in the two cultivars. We intend to continue proteomic work by focusing on identifying root and stem induced proteins and on the extracellular proteins.

STENOTROPHOMONAS – NEW INSIGHTS INTO A POTENT *VERTICILLIUM* ANTAGONIST

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The genus *Stenotrophomonas* is of high medical, ecological and biotechnological interest due to the versatility of the different species. The ability of *Stenotrophomonas* to associate with eukaryotic cells results in pathogenicity for humans, as in case of multidrug-resistant *S. maltophilia*, or in promotion of plant growth and antagonistic behaviour against fungal plant pathogens as in case of *S. maltophilia* and *S. rhizophila*. We analysed the plant-microbe interaction as well as the mechanisms behind using the type strains of *S. maltophilia* and *S. rhizophila*. Both strains showed a positive influence on plant growth and health, which was evaluated *in vitro* and in field studies. Strains of *S. maltophilia* are plant colonizers and can actively multiply in the rhizosphere, interestingly also those strains from clinical origins. Strains of *Stenotrophomonas* enhance plant productivity by several mechanisms, e.g., i) the production of the phytohormone indole-3-acetic acid, ii) nitrogen-fixation, and iii) oxidation of elemental sulfur which in turn provides sulfate to the plants. *In vitro*, both *Stenotrophomonas* isolates show an antifungal activity and they are also highly resistant to several antibiotics, which may help to compete in the rhizosphere. Furthermore, *Stenotrophomonas* strains are known for their extraordinary high hydrolytic potential and their production of volatile organic compounds (VOCs), which inhibit mycelial growth of the soil-borne pathogen *Verticillium dahliae* to more than 90%. Interestingly, *S. rhizophila* synthesises the compatible solutes trehalose and glucosylglycerol in response to salt stress, which may explain the highly positive effect, which was found for *Stenotrophomonas*-treated plants in salinated soils in Uzbekistan (Egamberdieyeva, pers. communication). However, due to the opportunistic character of some *S. maltophilia* strains it is necessary to evaluate their human pathogenic potential, e.g. by applying the *Caenorhabditis elegans* assay, and to understand molecular ecology. Strains with high mutation frequencies (hypermutators) were only found among isolates with a clinical origin; clinical environments might select bacterial populations with high mutation frequencies.

MODE OF ACTION OF A NON-PATHOGENIC *FUSARIUM OXYSPORUM* STRAIN AGAINST *VERTICILLIUM DAHLIAE*

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Numerous studies have examined the interaction of various non-pathogenic *Fusarium oxysporum* strains with different pathogenic *formae speciales* of *F. oxysporum*; however, there were no reports until recently about non-pathogenic *F. oxysporum* strains having a suppressive effect on *Verticillium dahliae*. In 2008, we reported the efficacy of a non-pathogenic *Fusarium oxysporum* strain, designated as F2, isolated from a suppressive compost amendment, to reduce Verticillium wilt symptom development in eggplants under greenhouse and field conditions; in addition, antibiosis or parasitism were ruled out by using a dual culture test. In the present study, the mode of action of F2 against *V. dahliae* was investigated. For this purpose, the F2 and *V. dahliae* strains were transformed with the EGFP and DsRed2 reporter genes, respectively, to facilitate visualization of their presence on eggplants root surface. In addition, the ramification of both fungi into the plant vascular system was monitored by Real Time qPCR analysis. It was shown that F2 colonizes the root surface along the intercellular junctions excluding *V. dahliae* from the same ecological niche. In parallel, qPCR analysis showed that application of F2 reduces the levels of *V. dahliae* vascular colonization along with the disease severity. In a split root experiment it was demonstrated that F2 does not trigger the defense mechanisms of eggplants against *V. dahliae*. Therefore, it seems that competition for space or nutrients on the root surface are the main mechanisms of action of F2 against *V. dahliae*.

CONTROL OF VERTICILLIUM WILT OF OLIVE CAUSED BY DEFOLIATING *VERTICILLIUM DAHLIAE* WITH THE *TRICHODERMA*-BASED BIOTEN® FORMULATION

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Verticillium wilt (*Verticillium dahliae*) is the main threat for the olive industry in Andalusia (southern Spain). Prevalence and severity of attacks by the diseases are associated with changes in cropping practices and the spread of the highly virulent, defoliating (D) pathotype across that region. Use of planting stocks certified free from *V. dahliae* and protection of their root system from infection by residual soilborne or incoming inoculum would be a suitable strategy to reduce the potential for severe disease in young trees. This would allow for the disease recovery phenomenon to express and contribute to control of Verticillium wilt if new infections do not take place thereafter. We tested that hypothesis using a *Trichoderma* spp.-based Bioten® formulation in artificial-inoculation experiments of highly susceptible 'Picual' olive plants with D *V. dahliae*. Repeated experiments were done in growth chambers under environmental conditions optimal for disease development, both by transplanting *Trichoderma*-treated plants ($3.2x10^6$ cfu/g root) to soil infested with D *V. dahliae* ($2x10^6$ cfu /g soil) or by root dip inoculation in a 10^7 conidia/ml suspension. Results indicated a consistent, significant reduction in the severity of symptoms after 2 month of incubation at 25ºC. The reduction in symptom severity lasted for additional 6 moths of incubation under natural environment in a shelter. Additionally, *Trichoderma*-treated plants were transplanted into solarized soil in field microplots and their root system was inoculated with D *V. dahliae* increased in corn meal-sand. Plants were treated again with the biocontrol formulation just after transplanting as well as 1 year later. After 2 years, disease incidence was 100% and 91% in untreated and treated plants ($P>0.05$), respectively, but 33% of untreated trees died from infection by the pathogen. However, both disease severity and the area under disease progress curve were reduced by 60% ($P<0.05$) by the biological treatment. This reduction in symptom severity over time is of significance since plants remained exposed to recurrent D *V. dahliae* inoculum in infected leaves falling from the affected trees over the 2 years of the experiment.

STUDY OF SOME COMMONALITIES BETWEEN THE ACTION MECHANISMS OF SELECTED GREEN MANURES USED TO CONTROL POTATO VERTICILLIUM WILT

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Canada milkvetch, oriental and yellow mustards and buckwheat are among the plant species that can be grown as green manures to replenish the soil and control Verticillium wilt. In the present study, we investigated the use, under controlled conditions, of these four plant species either as biomass incorporated into the soil or as seed-pieces coating agents at planting. All these tested green manures reduced disease severity under both application methods. Investigation of defense responses induced in potato cultivar 'Russet Burbank' revealed the ability of the tested green manure species to differentially trigger the accumulation of the phytoalexin rishitin, in both tubers and stems. An early accumulation of phenylamides such as hydroxycinnamoyltyramines was also recorded in tubers. Besides their role as phytoalexins, these secondary metabolites may also enhance lignin accumulation and cell wall strengthening. Our investigation revealed that either or both phenomena are occurring in the tuber.

MICROBIAL ANTAGONISTS AND COMPOST-BASED GROWING MEDIA AFFECT THE GROWTH OF OLIVE PLANTLETS AND THE INOCULUM DENSITY OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA

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Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae* Kleb., is one of the most important disease of olive worldwide. Microsclerotia, the resting structures produced by the pathogen, are critical factor in the epidemiology and control of the disease. Particularly, chemical control in the nursery is unfeasible, since the use of methyl bromide as fumigant phased out in 2005. Moreover, the implementation of organic farming system also in the olive nursery production, urgently requires new and eco-compatible control means. The new perspectives in controlling Verticillium wilt include the development of resistant cultivars or rootstocks, new phloem-mobile fungicides, and compounds able to induce resistance in the host plant. Among the environmentally friendly alternatives to control *V. dahliae*, the use of microbial antagonists and organic amendments has achieved much attention.

The objective of this study was to assess the effect of microbial antagonists and compost-based growing media on the growth of olive plantlets and on the inoculum density of *V. dahliae* microsclerotia, in comparison with a conventional soil mixtures. Growing media were treated or not with a fungal bioproduct ("Clonotri", based on *Trichoderma harzianum* Fv178, and *Clonostachys rosea* Fv114) or bacterial bioproduct ("Sublic", based on *Bacillus licheniformis* and *B. subtilis*). Organic growing media were composed of the same raw materials with a substitution of 30% of peat moss by a commercial certified compost. Before plant transplanting into pots, growing medium was artificially inoculated with *V. dahliae* microsclerotia (60-70 MS g^{-1}). Inoculum density of *V. dahliae* microsclerotia in the growing media, as well as, plant height, fresh weight and root weight were evaluated.

Olive plantlets grown in compost based growing medium treated with bioproducts showed a higher plant height, plant weight and root weight, being those treated with Clonotri the best. Clonotri and Sublic significantly reduced the inoculum density of *V. dahliae* microsclerotia in the rhizosphere of olive plantlets, as compared to the untreated control. These results demonstrate the potential of bacterial and fungal bioproducts for the protection of olive propagative material meeting all farming system requirements.

VERTICILLIUM WILT MANAGEMENT IN OUTDOOR FIELD CROPS: THE WATERMELON CASE IN GREECE

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Beyond cotton, potatoes and globe artichokes are the main field crops heavily infected by *Verticillium dahliae* in Greece. Solanaceous hosts, also vulnerable to the pathogen, suffer less deu to the plastic or glasshouse cultivation, where soil disenfestation takes place. Field crops such as cucurbitaceous hosts are also vulnerable to the pathogen. This becomme evident the last years when watermelon cultivation is drastically expanded in Greece as an out of season tunnel grown cultivation for export to Europe

Watermelon plants grafted on rootstocks resistant to *Fusarium oxysporum* f.sp. *niveum* could avoid Fusarium wilt. However, this protection is not extended to *Verticillium dahliae* infections. Indeed watermelon cultivations established in fields previously cultivated to potato or cotton, cause widespread infections of watermelons regardless of the use of *Fusarium resistant* rootstocks.

Application of strip soil solarization was carried out in 10 hectares sandy loam field previously cultivated to potatoes to evaluate effectiveness of soil solarization. A soil strip of 2 m wide was covered mechanically by impermeable transparent plastic films (actual width 2.80 m) while a between corridor 1.50 m wide was left uncovered. Machine application was carried out by a specifically regulated tractor in dry soil while irrigation tubes one m apart were also inserted during covering to be used to extensively water the soil. Two months after the application of soil solarization the plastic film was teared in the middle to allow rain water to water the field during winder. Beginning of February the soil was cultivated with a riper without soil mixing while the plantation was established in March and transparent plastic tunnels covered the plants. Enumeration of *Fusarium oxysporum* propagules in solarized or unsolarizad control strips revealed that two months soil solarization almost destroy all *Fusarium oxysporum* propagules. Regarding symptom development in unsolarized strips up to 50-60 % of the plants developed brown vascular discoloration indicating vascular wilt diseases. Laboratory examinations and pathogen isolation revealed that almost 80% of symptomatic plants were infected by *Verticillium dahliae*. On the contrary neither vascular wilt symptoms nor *Verticillium dahliae* was isolated form watermelon plants grown in solarized strips proving the effectiveness of the root stocks against Fusarium wilt.

Preliminary calculations of the effect of solarization on the number of produced watermelon fruits per plant with commercial value showed that the difference between treated and untreated plots was not significant (the corresponding figures were 2.5 to 3.2). However the mean weight of watermelon fruits from the untreated was per 8-10 Kg compared to 14-16 Kg from the solarized plots. This significant difference in th total production and the size of fruits justified the low cost extensive machine application of soil solarization in the Amaliada region of Helia county in Peloponnesus Greece (Cost of application 750 Euro per ha). Various examples of succesful soil solarization application refer to globe artichoke, lettuce, tomato and cucumber against several soilborne pathogens.

ATTEMPTS TO CONTROL VERTICILLIUM WILT ON OLIVES IN ISRAEL

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Verticillium dahliae represents one of the main limiting factors in intensively irrigated olive production in Mediterranean countries. Economic damage caused by the disease has increased during the last twenty years, due to extensive irrigation and intercropping with *V. dahliae*-susceptible hosts, such as potato and cotton. The most efficient means of controlling the disease is to simply prevent it. However, when outbreaks in the orchard do occur, it is necessary to look for potential ways to minimize the damage by reducing the disease. The objectives of the present study were to evaluate soil and seedlings treatments and application of fungicides to reduce the disease.

Field trials were conducted in an infested orchard located at Revivim in the southern part of Israel. Three types of trials were conducted in parallel. **1**) Soil treatment trial: soil solarization, solarization combined with cattle manure compost (6 kg/m2), solarization combined with soy meal (2 kg/m2) and solarization combined with cabbage residues (5 kg/m2). **2**) Fungicide treatment trials that involved injection under pressure into the tree trunks of: Miraz (prochloraz), Canon (phosphoric acid), TOG (8-hydroxyquinoline sulphate) and Bavistin (carbendazim). 3) Inoculating olive seedlings at the nursery with mycorrihzae. The effect of these treatments is discussed in detail. Following the first two trials, however, the differences between treated trees and untreated control trees were not significant. With amendments of mycorrihzae is too early to summarize. The major conclusion is that the best way to overcome Verticillium wilt on olive is to prevent it, by avoiding planting on infested soil, by using tolerant-resistant cultivars and by maintaining optimal management conditions.

A NEW STRAIN OF *VERTICILLIUM* THREATENS U.S. SUNFLOWER PRODUCTION: DISTRIBUTION AND SOURCES OF RESISTANCE

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Verticillium wilt or leaf mottle of sunflower has been a minor disease affecting U.S. sunflower production because nearly all oilseed hybrids use the single, dominant V-1 gene for resistance. Verticillium wilt in the U.S. sunflower crop generally occurs in less than 10% of fields, but in 2003 a multistate survey noted the disease in 19% of fields surveyed, with incidence ranging up to 54% of plants affected. Isolations from Verticillium samples collected in 2004 confirmed the existence of a new strain/race of *V. dahliae* that was able to overcome the V-1 gene, found in USDA line HA-89 and many others. This strain, designated as NA-V2, was found in 15% of 122 *Verticillium* samples collected over a two year period from North Dakota and Minnesota sunflower fields. Evaluation of sunflower germplasm for resistance to NA-V2 commenced with greenhouse trials of 109 commercial hybrids and 112 Plant Introduction (PI) accessions from the USDA-ARS National Plant Germplasm System's sunflower germplasm collection. Of these 221 entries, 27 were rated resistant and selected for field trials. Disease testing with artificial inoculation in 2005 and 2006 at two locations identified two entries with the highest level of resistance. PI 507901 and commercial hybrid "Interstate 4575NS' were then tested in Argentine field trials where both were moderately susceptible, suggesting the Argentine strain is different than NA-V2. Research continues on developing germplasm with Verticillium-resistance, with eventual public release and on investigations of VCGs of U.S. *Verticillium* sunflower isolates.

VERTICILLIUM RESISTANCE IN MAPLE

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Norway maple (*Acer platanoides*) mainly grown as cultivars grafted on seedling rootstocks is a major species in Dutch tree nursery industry. Both cultivars and seedlings are susceptible to *Verticillium dahliae* and annual losses are substantial. The only effective control method today is prevention of infection. With Verticillium being widely spread this strategy in practice is difficult and not very reliable. The use of resistant rootstocks would provide a much more reliable protection. This paper summarises more than ten years of research into developing a Verticillium resistant rootstock for *Acer platanoides* cultivars.

Research started in 1993 with development of efficient methods to select and screen for Verticillium resistance in maple. Following large-scale selection experiments resulted in selection of about 300 plants out of a total of nearly 20.000 seedling plants. Selected plants were propagated vegetatively (production of clones) to enable repeated testing of the same individual. During this stage many plants were lost because of failure to propagate. A first field experiment in 2000 showed a clear selection response with disease incidence (DI) in plants of the selected clones being 50% less than in the seedlings and randomly chosen clones. Also mortality in selected clones was about 50% less than that in non-selected clones. It was concluded that selection for resistance to Verticillium wilt in Norway maple is possible. Vegetative propagation was continued and in the period 2000-2003 three successive field tests with new plants of about 30 selection lines were performed with similar results.
Then in the period 2003-2007 the 8 best performing selections were tested as rootstocks for 3 commercial cultivars (Royal Red, Emerald Queen en Cleveland) in several experiments on the same experimental field. The same cultivars on seedling rootstocks served as control. Again two selections performed considerably better than the standard seedling rootstocks with a DI from 13-16% for the selections compared to 36% for the seedling rootstocks. Complete resistance however has not been found. Even in the cultivars on the best performing selections infection occurs (although considerably less) as was demonstrated by the presence of foliar symptoms and discoloration in the stem of some plants. The results of these experiments will be presented and the value and perspectives of the selections as a rootstock will be discussed.

PREDICTING THE EFFICACY OF SOIL FUMIGATION IN CONTROLLING VERTICILLIUM WILT: FROM RESEARCH TO A FARMER DECISION-MAKING TOOL

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The toxicity of fumigants in soil is affected by the rate of their degradation. The fate of methyl isothiocyanate (MITC) was studied in agricultural soils following metam-sodium (MS) application in a controlled system and under field conditions as it was related to disease control. Soil samples were collected from 34 field sites in Israel with no history of MS application. The generation and dissipation curves of MITC in these soils, under controlled conditions, varied significantly among the soils, as reflected by the concentration by time (C×T) product. Several field experiments were conducted in potato fields to validate the relation between the rate of MITC dissipation and the level of Verticillium wilt. The MS treatments significantly reduced Verticillium wilt incidence and severity in five and four experiments, respectively, out of seven. Combining MS with formalin was more effective for controlling disease than MS alone in most cases. A significant correlation was found between C_{MITC}×T products and the incidence of Verticillium wilt disease in the field.

During 2006-2009 The above described tests is used commercially for assessment of the potential MS efficacy. Soil samples are sent to a certified chemical laboratory for analysis of MITC dissipation rate. Each soil is tested and compared to a standard soil of which the rate of MITC dissipation and level of Verticillium control are know. The results which are sent to the farmer include the MITC dissipation curve and the C_{MITC} products of the tested soil and standard soil. These are followed by general recommendation of the advised fumigation treatment. Since 2006, over 300 soil samples were tested representing field scale over 7,000 hectare. Today the this approach is extended to other fumigants such as 1,3-Dichloropropene with similar results.

INITIAL SURVEY OF VERTICILLIUM WILT IN MANGO TREES (*MANGIFERA INDICA*) IN SOUTHERN SPAIN

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Verticillium wilt caused by both *V. dahliae* and *V. albo-atrum* has been reported as an increasing problem in areas where young trees are planted on land previously farmed with vegetables, especially tomatoes. In the province of Málaga in southern Spain more than 3000 hectares are dedicated to this crop. In this area Verticillium wilt symptoms were observed in a new mango grove of the 'Kent' variety planted on land previously farmed with potatoes and tomatoes. About 20% of 200 one-year-old trees presented branches with attached dead leaves in a characteristic "one-sided" fashion. In many of these trees the symptoms expanded followed by general decline and eventual death. In cross sections of affected branches brown vascular discoloration was evident. The fungus was isolated from 10 cm segments of symptomatic branches which were surface-sterilized and placed on acidic PDA. *V. dahliae* was initially identified on the basis of morphology and further confirmed by molecular methods. For the molecular characterization *V. dahliae* and *V. albo-atrum* specific primers were used. Only the *V. dahliae* specific primers generated a PCR product, which was also of the expected size. To further confirm our results primers ITS1 and ITS4 were used. The amplified ITS fragments were sequenced and showed a 100% homology with the sequence of the rDNA ITS of *V.dahliae*. *V. dahliae* was also isolated from symptomatic potato plants grown in a nearby field, and similarly characterized by molecular methods. Pathogenicity assays were then conducted using two lines of tomato differing in the presence or absence of the resistance *Ve* gene. Mango isolates were able to infect both lines and were re-isolated from infected plants but never from uninfected controls. Similar symptoms are observed in nearby orchards planted with mango trees of the 'Kent' and 'Osteen' varieties. We are currently analyzing samples from these trees for the presence of *V. dahliae*. With the increasing planting of mango in fields previously dedicated to horticultural crops in the subtropical-fruit-producing area of southern Spain, more problems with Verticillium wilt are expected.

MOLECULAR DETECTION OF *VERTICILLIUM DAHLIAE* IN SOIL

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Verticillium wilt is a serious problem in tree nursery and agricultural crops in the Netherlands. Losses due to this disease in tree nursery industry recently were estimated at around 5 million euro annually. Because *V. dahliae* is widely spread, nurserymen need to check the soil of new fields for the presence of this fungus before culturing susceptible crops. Methods for detection and quantification of *V. dahliae* in soil samples based on plating of subsamples on selective agar media are well known and commonly used. However, these methods are laborious, time consuming and the results are only indicative for the disease levels to be expected as some microsclerotia may remain dormant. For faster and more exact detection, a PCR method, preferentially a quantitative (real time) PCR, might be a better tool both to advise growers and for use in research.

The aim of this study is therefore to compare existing bioassays for detection of *V. dahliae* with a (quantitative) DNA test for application in detection and quantification of *V. dahliae* in soil samples.

The primers VerDITSF/VerDITSR were developed according to the ribosomal ITS sequence of *V. dahliae*. The sensitivity, due to the multi-copy ITS-sequences present in this fungus, under laboratory conditions was high; no cross-reaction was found with *V. albo-atrum* nor *V. tricorpus, Olpidium, Rhizoctonia*, *Pythium* spp. or other soil fungi as far as tested. A specific amplicon of approx. 300 bp was amplified under standard PCR-conditions. For real time PCR detection the primers VertBt-F/VertBt-R were developed which amplified an internal ITS-fragment of 115 bp; a good result was obtained using SYBRGreen detection with purified DNA of *V. dahliae.*

At this moment the extraction of soil samples containing *V. dahliae* is optimized to obtain DNA. Preliminary results indicate that a nested PCR might be needed to obtain a signal to be able to detect low amounts of DNA of this fungus.

DETECTION AND QUANTIFICATION OF *VERTICILLIUM DAHLIAE* AND *V. ALBO-ATRUM* IN SOILS TO DETERMINE RISK OF VERTICILLIUM WILT IN STRAWBERRY

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A three year study, funded by the UK's Horticultural Development Company, began in 2009 to improve disease control through the development of a rapid, affordable and accurate diagnostic test for verticillium wilt pathogens affecting strawberry. This will permit routine pre-planting soil testing for specific soil-borne *Verticillium* species. The current method for detecting and enumerating *Verticillium dahliae* in soils, commonly referred to as the 'Harris test', is relatively costly and takes 6-8 weeks from sample receipt to reporting. The proposed molecular diagnostic test will quantify the amount of target pathogen DNA in a few days for around half the price of the conventional test.

This work focuses on refining methods for extracting from large soil samples (c. 250 g) and designing real-time polymerase chain reaction (qPCR) assays for quantifying *Verticillium* species affecting strawberry. There was a reasonable relationship between levels of *V. dahliae* microsclerotia in naturally infested field soils, as enumerated by the Harris test, and by qPCR ($P<0.05$; $R^2 = 0.68$).

In addition, some *Verticillium* species, particularly *V. albo-atrum* (Vaa), survive in soil in the form of saprophytic mycelium. Currently no test exists for the detection and enumeration of Vaa in soil in the UK because the Harris test utilises a sieving method which detects only microsclerotia. Consequently, the role and importance of Vaa in causing strawberry wilt is poorly understood. The proposed molecular methods will detect microsclerotia and hyphal propagules of both target *Verticillium* species.

EFFECT OF AGRONOMICAL FACTORS ON THE IMPORTANCE OF VERTICILLIUM WILT OF OLIVE IN THE GUADALQUIVIR VALLEY IN ANDALUCÍA (SOUTHERN SPAIN)

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Verticillium wilt of olive (VWO) is nowadays the most destructive olive disease in the Guadalquivir Valley in Andalucía, the largest olive cultivation area in the world, where 1.5 million ha of olive orchards are concentrated. Disease surveys were conducted throughout the valley with the aim to update the importance and distribution of VWO, and to assess the influence of geographical, edaphical and agronomical factors in the current spread of the disease. Disease incidence (DI) was recorded in 90 olive orchards affected by VWO (27, 33 and 30, in Jaén, Córdoba and Seville provinces, respectively) in a one-hundred-tree rectangular plot, chosen arbitrarily inside plantations. VWO was widely distributed in the Guadalquivir Valley, with a mean DI reaching the 20.4% of 9000 inspected trees, but with significant differences between provinces (25.7, 23.7 and 12%, for Jaén, Córdoba and Seville, respectively). DI was significantly higher in irrigated (20.7%) than in dry-farming (18.3%) olive orchards, being differences particularly important in the central and the upper Valley. Also, non-tilled orchards showed higher DI (25.6%) than those regularly tilled (16.3%). DI was lower when the number of trees per ha in orchards was higher than 200. Moreover percentage of wilted plants was higher (21.5%) with the proximity of neighboring *V. dahliae* host crops to olive orchards, than if non-susceptible hosts surrounded them (11.9%). Finally, differences of DI were more stressed in plots where less than 25-year-old trees were grown and in those plantations closer to the Guadalquivir River (less than 10 km). 'Picual' was identified as the most susceptible cultivar to the disease, reaching 41.9% of DI in the plots in which this cultivar was identified. PCR-based molecular pathotyping of *V. dahliae* isolates recovered from wilted olive trees showed that defoliating (D) highly virulent isolates were presented at surveyed plantations in percentages significantly higher (67.7%) than non-defoliating isolates (32.3%), specially in lower (Seville province) and upper (Jaén province) valley. This fact could explain the important increasing of incidence and severity of VWO observed in the valley during the last decade.

VERTICILLIUM INTERSPECIFIC HYBRIDS MIGHT NOT BE SO RARE IN NATURE

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Molecular evidences for interspecific hybrid origin of *Verticillium dahliae* isolates of vegetative compatibility group 3 (VCG3) were found while analyzing the presence of multiple sequences of genes actin (*Act*), β-tubulin (*β-Tub*), calmodulin (*Cal*) and histone 3 (*H3*), and the internal transcribed sequences (ITS-1 and ITS-2) of the rRNA genes. Moreover, analyses of these sequences provided further consistent evidences of the hybrid origin of *V. dahliae* var. *longisporum* (VDLSP). Thus, analysis of the two alleles found for genes *Act* and *β-Tub* in *V. dahliae* VCG3 revealed that one allele was identical to previously characterized *V. dahliae* sequences, whereas the other was as unrelated as those homologues found in *V. nigrescens* or *V. albo-atrum*. However, for those genes for which unique sequences were identified, neither homology (*Cal*) nor full identity (ITS) to *V. dahliae* were found. None of the VCG3 sequences unrelated to *V. dahliae* showed complete homology in gene databases. On the other hand, none of the *Act*, *β-Tub* and *Cal* alleles found in VDLSP isolates were identical to their homologues in *V. dahliae* or *V. albo-atrum*. Moreover, the unique *H3* sequence found was identical to that existing in *V. dahliae*, but ITS sequences showed closer to *V. albo-atrum* ITS than to *V. dahliae* ITS. Additionally, a *V. dahliae*-specific polymorphic sequence was used to shed light about the putative parents of VCG3 and VDLSP isolates. Phylogenetic analysis of all these sequences suggested that the hybridization event originating some of the currently known VDLSP isolates might have happened between a *V. dahliae* of VCG1 and/or VCG4A and a closely-related *V. albo-atrum* as parents. On the other hand, phylogenetic analysis and PCR markers profiling of a *V. dahliae* VCG3 isolate suggested a VCG1B isolate as putative parent. However, the other parent appeared unrelated to *Verticillium* spp. and could not be identify according to the sequences available in gene databases. Therefore, the representative VCG3 isolate examined in this study must be considered as an interspecific hybrid between *V. dahliae* and as yet unidentified parent. These results suggest an important role for parasexuality in diversity and evolution in the genus *Verticillium*, and that interspecific hybrids within this genus might no be so rare in nature.

GENETICS AND PATHOGENIC CHARACTERIZATION OF *VERTICILLIUM DAHLIAE* ISOLATES FROM EGGPLANT IN TURKEY

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During 2005 and 2007, eggplant fields in 19 eggplant-growing provinces from five different regions (Aegean, Central Anatolia, Marmara, Mediterranean, Southeastern Anatolia regions) of Turkey were surveyed for Verticillium wilt. Sixty-seven isolates of *Verticillium dahliae* from wilted eggplants were collected and used for vegetative compatibility analysis using nitrate non-utilizing mutants and reference tester strains of vegetative compatibility groups (VCGs) 1A, 2A, 2B, 3, 4A and 4B. Among all isolates, 33 from 17 provinces were assigned to VCG2B, 23 from nine provinces to VCG2A, six from five provinces to VCG4B and five from three provinces to VCG1A while VCG3 and VCG4A were not defined among isolates. In order to test if there is a correlation between VCG and pathotype in V. dahliae, pathogenicity/ virulence of 30 isolates representing the four multimember VCGs were tested on S. melongena cvs. 'Kemer' and 'Aydın Siyahı' in the greenhouse. All isolates were found to be pathogenic on both cultivars. Isolates within each of VCGs 1A, 2A and 4B caused similar vascular discoloration index (VDI) on both cultivars. VCG1A isolates caused lower VDI comparing to the isolates of VCG4B on both cultivars. Most isolates of VGG4B led to higher VDI than those of VCG2A on 'Kemer', and most isolates of VCG2A led to lower VDI than those of VCG4B on 'Aydın Siyahı'. Isolates of VCG2B were found to vary in their VDI values on both cultivars. This is the first report of natural infections of eggplant by VCG1A in the world.

AN OVERVIEW OF VEGETATIVE COMPATIBILITY GROUPS OF *VERTICILLIUM DAHLIAE* FROM COTTON IN TURKEY, INCLUDING NEW ISOLATES

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Cotton (*Gossypium hirsutum* L.) is the most important field crop in the southern (Mediterranean part), southeastern and western Anatolia of Turkey. Considering the severe wilting of cotton plants observed and the presence of D pathotype in these regions and the role of VCGs proved for pathogenicity of the isolates, objective of this research was to strengthen a proper sight of the disease in all cotton areas of Turkey together with VCG results of 139 new and 254 previously reported isolates (not characterized or self-incompatible isolates were not included in these numbers). To summarize all findings of genetic diversity studies that have been conducted in the last 10 years, among 393 isolates, 42.0% of total belonged to VCG1A, 42.0% to VCG2B, 13.5% toVCG2A and 2.5% to VCG4B. The highly virulent form of *V. dahliae* resulting very severe disease in cotton consisted of VCG1/D and VCG2B/PD pathotypes and this type was detected in 84% of the affected fields. Mild-to-moderate disease in other fields was dominated by VCG2A/ND, with VCG4B/ND as a minor component. Newly collected isolates from some areas revealed no considerable difference in VCG pattern of *V. dahliae* isolates from the same locations over years. Majority of the isolates (61.1% of 113 isolates) from the Mediterranean part belonged to VCG2B, 28.3% to VCG2A, 5.3% to VCG1A and 5.3% to VCG4B. Among 198 isolates from the western Anatolia, 57.6% belonged to VCG1A, 32.3% to VCG2B, 8.1% to VCG2A and 2.0% to VCG4B. 54.9% of 82 isolates from the southeastern Anatolia belonged to VCG1A, 39.0% to VCG2B and 6.1% to VCG2A. A correlation between vegetative compatibility and geographical provenance of the isolates were observed: VCG1A was dominant in the western and southeastern Anatolia but rarely found in the Mediterranean region; on the other hand, VCG2B was dominant in the Mediterranean part but had lower provenance in the western and southeastern Anatolia regions.

VEGETATIVE COMPATIBILITY GROUPS OF *VERTICILLIUM DAHLIAE* ISOLATES OBTAINED FROM CULTIVATED AND WEED PLANTS IN CRETE

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A collection of one hundred and twenty two (122) isolates of *Verticillium dahliae* Kleb. obtained from 23 cultivated plant species (in open fields and under cover) belonging to nine botanical families, and seven weed species belonging to five botanical families, grown throughout Crete, were tested by vegetative compatibility group (VCG) analysis. Most of these isolates had been classified to pathotypes and races in previous works. The aim was to study the genetic relatedness of the isolates and to classify them in one of the four known VCGs (1, 2, 3, 4) by pairings of complementary nitrate-nonutilizing (*nit*) mutants induced on a chlorate-containing medium, using tester strains from the USA. Of these: one isolate originating from an olive tree cultivated at Messara valley was assigned to VCG1; 27 isolates (obtained from 10 cultivated species belonging to six families and two weed species belonging to two families) were assigned to VCG2, and 62 isolates (obtained from 17 cultivated species belonging to seven families and seven weed species belonging to five families) were assigned to VCG4. No isolates were assigned to VCG3. All isolates within a VCG were strongly compatible with at least one of the tester isolates but were not always completely incompatible with tester strains of other VCGs. Thirteen isolates (obtained from eight cultivated species belonging to 5 families and a weed species) were not assigned to a VCG because their *nit* mutants complmented with *nit* mutants of more than one tester strains. Four isolates (obtained from three cultivated and one weed species belonging to one family) were not assigned to VCG because they did not produce *nit* mutants; whereas two other isolates (obtained from two cultivated species belonging to two families) were not assigned to a VCG because they produced *nit* mutants that did not complement any of the tester strains. Of the 89 *V. dahliae* isolates that were finally assigned to VCGs, 68.9% belonged to VCG4, 30.0% to VCG2 and 1.1% to VCG1. This is the first attempt Cretan isolates of *V. dahliae* to be assigned to VCGs and the second report that VCG1 is recorded in our country.

DETERMINATION OF PATHOTYPES OF *VERTICILLIUM DAHLIAE* AND NEW HOSTS OF *V. DAHLIAE* RACE-2, AND SPREAD OF THE PATHOGEN IN CRETE

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Verticillium dahliae Kleb. is a soil-borne fungus infecting more than 400 cultivated and weed plant species, worldwide, causing usually severe disease and substantial damage. Two physiological races (1 and 2) and several pathotypes of the pathogen have been identified. A collection of 105 *V. dahliae* isolates obtained from 37 plant species (25 cultivated and 12 weed) belonging to 13 botanical families and grown on several areas of Crete was used in the present study, with the purpose to identify pathotypes and races.

Crassification of isolates to pathotypes was attempted by examining their pathogenicity, using the root-dipping technique on four differential plant species (eggplant, pepper, tomato and turnip). Of the 105 *V. dahliae* isolates: 71 obtained from 31 infected species (22 cultivated and 9 weed) belonging to 10 botanic families, were determined to the pathotype I (proved pathogenic to tomato, eggplant, and turnip), 26 obtained from 16 species (10 cultivated and 6 weed) belonging to 7 botanical families were classified to the pathotype II (proved pathogenic to eggplant and turnip), whereas the remaining 8, obtained from 2 cultivated species belonging to one botanical family were classified to the pathotype III (proved pathogenic to all four differential species). The conclusions are: a) the pathotypes I, II, and III of *V. dahliae* were greatly, moderately, and lightly spread, respectively, in Crete, and b) the pathotype IV (isolates pathogenic only to turnip, according to Japanese workers), lacks in Crete.

Crassification of isolates to races was attempted by examining their pathogenicity, using two differential tomato varieties: Earlypack No 7 (lacking the *Ve* resistance gene) and ACE 55 VF (possessing the *Ve* gene), using the root-dipping technique. Of these 105 *V. dahliae* isolates, 50 obtained from 21 cultivated species: bean*, broccoli*, cabbage*, cauliflower*, chickpea, chicory*, cucumber*, eggplant, endive*, melon, ochrus pea*, olive tree, pepper*, potato*, Romaine lettuce, radish*, summer squash, masigold*, tomato, vetch*, and watermelon, as well as 17 isolates obtained from 9 weed species: black nightshade, common groundsel*, *Erodium* sp.*, field bindweed*, hoary cress*, shepherd's purse*, senape*, *Trifolium* sp.*, and wild radish* proved to belong to race-2. Of the remaining 38 isolates, 12 obtaining from 10 species (9 cultivated and one weed) proved to belong to race-1, whereas 26 obtained from 16 species (10 cultivated and 6 weed) proved non-pathogenic to tomato. The conclusions are the identification of: a) 20 worldwide new hosts of race-2 (these which are listed with asterisks) and b) 10 known hosts of it (these which are listed without asterisks). Race 2 of the fungus proved widely spread in Crete infecting *Ve* tomatoes, vegetables, olive trees, etc (such as in the Lasithi plateau and the Messara valley areas). A 84.8% of the pathogenic to tomato isolates used belong to race-2, whereas the remaining 15.2% belong to race-1. Today because of its spread, race-2 is a serious problem to many vegetables in Crete.

FUNGAL ADHESION TO PLANTS – THE FIRST STEP OF EARLY INFECTION AND SYSTEMIC COLONIZATION OF *BRASSICA NAPUS* BY *VERTICILLIUM LONGISPORUM*.

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Verticillium longisporum is a major pathogen for oilseed rape (*Brassica napus*). This fungus induces early senescence and causes severe economic losses. V. longisporum infects host-plants via the roots and lives biotrophically in the xylem vessels of B. napus. Currently no fungicides are available to cure infected plants. Adhesion is a crucial step for the early infection and colonization of many pathogenic fungi. Proteins required for adhesion of plant fungal pathogens play important roles during pre-penetration, growth, biofilm formation as well as pathogenicity.

The goal of this project is to investigate adhesive proteins that may support successful infection and xylem vessel colonization of *V. longisporum*. Adhesion complementation assays in non-adherent yeasts Saccharomyces cerevisiae and bioinformatics analyses of the fungal cDNA library revealed ten genes encoding putatively secreted proteins that may be involved in adhesion of the fungus. We could show that eight of these putatively secreted proteins were up-regulated when the fungus interacted with roots of the host plant. In addition, two transcriptional regulators from *V. longisporum* induce strong adhesion of S. cerevisae to the agar surface. These transcription factors belong to the zinc finger family and were reported to regulate many essential genes in filamentous fungi such as genes for toxin production, for conidial formation or for cell wall biosynthesis, etc. We are currently analyzing the knockdowns of the corresponding genes in *V. longisporum*.

POTENTIAL OF LIGNIN INCORPORATION IN SOIL TO CONTROL VERTICILLIUM WILT OF CAULIFLOWER

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Verticillium wilt is an important problem in Belgian cauliflower production. The disease is caused by *Verticillium longisporum*, a soil-borne pathogen which can persist for many years in soil in the form of microsclerotia. Organic amendments, such as certain crop residues and side products have been used to control Verticillium wilt of other crops. Previous work in our lab has shown that incorporation of Kraft pine lignin, a side product of the paper industry, decreased the viability of *V. longisporum* microsclerotia in soil. In addition, lignin combined with introduced *Pseudomonas* spp. in soil was more effective than each treatment alone. These results raised the hypothesis that lignin incorporation in soil weakens the microsclerotia and makes them more susceptible for antagonists. Further investigation was carried out to test the effect of lignin on Verticillium wilt of cauliflower and explore the mechanisms underlying the disease control. Lower disease incidence and severity were observed on cauliflower plants grown in pots containing naturally infested soil amended with lignin (1% w/w) compared to the control plants, confirming the potential of lignin incorporation for managing the disease. However, the effect of lignin on microsclerotia seems to be soil dependent. When lignin was incorporated into two different soil types – Leest and Oppuurs, the number of viable *Verticillium* microsclerotia was reduced in Leest soil, but not in Oppuurs soil. Changes in microbial populations in these soils were studied using phospholipid fatty acid analysis. It revealed a similar increase in Gram negative bacteria upon lignin amendment in both soils and a higher increase in fungi and acitnomycetes in Leest soil than in Oppuurs soil. We suggest that the differential stimulation of fungi and actinomycetes that occurred in Leest soil was probably necessary for the successful result obtained in this soil type, but the decrease of microsclerotia viability might be the outcome of a complex microbial interaction involving Gram negative bacteria as well.

NON-CHEMICAL CONTROL OF VERTICILLIUM AND NEMATODES IN TREE NURSERY SOILS

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Verticillium wilt caused by *V. dahliae* is a serious problem in tree nursery industry in the Netherlands. Total loss of value due to Verticillium wilt recently was estimated to be about 5 million euro annually, mainly in the production of street trees and roses. The only effective control of Verticillium wilt in trees is to prevent the trees from being infected. *V. dahlia*, however, is widely spread in agricultural fields in the Netherlands and many important tree species are susceptible to Verticillium wilt. Therefore effective methods to eradicate the fungus from soil are strongly needed. The recent withdrawal of most soil fumigants due to their negative environmental effects enhanced the interest for new non-chemical techniques in the control of Verticillium and nematodes.

In 2009 PPO started a long-term field experiment to develop new strategies to control *Verticillium dahliae* and the nematode *Pratylenchus penetrans* in tree nursery soils. Biological methods applied are anaerobic biological soil disinfestation, growing marigold (*Tagetes patula*) combined with the application of compost, and growing a green manure crop (Sarepta or Indian mustard, *Sinapis juncea*), which will be incorporated in the soil to cause biofumigation. These treatments will be compared with two control techniques, fallow and chemical soil disinfestation (Metam-Sodium), and with growing white clover (*Trifolium repens*). In the clover treatment it is expected that both *Verticillium dahliae* and the nematode *Pratylenchus penetrans* will increase in the soil, since clover is a good host for both pathogens.

The treatments will be applied during 2009 to experimental plots on two different soil types. In 2010 on these plots roses (on sandy soil) and *Acer platanoides* (on clayey soil) will be grown to investigate the effectiveness of the different methods in the control of *Verticillium dahliae* and the nematode *Pratylenchus penetrans*. Besides growing these test crops, several techniques including plating of soil samples and the use of molecular detection techniques will be used to monitor the population densities of *Verticillium dahliae* and the nematode *Pratylenchus penetrans* in the soil during the whole project.

A KNOWLEDGE-BASED SYSTEM TO PREDICT PLOT INFESTATION WITH *VERTICILLIUM DAHLIAE* IN POTATO

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Intensive production of field crops in the south-west part of Israel implements a meticulous crop rotation scheme to minimize the damage from polyphagous soil-borne pathogens. Plot allocation and scheduling requires the consideration of various factors such as infestation and susceptibility to soil-borne pathogens, soil characteristics, and many other parameters. The objective of this study was to develop A knowledge-based system (KBS) to predict plot infestation with *Verticillium dahliae* in potato. A comprehensive historical database was characterized and constructed. Biotic and abiotic factors affecting the manifestation of *V. dahliae* were selected by a panel of three researchers and a potato grower. These factors were weighed and ranked using multi-criteria pairwise comparison. The ranking were combined using geometric-mean to a new ranking matrix which was found to be acceptable. The combined result was then used for the multi-criteria mechanism of the KBS. The multi-criteria mechanism implemented a weighted linear combination. A disease development model of *V. dahliae* in potato cultivars of differing susceptibility was proposed by one of the experts and was used to calibrate the KBS. The model was based on a hypothetic situation of growing potatoes consecutively on a disease free plot. Twenty-two plot records collected between 1998 and 2007 from an agricultural area in the south-west part of Israel and 23 records from studies conducted in Israel and in Idaho, USA were used for initial validation of the KBS. The KBS obtained more than 90% prediction accuracy with 0.91 Kappa coefficient and spearman correlation coefficient of 0.71. Based on the developed KBS a decision-support system (DSS) was then developed for crop allocation by ranking fields according to the predicted infection with *V. dahliae*.

Key words: multi-criteria pairwise comparison, KBS, DSS, modeling, V. dahliae

VERTICILLIUM WILT ON HOPS IN SLOVENIA: PHYTOSANITARY CONTROL AND DISEASE MANAGEMENT

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Verticillium wilt, caused by *Verticillium albo-atrum* Reinke & Barthold and *V. dahliae* Klebahn, is one of the most devastating diseases of hop (*Humulus lupulus* L.). In Europe, outbreaks of highly virulent pathotypes have been causing considerable economic damage to hop production; and isolates from hop are included in the II.A.II list of harmful organisms of Council Directive 2000/29/EC. Two disease syndromes (forms) are known on hops; fluctuating (mild) and progressive (lethal) wilt, the development of which is attributed to the pathogen virulence, the sensitivity of cultivars and ecological factors.

In Slovenia, an outbreak of the lethal form was first registered in 1997 in the western part of the Savinja valley and *V. albo-atrum* was identified as the infection agent. Virulence testing and molecular analysis of *V. albo-atrum* hop isolates collected in all Slovene hop growing regions identified two pathotypes, which were designated PG1 (mild) and PG2 (lethal). Over the next 12 years, new outbreaks caused by lethal pathotype PG2 were detected in a radius of 30 km from the first outbreak and, to date, more than 180 ha of hop gardens have been affected, representing 10 % of Slovenian hop production areas.

Since 1998, the Slovenian Institute of Hop Research and Brewing (SIHB), co-ordinated by the Phytosanitary Administration (PARS) of the Ministry of Agriculture, Forestry and Food (MAFF), has carried out a monitoring survey of hop gardens, which includes visual inspections of hop gardens, sampling, laboratory analysis and expert support. In addition, a certification scheme for the production of disease free planting material has been established and all other phytosanitary measures intended to prevent the further spread of Verticillium have been included in Slovenian legislation. Intensive research activities have focused on a wilt resistance breeding program and to soil disinfestation by using solarisation and organic amendments, with the aim of replanting infected areas.

EFFECTS OF ACIBENZOLAR-S-METHYL ON RESISTANCE INDUCTION AND STEM COLONIZATION BY *VERTICILLIUM DAHLIAE* IN NURSERY PRODUCED OLIVE PLANTING STOCKS

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Plants are continuously exposed to pathogen attacks, but they defend themselves with various mechanisms, including induced resistance. Acibenzolar-*S*-methyl (ASM), a functional analogue of salicylic acid, was found to behave as an effective resistance elicitor facilitating, in several crops, protection under field conditions against a wide range of pathogens.

Phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are key enzymes in phenylpropanoid/flavonoid pathway, leading to production of several compounds extremely important in plant defence strategy. A molecular method for monitoring PAL and CHS gene expression in olive plantlet leaves (cv Leccino), in response to ASM application, was developed. Specific primers were designed to quantify the relative transcript level of the two genes by quantitative reverse transcription-PCR. Gene expression was normalised to the housekeeping β-actin (*act1*) mRNA gene of *Olea europaea* L. Moreover, colonization of *Verticillium dahliae* in artificially inoculated stems (10^7 conidia ml^{-1}) of young olive plantlets treated with ASM (0.25 gl^{-1} a.i.) was also evaluated. Periodically, plantlets were inspected to evaluate ASM phytotoxicity and frequency of pathogen isolation was verified 1 month after inoculation by traditional technique and confirmed by real-time PCR. To correlate the increase of transcript level and the relative enzyme activity, PAL activity was determined 48 hours after ASM treatment.

Results indicated that ASM up-regulates the expression of the target genes. It was shown that PAL and CHS expression were strongly induced by ASM within six hours from the application. Moreover, the time-course revealed a persistence of gene up-regulation till the fifth day after the treatment. The increase of PAL transcript level was also confirmed by monitoring its enzyme activity which showed about one fold-increase, as compared to the untreated control, at 48 h after ASM application. Concerning pathogen progression, significantly lower stem colonization was found in ASM-treated plantlets than in the untreated control. The higher accuracy and sensitivity of real-time PCR detection method in comparison with the traditional isolation technique was confirmed.

CULTIVAR-SPECIFIC *PSEUDOMONAS* COMMUNITIES IN THE RHIZOSPHERE OF OLIVE TREES

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Bacteria from the genus *Pseudomonas* represent ubiquitous plant colonizer with distinct ability to antagonize fungal pathogens. This includes *Verticillium dahliae* a devastating pathogen causing dramatic damages in olive orchards across the Mediterranean region. Along with a study analyzing the cultivar specificity of rhizosphere-associated microbial communities, we drew special attention to *Pseudomonas* populations. Overall, 16 cultivars from different origins but grown at the same site in Andalusia, Spain, were studied. Rhizosphere samples from four trees each cultivar were analyzed applying a multiphasic approach including cultivation-dependent and –independent methods. Using the Most Probable Number (MPN) method with selective media, we observed 3 x 10^4 to 2 x 10^6 of culturable pseudomonads per gram root fresh weight. In addition to cultivation, *Pseudomonas* abundances were determined by quantitative Real-Time PCR from a total DNA extract. Structural as well as functional diversity of root-associated *Pseudomonas* communities was studied employing the fingerprint technique Single-Strand-Conformation-Polymorphism (SSCP) analysis based on 16S rDNA sequences and 2,4-diacetylphloroglucinol synthesis genes.

Although investigated olive cultivars were grown and managed at the same site, results indicate a significant cultivar and even an origin-specific colonization by pseudomonads. These findings could support development of plant protection strategies against Verticillium wilt by means of biological control agents.

MOLECULAR ANALYSIS OF ENDOPHYTIC COMMUNITIES OF WILD OLIVE TREES FROM DIFFERENT MEDITERRANEAN REGIONS

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Olea europaea L. is known as long-living tree species widespread in the Mediterranean region. The longevity of olive may accompanied with well established microbial populations. In this study, the structure of endophytic bacterial and fungal communities associated with wild trees from Cyprus and Greece were analyzed. In a cultivation-dependent approach, microorganisms were enriched from surface-sterilized and pestled leafs and stems by plating on nutrient poor media. Compared to other plant species, only 4 x 10^3 bacteria with a low diversity could be isolated from endosphere habitats. However, these isolates exhibited a strong inhibitory effect on the growth of *Verticillium dahliae*. In a second approach, we took advance of cultivation-independent, DNA-based methods. Single-Strand-Conformation-Polymorphism (SSCP) analysis was used to fingerprint bacterial and fungal communities in general and the taxonomic groups of *Pseudomonas* and *Bacillus* as well as *Ascomycota* in particular. Interestingly, analysis of the SSCP patterns revealed similarities of endophytic microbes not only between different individual trees at one site, but also between trees from different regions. Results reveal that wild olives are associated with highly specific and stable microbial endophytes with distinct functions. To confirm our findings, the study will be continued and extended by sampling wild olive trees from other regions and compared with cultivated trees.

UNRAVELLING THE OLIVE ROOTS COLONIZATION PROCESS BY VERTICILLIUM WILT EFFECTIVE BIOCONTROL *PSEUDOMONAS* SPP STRAINS

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Within a strategy aimed to promote plant growth and long-term protection against phythopathogens in olive (*Olea europaea* L.) by beneficial root-associated bacteria, we are studying the olive root tissues colonization processes of endophytic *Pseudomonas* spp. strains previously demonstrated to be effective biocontrol agents (BCA) against Verticillium wilt. A fluorescently-tagged derivative of strain *P. fluorescens* PICF7, whose ability to internally colonize root tissues has been established, was used to inoculate the roots of three-month-old nursery-produced potted olive plants (cv. Arbequina). The same strain was also used in root colonization bioassays of *in vitro* micropropagated olive plants (cv. Manzanilla). This gnotobiotic system provides an excellent environment for a rigorous analysis of the molecular mechanisms involved in the interaction PICF7-olive. By confocal laser scanning microscopy we were able to demonstrate that root hairs of *in vitro* micropropagated plants are the preferential entrance site for the internal root colonization by strain PICF7. This finding corroborates previous observations in PICF7-inoculated, nursery-produced olive plants grown under non-gnotobiotic conditions. Likewise, the BCA strain *P. putida* PICP2 showed root-hair internal colonization of *in vitro* micropropagated olive plants when tested under gnotobiotic conditions. Thus, olive root hairs seem to be essential structures for the internal olive root colonization by these beneficial *Pseudomonas* strains. These results demonstrate the suitability of the *in vitro* system to investigate endophytic colonization processes in olive.

Finally, to unravel the genetic and molecular basis of olive roots colonization by strain PICF7, suppression subtractive hybridization (SSH) libraries were separately generated from root and stem tissues of (non-inoculated *vs*. inoculated) olive plants grown either under non-gnotobiotic (potted, nursery-produced) or *in vitro* (micropropagated) conditions.

AN OUTBREAK OF VERTICILLIUM WILT IN HEDGEROW OLIVE ORCHARDS IN ANDALUCÍA (SOUTHERN SPAIN)

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Verticillium wilt of olive (VWO), caused by *Verticillium dahliae*, is nowadays the most destructive olive disease in the Guadalquivir Valley in Andalucía. In last years, some growers are establishing a new olive intensive cultivation system, based on high tree densities with 2000 tree/ha arranged in hedgerows. 'Arbequina' is the better-adapted olive cultivar to hedgerow system, but in some cases 'Picual' (major Spanish cultivar) has been used. Both cultivars are highly susceptible to the Defoliating pathotype of the pathogen in controlled conditions, although 'Arbequina' seems to be less susceptible than 'Picual' under field conditions. In this work, several agronomical and phytopathological parameters has been studied in six hedgerow olive orchards affected by VWO planted with 'Arbequina' (5 fields) or 'Picual' (1 field). In each field, inoculum density of *V. dahliae* in soil was determined before orchard establishment by wet sieving method. Thereafter, fields were inspected, from 14 to 64 months after planting, and disease incidence scored in two separate subplots (5 rows, 100 trees/row) of each field. Inoculum density in field soils before planting ranged from <0.4 to 35.5 microsclerotia per gram of soil and disease incidence ranged between 2.8 and 30.4% after a period from 19 to 64 months after planting in 'Arbequina' orchards. However, the field planted with 'Picual', reached 77.1% of wilted trees in observations carried out 14 months after planting, with an initial inoculum density in soil of 2.4 ppg. Results suggest that hedgerow cultivation system produces a shortening of disease onset period, favoring VWO, with more severe and faster disease development than those observed in traditional and intensive olive orchards planted with 'Arbequina'. It is also remarkable that epidemics can become devastating when very susceptible cultivars such as 'Picual' are used instead of 'Arbequina' in infested soils even with low inoculum density in soil. Among other agronomical factors favoring WVO development, higher density of superficial roots in the hedgerow system comparing with traditional or intensive systems, may account for a homogeneous exploration of the soil profile, increasing root infection by *V. dahliae*.

EFFECT OF CURED COMPOST AND BIOCONTROL AGENTS ON THE VIABILITY OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA IN THE RHIZOSPHERE OF NURSERY-GROWN OLIVE PLANTS

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Verticillium wilt is the most severe diseases of olive worldwide. The pathogen, *Verticillium dahliae* Kleb., is able to persist for several years in contaminated soils by its resting structures represented by the microsclerotia (MS). In the last decades the disease has occurred with increasing frequency and severity in most olive-growing areas, particularly on young plants in the nursery as well as in the field. The disease control by means of synthetic fungicides involves several risks and then safer and more eco-compatible control measures are necessary. Preventive methods (i.e. pathogen-free soil and planting material, suppressive substrates and biocontrol agents) have been shown as promising tools for an efficient control of the pathogen in the nursery. Recently, alternative plant growth substrates containing natural amendments or composted biomasses are being studied not only for agronomic properties, but also for their suppressiveness against soil-borne pathogens.

To prevent *V. dahliae* infections our investigations were conducted for one year in growth chamber and for two year in a commercial olive plant nursery with self-rooted olive plants, cv Leccino. Trials were aimed at evaluating the suppressive activity against *V. dahliae* MS of some organic amendments, obtained from vegetal by-products, and antagonistic bacteria isolated from these suppressive matrices. The experimental matrices at 15% (v/v) were mixed in different proportion with a standard substrate ("Sonnoli") while experimental bacteria were applied at a concentration of 10^9 cfu g^{-1}. The experimental substrates were artificially contaminated with 50-100 MS/g. In blind trial experiments, the pathogen was periodically monitored in the soil by either semi-selective medium or real-time Scorpion PCR. The effect of the treatments on the plant growth was also evaluated.

Results of experiments evidenced that some experimental matrices and antagonistic microorganisms significantly reduced the inoculums density of *V. dahliae* MS in the rizosphere of olive plants and did not interfere with the plant growth. The mechanisms of compost suppressiveness were mainly related to their natural content of antagonistic microflora. The new substrates and antagonists are worthy to be optimized as new and safer alternatives to chemicals for the prevention of *V. dahliae* infections in the rhizosphere of nursery-grown olive plants.

EFFECT OF GUANITO ON OLIVE PLANTLETS GROWTH AND ON THE INOCULUM DENSITY OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA

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In the last two decades Verticillium wilt of olive, caused by *Verticillium dahliae* Kleb., has occurred with increasing frequency and severity in most olive growing areas of the Mediterranean basin. The disease affects olive trees starting from the nursery and microsclerotia (MS) are critical factor in the epidemiology and control of the disease. Among the environmentally friendly alternatives to protect plantlets against *V. dahliae* infection, the use of organic amendments has received much attention. In this study, The effect of Guanito bio-fertilizer on the inoculum density of *V. dahliae* microsclerotia in organic growing medium, and on vegetative parameters of olive plantlets was assessed. Trials were carried out either on 8-months old self-rooted and 18-months old grafted olive plantlets, cv Leccino. The organic growing medium was amended with different concentrations of Guanito (1%, 2%, and 3%, v/v). Before plant transplanting into pots, growing medium was artificially inoculated with *V. dahliae* microsclerotia (60-70 MS g^{-1}).

A phytotoxic effect was observed on plantlets growing in medium amended with 2% and 3% of Guanito. At these bio-fertilizer concentrations *V. dahliae* microsclerotia were almost completely inactivated. These results were more evident on grafted than on self-rooted plantlets. Conversely, plantlets growing in 1% Guanito-amended medium showed a higher plant height than those growing in the unamended medium. Moreover, the inoculum density of *V. dahliae* microsclerotia in the medium amended with 1% Guanito was significantly lower than the unamended control.

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