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11th International Verticillium Symposium

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Chronicle of the Verticillium Symposium

11th	2013	Göttingen, Germany
10th	2009	Corfu Island, Greece
9th	2005	Monterrey, California, USA
8th	2001	Córdoba, Spain
7th	1997	Athens, Greece
6th	1994	Dead Sea, Israel
5th	1990	Leningrad, USSR
4th	1986	Guelph, Canada
3rd	1981	Bari, Italy
2nd	1978	Berkeley, California, USA
1st	1971	Wye College, Kent, UK

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Welcome address to the 11th International Verticillium Symposium in Göttingen 2013

Dear colleagues and friends, dear international Verticillium community,

on behalf of the International Verticillium Steering Committee, I would like to welcome you very cordially to this 11th International Verticillium Symposium. After this meeting has toured many countries in the world in the last 4 decades, we are pleased to host this high-ranked event in Germany for the first time.

The list of countries where this meeting has been hold until today reflects just an excerpt of those areas where diseases caused by Verticillium play a role and where this has triggered significant research activities to improve our knowledge into these particular plant pathogens. Since the early days of this symposium, a significant range of tools have evolved in plant pathology allowing for considerable advances of our knowledge on Verticillium. Among these, genome analysis, DNA-based bioanalytical detection methods and advanced metabolite-profiling and identification have played a major role. This has greatly improved our understanding of phylogenetic relationships, plant-pathogen interactions and ecological functions in the genus Verticillium.

Much of this has happened and developed since our last meeting on the splendid island of Corfu in November 2009. Together with all the colleagues from the international Verticillium community attending this symposium in Göttingen, I am very much looking forward to share the novel views and learn about the recent state of research into the fascinating world of these important vascular pathogens.

This meeting would have been impossible without the great support by a number of individuals to whom I have to express my sincere gratitude. A major share of these credits go to Birger Koopmann, who has been the center person of the whole local organization, the abstract handling, the logistics planning and the technical arrangements of the meeting.

I am also grateful to the members of the International Verticillium Steering Committee who actively contributed in many ways to this meeting, particularly by spending large support in evaluating and selecting papers, shaping the programme and chairing symposium sessions.

Not at least, the technical support by Falko Feldmann and Christian Carstensen from the German Society of Plant Protection and Plant Health (DPG) is gratefully acknowledged.

Thank you once more for coming and enriching this meeting with your presence and inspiring contributions.

Andreas von Tiedemann
Chair, Organizing Committee
University of Göttingen

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Local Organizing Committee

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- Birger Koopmann
- Martina Bode
- Christian Carstensen
- Falko Feldmann

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Programme 11th International Verticillium Symposium Göttingen 5-8 May 2013	
Session Topics:	Taxonomy and Genetics of Verticillium, Population Genetics and Virulence Host-Pathogen Interactions and Resistance, Epidemiology and Integrated Control, Biological Control and Microbial Ecology

Sunday, May 5th

15:00	**Arrival and registration** Poster set-up

Monday, May 6th

08:30	**Welcome, Introduction & Opening** *Convenor, chairman steering committee*
PLENARY SESSION	
Chair:	*Andreas von Tiedemann, Germany*
Keynote 1 09:00	**Verticillium diseases in Germany – history, significance and management** *Monika Heupel, Germany*
Keynote 2 09:30	**BioFung: First insights into the genome of the oilseed rape pathogen *Verticillium longisporum*** *Gerhard H. Braus et al., Germany*
10:00	**Tea/Coffee Break**
ORAL SESSION 1	**DEZ BARBARA MEMORIAL SESSSION** (TAXONOMY AND GENETICS I)
Chair:	*Steve Klosterman, USA*
10:30	**Tribute to Dez Barbara** *Krishna Subbarao, USA*
Keynote 3 10:50	**Taxonomic challenges - molecular evidence for species and sub-specific groups in Verticillium** *Patrick Inderbitzin, and Krishna Subbarao, USA*
11:20	**New insights on the phylogenetic relationships between strains of *Verticillium dahliae*** *Michael G. Milgroom, María del Mar Jiménez-Gasco, Concepción Olivares-García, and Rafael M. Jiménez-Díaz*
11:40	**Molecular diagnosis based on the VTA2 barcode marker to discriminate the hybrid lineages of *Verticillium longisporum* on the oilseed crop *Brassica napus*** *Van Tuan Tran, Susanna A. Braus-Stromeyer, Christian Timpner and Gerhard H. Braus*
12:00	**Classification and determination of similarity levels of *Verticillium dahliae* isolates, using FTIR-ATR spectroscopy** *Ami Pomerantz, Ahmad Salman, Elad Shufen, Leah Tsror, Raymond Moreh, Shaul Mordechai, and Mahmud Huleihal*
12:20	***Verticillium dahliae* hydrophobins: a multifunctional family** *Nadia P. Morales-Lizcano, and Katherine F. Dobinson*
12:40	**Lunch break**

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ORAL SESSION 2	TAXONOMY AND GENETICS II
Chair:	*Krishna Subbarao, USA*
13:40	**Genome-wide transcriptome analysis of defoliating and non-defoliating pathotypes of *Verticillium dahliae* Kleb. growing under axenic conditions** *Isabella Pentimone, Massimo Ferrara, Jesus Mercado-Blanco, Antonio Ippolito, and Franco Nigro*
14:00	**High throughput analysis of gene expression in microsclerotia of *Verticillium dahliae*** *Steven J. Klosterman, Dechassa Duressa, Amy Anchieta, Dongquan Chen, Anna Klimes, Maria D. Garcia-Pedrajas, and Katherine F. Dobinson*
14:20	**Transcriptome analysis of microsclerotia development in smoke-tree vascular wilt fungus *Verticillium dahliae*** *Dianguang Xiong,Yonglin Wang, Jie Ma, Xiaoshu Xiao, Chengming Tian*
14:40	**Comparative genomics of *Verticilium albo-atrum* strains reveals strain specific regions** *Jernej Jakse, Gregor Rot, Vid Jelen, Sebastjan Radisek, Stanislav Mandelc, Aljaz Majer, Blaz Zupan, Branka Javornik*

	10:00	**Tea/Coffee Break**

ORAL SESSION 3	HOST-PATHOGEN INTERACTIONS AND RESISTANCE (I)
Chair:	*Leah Tsror, Israel*
Keynote 5 15:30	**Spatial and temporal aspects of Verticillium in tree hosts and resistance against this fungus** *Jelle Hiemstra, The Netherlands*
Keynote 6 16:00	**Comparative pathogenomics of *Verticillium dahliae*** *Bart Thomma et al. The Netherlands*
16:30	**BioFung: Integrative study of biotrophic growth factors of the phytopathogenic fungus *Verticillium longisporum*** *Susanna Braus-Stromeyer et al., Germany*
16:50	**Verticillium infection triggers VASCULAR-RELATED NAC DOMAIN7–dependent de novo xylem formation and enhances drought tolerance in Arabidopsis** *Michael Reusche, Karin Thole, Dennis Janz, Jekaterina Truskina, Sören Rindfleisch, Christine Drübert, Andrea Polle, Thomas Teichmann and Volker Lipka*
17:10	**Verticillium manipulates RNA silencing to suppress host immunity** *Mireille van Damme, Emilie Fradin, Ursula Ellendorff and Bart Thomma*
17:30	**Studies of Verticillium-hop pathosystem** *Branka Javornik, Jernej Jakse, Aljaz Majer, Stanislav Mandelc, Sebastjan Radisek, Andreja Cerenak, Natasa Stajner, Gregor Rot, Vid Jelen, Marko Fljasman, Zlatko Satovic, and Blaz Zupan*

	17:50	**Tea/Coffee Break**
	18:10	**POSTER VIEWING I** Pauliner Church, Great Hall, 1st floor
	19:30	**WELCOME RECEPTION** Pauliner Church, Foyer 1st floor

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Tuesday, May 7th

ORAL SESSION 4		**HOST-PATHOGEN INTERACTIONS AND RESISTANCE (II)**
Chair:		*Gerhard Braus, Germany*
08:30		**Resistance in wild olive against the defoliating *Verticillium dahliae* pathotype** *Diana Jiménez-Fernández, José L.Trapero-Casas, David Gramaje, Blanca B. Landa, Giovanni Bubici, Matteo Cirulli, and Rafael M. Jiménez-Díaz*
08:50		**Phenolic accumulation in olive cultivars associated with resistance to defoliating or non-defoliating *Verticillium dahliae* pathotypes** *Emmanouil A. Markakis, Sotirios E. Tjamos, Polymnia P. Antoniou, Peter A. Roussos, Epaminondas J. Paplomatas, and Eleftherios C. Tjamos*
09:10		**Winter oilseed rape physiology, gene expression and agronomic performance during drought stress and infection with *Verticillium longisporum*** *Daniel Lopisso, Jessica Knüfer, Birger Koopmann, and Andreas von Tiedemann*
09:30		**A study of the infection and defense mechanism of *Medicago truncatula* inoculated by *Verticillium albo-atrum*** *Maoulida Toueni, Cécile Ben, Guillaume Matthieu, Laurent Gentzbittel, and Martina Rickauer*
09:50		**Coniferyl alcohol and not lignification plays an important role in the defense of *Arabidopsis thaliana* against *Verticillium longisporum*** *Stefanie König, Kirstin Feussner, Alexander Kaever, Manuel Landesfeind, Cornelia Herrfurth, Petr Karlovsky, Andrea Polle, Peter Meinicke, and Ivo Feussner*
	10:10	**Tea/Coffee Break**
ORAL SESSION 5		**VIRULENCE AND POPULATION GENETICS**
Chair:		*Branka Javornik, Slovenia*
10:40		**Biomolecules produced in vitro by *Verticillium dahliae* Klebahn and their role in Verticillium wilt of olive** *Giovanni L. Bruno, Samer Sermani, Rita Milvia De Miccolis Angelini, and Matteo Cirulli*
11:00		**Comparative analysis of *Verticillium albo-atrum* secretome** *Stanislav Mandelc, Sebastjan Radisek, and Branka Javornik*
11:20		**Identification of biotrophic and saprophytic growth factors by differential intra- and extra-cellular proteomics of *Verticillium longisporum*** *Anika Kühn , Harald Kusch, Clara Hoppenau, Christian Timpner, Oliver Valerius, Stefanie König, Katharina Michels, Kirstin Feussner, Ivo Feussner, Kathrin Aßhauer, Alexander Kaever, Manuel Landesfeind, Burkhard Morgenstern, Andreas Otto, Dirk Albrecht, Birgit Voigt, Dörte Becher, Michael Hecker, Susanna Braus-Stromeyer, and Gerhard Braus*
11:40		**The apoplastic life of *Verticillium longisporum* and plant consequences** *Christine Druebert, Bettina Otto, and Andrea Polle*
12:00		**The vascular pathogen *Verticillium longisporum* exploits a jasmonic acid-independent COI1 function in roots to enhance disease symptoms in *Arabidopsis thaliana* shoots** *Anjali Ralhan, Johanna Schmitz, Sonja Schöttle, Corinna Thurow, Tim Iven, Ivo Feussner, and Christiane Gatz*
	12:20	**Lunch Break**

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	14:00-17:30	**Excursion to KWS, Einbeck**	
	18:00-23:00	**Conference dinner at Hardenberg Castle**	

Wednesday, May 8th

ORAL SESSION 6	**EPIDEMIOLOGY AND INTEGRATED CONTROL (I)**
Chair:	*Andreas von Tiedemann, Germany*
Keynote 6 08:30	**Integrated management of Verticillium wilt in olives** *Rafael M. Jiménez-Diaz, Spain*
09:00	**Control of Verticillium in tree nurseries through biological soil disinfestation** *Jelle Hiemstra, Bart van der Sluis, Pieter van Dalfsen, Arjan Smits Johnny Visser, and Gerard Korthals*
09:20	**The power function explains the relationship *Verticillium dahliae* inoculum and sunflower wilt in a wide environmental range** *Ignacio Erreguerena, Facundo Quiroz, R Rojo, and Alberto Escande*
09:40	***Verticillium dahliae* pathotypes and olive cultivars determine geographic distribution and development of Verticillium wilt under current and future climate change scenarios in Southern Spain** *Carlos Lucena, José L. Trapero-Casas, Efrén Remesal, and Juan A. Navas-Cortés*
10:00	**Current problems in managing Verticillium wilt of olives in Greece and the prospective of biological control of the disease in olive orchards** *Polymnia P. Antoniou, Sotirios E. Tjamos, Sofia D. Kountouri, and Eleftherios C. Tjamos*
10:20	**Tea/Coffee Break**
ORAL SESSION 7	**EPIDEMIOLOGY AND INTEGRATED CONTROL (II)**
Chair:	*Rafael M. Jiménez-Díaz, Spain*
10:50	**Influence of irrigation frequency on the onset and development of Verticillium wilt of olive** *Mario Pérez-Rodríguez, Esteban Alcántara, M. Castillo, N. Serrano, Ignacio J. Lorite, Octavio Arquero, Francisco Orgaz, and Francisco Javier López-Escudero*
11:10	**Detecting *Verticillium dahliae* in olive plantation soils** *Jeff Peters, Jelle Hiemstra, Cristina Duran, and Irene Gonzalez*
11:30	**Potential efficacy of commercial chemicals to reduce water infestations by *Verticillium dahliae*** *Francisco Jesús Gómez-Gálvez, Antonio Santos-Rufo, and Dolores Rodríguez-Jurado*
11:50	**Olive mill wastes: A source of resistance for plants against *Verticillium dahliae*** *Fotios Papasotiriou, Kyriakos Varypatakis, Niki Christofi, Sotirios Tjamos, Epaminondas Paplomatas*
12:10	**Lunch Break**

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ORAL SESSION 8		**BIOLOGICAL CONTROL AND MICROBIAL ECOLOGY**
Chair:		*Maria del Mar Jimenez-Gasco, USA*
Keynote 7 13:30		**Recent advances in biological control of Verticillium diseases** *Gabriele Berg, Austria*
14:00		**Hidden endophytic interactions between *Verticillium dahliae* and monocotyledonous hosts** *María del Mar Jiménez-Gasco, and Glenna M. Malcolm*
14:20		***Pseudomonas fluorescens* PICF7 traits involved in the biocontrol of Verticillium wilt of olive** *María Mercedes Maldonado-González, Elisabetta Schiliro, Peter A.H.M. Bakker, Pilar Prieto, Antonio Valverde-Corredor, and Jesús Mercado-Blanco*
14:40		**Molecular insights in the biocontrol interaction of *Paenibacillus alvei* strain K165 with *Verticillium dahliae* and the host plant** *Sotirios Tjamos, Silke Lehmann, Epaminondas Paplomatas, and Jean Pierre Metraux*
15:00		**Interaction of the wilt pathogen *Verticillium longisporum* and the biological control agent Verticillium Vt305 in cauliflower plants** *Lien Tyvaert, Soraya C. França, Jane Debode, and Monica Höfte*
15:20		**Unraveling the nature of suppressiveness to Verticillium wilt of specific olive orchard soils** *Miguel Montes-Borrego, and Blanca B. Landa*
15:40		**Biofumigation - a method to control Verticillium?** *Christian Neubauer, Benedikt Heitmann, and Caroline Müller*
	16:00	**Tea/Coffee Break**
	16:30	**POSTER VIEWING II** Pauliner Church, Great Hall, 1st floor
		Assembly of the Steering Committee
17:30		**Closing & Farewell** Great lecture hall

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PLENARY SESSION

Chair: Andreas von Tiedemann, Germany

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Verticillium diseases in Germany – history, significance and management

Monika Heupel

Chamber of Agriculture North Rhine-Westphalia, Plant Protection Service, Bonn, Germany

The Verticillium fungus has a big and substantial impact on numerous crops in Germany. Depending on the crop, the species *Verticillium dahliae, Verticillium longisporum* and *Verticillium albo-atrum* are of economic relevance.

In rapeseed cultivation, management of *V. longisporum* is a special challenge for farmers. In 2011, rapeseed was cultivated on 1.4 million hectares of the 12 million hectares of arable land in Germany. With about 6 million tons, Germany is Europe's biggest rapeseed producer. The most important engine for German rapeseed production is the biodiesel production induced by the biodiesel quota for motor vehicles. The economic impact of damage caused by Verticillium on rapeseed is rather big, particularly in areas with low crop rotation. The symptoms often appear quite late during maturation and are characterized by discolorations on stalks and yellowing of leaves as well as the typical microsclerotia under the stalk epidermis. For controlling it there are no authorised chemical plant protection products. Moreover, such control would be rather difficult as the fungus grows only submerged in the ground; in other words, infestation takes place only via the roots. Varieties that are resistant to the fungus are currently not available, although cultivation efforts are underway and varieties with less susceptibility are used. In addition, managing the disease by varying sowing times is being attempted.

In potato-growing the fungus *Verticillium dahliae* has been of increasing importance in the last few years. The wilting symptoms are often masked, particularly with irrigated cultures, while the overall impact on yields is still small. However, an increase of the economic impact over the next years could take place due to both increasing temperatures and increasing soil infestation. Nevertheless with potato cultivation the Verticillium soil infestation is increasing and spreading and so on a challenge for following cultures

With sugar beet, symptoms of *Verticillium dahliae* were for the first time diagnosed in cultivation areas in the state of North Rhine-Westphalia. Half-sided wilting of leaves and the parchment-like necrosis of the leafage were identified as typical symptoms. The number of affected areas is still very low and yield impacts are negligible. However, depending on annual weather conditions, an increase in affected areas is anticipated.

In German vegetable cultivation, *Verticillium longisporum* is of economic relevance primarily in cauliflower-growing areas in the state of Rhineland Palatinate. Brussels sprouts and Chinese cabbage crops are also affected in these regions. In Bavaria Verticillium is of economic relevance in horseradish. Typical wilting symptoms and discolouration of the veins due to microsclerotia production can be observed. In other vegetable cultures such as tomatoes or cucumbers, Verticillium infection is insignificant. Tools for managing the fungus are crop rotation and the application of resistant varieties. Chemical plant protection options are not available. Treatment with Shell DD and also with Basamid has no longer been permitted for more than a decade.

In German fruit cultures, *Verticillium dahliae* is of great importance especially in strawberry crops. Strawberry cultivation has expanded in Germany over the last few years and in 2011 covered 13,000 hectares. Besides *Phytophthora cactorum*, *Verticillium dahliae* is the most important pathogen for strawberry crops. Like in vegetable cultivation, chemical treatment is no longer permitted. Managing the fungus has been done for more than a decade by using the well-established method of wet-screening soil tests with the prediction of infestation probability. Apart from crop management and weed control, irrigation in particular plays an

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important role in reducing damage by *Verticillium dahliae*. Also less susceptible strawberry varieties can be used as a control method.

Verticillium infection is of lesser importance in other fruit crops than strawberries.

With respect to the cultivation in nurseries of woody plants such as Acer, Catalpa, Fraxinus, Robinia or Tilia, *Verticillium dahliae* infection has attained greater importance following the ban of chemical soil treatments and the decrease in land availability. Like in fruit cultures, management is done by using the wet-screening soil test with prediction of infestation probability.

In ornamental plant cultures Verticillium infection is rather insignificant. Wilting symptoms are observed from time to time in susceptible pot plants but are of no economic relevance.

In German hop cultures, *Verticillium albo-atrum* infection is of major importance. 0.17 % of agricultural land in Germany is cultivated with hop, whereby the most important region is the Hallertau. In this region, hop is typically grown as monoculture. Infection by *Verticillium albo-atrum* has been increasing over the past few years. It has now been confirmed that both the aggressive lethal isolates of *Verticillium albo-atrum* and the less aggressive ones can be found. As chemical protection measures are not available, preventive Verticillium management is focussing on fertilization and soil cultivation methods. In addition, farmers are experimenting with the application of bioantagonists and cultivation of breeding lines. Methods for preventive soil testing are being applied. In hop cultivation, the recirculation of plant biomass after harvest is common practice, thus promoting further infection by *Verticillium albo-atrum*. Meanwhile, several techniques of hygienising the organic waste are under investigation in order to reduce new infections. In terms of successful hop production in Germany, *Verticillium albo-atrum* is expected to be the biggest agronomic challenge in the coming years.

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BioFung: First insights into the genome of the oilseed rape pathogen *Verticillium longisporum*

BioFung Consortium
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AG Feussner[2]: Kirstin Feussner[2], Stefanie König[2], Katharina Michels[2], Mareike Possienke[2], Pablo Tarazona[2], Ivo Feussner[2].
AG Morgenstern[1]: Kathrin P. Aßhauer[1], Alexander Kaever[1], Manuel Landesfeind[1], Peter Meinicke[1], Burkhard Morgenstern[1].
AG Daniel[1]: Andrea Thürmer[1], Rolf Daniel[1].
AG Stanke[3]: Ingo Bulla[3], Katharina J. Hoff[3], Tonatiuh Pena Centeno[3], Mario Stanke[3], Minou Nowrousian[4].
AG Hecker[5]: Dirk Albrecht[5], Dörte Becher[5] , Andreas Otto[5], Birgit Voigt[5], Michael Hecker[5] Stefanie Pöggeler[1].

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Verticillium longisporum infects predominantly *Brassicaceae* such as *Brassica napus* (oilseed rape, Canola), *B. oleracea* (broccoli, cabbage, cauliflower, etc.), *Armoracia rusticana* (horseradish) and the model plant *Arabidopsis thaliana*. *B. napus* plays a major role as vegetable oil producing plant in Germany but also in Northern Europe, Canada and China. *V. longisporum* is an allodiploid hybrid with long spores and almost double the amount of nuclear DNA compared to other Verticillium species. Two genomes of a virulent and an avirulent *V. longisporum* isolate from Mecklenburg (Germany) were sequenced by a combined approach via pyro-sequencing (454-Roche) and paired-end library sequencing of 0.5, 3, 8, and 20kb (454-Roche and Illumina). Gene prediction was performed by AUGUSTUS and additional hints were integrated such as predicted proteins derived of the sequences of the putative parents, peptide information derived from proteome analysis, ESTs and RNA-Seq data. The resulting gene sets were visualized in GBrowse, imported into Pathway Tools and integrated into a VertiBase web interface for the BioFung consortium. Although both genomes show high sequence identities, we have evidences that both strains could originate from separate hybridization events. Phylogenetic analysis revealed significant differences between the parental strains of the two hybrids. Both *V. longisporum* isolates contain two alleles of the MAT-1-1-1 gene and the MAT1-1-3 gene. BLAST searches failed to detect any homologs of MAT1-2-1 sequences. In depth analysis of the mating-type genes favored a hybridization event in contrast to a possible mating. Currently, we analyze which genes have two or more alleles to identify possible gene duplication events during or after the hybridization.

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Oral Session 1

DEZ BARBARA MEMORIAL SESSSION (TAXONOMY AND GENETICS I)

Chair: Steve Klosterman, USA

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A Tribute to Dez Barbara

Krishna V. Subbarao
University of California, Davis, USA

Dr Derek John Barbara (1948 – 2012) or Dez Barbara as he was popularly known, passed away on July 15, 2012 following a short period in the hospital. In his death, the *Verticillium* community lost one of the unsung heroes and I personally lost a good friend, colleague and a consummate professional. I first came across Dez's name as I was reviewing the literature on *Verticillium* when I began working with this group of important pathogens, and was immediately struck by the clarity and utility of his papers. Dez's career spanning nearly 35 years was spent nearly equally at two institutions and his contributions to two major groups of plant pathogens also reflect this dichotomy. After obtaining a Ph.D. in virology from the University of Birmingham, Dez joined the famed virology group at the East Malling Research Station. Over the next two decades, he developed a series of serological and nucleic acid-based diagnostic tests to enable the sensitive detection of viruses, viroids and phytoplasmas in hops, strawberry, raspberry, apple, pear and woody ornamentals. He developed and refined ELISA techniques for routine and highly specific virus disease diagnosis. In the late 1980s, Dez also spent time at Purdue University in the USA working on Barley Yellow Dwarf Virus expanding his tool-box of expertise in molecular biology and diagnostics. These tools came in handy towards the end of his stay at the East Malling Research Station as Dez made the switch to working on soilborne fungal diseases of horticultural crops. His substantial and creative contributions to the Verticillium literature came from his development of new molecular approaches to the detection and study of *Verticillium* species; the determination that the origin of *V. longisporum*, the pathogen of crucifer crops, occurred out of two or possibly three different hybridization events; that these hybridization events potentially occurred on unusual hosts for both parents, increasing the chances for the hybrid to outcompete its parents; and narrowing down the number of species in *Verticillium* to six. Beyond his research, Dez had many other interests including history and especially the Egyptian history, music and geology. He was also a skilled carpenter. His wife, Anne Morton reminisces that 'Dez was interested in so many things and brought his scientific thinking to everything he did'. We the Verticillium community deeply mourn the passing of Dez Barbara.

References

Clewes, E., Edwards, S. G., Barbara, D. J. 2008. Direct molecular evidence supports long-spored microsclerotial isolates of Verticillium from crucifers being interspecific hybrids. Plant Pathol 57: 1047–1057.

Clewes, E., Barbara, D. J. 2008. Two allopolyploid ascomycete fungal plant pathogens were not rescued by vertical transmission. New Phytol. 177: 583–585.

Barbara, D. J., Clewes, E. 2003. Plant pathogenic Verticillium species: how many of them are there? Molecular Plant Pathology 4: 297–305.

Collins, A., Okoli, C.A., Morton, A., Parry, D., Edwards, S.G., *et al*. 2003. Isolates of *Verticillium dahliae* pathogenic to crucifers are of at least three distinct molecular types. Phytopathology 93: 364–376.

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Taxonomic challenges - molecular evidence for species and sub-specific groups in Verticillium

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The genus *Verticillium* encompasses phytopathogenic species that cause vascular wilts of plants and has had a long taxonomic history with the first species described in 1816. Nearly 190 species have been erected. In 2007 *Verticillium* in the strict sense was limited to the monophyletic group of *V. dahliae* and four other species, but even with only five species, boundaries between species were ill defined. Species identification was largely based on the kind of resting structures produced, i.e., microsclerotia in *V. dahliae*, resting mycelium in *V. albo-atrum*, chlamydospores in *V. nubilum*, and all three kinds of resting structures in *V. tricorpus.* Species names provide an ideal framework for storage and retrieval of relevant information, a system that is contingent on a clear understanding of species boundaries and consistent species identification. Molecular data indicated that resting structure morphology might be a poor indicator of species limits, as *V. albo-atrum*-like morphology was present in at least two different, unrelated phylogenetic groups. Using phylogenetic analyses, morphological investigations and comparisons to herbarium material and the literature, a new taxonomic framework has been established for *Verticillium* comprising ten species, five of which are new to science. We used a collection of 74 isolates representing much of the diversity of *Verticillium*, and phylogenetic analyses based on the ribosomal internal transcribed spacer region (ITS), partial sequences of the protein coding genes *actin* (*ACT)*, *elongation factor 1-alpha* (*EF*), *glyceraldehyde-3-phosphate dehydrogenase* (*GPD*) and *tryptophan synthase* (*TS*). Combined analyses of the *ACT*, *EF*, *GPD* and *TS* datasets recognized two major groups within *Verticillium*, Clade Flavexudans and Clade Flavnonexudans, reflecting the respective production and absence of yellow hyphal pigments. Clade Flavexudans comprised *V. albo-atrum* and *V. tricorpus* as well as the new species *V. zaregamsianum*, *V. isaacii* and *V. klebahnii*, of which the latter two were morphologically indistinguishable from *V. tricorpus* but may differ in pathogenicity. Clade Flavnonexudans comprised *V. nubilum*, *V. dahliae* and *V. longisporum*, as well as the two new species *V. alfalfae* and *V. nonalfalfae*, which resembled the distantly related *V. albo-atrum* in morphology. Apart from the diploid hybrid *V. longisporum*, each of the ten species corresponded to a single clade in the phylogenetic tree comprising just one ex-type strain, thereby establishing a direct link to a name tied to a herbarium specimen. Sub-specific diversity has also been documented in *Verticillium*, including six vegetative compatibility groups and two races. Recent developments on these sub-specific groups will also be reviewed.

References

Inderbitzin, P., Davis, R. M., Bostock, R. M., Subbarao, K. V. 2011: The ascomycete *Verticillium longisporum* is a hybrid and a plant pathogen with an expanded host range. PLoS ONE 6: e18260.

Inderbitzin, P., Bostock, R. M., Davis, R. M., Usami, T., Platt, H.W., Subbarao, K. V. 2011: Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the descriptions of five new species. PLoS ONE 6: e28341.

Klosterman, S. J., Subbarao, K. V., Kang, S., Veronese, P., Gold, S. E., *et al*. 2011: Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. PLoS Pathog 7: e1002137.

de Jonge, R., van Esse H. P., Maruthachalam K., Santhanam P., Usami T., Subbarao K. V., Thomma B. P. H.J. 2012. Tomato immune receptor Ve1 recognizes effector of multiple fungal wilt pathogens uncovered by genome and RNA sequencing. PNAS 109: 5110-5115.

Inderbitzin, P., Bostock, R. M., Subbarao, K. V. 2012: *Cylindrocladiella hahajimaensis*, a new species of *Cylindrocladiella* transferred from *Verticillium*. MycoKeys 4: 1–8.

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New insights on the phylogenetic relationships between strains of *Verticillium dahliae*

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Understanding genetic variability in *Verticillium dahliae* is key for the management of Verticillium wilts by use of resistant cultivars. Genetic variation in this fungus can develop through accumulation of mutations, but it could also result from cryptic sexual reproduction or parasexual recombination. The widespread occurrence of a highly virulent defoliating (D) *V. dahliae* pathotype in southern Spain has raised the question of whether such spread is a result of independent development of new strains from native populations or migration of D strains from the original sites. The D pathotype is considered indigenous to southern North America but now it has a widespread distribution in America, Asia and some European countries. However, D isolates show high variation in virulence on different hosts and amplification of specific PCR markers, which would not be concordant with a single origin and subsequent migration but rather suggests that the D pathotype may have arisen multiple times. *V. dahliae* populations are considered to have clonal structure, which has been described mainly by vegetative compatibility groups (VCGs). All D isolates belong to VCG1A. Studies on the evolutionary relationships among *V. dahliae* VCGs using different molecular markers of low resolution for intra-clonal variation have led to contrasting results. To understand the evolution of *V. dahliae*, we genotyped isolates for thousands of single-nucleotide polymorphisms (SNPs) using a high-throughput genotyping method. Genotyping by sequencing is a method that reduces genome complexity by digesting genomic DNA with a restriction enzyme and then pyrosequencing the ends of restriction fragments. By comparing sequence reads to the reference genome, we discovered more than 20,000 SNPs in a sample of 85 *V. dahliae* isolates of diverse host and geographic origins, representing all the known VCGs. Not surprisingly, an analysis of SNP genotypes showed clear clustering that correlated almost perfectly with VCGs, which are known to represent clonal lineages. No correlation was evident with respect to hosts or geographic origins. However, VCG2B is polyphyletic, with some clades appearing to have arisen by recombination. VCG1A is indistinguishable from VCG1B (nondefoliating), which together form a single cluster. These results are consistent with the hypothesis that the defoliating strains in VCG1A arose recently and share a common origin.

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Molecular diagnosis based on the *VTA2* barcode marker to discriminate the hybrid lineages of *Verticillium longisporum* on the oilseed crop *Brassica napus*

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The cruciferous fungal pathogen *Verticillium longisporum* represents an allodiploid hybrid with long spores and almost double the amount of nuclear DNA compared to other *Verticillium* species. In Europe, both virulent and avirulent lineages of *V. longisporum* corresponding to hybrids A1xD1 or A1xD3 were isolated from the oilseed crop *Brassica napus*. Parental A1 or D1 species are yet unknown whereas the D3 represents *Verticillium dahliae*. Only one single characteristic type of ribosomal DNA (rDNA) could be assigned to each hybrid lineage. The avirulent A1xD3 isolates carry D3 rDNA, which corresponds to *V. dahliae*, whereas the rDNA of the virulent A1xD1 isolates presumably originates from A1. Both hybrid lineages carry distinct isogene pairs of conserved regulatory genes corresponding to either A1 or D1/D3. D1 and D3 paralogues show high identities but differ in several single nucleotide polymorphisms. Distinct signatures of the *VTA2* regulatory isogene pair allow the identification of *V. longisporum* hybrids by only a single PCR and the separation of these hybrids from the haploid species as A1 or D1/D3. The combination between the *VTA2* marker as a barcode marker and differentiation of the rDNA type represents an attractive diagnostic tool to discriminate the allodiploid from haploid *Verticillia* and to distinguish between A1xD1 and A1xD3 hybrids, which differ in their virulence towards *B. napus*. Furthermore, the *VTA2* gene encoding a nuclear protein conserved within filamentous fungi was demonstrated to be a virulence factor that is required for fungal morphogenesis, oxidative stress tolerance and plant host infection.

References

Tran, V. T., Braus-Stromeyer, S. A., Timpner C. Braus, G. H. 2012: Molecular diagnosis to discriminate pathogen and apathogen species of the hybrid *Verticillium longisporum* on the oilseed crop *Brassica napus*. Appl. Microbiol. Biotechnol. doi: 10.1007/s00253-012-4530-1.

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Classification and determination of similarity levels of *Verticillium dahliae* isolates, using FTIR-ATR spectroscopy

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Verticillium dahliae is the causal agent of Verticillium wilt on various crops worldwide. Knowledge of the extent of similarity between different *V. dahliae* isolates is helpful in tracking the origin of the fungus and assessing disease severity. DNA sequencing can determine differences and similarity between fungal isolates based on their genome; however, the process is complicated and expensive. In this study we suggest an alternative method of Fourier transform infrared spectroscopy (FTIR) attenuated total reflectance (ATR) combined with mathematical and statistical tools as a method for genus, species and isolate classification. FTIR is an inexpensive and reagent free technique which gives accurate results in only a couple of hours. It is based on measuring the fungus infrared light absorption and constructing an absorbance spectrum of each fungus. Using FTIR spectroscopy and analysing the absorption spectra of fungi we have managed to classify successfully isolates of three different genera; *Verticillium*, *Colletotrichum* and *Fusarium*. By careful analysis of the FTIR spectra, we can determine the similarity level of different isolates within a certain species. We examined different isolates of *V. dahliae* and determined the similarity levels of these isolates. Our results are in high compliance with the biological classification methods. Vegetative Compatibility Groups (VCGs) are composed of isolates that tend to be similar due to a common genetic pool. Vegetative compatible isolates may form subpopulations that are similar in pathological and physiological characteristics, differing from isolates that are not assigned to the same VCG. Relying on a research performed in our laboratory on classification of different *Colletotrichum coccodes* VCGs, we believe that FTIR can be also used for assignment of each *V. dahliae* isolate to its VCG. FTIR may turn into an important in situ and in vivo alternative diagnostic tool in agricultural research.

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Verticillium dahliae hydrophobins: a multifunctional family

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The broad host range, soil borne fungus *Verticillium dahliae* Kleb. is the causal agent of an economically significant vascular wilt disease. *V. dahliae* produces persistent resting structures, known as microsclerotia (MCS), which are the primary source of disease inoculum in the field. Five hydrophobin-like proteins (VDH1 to 5) have been identified in the genome of *V. dahliae*. The results of bioinformatics analyses suggested secretion of these proteins, and that they are all class II hydrophobins. VDH1 was previously studied and was found to be highly express and to play an important role in microsclerotia development and pathogenicity. Specifically, the vdh1 knockout (KO) strain is amicrosclerotial, and produces disease more rapidly than does the wild type. Preliminary gene expression analyses of Vdh1 to 5 indicate that the transcript levels of the individual genes vary under different growth conditions. Additionally, the transcript levels from these genes are different from one another. These results suggest that the members of this protein family in V. dahliae have different roles. For further functional analyses Agrobacterium tumefaciens-mediated transformation is being used to generate gene deletion mutants. Such a KO strain has been produced for VDH5, and production of strains having KOs of VDH2, VDH3 or VDH4 are currently in progress. The vdh5 strain shows delayed and reduced MCS production, as well as a less aggressive pathogenic phenotype. These data and the gene expression results obtained to date support the hypothesis that the hydrophobin genes in *V. dahliae* have different functions in development and pathogenicity.

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Oral Session 2

TAXONOMY AND GENETICS II

Chair: Krishna Subbarao, USA

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Genome-wide transcriptome analysis of defoliating and non-defoliating pathotypes of *Verticillium dahliae* Kleb. growing under axenic conditions

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A deep sequencing transcriptome analysis of *Verticillium dahliae* has been carried out by comparing the expression profile of two different isolates, previously characterized as non-defoliating (V111) (ND) and defoliating (V937I) (D) pathotypes in olive and cotton. Total RNA was extracted from mycelium growth in a synthetic simulated xylem medium at 8, 24, and 96 hours post-incubation (hpi), and the corresponding expression libraries were constructed. The high-throughput sequencing of the libraries (RNA-seq) was performed on a HiScanSQ Illumina platform. Data were analyzed by using CLC Genomics Workbench 6. Moreover, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed to identify genes showing differential expression. A total of 127 million single-end 51 bp reads of high quality score (Q40 =1 error/10^4 bases) were mapped on the reference genome of *V. dahliae* (http://www.broadinstitute.org/). Relying on the normalized gene signals, the average Log2 fold-changes between the D and ND pathotype was assessed for 10.535 predicted protein-coding genes of *V. dahliae*. Some 1.250 genes differentially expressed at a significant level ($P \leq 0.05$) in the D and ND pathotype were found. Among these, 44.7% and 70% for the D and ND pathotype sequences, respectively, had GO annotation, whereas the remaining were identified as hypothetical proteins. At each time-point, a high proportion of annotated up-regulated genes in the D and ND isolate referred to binding and catalytic activity, nucleic acid binding transcription factor activity, transporter activity, and molecular transducer activity. The comparative transcript profiling of synchronized cultures revealed differential gene expression patterns. The number of dissimilarly-transcribed genes was higher at 96 hpi (840 and 927 up-regulated genes in ND and D pathotypes, respectively) than at other time points. In the D pathotype, 18 genes resulted significantly up-regulated at all time-points, compared to the ND. Among these, transcription initiation protein (SPT5), transmembrane protein, protein kinase-like domain, MYB DNA-binding domain protein, mannose-6-phosphate isomerase-like protein, and amine oxidase are included. Nine significantly abundant transcripts, found to be constantly up-regulated at all time-points were identified in ND pathotype as compared to the D isolate. This group included genes, among others, such as mitochondrial chaperone BCS1, NADH-dependent D-xylose reductase, phosphotransferase family protein, ankyrin repeat and SOCS box protein. Finally, 8 and 9 genes were exclusively expressed in the ND and D pathotype, respectively. Results reveal the difference in gene expression between D and ND *V. dahliae* isolates cultivated under axenic conditions by transcriptome analysis, and add valuable insight to unravel the different life-style of the two pathotypes.

Work carried out in the framework of the Project No. 14 "Laboratory network for the selection, characterization and conservation of germplasm and for preventing the spread of economically-relevant and quarantine pests (SELGE)", founded by the Apulia Region, PO FESR 2007-2013 - Axis I, Line of intervention 1.2., Action 1.2.1.

References

Klosterman, S. J., Subbarao, K. V., Kang, S., Veronese P., Gold, S.E., Thomma, B.P.H.J., Chen, Z., Henrissat, B., Lee, Y-H., Park, J., Garcia-Pedrajas, M.D., Barbara, D.J., *et al*. 2011: Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. PLoS Pathogens 2011; 7:e1002137; PMID:21829347; http://dx.doi.org/10.1371/journal.ppat.1002137.

Neumann, M. J., Dobinson, K. F. 2003: Sequence tag analysis of gene expression during pathogenic growth and microsclerotia development in the vascular wilt pathogen *Verticillium dahliae*. Fungal Genet. Biol. 38: 54-62.

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High throughput analysis of gene expression in microsclerotia of *Verticillium dahliae*

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The processes of microsclerotia (MS) formation, maintenance, and germination are critically important in the disease cycle of V. dahliae. To shed additional light on the molecular processes involved in MS biogenesis and melanin synthesis in V. dahliae, three replicate RNA-seq libraries were prepared from 10 day-old MS-producing cultures of V. dahliae (ave = 52.2 million reads), and those not producing microsclerotia (NoMS, ave = 50.6 million reads) and analyzed for differential gene expression. The comparisons revealed up-regulation of MS library genes involved in melanogenesis, including tetrahydro-xynaphthalene reductase (344-fold increase) and scytalone dehydratase (231-fold increase), and additional genes located in a 48.8 kilobase melanin biosynthetic cluster. Numerous hypothetical protein-encoding genes were also identified as differentially expressed in the MS library. Differential expression of selected genes identified as up- or down-regulated by RNA-seq were analyzed by RT-qPCR from several MS and NoMs culture types, including MS cultures that were stored for 6 months, and a 7 day culture having an intermediate level of fully melanized MS. These data provide further insight into gene expression during melanin biosynthesis and MS formation in V. dahliae and potentially insight on disease control targets.

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Transcriptome analysis of microsclerotia development in smoke-tree vascular wilt fungus *Verticillium dahliae*

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Smoke-tree vascular wilt fungus Verticillium dahliae is a causal agent of Verticillium wilt disease, which is widespread and devastating to plants. V. dahliae can infect more than 200 plant species including important economical crops, flowers, vegetables, trees, and shrubs, causing great economic loss every year. The smoke-tree, Cotinus coggygria, is one of the most important tree species used in ecological and landscape plantings in China and it is also the main component of the red leaf scenery of the Beijing region during autumn. Smoke-tree vascular wilt was caused by V. dahliae. The disease causes stunted growth of stems, early senescence of leaves, and severe mortality of trees, with seriously detrimental effects on the red leaf scenery in Beijing region. The life cycle of V. dahliae includes three vegetative phases: parasitic, saprophytic and dormant. In the dormant stage, V. dahliae formed a resting structure called microsclerotia, which can survived in the soil for more than 10 years. In this study, we applied mRNA-Sequencing to gain comprehensive understanding of transcriptional processes during microsclerotia development of V. dahliae. Analysis global pattern of gene expression, important genes and processes may contribute to the microsclerotia formation. We identified that 600 genes are significantly up-regulated during the stage of microsclerotia development. We also revealed that melanin biosynthesis, proteolysis, carbohydrate hydrolysis, lipid metabolism and signal pathways may involve in the microsclerotia development. These results may help us to understand the mechanism of microsclerotia formation and the Verticillium wilt disease, and may provide better guidance for disease prevention and cure.

References

Neumann, M.J., Dobinson, K.F. 2003: Sequence tag analysis of gene expression during pathogenic growth and microsclerotia development in the vascular wilt pathogen *Verticillium dahliae*. Fungal Genetics and Biology. 38:54–62.

Klimes, A., Amyotte, S.G., Grant, S., Kang, S., Dobinson, K.F. 2008: Microsclerotia development in *Verticillium dahliae*: Regulation and differential expression of the hydrophobin gene VDH1. Fungal Genet Biol. 45:1525-1532.

Dangl, J.L., Klosterman, S.J., Subbarao, K.V., Kang, S., Veronese, P., Gold, S.E., Thomma, B.P.H.J., Chen, Z., Henrissat, B., Lee, Y-H., *et al*. 2011: Comparative Genomics Yields Insights into Niche Adaptation of Plant Vascular Wilt Pathogens. PLoS Pathogens. 7:e1002137.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimente, I. H., Salzberg, S.L., Rinn, J.L., Pachter, L. 2012: Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocols 7: 562-578.

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Comparative genomics of *Verticilium albo-atrum* strains reveals strain specific regions

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To understand the mechanisms behind the development of the increased virulence of the lethal strains of *Verticillium albo-atrum* and to detect virulence-associated factors, we sequenced six hop isolate genomes with different virulence and from three different geographical regions, using Illumina technology. For the reference strain, the Slovenian lethal strain was selected, showing the largest genome as confirmed by flow cytometry measurements. Three different insert size libraries (370 bp, 500-600 bp and 1000 bp) were sequenced, producing a total of 76.3 M reads. Another five strains were sequenced to a depth of 4.8 up to 11.5 M reads. For annotation of the transcribed part of the genome, 38.3 M RNA-seq reads were produced from one mild and one lethal transcriptome in three biological replicates. *De-novo* assembly of the reference genome and reference mapping of the other five genomes was performed using CLC software. The reference genome was also scaffolded, using SSPACE and paired-end data information. The final assembly resulted in 715 contigs with a total length of 33.59 Mb, of which 0.5 Mb of DNA was present only in lethal strains. Gene prediction tools supported by Exonerate protein alignments and RNA-seq analysis resulted in 9858 gene models. In the lethal specific region, 91 gene models were predicted, with additional evidence of a few regions being expressed but not predicted by the software tools. Masking of the assembled genome with RepBase models revealed 1.53% of the genome to be associated with the repetitive type of DNA, wheras *de-novo* building of the models masked 5.86% of the genome. The acquired *V. albo-atrum* genome data provide an important genomic resource for studying the virulence of non-alfalfa *V. albo-atrum* strains.

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Oral Session 3

HOST-PATHOGEN INTERACTIONS AND RESISTANCE (I)

Chair: Leah Tsror, Israel

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Spatial and temporal aspects of Verticillium distribution in tree hosts and resistance against this fungus

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The wide range of plants that can be affected by Verticillium includes many woody hosts (Sinclair *et al.*, 1987). As a result Verticillium wilt is a major problem in shade tree nurseries and fruit production with olive being the most important host with very serious annual losses (Jiménez-Díaz *et al.*, 2012). The size and long life of tree hosts results in several unique aspects of disease, including the possibility of recovery and repeated infections. Starting from the disease cycle as drawn for tree hosts (Hiemstra & Harris, 1998) in this keynote the processes of infection, and colonization of woody hosts will be discussed based on a review of the available literature and illustrated by examples from research on maple (*Acer spp.*), ash (*Fraxinus excelsior*) and olive (*Olea* europaea).

Verticillium wilt is considered a single-cycle disease (Smith *et al.*, 1988) since inoculum produced in one year rarely results in infection in the same year. However, the multi-annual character of tree hosts makes them vulnerable to this inoculum in the following years. In this way a very low infection percentage in the planting stock, after build-up of soil inoculum through newly formed inoculum, may result in considerable disease over a period of years. Therefore use of Verticillium-free planting stock is an essential element in strategies to control Verticillium wilt in tree plantations.

The multi-annual character of tree hosts in combination with their compartmented structure and the periodic formation of new xylem provides them with the opportunity to isolate and replace infected parts (Shigo, 1984). In the case of Verticillium in susceptible hosts after infection of the root the pathogen enters the xylem. Upward spread starts and the pathogen may colonizes large parts of the xylem in stem and branches. However, until the plant starts to deteriorate the pathogen mainly lives within the xylem vessels and directly associated tissues. As long as a tree host can keep its cambium alive and free from Verticillium it is able to form new, healthy xylem. If this can be kept free from new infections, an infected tree may recover simply by outgrowing the infection. The efficacy of these processes varies with host species but in some species such as *Fraxinus excelsior* this mechanism can be very effective leading to complete recovery in the year after symptom onset.

The above mechanisms also contribute to resistance in tree hosts. The information on the reactions of different tree hosts in response to infection by *V. dahlia* and the implications for disease development versus resistance will be discussed. Finally the implications on selection for resistance and on resistance testing will be discussed and the very limited sources of resistance in woody hosts will be summarized.

References

Hiemstra J. A., Harris D. C. 1998: A compendium of Verticillium wilts in tree species. CPRO-DLO/HRI-EM.

Jiménez-Díaz R. M., Cirulli M., Bubici G., Jiménez-Gasco M., Antoniou P. P., Tjamos E.C. 2012: Verticillium wilt, a major threat to olive production: current status and future prospects for its management. Plant Disease 96: 304-329.

Shigo A.L. 1984: Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. Ann. Rev. Phytopathology 22: 189-214.

Sinclair W. A., Lyon H. H., Johnson W. T. 1987: Diseases of trees and shrubs. Cornell University Press.

Smith I.M., Dunez J., Phillips D.H., Lelliott R.A., Archer S.A. 1988: European Handbook of Plant Diseases. Blackwell Scientific Publications.

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Comparative pathogenomics of *Verticillium dahliae*

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The tomato immune receptor Ve1 governs resistance to race 1 strains of Verticillium, while race 2 strains are not recognized. Until recently, the Verticillium effector that is detected by Ve1 remained unknown. By high-throughput population genome sequencing, the gene that encodes the Ave1 effector (for Avirulence on Ve1 tomato) was identified. Interestingly, Ave1 homologs were also found in the fungal pathogens *Cercospora beticola*, *Colletotrichum higginsianum* and *Fusarium oxysporum* f. sp. *lycopersici*, some of which are recognized by Ve1. Based on the differential recognition of the Ave1 homologs, the epitope of the Ave1 protein has been identified. The identification of Ave1 facilitates functional analysis of the Ve1 immune receptor.

Strictly asexual microorganisms, such as *V. dahliae*, are often considered as evolutionary dead ends as it remains unknown how they can generate the genetic variation that is required for coevolution with their hosts. Based on comparative population genomics we show that extensive chromosomal rearrangements establish highly dynamic 'plastic' genomic regions that act as a source for genetic variation to mediate aggressiveness and that are enriched for in planta-expressed effector genes. We propose that *V. dahliae* evolves by prompting chromosomal rearrangements, enabling rapid development of novel effector genes.

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BioFung: Integrative study of biotrophic growth factors of the phytopathogenic fungus *Verticillium longisporum*

BioFung Consortium
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The oilseed rape (*Brassica napus*) pathogen *Verticillium longisporum* is an emerging threat in Europe. The fungus can survive in the form of resistant microsclerotia in the soil for years. It infects the host through the roots and invades the vascular system.

V. longisporum is an allodiploid hybrid derived from parents showing different host specificities. Our goal is to identify key metabolic steps and effectors (virulence factors) which could be used as targets against the economically important pathogen.

As basis for a functional genomics approach the genome of *V. longisporum* was sequenced and analyzed by pyro- and paired-end sequencing (454-Roche and Illumina) and optical mapping. Resulting datasets were fed into a bioinformatic annotation pipeline and are accessible through a Verticillium genome database (VertiBase). The genome sequence database in combination with proteomic and metabolomic approaches is currently being used to identify biotrophic growth factors. In a first attempt xylem sap of oilseed rape plants was extracted, sterile filtered and used as growth medium for *V. longisporum* cultures. Resulting samples were in parallel analyzed by transcriptomics (RNA-seq deep sequencing), proteomics (shotgun LC-MSMS, 2D-gel electrophoresis) and metabolomic fingerprinting (untargeted UPLC-MSTOF). Evaluation and integration of the datasets made use of the MarVis Suite which enabled a specifically adapted and optimized statistical analysis. Experimental results were further used to optimize ORF prediction, genome annotation and reconstruction of biochemical pathways. By this approach a characteristic set of xylem sap specific growth factors could be identified. These are currently analyzed regarding their detailed roles in the host-pathogen interaction.

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Verticillium infection triggers VASCULAR-RELATED NAC DOMAIN 7-dependent *de novo* xylem formation and enhances drought tolerance in Arabidopsis

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The soil-borne fungal plant pathogen *Verticillium longisporum* invades the roots of its *Brassicaceae* hosts and proliferates in the plant vascular system. Typical above-ground symptoms of *Verticillium* infection on *Brassica napus* and *Arabidopsis* are stunted growth, vein clearing and leaf chloroses. Here, we provide evidence that vein clearing is caused by pathogen-induced transdifferentiation of chloroplast-containing bundle sheath cells to functional xylem elements. Additionally, our findings suggest that re-initiation of cambial activity and transdifferentiation of xylem parenchyma cells results in xylem hyperplasia within the vasculature of *Arabidopsis* leaves, hypocotyls and roots. The observed *de novo* xylem formation correlates with *Verticillium*-induced expression of the *VASCULAR-RELATED NAC DOMAIN (VND)* transcription factor gene VND7. Transgenic *Arabidopsis* plants expressing the chimeric repressor VND7-SRDX under control of a Verticillium infection-responsive promoter exhibit reduced *de novo* xylem formation. Interestingly, infected *Arabidopsis* wild-type plants show higher drought stress tolerance compared to non-infected plants, whilst this effect is attenuated by suppression of VND7 activity.

Together, our results suggest that *Verticillium longisporum* triggers a tissue-specific developmental plant program that compensates for compromised water transport and enhances the water storage capacity of infected *Brassicaceae* host plants. In conclusion, we provide evidence that this natural plant-fungus pathosystem has conditionally mutualistic features

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Verticillium manipulates RNA silencing to suppress host immunity

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RNA silencing is a eukaryotic mechanism that exploits small non-coding RNAs (small-RNAs) to regulate gene expression in a sequence-specific manner in many cellular processes, including innate immunity. The plant immune system against viruses and bacteria is a well-known target of RNA silencing. Data from our laboratory indicate that the fungus *Verticillium dahliae* also targets the plant RNA silencing pathway, presumably by secreted effectors, to suppress host defence (Ellendorff *et al*., 2009). How *Verticillium* manipulates the RNA silencing pathway to suppress host immunity is still unknown. Arabidopsis is susceptible towards *Verticillium*, and a model plant to study epigenetics. We plan to identify the secreted *Verticillium* effectors and the Arabidopsis components that play a role in RNA silencing and are essential for *Verticillium* wilt disease. We have identified *Verticillium* regulated gene transcripts and small-RNAs of the host by combining transcriptomics and small-RNA profiling. We are currently investigating if the identified small-RNAs and putative gene targets are contributing to *Verticillium* immunity, the obtained results will be presented.

References

Ellendorff U., Fradin E.F., de Jonge R., Thomma B.P. 2009: RNA silencing is required for Arabidopsis defence against *Verticillium* wilt disease. J Exp Bot. 60(2):591-602.

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Studies of Verticillium-hop pathosystem

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Our group has been studying the hop-Verticillium pathosystem in order to decipher hop resistance towards *Verticillium albo-atrum,* the main causative agent of the devastating hop wilt spread through European hop gardens, and to search for virulence-associated factors that might explain the increased aggressiveness of the *V.albo-atrum* lethal pathotype. Various research approaches have been employed in these studies, from genetic mapping of resistance gene(s) and QTLs, gene expression studies of compatible and incompatible interactions (proteomics and transcriptomics approach) to whole genome sequencing of six different *V.albo-atrum* pathotypes. A short overview of our work will be given in this presentation, with an emphasis on the gene(s) conferring resistance to hop wilt and on studies of *V.albo-atrum* virulence factors.

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Oral Session 4

HOST-PATHOGEN INTERACTIONS AND RESISTANCE (II)

Chair: Gerhard Braus, Germany

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Resistance in wild olive against the defoliating *Verticillium dahliae* pathotype

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Rootstocks highly resistant to the highly virulent *Verticillium dahliae* defoliating (D) pathotype would be of much interest for the management of Verticillium wilt in olive and growing of Verticillium-susceptible olive cultivars in geographic areas where D *V. dahliae* prevails. Recently, research done at the University of Bari, Italy, and University of Córdoba, Spain, have led to the identification of some wild olive genotypes that could be of use as resistant rootstocks, including the currently patented clones STOPVERT and OUTVERT, AC13 and AC18. To further characterize the resistance reaction shown by those genotypes in previous studies, we have carried out a series of experiments using standardized protocols and controlled conditions optimal for development of Verticillium wilt. Own-rooted plants of a range of ages were inoculated with a range of high inoculum concentrations of selected, highly virulent D isolate V138I by root dipping and/or transplanting in an artificially infested soil mixture. Plants were inoculated once or twice in a sequence, and incubated in the growth chamber for 3 to 4 months under optimal conditions for disease development. Disease reaction was assessed by the development of foliar symptoms, isolation of the pathogen from the lower, middle, and upper main stem, and molecular quantification of the fungus in the sampled tissues using a real-time quantitative PCR (qPCR) protocol with a detection limit of 18 fg of *V. dahliae* DNA in infected, symptomless tissues. Clones STOPVERT and OUTVERT showed a symptomless reaction to inoculation compared with 100 % dead plants in susceptible 'Picual' olive and mild disease reaction in tolerant 'Frantoio'. *V. dahliae* was isolated from middle stem parts of STOPVERT and OUTVERT plants to a lesser extent than from the lower stem, but isolations from 'Frantoio' plants yielded the fungus from all stem parts at similar proportions. On average, the concentration of *V. dahliae* DNA per 100 ng of stem DNA ranged from 5.6 to 41.1 pg in STOPVERT plants, from 13.7 to 80.9 pg in OUTVERT plants, and from 94.6 to 141.6 pg in 'Frantoio' plants. The larger of those concentrations is 120 times lower than that found in susceptible 'Picual' olive. Extending the time of incubation of infected STOPVERT and OUTVERT plants reduced the frequency of successful isolations and quantification from previously infected tissues. Clones AC13 and AC18 also showed a highly resistant reaction to root-dip inoculation with *V. dahliae* 138I, though slight symptoms developed in some 'AC18' plants. Average *V. dahliae* DNA concentration per 100 ng of stem DNA was 10,9 and 86,7 pg in 'AC13' and 'AC18' plants, respectively, compared with 42,9 pg and 16,6 ng in 'Frantoio' and 'Picual' olives. Histopathological assessment of the plants reaction is in progress. Also, experiments are being conducted to determine the influence of genetic and geographic diversity of D *V. dahliae* isolates on the resistant reaction of the wild olive clones.

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Phenolic accumulation in olive cultivars associated with resistance to defoliating or non-defoliating *Verticillium dahliae* pathotypes

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Verticillium wilt is the most serious olive disease worldwide. The olive-infecting *Verticillium dahliae* pathotypes have been classified as defoliating (D) and nondefoliating (ND), and the disease is mainly controlled in olive orchards by using resistant or tolerant cultivars. Limited information is available about the nature of resistance in most of the olive cultivars. In the present study, the phenolic responses of the susceptible to *V. dahliae* olive cv. Amphissis and the resistant cv. Koroneiki upon D and ND *V. dahliae* infection were monitored in relation to the fungal DNA levels in the vascular tissues with the purpose to explore the defence mechanisms of olive trees against *V. dahliae*. Quantitative polymerase chain reaction revealed that the decrease in symptom severity shown in Koroneiki trees was associated with significant reduction in the growth of both *V. dahliae* pathotypes in the vascular tissues compared with Amphissis. In Koroneiki trees, the levels of o-diphenols and verbascoside were positively associated with the DNA levels of the D and ND pathotypes. In addition, a positive association was observed between the levels of verbascoside and the fungal DNA level in Amphissis trees, whereas a negative association was revealed between the fungal DNA level and the total phenols and oleuropein content in both cultivars. The levels of verbascoside were clearly higher in Koroneiki trees compared with Amphissis trees, indicating for the first time in the literature the involvement of verbascoside in the defence mechanism of olive trees against *V. dahliae*.

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Winter oilseed rape physiology, gene expression and agronomic performance during drought stress and infection with *Verticillium longisporum*

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Oilseed rape (*Brassica napus*) is the leading oil crop grown in temperate regions of the world. The host-specific fungus *Verticillium longisporum* (VL) is one of the economically important pathogens in oilseed rape (OSR). It causes foliar chlorosis, reduced growth, and premature senescence and ripening which ultimately leads to substantial yield losses. Unavailability of VL-effective fungicides and production of abundant and highly durable microsclerotia contributing to the soil inoculum are among the major factors that greatly hampered control of VL. The only possible alternative control measure available at present is the use of genotypes with enhanced resistance. However, studies regarding OSR-VL interaction as a whole and VL-resistance mechanisms in particular are limited. Moreover, the stability of VL resistance during drought periods is not known. A greenhouse experiment was conducted with winter OSR cultivars to investigate the combined effects of drought stress and VL infection on plant performance, physiology and expression of drought-responsive genes. Vernalized seedlings of the susceptible cultivar Falcon and the tolerant genotype SEM 05-500256 were inoculated with VL and received three watering levels (optimum, moderate deficiency and severe deficiency i.e. watering at 100, 60 and 30% field capacity). Mock-inoculated plants supplied with water at 100% field capacity were used as control. The results showed that resistance of genotype SEM 05-500256 as expressed by disease development (AUDPC), fungal DNA in the hypocotyl, stunting, impact on stem thickness and dry matter yield was confirmed under all watering conditions. Regardless of VL infection and genotype, drought stress significantly reduced stomatal conductance, transpiration and photosynthesis rates, leaf relative water content and dry matter yield. Moreover, up to five fold increase in expression of drought responsive genes (CIPK1, DREB2-23, P5SC1, P5SC2 and HB6) and significantly higher accumulation of proline in leaf and hypocotyl tissues were induced by severe drought stress. On the other hand, drought stress alone and when combined with VL infection had no substantial effect on disease parameters. More importantly, internal resistance of OSR to VL by formation of vascular occlusions did not significantly affect plant water relations under drought stress conditions. In conclusion, phenotypic and molecular disease evaluation results revealed that resistance of OSR to VL is not affected by drought stress indicating stability of resistance under drought stress conditions. Similarly, resistance to drought was not influenced by VL infection as evidenced by physiological and gene expression analysis. Overall, the results of the present study provided evidence that VL induced vascular occlusions, which are significantly more accumulated in resistant OSR cultivars, do not interfere with vascular transport of water and nutrient elements but they may selectively restrict VL in the hypocotyl tissue and inhibit further colonization of the shoot.

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A study of the infection and defense mechanism of *Medicago truncatula* inoculated by *Verticillium albo-atrum*

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Verticillium albo-atrum (Vaa) is a major pathogen of alfalfa (*M. sativa*). We study the interaction between *Medicago truncatula*, a near parent of alfalfa, and Vaa V31-2, a strain isolated from alfalfa, to understand the mechanisms involved in the response to this root pathogen. Root inoculation of *M. truncatula* line F83005.5 with a spore suspension of V31-2 induces typical wilt symptoms whereas line Jemalong A17 is resistant to the fungus (Ben *et al*., 2013).

The role of salicylic acid (SA), methyl jasmonate (MeJA), abscissic acid (ABA), auxin and ethylene in the interaction were studied with exogenous treatments prior to inoculation. Our results showed that pretreatments with SA and ABA protected *M. truncatula* against Vaa. The DNA of Vaa in planta is currently quantified by qPCR.

We introduced the GFP marker gene into Vaa V31-2 by *Agrobacterium tumefaciens*-mediated transformation (Eynck *et al*., 2007) and selected a GFP-expressing monosporic strain with the same level of pathogenicity as the wild type and a high level of fluorescence. Root colonization of A17 and F83005.5 was studied with the aid of laser scanning confocal and epi-fluorescence microscopy. Results showed that the resistant line A17 was able to suppress Vaa V31-2 from its vessels after initial growth of the fungus.

To determine the plant defense mechanisms triggered by Vaa in roots of the two *M. truncatula* lines A17 and F83005.5, a transcriptomic analysis was performed by new generation sequencing. RNA was extracted at various stages of infection, from two independent experiments. RNA sequencing was performed with the MACE (massive analysis of cDNA ends) technology (GenXPro GmbH, Frankfurt, Germany). First results of this transcriptomic analysis will be presented.

We thank Björn Rotter at GenXpro for transcriptomic analysis and the team of the FR40 microscopy platform for help with confocal laser scanning microscopy.

References

Ben C., Toueni M., Montanari S., Tardin M.C., Fervel M., Negahi A., Saint-Pierre L., Mathieu G., Gras M.C., Noël D., Prospéri J.M., Pilet-Nayel M.L., Baranger A., Huguet T., Julier B., Rickauer M., Gentzbittel L. (2013) J Exp Bot. 64: 317-332.

Eynck C., Koopmann B., Grunewaldt-Stoecker G., Karlovsky P., von Tiedemann A. 2007: European Journal of Plant Pathology 118: 259-274.
http://www.genxpro.info/media/Massive%20Analysis%20of%20cDNA%20Ends.pdf

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Coniferyl alcohol and not lignification plays an important role in the defense of *Arabidopsis thaliana* against *Verticillium longisporum*

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Verticillium longisporum is a soil borne vascular pathogen with economical significance in oilseed cultivation. Using the model plant *Arabidopsis thaliana* this study analyzed metabolic changes upon fungal infection in order to identify defense strategies of *Brassicaceae* against this fungus. Metabolite fingerprinting was used to detect metabolic changes in leaves of Arabidopsis upon infection. The most prominent identified metabolites derived from the phenylpropanoid pathway which was confirmed by targeted analysis. From early stages of infection on an accumulation of sinapoyl glucosides, coniferin, lignans, sesamins and amino acids was detected. To test the contribution of the phenylpropanoid pathway on the interaction of Arabidopsis with V. longisporum, different mutants in the pathway were analyzed. In line with this, the sinapate ester deficient mutant fah1-2 showed stronger infection symptoms than wild-type plants whereas the coniferin accumulating mutant UGT72E2-OE was less susceptible. Comparison of cell wall composition and amount of both mutants with wild type revealed no differences. However, free coniferyl but not sinapyl alcohol inhibited fungal growth. In summary these data show that specific soluble phenylpropanoids are important for the defense response of Arabidopsis against *V. longisporum* and that metabolite fingerprinting is a valuable tool to identify infection relevant metabolic markers.

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Oral Session 5
VIRULENCE AND POPULATION GENETICS

Chair: Branka Javornik, Slovenia

Biomolecules produced *in vitro* by *Verticillium dahliae* Klebahn and their role in Verticillium wilt of olive

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Two strains of *Verticillium dahliae* Klebahn characterized as defoliating (VD312D) and non defoliating (VD315ND) pathotypes from olive were grown (28 days, 25±2°C, in the dark) in four different liquid media (VdM, PDB, LME, and Corn-meal-Czapec) containing inorganic and organic components. After filtration on Miracloth and centrifugation (5500×*g*, 4°C, 20 min), the culture filtrates (CFs) and their 1:10, 1:100 and 1:1000 dilutions with sterile distilled water were assayed under controlled environmental conditions (RU: 40%, T: 23°C, continuous photoperiods, light: 150 µE cm^{-2} sec^{-1}) on cuttings of *Olea europea* cvs Leccino and Frantoio (respectively susceptible and resistant to *V. dahliae*) and the wild olive StopVert (a new resistant rootstock found in Italy; Bubici and Cirulli, 2011). CFs collected from VdM media and their dilution induced symptoms of curling, wilting and defoliation on the three olive cultivars. Each CF was extracted with ethyl acetate, chloroform, diethyl-ether or precipitate with absolute ethanol. The material recovered after ethanol precipitation and further washing with methanol confirmed the toxicity on susceptible and resistant olive cuttings. The purified fraction assayed at 50-100µg ml^{-1} increased transpiration flow and total phenol concentrations on leaves of treated cuttings and enhanced of root formation in Frantoio. The purified fraction had no effects on chlorophyll, total proteins, reactive oxygen species, and cellular antioxidant (ascorbate/glutathione) systems. Next generation sequencing (Illumina technology) has been applied to study the gene expression profiles of VD312D and VD315ND strains in two liquid media: VD-media enhancing toxin production and PDB encouraging fungal growth but not inducing toxin production. Sequencing data were analyzed using CLC Genomics Workbench. The following reads (50 bases), were generated from each of the four libraries, and matched the *V. dahliae* reference genome (http://www.broadinstitute.org/): 15,552,029 for VD312D on VD-media, 14,647,196 for VD312D on PDB, 7,531,321 for VD315ND on VD-media, and 15,674,042 for VD315ND on PDB. Normalized expression values (RPKM) of a total of 10,535 annotated genes were assessed and compared in the four tested conditions. Cell free CFs obtained from the strains VD312D and VD315ND on VD-media were tested for their activity against *V. dahliae* isolates obtained from different plant species. The CFs were tested on PDA medium amended with at concentration of 500 ml l^{-1} or 666 ml l^{-1}. Plates containing PDA medium and PDA with VD-medium (666 ml l^{-1}) were used as controls. All plates were inoculated with a hyphal fragment of a colony of 1-2 weeks of each fungal isolate and their growth at 25±2°C, in the dark was recorded every 3-days. CF obtained from VD315ND strain reduced or inhibited the growth of colonies of the tested isolates.

References

Bubici, G., Cirulli, M. 2011: Verticillium wilt. In: Olive diseases and disorders. Schena L., Agosteo G.E. and Cacciola S.O. (Eds): 191-222.

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Comparative analysis of *Verticillium albo-atrum* secretome

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Proteins secreted by fungal plant pathogens are critical to the success of an infection. The most important of these proteins are cell wall-degrading enzymes, specific toxins and effectors. Verticillium wilt of hop is a vascular wilt disease caused by *Verticillium albo-atrum*. Based on aggressiveness, fungal isolates from hop have been classified into mild and lethal pathotypes. To determine the differences in secretome composition between mild and lethal pathotypes, six hop isolates (mild and lethal isolates from three countries) were grown in a simulated xylem medium designed to resemble nutritional conditions in xylem sap. Proteins secreted into the medium were analyzed by two-dimensional difference gel electrophoresis (2D-DIGE). Approximately 850 protein spots were reproduced among the samples, of which 191 were identified by mass spectrometry. The secretome contained an arsenal of hydrolytic enzymes capable of degrading various cell wall components, such as pectin, cellulose, hemicellulose and proteins. In addition, lipases, various oxidoreductases and potential effectors were identified. Protein spots specific to lethal or mild isolates were not observed. Quantitative comparison between the samples revealed that lipases, carboxylesterases, endoglucanase and rhamnogalacturonan acetylesterases were more abundant in the secretome of lethal isolates from all three countries. Other significant differences in secreted protein levels between mild and lethal isolates were specific to individual isolates. The results indicate that mechanisms shared among isolates and mechanisms specific to each isolate both contribute to the aggressiveness of lethal isolates.

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Identification of biotrophic and saprophytic growth factors by differential intra- and extra-cellular proteomics of *Verticillium longisporum*

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Verticillium longisporum can invade oilseed rape (*Brassica napus*) roots and colonize the plant vascular system in its biotrophic state leading to premature aging of the host. Concurrently with plant senescence and flowering the fungus builds spores and long time surviving resting structures (microsclerotia) that persistently contaminate agricultural soil. Both yield reduction by premature senescence and microsclerotial contaminations turn *Verticillium longisporum* infections into a severe economical problem.

In this study we aimed to identify proteins that are key factors for colonization and biotrophic conditions. In extracted xylem sap of oilseed rape (biotrophic model) cultivated fungal proteomes were compared to growth on conventional growth media (saprophytic model). Xylem sap cultivation revealed a characteristic and reproducible intra- and extra-cellular proteome signature divergent from growth on saprophytic media. Xylem sap specific and intracellularly we detected an enrichment of proteins involved in degradation of exogenous carbohydrates and peptides. Also increased are components of amino acid and lipid metabolism as well as stress response. A parallel RNA-Seq approach by deep sequencing of the same fungal samples revealed a partial overlap of induced corresponding transcripts and peptides involved in carbohydrate-, amino acid and lipid metabolism.

In xylem sap specific exoproteomes we identified putative adhesins with predicted carbohydrate-binding domains and carbohydrate-active enzymes like polysaccharide lyases and glycosyl hydrolases. These enzymes are probably needed for the degradation of structurally complex molecules of the plant and might play a role during the penetration of roots and cell walls and distribution within the host. In addition various members of peptidase families were enriched, which might be important for proteolysis of host substrates or host defence proteins. Furthermore several small cysteine-rich proteins, necrosis and ethylene-inducing-like proteins (NLP) and proteins of unknown function were identified, which resemble potential effectors in pathogenicity. Candidate genes and proteins are currently analyzed regarding their importance during plant infection.

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The apoplastic life of *Verticillium longisporum* and plant consequences

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Growth and development of plants critically depend on root uptake and xylemtransport of water and nutrients to above-ground tissues. Verticillium fungi colonize the xylem and may partially block this transport path leading in some plant species to typical wilting symptoms in addition to stunting and chlorosis. It has therefore been speculated that Verticillium infection may cause nutrient deficiencies, thus, influencing plant performance. Our studies show that *Brassicaceae* infected with *Verticillium longisporum* strain VL43 did neither show wilting symptoms nor premature nutrient deficiencies. Instead P concentrations were even increased in infected compared with non-infected plants. We also analysed xylem saps of infected and non-infected *Brassica napus* plants and did not observe any suspicious decreases in nutrient compounds such as mineral nutrients, carbohydrates, amino acids, or proteins in response to VL43. By fractionation of low molecular compounds and protein we found that protein-free xylem strongly stimulated VL43 growth, whereas protein-containing xylem sap had antimicrobial activities. Proteome analysis of xylem sap of *Brassica napus* as well as of apoplastic washing fluids of *B. napus* and *Arabidopsis thaliana* revealed the presence of enzymes with antifungal activities such as proteases, glucanases, peroxidases and others. Some of these enzymes were specifically induced in response to VL43 infection. In Arabidopsis leaves, six VL-responsive proteins were identified. Analyses of Arabidopsis T-DNA lines of these proteins suggested that a germin-like protein was mainly responsible for inhibition of proliferation of VL43. Furthermore, overexpressing germin-lines were more resistant towards VL43 than the wildtype. Overall, our data suggest that the composition of low molecular weight compounds renders apoplastic fluids an ideal environment for microbes but that their uncontrolled proliferation is prevented by the protein complement.

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The vascular pathogen *Verticillium longisporum* exploits a jasmonic acid-independent COI1 function in roots to enhance disease symptoms in *Arabidopsis thaliana* shoots

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The soil-borne vascular pathogen *Verticillium longisporum* causes reduced shoot growth and early senescence in *Arabidopsis thaliana*. Analyses of plant mutants in the jasmonic acid (JA)-dependent signaling pathway revealed that disease symptoms are less pronounced in plants lacking the receptor of JA, CORONATINE INSENSITIVE 1 (COI1). Initial colonization of the roots was comparable in wild-type and coi1 plants and fungal DNA accumulated to almost similar levels in petioles of wild-type and coi1 plants at 10 days post infection. At late disease stages the number of plants with microsclerotia was reduced in coi1, indicating that completion of the fungal life cycle is impaired. Contrary to the expectation that the hormone receptor mutant coi1 should display the same phenotype as the corresponding hormone biosynthesis mutant dde2, dde2 plants developed wild-type-like disease symptoms. Induction of marker genes of the JA and the JA/ethylene defense pathway in wild-type petioles but not in dde2 petioles indicated absence of fungal compounds that would activate the known COI1-dependent signal transduction chain. Grafting experiments revealed that the susceptibility-enhancing COI1 function acts in the roots. Moreover, we showed that the coi1-mediated tolerance is not due to the hyper-activation of the salicylic acid pathway. In combination with previously reported results on the *Fusarium oxysporum*/Arabidopsis interaction, this study points at a conserved strategy of two vascular pathogens to weaken the host tissue through an unknown JA-Ile-independent but COI1-dependent mechanism in the roots which influences disease-promoting processes in the shoot.

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Oral Session 6

EPIDEMIOLOGY AND INTEGRATED CONTROL (I)

Chair: Andreas von Tiedemann, Germany

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Integrated management of Verticillium wilt in olive

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Verticillium wilts are amongst the most devastating diseases in agricultural production worldwide. Collectively, these diseases cannot be effectively controlled by applying a single control measure, but rather are best managed by an integrated disease management (IDM) strategy. Integrated disease management strategies and minimum use of chemicals will be enforced in the EU member countries by year 2014, according to Directive 2009/128/CE of the European Parliament and the Council of 11/24/2009. However, IDM is not a panacea for the control of plant diseases. It is an ecology-based approach aiming minimizing damage caused by diseases through the combined use of all available disease control measures, either simultaneously or in a sequence, through actions taken prior and after establishing the crop. The integrated management of Verticillium wilt diseases is difficult because complexities of the management strategy itself are overlaid on the inherent complexities of target pathosystems. Verticillium wilt of olive caused by *Verticillium dahliae*, the most important soilborne disease of olive worldwide, is an example of such complexities. Control of this disease is made difficult by: (i) the long survival of the pathogen in soil; (ii) its ability to infect hundreds of plants confined within the xylem during its parasitic phase; (iii) the genetic and virulence diversity of *V. dahliae* populations, including a highly virulent, defolianting (D) pathotype; and (iv) the easy spread of the pathogen within and among orchards by means of: (a) infected planting material; (b) infested soil; (c) infected debris from cultivated and alternative hosts; (d) irrigation water; and (e) leaves fallen from trees infected with the D pathotype. An IDM strategy for the management of Verticillium wilt in olive that combines the use of pre-planting and post-planting control measures includes: (i) site selection to avoid planting into high risk soils; (ii) use of *V. dahliae*-free planting material; (iii) reduction or elimination of *V. dahliae* inoculum in soil; (iv) protection of healthy planting material from infection by residual or incoming inoculum; (v) use of resistant cultivars and rootstocks; (vi) cultural practices; (vii) soil solarization; and (viii) organic or biological amendments. The efficiency of those control measures may be compromised in olive-growing areas where the D pathotype prevails (such as at southern Spain and the Aegean coastal region in Turkey) because: (i) the lowered threshold inoculum density (ID) for disease by D isolates compared with that by ND; (ii) the role of leaves fallen from infected trees as a source of inoculum for secondary infections; and (iii) the increased susceptibility of olive cultivars to infections by D *V. dahliae* and the lessened ability to recover from them. The recovery phenomenon is an intriguing feature of olive wilt whereby the plant is able to recover from disease over time, thus determining that new infections through the root system are needed for disease to develop in a tree on successive years. The recovery phenomenon should facilitate an integrated management of the disease aimed at reducing the potential for severe disease in young trees and protecting the root system of recovered trees from new infections. The practice of IDM of Verticillium wilt in olive requires involvement of well-trained professional plant pathologists able to implement the tenets of that concept at the local level, as well as to incorporate into decision-making frameworks new knowledge and technologies that may be developed from scientific research. This requirement might be at risk as institutional support has been reduced through declining or even despairing University education in Plant Pathology.

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Control of Verticillium in tree nurseries through biological soil disinfestation

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Verticillium wilt caused by *V. dahliae* is a serious problem in tree nursery industry in the Netherlands especially in the production of street trees and roses. The only effective way to control Verticillium wilt in nursery stock is to prevent the plants from being infected. *V. dahlia*, however, is widely spread in agricultural fields in the Netherlands. Therefore effective methods to eradicate the fungus from soil are strongly needed. The withdrawal of most soil fumigants due to their negative environmental effects enhanced the interest for non-chemical techniques in the control of Verticillium and nematodes.

In 2009 PPO started a long-term field experiment to test (anaerobic) biological soil disinfestation (BGO) as a method to control *Verticillium dahliae* and the nematode *Pratylenchus penetrans* in tree nursery soils. As a comparison several other treatments were carried out including growing marigold (*Tagetes patula*) combined with the application of compost, biofumigation with Sarepta or Indian mustard (*Sinapis juncea*), a fallow treatment, chemical soil disinfestation with Metam-Sodium, and finally growing white clover (*Trifolium repens*) that was expected to increase both *V. dahliae* and *P. penetrans* in the soil, since clover is a good host for both pathogens.

The treatments were applied in 2009 to two experimental plots on different soil types. In 2010 and 2011 on these plots a test crop of roses (on sandy soil) and *Acer platanoides* (on clayey soil) were grown to investigate the effectiveness of the different treatments. In this paper the effects of the different treatments on disease incidence and growth of the test crops and on the soil populations of *V. dahliae* and *P. penetrans* will be discussed. It is concluded that on sandy soils BGO may be an effective way of controlling Verticillium wilt in nursery stock. However, on clayey soils the remaining pathogen populations were of such a level that susceptible hosts still may be infected. Further investigations to improve the method on clayey soils therefore are needed. A field test with the application of BGO in a commercial nursery on a sandy soil has been started.

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The power function explains the relationship *Verticillium dahliae* inoculum and sunflower wilt in a wide environmental range.

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Sunflower wilt (SW) is the most important disease of sunflower in Argentina. The disease is monocyclic and microsclerotia (ME) constitute the primary inoculum. Many authors related ME with Verticillium wilt in different crops (artichoke, cotton, cauliflower, eggplant, horseradish, olive, potato and tomato) and established regression functions to explain that relationship (lineal, quadratic, sigmoid, power, negative exponential or logarithmic). These functions or models could be affected by factors such as soil characteristics (physicals or biologicals), pathotypes, host genetic or meteorological factors. In sunflower, our group has studied the relationship ME-SW in diverse environmental conditions (pasteurized substrates or substrates without pasteurization; pots or field sampling; natural soil-borne inoculum or amending ME; field or greenhouse; different susceptible sunflower varieties). Based on these previous studies, in the present work we look for the function that better explain the relation ME-SW in each experiment. Functions found for other crops in the literature were tested using the data of our previous experiments with sunflower. The selection criteria to find the best model were the comparison of R^2, the residuals and the Akaike index. The power function ($SW=a*ME^b$) was the best to explain the relationship ME-SW for all the experiments, but the parameters "a" and "b" varied substantially among experiments ($11.6<a<43.9$; $0.1<b<0.3$; averages: 21.1 and 0.2 for "a" and "b" respectively). To use data from experiments run under different environments was strategic to identify a function that would not be affected by these different conditions. In the range of low densities of inoculum, the power function showed that small changes in ME in soil represent large variations in SW level. This also qualifies the strategy of health management based on diminishing soil inoculum as a non-promising one. This work shows that independently of the experimental conditions or environments, the power function was the one that better explain the relationship ME-SW in sunflower.

References

Erreguerena I. A., Clemente G. E., Rojo R., Quiroz F. J., Escande A. 2010: "Inóculo, relación con la enfermedad y variabilidad patogénica en la interacción *Verticillium dahliae*-girasol". Libro de Actas de 5to Congreso Argentino de Girasol.

Erreguerena I. A., Quiroz F. J., Escande A. 2012: Relationship between *Verticilium dahliae* inoculum and sunflower wilt in three undisturbed soils of Argentina. Acta de XXXV Congreso Paulista de Fitopatología, San Pablo,Brazil.

Khan A., Atibalentja N., Eastburn D. M. 2000: Influence of inoculum density of *Verticillium dahliae* on root discoloration of horseradish. Plant Disease 84: 309-315.

López-Escudero F. J., Blanco-López M. A. 2007: Relationship between the inoculum density of *Verticillium dahliae* and the progress of Verticillium wilt of olive. Plant Dis. 91: 1372-1378.

Nagtzaam M.P.M., Termorshuizen A.J., Bollen G.J. 1997: The relationship between soil inoculum density and plant infection as a basis for a quantitative bioassay of *Verticillium dahliae*, European Journal of Plant Pathology 103: 597–605.

Pullman G.S., DeVay J.E. 1982: Epidemiology of Verticillium wilt of cotton: A relationship between inoculum density and disease progression. Phytopathology 72:549-554. [4]

Rojo R. 2007: Efecto de la densidad de inóculo de *Verticillium dahliae* sobre el abigarrado de girasol en suelos del sur y oeste de la provincia de Buenos Aires, Argentina. Tesis de Magister Scientiae, Posgrado de Producción Vegetal, FCA UNMdP.

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Verticillium dahliae pathotypes and olive cultivars determine geographic distribution and development of Verticillium wilt under current and future climate change scenarios in Southern Spain

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Global climate variability and change caused by natural processes as well as anthropogenic factors are major environmental issues in the 21st century. The increase in mean temperatures, change in precipitation regimes, and a continuous increase in CO_2 concentration are likely the main scientific evidence of climate change in recent decades. Plant disease epidemics result from specific interactions of a susceptible host plant, a prevalent and virulent pathogen and a conducive environment. Consequently, shifts in any one of these components can change geographic distribution and disease expression in a given pathosystem. Verticillium wilt (VW) of olive caused by the soil-borne fungus *Verticillium dahliae* is of major concern for the olive industry in the Mediterranean basin, mainly due to the rapid and wide spread of the highly virulent defoliating (D) pathotype. In this work, we have evaluated the effect of temperature and CO_2 concentration on virulence of *V. dahliae* pathotypes and olive cultivars interactions.

We carried out experiments under controlled conditions using 9 month old olive plants of cvs. Picual and Arbequina, which grew in soil infested by the defoliating (D) or non-defoliating (ND) pathotype of *V. dahliae* and incubated at 20 to 28ºC and each of three CO_2 levels of 386, 550 and 750 ppm. These conditions are representative of current and future levels of both environmental variables estimated by general climate circulation models for the SRES-IPCC A2 and B2 scenarios for southern Spain. Disease reactions were assessed by the incidence of infection and severity of symptoms, and various plant growth parameters. We used surface response regression models to quantify the combined effects of temperature and CO_2 concentration on olive cultivar x *V. dahliae* pathotype combinations. Spatial regression and Geographic Information Systems-based methods were used to estimate impacts of climatic factors on pathogen distribution and Verticillium wilt intensity.

Results showed a differential effect of both, biotic and abiotic factors included in the study. Thus, under current CO_2 concentration, optimum VW development occurred at 20-24°C, being faster and more severe in the most favorable combination 'Picual' / D as compared to that developed in 'Arbequina' / D, and delayed and lowest infection levels in the less favorable 'Picual' / ND. Furthermore, for this same optimum temperature range, an increase in CO_2 concentration determined a reduction in the rate of disease increase and infection level. Extreme values of temperature (28°C) and CO_2 (750 ppm) limited infection level by the pathogen and disease intensity. Models developed in this research allowed to establish risk prediction maps of Verticillium wilt in olive under current and future climate-change change scenarios for Southern Spain.

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Current problems in managing Verticillium wilt of olives in Greece and the prospective of biological control of the disease in olive orchards

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Verticillium wilt of olive trees is considered as the most serious and destructive disease of olive trees in Greece particularly in regions where the susceptible variety Amphissis is cultivated. During the last two decades, the extensive replacement of Amphissis with the tolerant variety Kalamata did not resolve the problem of the disease. Indeed the cultivation of Kalamata in fields that had been previously cultivated with cotton or at the first stages of olive plantation were co-cultivated with cotton or other Verticillium susceptible vegetables, increased the inoculum level of microsclerotia in the soil so that the olive trees developed severe symptoms. Kalamata variety in the regions of Aitoloakarnania and Fthiotida and Amphissis and Chalkithikis in Magnesia suffered dramatically from the disease. The disease is currently spreading in traditional olive-growing regions of Chalkidiki, Magnesia and Kalampaka. More recent data in the regions of Western Macedonia and Thrace report heavy symptoms in olives orchards of the variety Chalkidiki. And here it appears that the source of the inoculum came from cotton fields from the period of extensive culture of cotton in the regions. Recent establishments of olive tree orchards in dense or hyper-dense planting both in Aitoloakarnania and in Ilia regions could potentially create respectively similar problems. The plantations of sensitive variety Manzanillo in Ilia in former potato fields probably cause similar problems.

Biological control of Verticillium wilt was attempted in heavily infested olive orchards of Kalamata variety by drenching the soil around the trees with bacterial suspensions of a *Paenibacillus alvei* strain K-165, effective against Verticillium wilt of potatoes. It was shown that three soil drenchings of the biocontrol agent applied at 4 month intervals mitigated significantly symptoms of the disease and promoted recovery of diseased trees or prevented infection of healthy ones. However, new infestation of the experimental orchards by the dispersal and incorporation of leaves from diseased trees restricted the efficacy of the biocontrol agent during the next years after the treatments and underlined the importance of the inoculum level in overcoming the effectiveness of biological control agents.

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Oral Session 7
EPIDEMIOLOGY AND INTEGRATED CONTROL (II)

Chair: Rafael M. Jiménez-Díaz, Spain

Influence of irrigation frequency on the onset and development of Verticillium wilt of olive

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Verticillium wilt of olive losses are particularly high under irrigation regimes as occurs in other hosts of *Verticillium dahliae*, the causal agent of these wilt disease. However, there is an important lack of information regarding how this culture practice influences on the onset and disease development. Therefore, studies in an experimental plot consisting of a line of microplots (1 m^2 by 70 cm deep), open at the bottom and protected from rain by a cover, and filled with a naturally clay-loam infested soil (10 microsclerotia per g), are being conducted since December 2011 to determine the effect of watering frequency on the disease. One-year-old olive rooted cuttings of cultivars 'Picual' (susceptible), 'Arbequina' (moderately susceptible) and 'Frantoio' (resistant) were planted according to a split-plot design for studying four irrigation frequency treatments: daily (T1), weekly (T2), two-weekly (T3) and deficit irrigation (T4) (3 microplots per cultivar and treatment, and 9 plants per microplot). As control, two microplots per cultivar filled with sterilized soil and irrigated with the daily frequency were used. The irrigation system of each microplot consisted of a stopcock connected to six branch lines (16.5 cm separate) that bore six 2 l/h compensating drippers each one. The volumetric water content was continuously recorded by four sensors connected to a one data logger per microplot (1 microplot per irrigation treatment and cultivar, and one microplot control per cultivar). For the irrigation calendar scheduling, each treatment was calculated according to the 'Relative Soil Water Deficit' (RSWD) parameter (RSWD was 0 for T1, 0.4 for T2, 0.6 for T3, and 0.9 for T4). First symptoms (chlorosis, necrosis and/or defoliation of stem and leaves) started 20 weeks after planting. Disease incidence and severity increased mainly during fall 2012. The inoculum density in soil of microplots has remained constant along the recording period. After one year of observations, the disease has significantly developed in plants of 'Picual' and 'Arbequina'. In plants of 'Picual' subjected to the daily irrigation treatment (T1), disease incidence has reached 55.5%, with 18.5% of mortality. On the contrary, number of affected trees in the rest of treatments of this susceptible cultivar remains still lower than 22.2%. Up to now, no differences between irrigation treatments have been observed in plants of 'Arbequina', although disease incidence has also consistently progressed, ranging from 25.9% of affected plants in T1 to 51.8% in T3. Parameters regarding vegetative growth of olive plants (total shoot length and trunk diameter) did not differ between cultivar and irrigation treatments. Stomatal conductancy was recorded periodically on leaves of symptomatic and non-symptomatic trees planted on infested microplots of the T1 and T3 treatments. No differences were found between cultivars. Nevertheless, the two-weekly irrigation schedule (T3) provoked a significant reduction (nearly to 50%) of this parameter compare with the daily irrigation treatment (T1). Moreover, affected plants exhibited this reduction in symptomatic and non-symptomatic leaves.

References

García-Cabello S., Pérez-Rodríguez M., Blanco-López M.A., López-Escudero F. J. 2012: Distribution of *Verticillium dahliae* watering systems in widely irrigated olive growing areas in Andalucia (southern Spain). Eur J. Plant Pathol 133: 877-885.

López-Escudero F. J., Mercado-Blanco J., 2011. *Verticillium wilt* of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344: 1-50.

López-Escudero F. J., Blanco-López M.A. 2005: Effects of drip irrigation on population of *Verticillium dahliae* in olive orchards. Journal of Phytopathology 153: 238-9.

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Detecting *Verticillium dahliae* in olive plantation soils

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Verticillium dahliae is a major cause of olive wilt where crops have been planted in soils contaminated with pathogen inoculum. This is common in many European regions where crops susceptible to *V. dahliae*, particularly cotton, have been grown. A three year EU funded project, VERTIGEEN (www.vertigeen.eu), is underway to improve the detection and surveillance of *V. dahliae* in soils and plants from these soils throughout a number of European countries.

Work carried out in recent years by Fera has developed methods for extracting nucleic acids from soil samples of biologically meaningful volumes (50 g to 1000 g) (Woodhall et al. 2012). The Fera extraction method coupled with a real-time PCR assay (Bilodeau et al, 2012) has enabled the detection of *V. dahliae* down to below 1 microsclerotia/g soil. To date, seven intensively sampled sites (total of 300 soil extracts) in locations where olive wilt was suspected, as well as 28 soil samples submitted by growers, have been tested for *V. dahliae* levels using real-time PCR. *Verticillium dahliae* was detected in all soils, ranging from between 5% to 90% of samples. Laboratory tests confirmed that wilt symptoms were caused by *V. dahliae* (results presented by Hiemstra et al. elsewhere in this Symposium). Geo-statistical analyses done on the soil data showed that the presence of pathogen in soil cores were in broad agreement with locations associated with symptoms detected in trees. The data suggest that it should be possible to use on-site molecular detection methods (such as recent developments in isothermal methods such as LAMP) to predict which soils pose a risk in terms of *V. dahliae* inoculum to crops prior to planting with propagation material. The ultimate aim is to ensure that planting stock is not at risk from soil-borne *V. dahliae*.

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References

Woodhall JW, Webb KM, Giltrap PM, Adams IP, Peters JC, Budge GE, Boonham N. 2012. A new large scale soil DNA extraction procedure and real-time PCR assay for the detection of Sclerotium cepivorum in soil. European Journal of Plant Pathology 134: 467-473

Bilodeau GJ, Koike ST, Uribe P & Martin FN (2012). Development of an assay for rapid detection and quantification of Verticillium dahliae in soil. Phytopathology 102, 331-343.

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Potential efficacy of commercial chemicals to reduce water infestations by *Verticillium dahliae*

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Irrigation water is one of the factors implicated in the dispersal of *Verticillium dahliae* propagules in Andalusia (Spain). *V. dahliae* isolates obtained of irrigation water in different crops are defoliating and non-defoliating to olive and cotton plants. Water irrigation treatment is imperative: to reduce pathogen inoculum levels in water, to prevent the spread of Verticillium wilts and for more efficient management of these diseases in Andalusia. Chemicals with oxidative agents as active ingredient are authorized for applying to the water for other uses and could be registered to disinfest irrigation water in the crops in the future. Therefore, the efficacy *in vitro* of two commercial chemicals with sodium hypochlorite (hp) or hydrogen peroxide (px) as active agent was evaluated to reduce the inoculum viability of *V. dahliae* in the water. In some experiments (suppressive efficacy, SE), sterile water infested with conidia or sclerotia was mixed with 5 concentrations of each chemicals (3 of them authorized for applying to the water for other uses) or with sterile water (control) and was incubated for 30 days at 24 °C under artificial light. In other experiments (suppressive persistence, SP), sterile water (control) or water treated with 5 concentrations of each chemical was incubated for 30 days as before and periodically infested with conidia. Six *V. dahliae* isolates recovered from irrigation water belonging to defoliating (3) and non-defoliating (3) pathotypes were tested. Aliquots of each repetition were extracted at 4 times during incubation and conveniently diluted to determine the inoculum viability on semiselective medium. The chemical consisting of px presented higher SE and SP against conidia and higher SE against sclerotia than the other chemical evaluated. The chemical with hp as agent active did not delay water infestations by sclerotia and its SP against conidia was low at authorized concentrations. The SE and SP of biodegradable chemical consisting of px at two authorized concentrations were 100% in water infested by conidia of all isolates compared to their controls (Dunnett's test) at all times studied. This biodegradable chemical at one authorized concentration also reduced the sclerotial viability of all isolates by 100% compared to their controls until 15 days post-incubation. Research support by project RTA2011-00019-00-00 from INIA and project PEI.PEI2011.1 from IFAPA, both partially funded by European Regional Development Fund (ERDF).

References

García-Cabello, S., Pérez-Rodríguez, M., Blanco-López, M. A., López-Escudero, F. J. 2012: Distribution of *Verticillium dahliae* through watering systems in widely irrigated olive growing areas in Andalucía (southern Spain). Eur. J. Plant Pathol. doi:10.1007/s10658-012-0011-8.

Jiménez Díaz, R.M., Olivares García, C., Landa, B.B., Jiménez Gasco, M.M. Navas Cortés, J.A. 2011: Region-Wide analysis of genetic diversity in *Verticillium dahliae* populations infecting olive in southern Spain and agricultural factor influencing the distribution and prevalence of vegetative compatibility groups and pathotypes. Phytopathology 101: 304-315.

Moraño-Moreno, R., Bejarano-Alcázar, J., Rodríguez-Jurado, D. 2008: Propágulos de *Verticillium dahliae* de diferente tamaño infestan el agua utilizada en distintas campañas de riego del olivar en Andalucía. XIV Congreso de la Sociedad Española de Fitopatología. Lugo: 251. http://www.sef.es/congresos_sef.php?id_aplic=9&id_area=3

Rodríguez-Jurado, D., Bejarano-Alcázar, J. 2007: Dispersión de *Verticillium dahlie* en el agua utilizada para el riego de olivares en Andalucía. Bol. San. Veg. Plagas 33: 547-562. http://www.magrama.gob.es/fr/ministerio/servicios/informacion/plataforma-de-conocimiento-para-el-medio-rural-y-pesquero/biblioteca-virtual/articulos-de-revistas/art_datos.asp?articuloid=1592&codrevista=Plagas

Rodríguez-Jurado, D., Moraño-Moreno, R., Bejarano-Alcázar, J. 2008: Dispersion of defoliating and non-defoliating pathotypes of *Verticillium dahliae* in host crops by irrigation water in southern Spain. J. Plant Pathol. 90 (S2): 419-420.

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Olive mill wastes: A source of resistance for plants against *Verticillium dahliae*

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The olive producing countries face every year the challenge of managing the olive mill wastes (OMW). Recycling through composting constitutes a feasible and agronomicaly valuable strategy of dealing with the OMW. Therefore, it is of vital importance for the Mediterranean countries to promote the usage of OMW composts in agriculture. In the present study, the microbial nature involved in the suppressiveness of an OMW compost amendment (GR9) against *V. dahliae* was investigated. It was revealed that heat sterilisation of GR9 resulted in partial loss of its suppressiveness pointing out the presence of microorganisms antagonistic to *V. dahliae*. For this purpose, several microbes were isolated from the rhizosphere of eggplants grown in the compost and tested in vitro against *V. dahliae*. One bacterial and one yeast like fungus, identified as members of the Arthrobacter and Blastobotrys genera, respectively, were selected for further evaluation under glasshouse conditions. The ability of the microbial agents to reduce Verticillium wilt symptoms was demonstrated in a single root and split root experiment; indicating the possible triggering of induced systemic resistance. Furthermore, it was observed that application of the two microorganisms or the OMW compost reduced the percentage of *V. dahliae* microsclerotia germination and the number of hyphae per germinated microsclerotium in planta.

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Oral Session 8

BIOLOGICAL CONTROL AND MICROBIAL ECOLOGY

Chair: Maria del Mar Jimenez-Gasco, USA

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Recent advances in biocontrol of Verticillium diseases

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Verticillium species causes serious diseases in a broad variety of crops; they are often connected with high yield losses. Due to the specific ecological behaviour, especially their persistent resting structures in soil, it is difficult to control the pathogen. Negative impact on the environment was reported for more general protection strategies against soil-borne pathogens, e.g. by chemical fumigants. Therefore, Verticillium is an interesting target for biological control, which presents an environmental friendly plant protection strategy.

During the last years, interesting biological approaches were suggested to control Verticillium diseases. They can be grouped into the general strategies: i) biofumigation, ii) biocontrol using naturally antagonistic counterparts (bacteria & fungi) and, iii) biocontrol using suppressive soil communities. Although all strategies resulted in notable suppression rates, none of these approaches was transferred into commercial plant protection.

Due to next generation sequencing (NSG) techniques, there is a huge progress in our understanding of the mode of action and interaction, which will result in more consistent biocontrol effects (1). In addition to an overview about different biocontrol approaches, examples for applying NSG techniques for the selection and optimization of biological control agents will be given. One example explains biocontrol strategies developed especially for desert agriculture; here emerging problems with *Verticillium dahliae* strongly limit the yield. Endophytic biological control agents were selected as promising candidates for plant protection under arid conditions (2). The second example shows transcriptomic studies for *Stenotrophomonas rhizophila*, which is a potent Verticillium antagonist especially under salinated conditions.

References

Köberl, M., Schmidt, R., Ramadan, E.M., Bauer, R., Berg, G. 2012: Biocontrol strategies and next generation sequencing: organic desert agriculture in Egypt. iConcept, online.

Köberl, M., Müller, H., Ramadan, E.M., Berg, G. 2011: Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. PLoS ONE 69: e24452.

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Hidden endophytic interactions between *Verticillium dahliae* and monocotyledonous hosts

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Verticillium dahliae causes vascular wilt diseases on over 400 host plants. From a phytopathological perspective, plants on which disease symptoms have not yet been observed are considered to be non-hosts. Monocotyledonous crops have been traditionally considered to be non-hosts of *V. dahliae,* and cereal species have been used in crop rotations as a cultural measure for the management of Verticillium wilts by reducing the amount and/or efficiency of pathogen inoculum, but have had limited or inconsistent success. However, isolations from surface-disinfested internal stem tissue of asymptomatic oat plants grown as rotational crops of potatoes in Pennsylvania fields often yielded *V. dahliae*, indicating endophytic infections of those plants. That is, the fungus can establish endophytic relationships with oat plants. To determine any potential relationships between pathogenic and endophytic *V. dahliae*, we followed a population genetics approach using microsatellite markers to examine *V. dahliae* isolated from potato (cvs. Reba and Snowden) and oats grown in rotation in the same fields that had a history of Verticillium wilt of potato. Results indicated that the populations from symptomatic and asymptomatic hosts were significantly different. While at least five distinct genotypes were differentiated in the *V. dahliae* populations obtained from potato, only one of the genotypes was consistently found associated with oats, in different sampling years and grown in different fields. These results indicate that the interaction between *V. dahliae* and oats is highly specialized; that is, only selected *V. dahliae* genotypes seems to be able to establish endophytic relationships with oats and possibly other monocots. The potential dual role of *V. dahliae*, pathogenic on certain plants and endophytic on others, raises interesting questions about the biology, ecology, persistence, and spread of this fungus, which have important potential implications in the management of Verticillium wilts in agroecosystems. We propose to embrace the broader ecology of *V. dahliae* by differentiating between “symptomatic hosts” as those plants in which the infection and colonization by the fungus results in disease, and “asymptomatic hosts” as those plants that harbor the fungus endophytically, and are different than true non-hosts.

References

Malcolm, G. M., Kuldau, G. A., Gugino, B. K., Jiménez-Gasco, M. M. 2013: Letter to the Editor: Hidden host plant associations of soilborne fungal pathogens: An ecological perspective. Phytopathology doi.:10.1094/PHYTO-08-12-0192-LE.

Rowe, R. C., Powelson, M. L. 2002: Potato early dying: Management challenges in a changing production environment. Plant Dis. 86: 1184-1193.

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Pseudomonas fluorescens PICF7 traits involved in the biocontrol of Verticillium wilt of olive

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Verticillium wilt of olive (VWO), caused by *Verticillium dahliae* Kleb., is one of the most important biotic constrains affecting olive. The incidence and severity of this disease has increased during the last decades and its effective control requires implementation of an integrated disease management strategy, with emphasis on preventive measures. The use of biological control agents (BCA) appears as an excellent before-planting measure since they can be applied during the nursery production stage. *Pseudomonas fluorescens* PICF7 is a natural inhabitant of olive roots and an effective BCA against *V. dahliae*. Strain PICF7 displays some traits *in vitro* which may explain its biocontrol activity. For instance, growth inhibition of *V. dahliae* and production of salicylic acid and the siderophore pyoverdine have been demonstrated, as well as swimming motility which can be important for rhizosphere colonization. Moreover, PICF7 is able to endophytically colonize olive roots and induces a broad range of defense responses. However, mechanism(s) underlying its biocontrol activity remain mostly unknown. To unravel traits involved in the suppressive effect against VWO, a mutant bank of *P. fluorescens* PICF7 was generated (>9.000 insertions) by random transposon Tn5 mutagenesis. Phenotypes affected in swimming motility, siderophore production and growth inhibition of *V. dahliae* were screened. A collection of selected mutants altered in one of these phenotypes were analyzed by nested-PCR to localize the Tn5 insertion site. Adjacent DNA regions to the insertion point were sequenced and compared against available databases. Four mutants were finally chosen to conduct *in planta* bioassays: ME424, affected in motility (insertion in *fli*l homologue); ME589, a siderophore mutant with insertion located in a gene coding for a putative pyoverdine non-ribosomal peptide synthetase; ME419, showing enhanced growth inhibition of *V. dahlia*e (undetermined Tn5 insertion); and ME1508, a mutant displaying diminished growth inhibition of the pathogen (insertion in a gene encoding a putative sulfite reductase). Biocontrol bioassays using nursery-produced olive plants (cv. Picual) have consistently shown that mutant ME1508 has significantly lost the ability to control *V. dahliae* (defoliating [D] pathotype). However, the remaining mutants displayed variable biocontrol performance among bioassays. *Arabidopsis thaliana* has been also used to evaluate these mutants. This model plant was first checked for: i) Verticillium wilt symptoms development upon inoculation with an olive *V. dahliae* D isolate; ii) successful rhizosphere colonization by strain PICF7; and iii) effective control of the disease by the BCA. Results were similar to those observed in olive plants: mutant ME1508 had significantly lost the biocontrol ability compared to PICF7. Thus, the gene disrupted in ME1508 seems to play an important role in biocontrol of PICF7 against *V. dahliae*. However, phenotypes such as motility and siderophore production either do not participate in the suppressive effect or are greatly influenced by the experimental conditions used.

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References

López-Escudero, F. J., Mercado-Blanco, J. 2011: Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344: 1–50.

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Molecular insights in the biocontrol interaction of *Paenibacillus alvei* strain K165 with *Verticillium dahliae* and the host plant

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Control of Verticillium wilt has relied heavily on soil fumigation a protective measure that is contingent on the economic returns from the crop. The use of methyl bromide has been banned in European Union since 2010 creating a strong demand for novel crop protectants. Therefore, the development and use of biocontrol agents (BCAs) against Verticillium seems an appealing management strategy for the conventional and the organic farming industry.

Several bacteria and fungi have been reported as BCAs against *V. dahliae*. Among these BCAs is the bacterial strain *Paenibacillus alvei* K165 isolated from a suppressive field of *V. dahliae*. In the present study, we have explored aspects of the mode of action of K165 against *V. dahliae* using the model plant *Arabidopsis thaliana*. We showed that the plant receptor FLS2 (flagellin receptor) has a key role in this biocontrol interaction since application of K165 on *A. thaliana* plants impaired in flagellin perception (fls2 mutants) did not result in reduced Verticillium wilt symptom development. Furthermore, plant genes downstream of FLS2 (such as PR1, PR2, PR5, WRKY22, WRKY29) were not induced in the K165-treated fls2 in contrast to the wild type plants.

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Interaction of the wilt pathogen *Verticillium longisporum* and the biological control agent *Verticillium* Vt305 in cauliflower plants

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In Belgium, Verticillium wilt, caused by *V. longisporum*, results in important losses to cauliflower. In previous research in Belgian fields, the presence of *V. tricorpus*-like isolates in soil was negatively correlated with Verticillium wilt of cauliflower. The ability of *V. tricorpus* to protect potato and lettuce against virulent isolates of *V. dahliae* has been reported in the literature. To investigate the role of the indigenous *Verticillium* in the interaction of *V. longisporum* with cauliflower, the *Verticillium* isolate Vt305 was obtained from the field. In 2010, sequencing of rDNA ITS region confirmed its identity as *V. tricorpus.* More recently, the sequence of the isolate was reanalysed and the result indicated that, according to the new classification proposed by Inderbitzin *et al*. (2011), it would be classified as *Verticillium isaacii*. In controlled conditions, cauliflower seedlings were inoculated with *Verticillium* Vt305 and *V. longisporum*, using the root-dip method. Both *Verticillium* species were inoculated in two concentrations (1 x 10^4 spores/ml and 1 x 10^6 spores/ml). Disease symptoms were recorded and fungal DNA of both *Verticillium* species in plants was quantified using real-time PCR. *Verticillium* Vt305 did not cause symptoms, although it was detected in roots, hypocotyls and stems. *V. longisporum* caused stunted growth, vascular discoloration and yellowing of the leaves. Symptoms and colonization of *V. longisporum* were clearly reduced by pre-inoculation with *Verticillium* Vt305. Pre-inoculation of *Verticillium* Vt305 at the highest concentration completely prevented vascular discoloration and stunted growth of the cauliflower plant when inoculated with *V. longisporum* at the lowest concentration. *Verticillium* Vt305 reduced the amount of *V. longisporum* DNA in the root and the incidence of detection of *V. longisporum* in the stem as compared with single inoculation of *V. longisporum*. These findings indicate that *Verticillium* Vt305 behaves as an endophyte in cauliflower with potential as biological control agent against Verticillium wilt in cauliflower. Currently, we are investigating the mechanisms involved in the control and the interaction of *Verticillium* Vt305 with other crops.

References

Interbitzin, P., Bostock, R.M., Davis, R.M., Usami, T., Platt, H.W., Subbarao, K.V. 2011: Phylogenetics and Taxonomy of the Fungal Vascular Wilt Pathogen *Verticillium*, with the Description of Five New Species. PLoS ONE 6(12): e28341

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Unraveling the nature of suppressiveness to Verticillium wilt of specific olive orchard soils

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Plants have evolved strategies of stimulating and supporting specific groups of antagonistic microorganisms in the rhizosphere as a defense against diseases caused by soilborne plant pathogens. Disease suppressive soils provide the best examples of this strategy. Suppressive soils have been described worldwide for many different pathogens, however for the fungal pathogen *Verticillum dahliae* the information is very scarce. Olive (*Olea europaea* L.) is one of the most important crops in Spain with > 2.4 million ha. During the last two decades the phytosanitary status of olive orchards is being threatened mainly due to Verticillium wilt. In previous studies a collection of rhizosphere soils from 90 olive orchards under different management systems and three rhizosphere soils from wild olive havens in Andalusia were characterized by their level of suppressiveness to Verticillium wilt. Results indicated that at least 25% of soils showed a high level of suppressiveness to Verticillium wilt. The objective of this study was to unravel the biotic and abiotic factors that may be associated with this phenomenon. For that purpose we selected a set of suppressive and conducive soils and have performed some “Classical” approaches to identify the biotic factors (microorganisms) involved in this specific suppression that included: 1) Transferring suppressiveness by adding small amounts of suppressive soil to conducive soil which have confirmed its biological nature. 2) Treating suppressive soil with heat to eliminate specific groups of microorganism which have demonstrated loss of suppressiveness at a certain level. 3) Isolating different microbial groups and correlating their presence with suppressiveness. 4) Screening representatives of microbial groups for in vitro and biocontrol activity against the target pathogen. In a second step using molecular approaches such as bar-coded pyrosequencing we have characterized all bacterial communities associated to the rhizosphere of plants growing in those selected soils. Sequences generated from pyrosequencing of rRNA gene amplicons with the GS Junior system (Roche) were processed using the Quantitative Insights Into Microbial Ecology (QIIME 1.6.0) pipeline. Flowgrams were clustered into OTUs at 97% pairwise identity using the seed-based UCLUST algorithm, and representative sequences from each OTU were aligned to the Greengenes bacteria database using PyNAST. In addition, α diversity and β diversity metrics together with rarefaction plots were also calculated to determine the bacterial population structure in those soils. Different multivariate analyses have allowed identifying some climatic parameters and physicochemical soil characteristics that are differentially associated to the level of suppressiveness of those soils as well as to determine which OTUs are specially enriched in the suppressive soils. Furthermore, specific OTUs have been identified as being transferred from the suppressive into the conducive soils in the ‘transferability experiment’ which might be associated as the OTUs being responsible of this transferable suppressive effect. Finally, experiments are being conducted to determine the influence of the indigenous microbiota on inducing plant defense mechanisms in olive plants grown in those suppressive soils. For that purpose both microarray and metagenomic sequencing of total DNA will be used to unravel genes differentially expressed or present under the suppressive conditions.

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Biofumigation – a method to control Verticillium?

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Verticillium dahliae causes in Germany severe economic losses in production of strawberry and trees. Because the use of synthetic soil fumigants is restricted alternative management strategies for control of *Verticillium* are discussed, like the use of natural, plant-derived compounds. Biofumigation is such an approach, which originally is defined as the suppression of soil pests and diseases resulting from hydrolysis products, especially isothiocyanates (ITCs), released in the soil after incorporation of brassicaceous green manures containing glucosinolates (GSLs). The main objective of a research project was by following a systematic approach to assess the biofumigation potential of different genotypes of Brassicacae to *Verticillium*. Exemplary data of the Indian mustard, *B. juncea,* are presented. This species is considered worldwide as the most promising biofumigation crop, which is producing the single GSL Sinigrin and 2-propenyl-ITC respectively. In a first step the toxicity of 2-propenyl-ITC and other commercial available ITCs to *Verticillium* was evaluated using a standardized test system. ITCs were added in sealed flasks to an artificially with microsclerotia infested sterile quartz sand. After an incubation time of 48 h the viability of the microsclerotia was measured using a wet-sieving and plating detection method. For 2-propenyl-ITC a LD_{90} value of 88.7 nmol/g sand was determined. With the same method the toxicity of 2-propenyl-ITC was evaluated in 22 naturally infested soils. Results indicating that in natural soils a concentration in excess of 150 nmol/g soil is necessary to eliminate 80-90% of microsclerotia, considering that ITCs are prone to rapid microbial degradation and sorption to organic matter.

In a next step the biofumigation potential of different *B. juncea* cultivars was assessed. Field trials are very extensive. Also it is difficult to provide reproducible results because of many influencing soil factors. Therefore an efficient lab bioassay was developed to assess the biofumigation potential of biomasses of different *Brassica* genotypes under standardized conditions with the aim to select the best one for further field trails. First of all cultivars of *B. juncea* were grown on the field and biomass were sampled. Freeze dried, ground shoot tissue of the cultivars was mixed into infested quartz sand filled in glass flasks considering the field situation. After 48 h incubation the sand was analyzed for viable microsclerotia using the wet sieving detection method. Additionally the GSL concentration of the biomass was analyzed by HPLC.

Under the optimal conditions of the bioassay the tissues of *B. juncea* reduced the number of viable microsclerotia significantly. For the cultivars efficiencies between 69.3-81.3% could be determined. The potential released 2-propenyl-ITC concentrations calculated on the Sinigrin contents of the amended biomass ranged from 50.6 to 78.1 nmol/g sand. The effects of the *B. juncea* amendments and the total liberated amounts of 2-propenyl-ITC are corresponding well with the toxicological data of 2-propenyl-ITC. Therefore a clear ITC-related biofumigation effect is assumed. But in practice the ITC concentrations are even more reduced so that no adequate effect can be expected, because of the low release efficiency of ITCs. This results from un-hydrolysed GSLs in the plant tissue due to incomplete tissue pulverization, whereas in the bioassay freeze tissue with a maximum disruption on cell level was used. Therefore on base of lab data it can be expected that under practical conditions the biofumigation potential of the tested *B. juncea* cultivars is too low. This assumption is confirmed by results of field trails in 2011. The performance of other tested Brassicaceae species was also poor. Therefore the conclusion is drawn that biofumigation is currently no effective method to control *Verticillium*.

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Poster
TAXONOMY AND GENETICS

P-01 Analysis of the intergenic spacer (IGS) region of the nuclear rDNA of *Verticillium dahliae* for VCG discrimination and phylogenetic studies

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Implementation of effective management strategies for the control of *V. dahliae* has been restricted by our limited understanding of the genetic diversity of the fungus. Vegetative compatibility group (VCG) analysis has been traditionally used for examining fungal population structures. In this study, we performed initially an extended *in silico* analysis of 201 publicly available IGS sequences of *V. dahliae* and determined the most variable IGS sub-region, which was characterized by the presence of several indels of varying length as well as single nucleotide polymorphisms. This sub-region was PCR-amplified from 59 *V. dahliae* isolates covering all VCGs, 4 *V. albo-atrum* and 5 *V. longisporum* isolates, and the amplicons were cloned, sequenced and structurally and phylogenetically analyzed. Variation was largely due to different copy numbers of four classes of short repetitive DNA elements, organized in higher-order repetitive structures or commonly encountered composite blocks. Structural and phylogenetic analyses of sequences of this sub-region were consistent and allowed the identification of two main lineages in *V. dahliae*, i.e., cluster I including VCGs 1A, 1B, 2A, 4B and 3, and cluster II containing VCGs 2B, 4A and 6. IGS sequence analysis is a highly suitable molecular tool for (a) the study of IGS sequence molecular evolution in fungi, (b) rapid inter-specific differentiation, (c) intra-specific discrimination among VCGs of *V. dahliae*, allowing high-throughput VCG confirmation and prediction/profiling, and (d) phylogenetic analysis within and among *V. dahliae* VCGs.

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References

Collado-Romero, M., Mercado-Blanco, J., Olivares-García, C. Jiménez-Díaz, R. M. 2008: Phylogenetic analysis of *Verticillium dahliae* vegetative compatibility groups. Phytopathology 98: 1019-1028.

Jiménez-Gasco, M. M., Berbegal, M., Malcolm, G. M., Armengol, J. Jiménez-Díaz, R. M. 2009: New insights on the phylogenetic relationships among vegetative compatibility groups in *Verticillium dahliae*. In Abstracts of the 10th International Verticillium Symposium, 16-20 November, Corfu, Greece.

Pantou, M. P., Mavridou, A. Typas, M. A. 2003: IGS sequence variation, group-I introns and the complete nuclear ribosomal DNA of the entomopathogenic fungus *Metarhizium*: excellent tools for isolate detection and phylogenetic analysis. Fungal Genet. Biol. 38: 159-174.

Pramateftaki, P. V., Antoniou, P. P. Typas, M. A. 2000: The complete DNA sequence of the nuclear RNA gene complex of *Verticillium dahliae*: intraspecific heterogeneity within the intergenic spacer region. Fungal Genet. Biol. 29: 19-27.

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P-02 Group-I introns in the nuclear SSU gene of *Verticillium dahliae*: structural characterization and study of distribution

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Group-I introns are autocatalytic elements that mediate their own cleavage from precursor transcripts through a two-step splicing pathway. They are encountered in the nuclear and organelle genomes of eukaryotic organisms, mainly of fungi, plants and algae, and less frequently in viruses, bacteria and phages. A group-I intron has been previously identified in the nuclear SSU gene of *V. longisporum* and has recently been used as a *V. longisporum*-specific molecular marker. Here, we report the presence of this intron in the SSU gene of *V. dahliae* isolates, as well as of another, highly similar group-I intron at the same position in *V. dahliae*. SSU amplicons containing these introns were cloned and sequenced, and the sequences were used to predict secondary structures and to analyze their characteristics. A population of 110 isolates of *V. dahliae* and relative species was PCR-screened with SSU- and intron-specific primer pairs, and the majority were found to possess one or both the intron types. The location of the introns in the SSU gene was confirmed with the application of suitable PCR primer pairs, and intron cleavage from precursor ribosomal RNAs was shown by RT-PCR. Interestingly, remarkable heterogeneity within the rDNA of individual isolates was demonstrated by the PCR screenings and confirmed with quantitative real-time PCR copy number analysis. Our results (a) demonstrate that the group-I introns are not specific to *V. longisporum* and their use as inter-specific discrimination markers should be discontinued, (b) show that group-I introns may remain undetected by conventional SSU PCR-screenings, which possibly renders any phylogenetic conclusions incomplete or even misleading, (c) prove that the rDNA is considerably heterogeneous with regard to intron presence, and (d) provide evidence for evolution of the group-I introns in the *Verticillium* species group through vertical transmission and possibly horizontal transfer.

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References

Cannone, J. J., Subramanian, S., Schnare, M. N. *et al*. 2002: The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics 3: 2 [Correction: BMC Bioinformatics 3: 15.].

Collins, A., Okoli, C. A. N., Morton, A., Parry, D., Edwards, S. G., Barbara, D. J. 2003: Isolates of *Verticillium dahliae* pathogenic to crucifers are of at least three distinct molecular types. Phytopathology 93: 364-376.

Haugen, P., Simon, D. M., Bhattacharya, D. 2005: The natural history of group I introns. Trends Genet. 21: 111-119.

Karapapa, V. K., Typas, M. A. 2001: Molecular characterization of the host-adapted pathogen *Verticillium longisporum* on the basis of a group-I intron found in the nuclear SSU-rRNA gene. Curr. Microbiol. 42: 217-224.

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P-03 Targeted knock-out transformants of *Verticillium albo-atrum*

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One of the most powerful approaches for dissecting the gene function in phytopathogens is the study of the phenotypes of mutants in which a genomic locus has been altered by insertion of (gene disruption) or replacement with (gene replacement) heterologous DNA. This is a high throughput reverse genetics approach, which greatly contributes to understanding the gene function of fungal pathogens. The knock-out technique is a reverse genetic tool for functional analysis of various phytopathogens. Such reverse genetic approaches have become even more straightforward since increasing amounts of genomic sequence information have become available for an ever larger number of phytopathogen species. The genome of *Verticillium albo-atrum*, which is a destructive soil-borne fungal pathogen that causes vascular wilt diseases, has already been sequenced. Translation of genome sequence information into biological functions is therefore possible.

We successfully established a protocol for generating knock-outs of *V. albo-atrum*, which comprises two methods. The first is the creation of knock-out plasmids by the USER Friendly cloning technique. In this technique, the cloning event relies on the ability of complementary 3´ overhangs generated at the ends of, respectively, a PCR amplified DNA fragment and a linearized destination vector, to make a stable hybridization product. pRF-HU2 plasmid, containing a hygromycin resistance gene, was used for the USER Friendly cloning of knock-out plasmids. Two PCR amplicons about 1000 bp long, containing homologous recombination sequences flanking a deletion gene, were inserted into the vector and the entire USER-treated reaction mixture was used to transform chemically competent *E. coli* cells by heat shock. Two knock-out plasmids for two genes (EEY18971 V. albo-atrum predicted protein and Versatile peroxidase VPL1), found to be highly expressed at the protein level in the xylem of infected hop plants, were constructed. The second method is transformation of the fungal pathogen by *Agrobacterium tumefaciens*-mediated transformation (ATMT). Isolated KO plasmids from *E. coli* were transformed into electro-competent *A. tumefaciens* LBA4404 by electroporation. *A. tumefaciens* LBA4404 culture (OD600=0.5) and diluted *V. albo-atrum* conidiospores ($1*10^8$ spores mL-1.) were mixed in a 1:1 (v:v) ratio and spread onto solid IMAS plates, covered with sterile cellophane filters. The plates were incubated on selection medium. *V. albo-atrum* knock-out transformants were verified by PCR testing and Southern blot analysis, which confirmed that deletion of the target gene had been successful.

A major advantage of gene knock-out is its capacity to target a specific genetic region. Using targeted gene disruption, many genes implicated in the virulence and pathogenicity of the phytopathogen *V. albo-atrum* can be characterized. To the best of our knowledge, this is the first report of the creation of *V. albo-atrum* targeted gene knock-outs. It demonstrates that knock-out transformants of this fungal pathogen can be efficiently made.

References

Bhadauria V., Banniza S., Wei Y., Peng Y.L. 2009: Reverse genetics for functional genomics of phytopathogenic fungi and Ooomycetes. Comparative and Functional Genomics, Article ID 380719: 1-11.

Fradin E.F., Thomma B.P.H.J. 2006: Physiology and molecular aspects of Verticillium wilt diseases caused by V. dahliae and V. albo-atrum. Molecular Plant Pathology 7:, 71–86.

Frandsen R. J. N., Andersson J. A., Kristensen M. B., Giese H. 2008: Efficient four fragment cloning for for the construction of vectors for targeted gene replacement in filamentous fungi. BMC Molecular Biology: 9, 70-81.

Knight C.J., Bailey A.M., Foster G.D. 2009: Agrobacterium-mediated transformation of the plant pathogenic fungus, Verticillium albo-atrum. Journal of Plant Pathology 91: 745–750.

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Poster
HOST PATHOGEN INTERACTIONS AND RESISTANCE

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P-04 Present status of the University of Córdoba (Spain) olive breeding program for resistance to Verticillium wilt

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Verticillium wilt of olive (*Olea europaea*), caused by the fungus *Verticillium dahliae*, is the most serious disease of this crop and affects it worldwide (López-Escudero & Mercado-Blanco, 2011). The use of resistant cultivars or rootstocks is one of the most efficient measures, although there is a lack of this kind of genotypes. A breeding program aimed to obtain plant material resistant to the disease is carried out since 2008 by the University of Cordoba, Spain (Rallo *et al*., 2007). Screening a great number of olive genotypes for resistance to V. dahliae is the first step of this breeding program, and therefore during the last 4 years it has been developed a method to screen olive seedlings for resistance to the disease (Trapero *et al*., 2011). This method involves the root-dip inoculation of young seedlings with a highly virulent isolate of the fungus, and their assessment under controlled conditions. The screening test has allowed evaluating for *V. dahliae* resistance more than 8,000 genotypes up to date, from which 500 have been selected as resistant to the disease and are being evaluated in the field under a high disease pressure in different locations. The genotypes evaluated have been obtained from crosses and open pollination of different plant material made during the last few years: olive cultivars, wild olives collected in surveys in southern Spain, and other Olea species such as *O. capensis* and *O. exasperata*, collected in Croatia, Pakistan and South Africa. Results have also provided important information about inheritance of resistance. Current and future evaluation and selection of genotypes under controlled and field conditions will probably lead to the development of new cultivars or rootstocks resistant to Verticillium wilt in the next years.

References

López-Escudero, F.J., Mercado-Blanco, J. 2011: Verticillium wilt of olive: A case study to implement an integrated strategy to control a soil-borne pathogen. Plant and Soil 344: 1-50.

Rallo, L., Barranco, D., De la Rosa, R., León, L. 2007: The olive breeding program of Cordoba, Spain. HortScience 42: 988-988.

Trapero, C., Muñoz-Díez, C., Rallo, L., López-Escudero, F.J., Barranco, D. 2011: Screening olive progenies for resistance to Verticillium dahliae. Acta Horticulturae 924: 137-140.

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P-05 Screening of olive breeding progenies for Verticillium wilt resistance

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The use of resistant cultivars is one of the most important measures to control Verticillium wilt of olive (Olea europaea L.) in the framework of an integrated disease management strategy. However, few cultivars with resistance to the development of symptoms have been so far reported. Therefore, the objective of this work was to select new olive genotypes showing higher levels of resistance to Verticillium wilt. Thus, 39 seedlings coming from open pollination progenies of different cultivars were screened for resistance to Verticillium dahliae in growth chamber under controlled environmental conditions. Plants were inoculated by dipping roots in a conidial suspension of a highly virulent defoliating V. dahliae isolate or were immersed in sterile water (non-inoculated control plants) and kept in growth chamber at 22±2 °C for 112 days. Cultivars 'Picual' (susceptible) and 'Frantoio' (resistant) were used as reference controls of two resistance levels. Disease reaction was evaluated weekly by external symptoms using a 0-4 severity scale and by the isolation of the fungus at the end of the experiments. Four genotypes of total evaluated (10.3%) were showed similar resistance than the resistant reference control 'Frantoio' and were significantly different from 'Picual', according to the values of final disease intensity index and standardized area under the disease progress curve. One of these resistant genotypes coming from cultivar 'Frantoio' open pollination, showed significantly lower root colonization than the resistant reference control 'Frantoio' and remained symptomless during the experiment. The most resistant ones will be propagated for future trials to confirm their resistance level under controlled and field conditions. Their possible use as rootstocks or genitors in breeding programs is discussed. Funding source: INIA project RTA2010-00036 and IFAPA project PEI.PEI2011.1, both partially funded by European Regional Development Fund (ERDF).

References

López-Escudero, F.J., Mercado-Blanco, J. 2011: Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344: 1-50.

Rodríguez-Jurado D. 1993: Interacciones huésped-parásito en la marchitez del olivo (Olea europaea L.) inducida por Verticillium dahliae Kleb. PhD Thesis, University of Córdoba, Spain.

Trapero, C., Muñoz-Díez, C., Rallo, L., López-Escudero, F.J., Barranco, D. 2011: Screening olive progenies for resistance to Verticillium dahliae. Acta Hort. 924: 137-140.

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P-06 Evaluation of resistance of Spanish olive cultivars to *Verticillium dahliae* in inoculations conducted in greenhouse

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The resistance of twenty-eight Spanish olive cultivars to Verticillium dahliae was evaluated in an experiment conducted under greenhouse conditions, by soaking plant roots with a semisolid fluid mass composed of culture medium, conidia and mycelium of the fungus. Five-months-old rooted olive cuttings were inoculated with a cotton-defoliating (highly virulent) isolate of V. dahliae. 'Frantoio' and 'Picual' were used as resistant and susceptible reference cultivars, respectively. Disease was assessed on the basis of final values of the area under disease progress curve, mean severity of symptoms, and mortality at twenty-six weeks after inoculations. Verticillium wilt disease developed slower and disease parameters reached lower values than those normally recorded in studies conducted in growth chamber, using root-dip inoculation with a conidial suspension of the pathogen. However, most of the evaluated cultivars had susceptible or moderately susceptible reactions to the infections caused by V. dahliae. Particularly, eight cultivars, together with 'Picual', such as 'Manzanilla de Abla', 'Manzanilla del Centro' or 'Negrillo de Iznalloz', were significantly more susceptible than 'Frantoio'. On the contrary, cultivars 'Escarabajillo', 'Menya' and 'Sevillana de Abla' performed high level of disease resistance, showing no dead plants and vegetative recovery. Field experiments are currently being carried out to confirm the resistance of these latter genotypes. They might be potential resistant genitors for being included in olive breeding programs or used as resistant rootstocks.

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P-07 Influence of the photoperiod on the evaluation of resistance of olive cultivars to Verticillium wilt

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The influence of the photoperiod during the process of evaluation of olive cultivars to infections caused by *Verticillium dahliae* was evaluated for 14 olive cultivars at two greenhouse conditions. Five-month-old olive rooted cuttings were inoculated by root dipping in a conidial suspension (10^7 conidia/ml) of a highly virulent cotton defoliating isolate of the pathogen. Plants were arranged on greenhouse benches according to a complete randomized block design. Cultivars 'Picual' (susceptible) and 'Frantoio' (resistant) were used as reference. After inoculations, four out of eight blocks were incubated in a greenhouse with natural lighting (NL) (minimum 12 h in March and maximum 14.8 h of light in June 2011). The other four blocks were incubated in an adjacent greenhouse in which natural illumination was supplemented with lamps which provided continuous lighting (CL). In both cases maximum and minimum temperatures ranged between 23±5ºC and 16±5ºC during the day and the night, respectively. Disease was weekly assessed on a basis of a 0-4 scale of necrosis, defoliation and chlorosis of leaves and stems. At the end of experiments, both lighting treatments were compared in terms of mean symptom severity, disease incidence, mortality, area under disease progress curve (AUDPC) and vegetative growth of plants. Most of cultivars exhibited susceptibility levels similar to 'Picual', with the exception of 'Marsaline', which showed a slightly higher resistance than 'Frantoio'. Incubation period was shortened between 3 to 4 weeks in inoculated plants subjected to CL treatment compared with plants incubated under NL conditions. Continuous lighting also favored the disease development, probably due to temperature increments that occurred during the night. However, these differences were clearer in susceptible cultivars, but these were not so noticeable in extremely susceptible and resistant cultivars. Differences of accumulated hours between 20º and 24ºC (optimum temperature for disease development) between two lighting treatments were not significant, reaching an average of 744 h for the whole evaluating period. The CL treatment also encouraged vegetative plant growth regarding to the NL one. In both treatments, inoculated plants showing symptoms did not grow or their growth was strongly lower than non-inoculated control plants. On the contrary, asymptomatic inoculated plants grew at similar rate than non-inoculated control ones. Continuous lighting during incubation at greenhouse conditions after inoculating olive plants with *V. dahliae* could be useful to reduce incubation period and to shorten the assessment period. This will be an important tool to be used in olive breeding programs in which is necessary to evaluate lots of genotypes in short periods of time.

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P-08 Genetic responses induced in aerial olive tissues during the interaction in roots with the defoliating pathotype of *Verticillium dahliae*

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Verticillium dahliae Kleb is the causal agent of Verticillium wilt of olive (*Olea europaea* L.) (VWO), one of the most threatening diseases in many areas where this tree is cultivated, particularly in the Mediterranean basin. The current spread of the disease and the severity of its attacks need of an integrated disease management strategy to achieve effective control. The use of resistant olive cultivars is one of the most plausible measures for controlling VWO within such framework. While diverse studies aimed to identify VWO resistant or tolerant genotypes under diverse inoculation conditions have provided some promising results, knowledge on the genetics of resistance to VWO is still very limited. One of our current lines of research aims to shed light on the genetic responses taking place during *V. dahliae*-olive interaction. In the present study, we have conducted a functional genomics analysis to unravel responses that could be induced in aerial tissues of the resistant olive cultivar Frantoio. We particularly aimed to elucidate whether early systemic responses can be differentially triggered in above-ground tissues upon artificial inoculation of *V. dahliae* in the root system. A suppression subtractive hybridization cDNA library, enriched in up-regulated genes, was generated from aerial olive tissues of 'Frantoio' nursery-produced plants, sampled at different time points along 21 days after root-dip inoculation with a representative isolate of the defoliating pathotype. This strategy has enabled the identification of 612 ESTs (71 contigs and 541 singlets) expressed in aerial tissues during the interaction of *V. dahliae* with roots. Querying (Blastx) the non-redundant NCBI database allowed the attribution of homologous hits for transcripts (approximately 43.8%) with coding sequences present in genomes of woody plants such as *Vitis vinifera*, *Populus trichocarpa* and *Ricinus communis*. Moreover, 2.3% of up-regulated genes matched to genes already identified in olive. Finally, nearly 24% of detected olive transcripts corresponded to unidentified genes. Computational analysis showed a number of transcripts involved, among other processes, in plant defense response to biotic and abiotic stresses (i.e., formamidase, phosphatase 2c, PR proteins such as thaumatin-like protein belonging to PR-5 family and lipoxygenases), phenylpropanoid biosynthesis (i.e., caffeoyl-O-methyltransferase) or terpenoids and hormones biosynthesis (i.e., acetone-cyanohydrin lyase and malate dehydrogenase). Similarly, different classes of transcription factors such as GRAS1 and WRKYs were shown to be up-regulated in aerial tissues after *V. dahliae* inoculation in roots. Thus, a broad range of defensive responses seems to be induced in tissues located far away from where the pathogen colonization process is taking place in a resistant olive cultivar.

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References

López-Escudero, F. J., Mercado-Blanco, J. 2011: Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344:1–50.

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P-09 Disease resistance and pathogen colonization of olive cultivars inoculated with *Verticillium dahliae*

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The fungus Verticillium dahliae is the causal agent of Verticillium wilt of olive (Olea europaea), which is nowadays the main disease in most olive-producing countries. Control of the disease is very difficult to achieve, and the use of resistant cultivars is currently the most efficient measure (López-Escudero & Mercado-Blanco, 2011; Markakis *et al*., 2010). In this study, olive plants of susceptible cultivars 'Arbequina' and 'Picual', together with plants of resistant ones 'Changlot Real', 'Empeltre' and 'Frantoio', were inoculated with a highly virulent isolate (defoliating) of V. dahliae. Disease severity and amount of pathogen DNA in roots and stems of infected plants were measured every 2 weeks after inoculation, starting one week after inoculation and ending at 15 weeks after inoculation. Disease severity was very high in susceptible cultivars and most of the plants were killed 90 days after inoculation; while almost no symptoms were scored in the resistant ones. The amount of pathogen DNA in the roots decreased over time from the first week after inoculation, and there were only slight differences between susceptible and resistant cultivars. Conversely, amount of pathogen DNA in stems increased over time, especially in susceptible cultivars. The amount was significantly lower in stems of resistant cultivars compared with stems of susceptible cultivars. Disease severity and the amount of pathogen DNA in stems were significantly correlated, although the value of the correlation coefficient was low ($r < 0.6$) for all cultivars. Based on these results, it appears that V. dahliae is able to colonize the roots of all cultivars during the first weeks after the inoculation, but not to extensively colonize the stem xylem of resistant cultivars, as reported before (Mercado-Blanco *et al*., 2003). Histological changes related with pathogen resistance are being studied.

References

López-Escudero, F.J., Mercado-Blanco, J. 2011: Verticillium wilt of olive: A case study to implement an integrated strategy to control a soil-borne pathogen. Plant and Soil. 344:1-50.

Markakis, E.A., Tjamos, S.E., Antoniou, P.P., Roussos, P.A., Paplomatas, E.J., Tjamos, E.C. 2010: Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and nondefoliating Verticillium dahliae pathotypes. Plant Disease 94: 1156-1162.

Mercado-Blanco, J., Collado-Romero, M., Parrilla-Araujo, S., Rodríguez-Jurado, D., Jiménez-Díaz, R.M. 2003: Quantitative monitoring of colonization of olive genotypes by Verticillium dahliae pathotypes with real-time polymerase chain reaction. Physiological and Molecular Plant Pathology 63: 91-105.

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P-10 Host responses and metabolite changes in tomato induced by *Verticillium dahliae* infestation

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Soil-borne fungal diseases cause damage to agriculturally important crops worldwide. *Verticillium dahliae*, for example, belongs to the group of vascular fungal pathogens that cause foliar wilting by infecting the root. This fungus further penetrates immature xylem elements where conidia are produced to be spread systemically into the shoot. Disease management is complicated since *V. dahliae* form microsclerotia as resting structures surviving several years in soil and plant debris.

It is generally known that infected plants have to cope with high energy demands, originated by plant defense responses and plant-pathogen interactions. Compared to studies focused on the impact of foliar pathogens, metabolic responses that occur in root and above-ground plant organs upon soil-borne pathogen infection have been poorly investigated.

The aim of our study is to provide information about effects of *V. dahliae* on i) tomato photosynthesis, ii) root morphology, and iii) metabolite composition in leaves and roots. Assimilation rates in *V. dahliae*-infected tomato plants decrease significantly as previously shown (Bollig *et al.,* 2013). Root morphological parameters including total length and root area decrease at 21 days post inoculation (dpi). To monitor changes in primary metabolism at different time points after inoculation, metabolic profiling by gas chromatography coupled with mass spectrometry (GC-MS) was used. Therefore, mass spectral data processing and metabolite annotation was performed using MetAlign (Lommen, 2009) and TagFinder software (Lüdemann *et al.*, 2008) by matching retention time indices and fragment mass spectral tags with a reference compound library. Our studies have shown a reduction of different carbohydrates such as fructose and glucose at later sampling time points (14 or 21 dpi), whereas different amino acids like proline and serine were increased. Further organic acids were found to be affected after *V. dahliae* infection. Relative levels of identified metabolites from samples of infected and healthy plants were compared and statistically analysed to determine differential metabolites. Our results help to explain the high complexity of physiological host responses and could provide insight into metabolite distribution to root-infecting fungal pathogens.

References

Bollig, K., Specht, A., Myint, S. S., Zahn, M., Horst, W. J. 2013: Sulphur supply impairs spread of *Verticillium dahliae* in tomato. Eur. J. Plant Pathol. 135: 81-96.

Lommen, A. 2009: MetAlign: Interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. Anal. Chem. 81: 3079-3086.

Lüdemann, A., Strassburg, K., Erban, A., Kopka, J. 2008: TagFinder for the quantitative analysis of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling experiments. Bioinformatics 24: 732-737.

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P-11 Hog1-MAPK gene VdHOG1 regulates microsclerotial formation and stress response in smoke-tree vascular wilt fungus *Verticillium dahliae*

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Smoke-tree wilt, caused by a soilborne fungus *Verticillium dahliae*, is a destructive disease on smoke-tree *Cotinus coggygria* in Beijing region. Mitogen-activated protein kinase (MAPK) signaling pathway plays an essential role in regulating growth, development, pathogenesis and stress response, such as salt, osmotic and hydrogen peroxide in phytopathogenic fungi. HOG1-MAPK pathway in fungi has led to different cellular reactions. However, the function of HOG1-MAPK is less characterized in *V. dahliae*. Thus, we first identified VdHOG1 and analyzed the transcriptional expression in vegetative growth and microsclerotial formation. Real time qPCR analysis showed that VdHOG1 expression was upregulated in conidia stage. To elucidate the function, VdHOG1 was deleted by homologous recombination. We find that the lack of *V. dahliae* Hog1 inhibits the microslerotial formation apparently, the △VdHOG1 mutant strain forms microslerotia later than *V. dahliae* wild type and produces less microslerotia, while it is normal in conidia stage. In detail, the WT strain forms microslerotia in 4 to 5 days, however the △VdHOG1 mutant in 9 to 10 days, and it couldn't be seen any microslerotia on PDA in 10 days. Moreover, it is indeed stressed by NaCl and Sorbitol, which is proportional to their concentration respectively. Interestingly, the △VdHOG1 mutant strain grows faster than WT on CM lack of carbon element. In summary, the results demonstrate that VdHOG1 plays a significant role in microslerotial formation and stress response.

References

Hamel, L.P., Nicole, M.C., Duplessis, S., Ellis, B.E. (2012): Mitogen-activated protein kinase signaling in plant-interacting fungi: distinct messages from conserved messengers. Plant Cell 24: 1327-1351.

Klimes, A., Amyotte, S.G., Grant, S., Kang, S., Dobinson, K.F. (2008): Microsclerotia development in Verticillium dahliae: Regulation and differential expression of the hydrophobin gene VDH1. Fungal Genet Biol 45: 1525-1532.

Klosterman, S.J., Subbarao, K.V., Kang, S., Veronese, P., *et al* (2011): Comparative Genomics Yields Insights into Niche Adaptation of Plant Vascular Wilt Pathogens. PLoS Pathog 7: e1002137.

Wang, Y., Xiao, S., Xiong, D., Tian, C. (2012): Genetic transformation, infection process and qPCR quantification of Verticillium dahliae on smoke-tree Cotinus coggygria. Australasian Plant Pathol 42, 33-41.

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P-12 Genes and pathways controlling *Verticillium longisporum*-resistance in *Arabidopsis thaliana*

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Verticillium longisporum is causing increasing problems in the cultivation of oilseed rape in Central and Northern Europe. It colonises susceptible cruciferous hosts systemically during flowering and causes premature ripening and yield loss in the field. So far only quantitative resistance relying on several, mostly unidentified genes is known in Brassica. Natural genetic variation with respect to *V. longisporum* resistance exists also in *A. thaliana*, which can be used as a model. QTL-mapping in a new recombinant inbred line (RIL-) population originating from a cross between ecotypes Bur and Ler revealed that different resistance traits were controlled by different sets of QTL. A QTL- region on chromosome 2 around the Erecta gene was found to control resistance traits like systemic colonisation, resistance to V. longisporum-induced stunting and to chlorosis. The role of Erecta in this pathosystem had been investigated by testing several erecta mutants for their reaction to V. longisporum. While loss of Erecta function aggravated V. longisporum-induced stunting, no effect on systemic colonisation by the fungus could be detected. A locus controlling systemic colonisation was closely linked, but not identical to Erecta. To identify the corresponding gene(s), a population of near-isogenic lines (NILs) has been developed that differ only in the region of interest on chromosome 2. The fine-mapping approach in NILs will be complemented by studying gene expression and contents of phytohormones with a potential role in the host-pathogen-interaction; first results of these experiments will be presented.

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P-13 Analysis of a putative JA-Ile-independent COI1 function in roots of *Arabidopsis thaliana* which mediates susceptibility to *Verticillium longisporum*

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Verticillium longisporum is a soil-borne vascular pathogen that causes reduced shoot growth and early senescence in *Arabidopsis thaliana*. We have shown that these disease symptoms are less pronounced in plants, which lack the receptor of the plant defense hormone jasmonic acid (JA), CORONATINE INSENSITIVE 1 (COI1). Initial colonization of the roots was comparable in wild-type and *coi1* plants and fungal DNA accumulated to almost similar levels in petioles of wild-type and *coi1* plants at 10 days post infection. Contrary to the expectation that the hormone receptor mutant *coi1* should display the same phenotype as the corresponding hormone biosynthesis mutant *aos*, *aos* plants developed wild-type-like disease symptoms. Grafting experiments revealed that the susceptibility-enhancing COI1 function acts in the roots. Together, our results have unravelled a novel COI1 function in the roots, which acts independently from JA-Ile or any JA-Ile mimic. This independent COI1 function was supported with first results of transgenic plants that express COI1 with an altered ligand binding pocket in the *coi1-16* background. These plants are no longer able to bind JA-Ile and showed susceptibility after infection with *Verticillium longisporum*. To identify genes under the control of this novel COI1 function, RNA from infected wild-type, *coi1* and *aos* roots is collected for microarray analysis.

References

Ralhan, A., Schöttle, S.; Thurow, C., Iven, T., Feussner, I., Polle, A. Gatz, C. 2012: The vascular pathogen *Verticillium longisporum* requires a jasmonic acid independent COI1 function in roots to elicit disease symptoms in *Arabidopsis* shoots. Plant. Physiol. 159 (3):1192-1203

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P-14 Towards the identification of fungal effector molecules involved in host plant developmental reprogramming

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Typically, Verticillium infections lead to severe wilting symptoms on their respective host plants, with the remarkable exception of *V. longisporum* infected Brassicaceae, which maintain their water status. Recently, we could show that water status maintenance correlates with a substantial *V. longisporum*-induced developmental reprogramming on its host plants *Brassica napus* and *Arabidopsis thaliana* (Reusche *et al*., 2012). Together, our data suggest that de novo formation of xylem by hyperplasia and transdifferentiation in response to infection by *V. longisporum* is likely to be a compensatory plant response resulting in increase of plant water storage capacity. Interestingly, tomato races of *V. dahliae* have been shown to be vascular pathogens of Arabidopsis, too, and have been used for functional studies of tomato resistance genes by interfamily transfer (Fradin *et al*., 2011). However, comprehensive comparative analyses of *V. dahliae*-induced symptom development on Arabidopsis thaliana have not been conducted to our knowledge. To take remedial action we recently analyzed symptom development induced by 22 different *V. longisporum* and 43 *V. dahliae* strains on *Arabidopsis thaliana* ecotype Col-0 (Thole *et al*., unpublished). Interestingly, we identified three *V. dahliae* strains that phenocopy the disease symptoms typically associated with *V. longisporum* infection. On the other hand, we found several *V. dahliae* strains which induce strong wilting phenotypes and lateral branching, but lack the capacity to induce developmental reprogramming of vascular tissues. Comparative genomic sequence analyses of these haploid *V. dahliae* strains will be conducted in order to identify and functionally characterize effector molecule candidates underlying the distinct infection symptoms.

References

Reusche, M., Thole, K., Janz, D., Truskina, J., Rindfleisch, S., Drübert, C., Polle, A., Lipka, V., Teichmann, T. 2012: Verticillium infection triggers VASCULAR-RELATED NAC DOMAIN7–dependent de novo xylem formation and enhances drought tolerance in Arabidopsis. The Plant Cell, 24: 3823–383.

Fradin, E. F., Abd-El-Haliem, A., Masini, L., van den Berg, G. C. M., Joosten, M. H.A.J., Thomma, B. P.H.J. 2011: Interfamily transfer of tomato Ve1 mediates Verticillium resistance in Arabidopsis. Plant Physiol.156: 2255–2265.

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P-15 Stabilization of cytokinin levels enhances Arabidopsis resistance against *Verticillium longisporum*

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Verticillium longisporum is a vascular pathogen that infects the *Brassicaceae* host plants *Arabidopsis thaliana* and *Brassica napus*. The soil-borne fungus enters the plant via the roots and colonizes the xylem of roots, stems and leaves. During late stages of infections, *Verticillium* spreads into senescing tissue and switches from biotrophic to a necrotrophic life style. Typical symptoms of *Verticillium longisporum*-induced disease are stunted growth and leaf chloroses. Expression analyses of the senescence marker genes *SENESCENCE ASSOCIATED GENE12, SENESCENCE ASSOCIATED GENE13* and *WRKY53* revealed that the observed chloroses are a consequence of premature senescence triggered by *Verticillium* infection. Our analyses show that concomitant with the development of chloroses levels of *trans*-zeatin decrease in infected plants. Induction of cytokinin oxidase expression by Verticillium-infection may be one factor that contributes to the observed decreases in cytokinin levels. Stabilisation of *Arabidopsis* cytokinin levels by both pharmacological and genetic approaches prevents development of chloroses and inhibits *Verticillium* proliferation but does not influence pathogen-induced growth reduction. In summary, our results indicate that *Verticillium* triggers premature plant senescence for efficient host plant colonization.

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P-16 Association of phenylpropanoid metabolites with resistance of rapeseed to *Verticillium longisporum*

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Verticillium longisporum is a major threat to production of oilseed rape (*Brassica napus*) in Europe. Control of *V. longisporum* by fungicide treatment is not effective. The most promising long-term measure to control the disease is breeding of cultivars exhibiting effective resistance. Genetic analysis revealed a major quantitative trait locus (QTL) for resistance in a doubled haploid (DH) rapeseed mapping population on chromosome C5 and a minor QTL on chromosome C1. Resistance was correlated with the concentration of soluble (Obermeier *et al*. 2013) and cell-wall bound phenolics in the hypocotyl of the mapping population after infection and some of the corresponding QTL were co-localizing with resistance QTL. Some of these phenolics were identified as key compounds of the phenylpropanoid pathway which are known to be involved in cell-wall esterification and enhancement of plant resistance against fungal enzymes. Also, the lignin monomer composition showed a significant change in the hypocotyls of susceptible, but not in resistant plants in the mapping population at 28 days after inoculation. Similar observations have been made in studies using a limited number of genotypes for different host-pathogen interactions including *V. dahliae* interaction with susceptible and resistant pepper and tomato genotypes (Pomar *et al*. 2004; Gayoso *et al*. 2010). In these studies it was suggested that lignification and reinforcement of cell walls by crosslinking is a major factor involved in resistance reactions of plants against fungal infections. However, in contrast to these studies we found an increase of the G/S lignin monomer ratio and significant correlation with susceptibility. Increase of the G/S ratio was only observed in susceptible genotypes. This might suggest that these changes are rather indirect effects of successful fungal invasion of the hypocotyl tissue and not directly causally linked to resistance. Further genetic, metabolome and transcriptome studies are underway to evaluate the contribution of phenylpropanoid metabolites and genes from the phenylpropanoid pathway to resistance against *V. longisporum* in rapeseed and to develop markers which are useful for broad application in marker-assisted breeding.

References

Gayoso C., Pomar F., Nova-Uzal E., Merino F., Martínez de Ilárduya O. 2010: The *Ve*-mediated resistance response of the tomato to *Verticillium dahliae* involves H_2O_2, peroxidase and lignins and drives *PAL* gene expression. BMC Plant Biol. 10: 232.

Obermeier C., Hossain M. A., Snowdon R, Knüfer, J, von Tiedemann A, Friedt W. 2013: Genetic analysis of phenylpropanoid metabolites associated with resistance against *Verticillium longisporum* in *Brassica napus*. Mol. Breeding 31:347-361.

Pomar F., Novo M., Bernal M. A., Merino F., Ros Barceló A. 2004: Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt in *Capsicum annuum*. New Phytol. 163: 111-123.

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P-17 Phenylpropanoid metabolism and its role in the resistance of *Brassica napus* against the vascular pathogen *Verticillium longisporum*

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Verticillium longisporum is a soilborne vascular fungal pathogen of oilseed rape and poses a major threat to its cultivation. Lignification of cell walls is a common defense response and has been shown to enhance resistance against vascular pathogens. In the present investigation, we compared the expression of resistance in respect to the phenylpropanoid metabolism in a susceptible ('Falcon') and a resistant genotype (SEM 05-500256) of *Brassica napus*. Our earlier work on β- aminobutyric acid (BABA) induced resistance in *B. napus* against *V. longisporum* demonstrated early and significant increase in phenylalanine ammonia lyase (PAL) activity in hypocotyls suggesting higher synthesis and accumulation of phenylpropanoids. This increase in PAL activity was found to correlate with large numbers of phenol storing cells surrounding the vessels. A similar kind of defense responses was also reported from genotypic resistance in *B. napus* against *V. longisporum* wherein resistance was correlated with higher levels of soluble and cell wall bound phenolics, phenol storing cells and lignin formation in hypocotyl tissues. We also observed a strong and significant increase in salicylic acid (SA) during susceptible interaction of *B. napus* with *V. longisporum* which correlated with higher amounts of pathogen DNA found in the hypocotyl. These results suggested the possibility of pathogen mediated diversion of the cinnamic acid pool (a common precursor for both SA and lignin biosynthesis) towards SA, to the expense of a rapid and effective lignin biosynthesis hence weakening the plant defense response against fungal invasion. Based on this hypothesis we are investigating the role of SA as a negative regulator of resistance in *B. napus* response to *V. longisporum*. Our study suggested significant increase in activities of PAL, peroxidase and polyphenol oxidase enzymes, differential accumulation of phenolic compounds and differential expression of lignin biosynthesis genes in susceptible and resistant genotypes of *B. napus* in response to the pathogen.

References

Eynck, C., Koopmann, B., Karlovsky, P., v. Tiedemann, A. 2009: Internal resistance in winter oilseed rape inhibits systemic spread of the vascular pathogen *Verticillium longisporum*. Phytopathol. 99: 803–11.

Kamble, A., Koopmann, B., v. Tiedemann, A. 2012: Induced resistance to *Verticillium longisporum* in *Brassica napus* by β-aminobutyric acid. Plant Pathol. Doi: 10.1111/j:1365-3059.2012.02669.x

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P-18 Pre-symptomatic detection of *Verticillium longisporum* in field grown winter oilseed rape

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Field screening for *Verticillium longisporum* resistant oilseed rape (OSR) plants is commonly performed by visual scoring of microsclerotia on stubbles. This method enables a rough classification of the plants in terms of disease severity. Typically, screening time points are chosen close to harvest or after harvest, owing to the absence of symptoms before maturation stage. We here demonstrate that microsclerotia formation and symptom expression depend on the ripening stage of the crop, implying that a precise determination of resistance levels by stubble screening is difficult - in particular when early and late ripening genotypes are compared. A robust method for detection and quantification of the fungus in the plant before symptom outbreak is therefore required. We present a quantitative real-time PCR (qPCR) method to quantify *V. longisporum in planta* in field-grown winter OSR. Two different primer pairs targeting different gene loci were evaluated with regard to specificity and sensitivity towards *V. longisporum*. The primers OLG 70/71, targeting the internally transcribed spacer (ITS) region of fungal ribosomal DNA were less specific towards *V. longisporum* isolates compared to tubulin targeting primers VlTubF2/R1 (Debode *et al*, 2011), but outperformed the latter in terms of sensitivity. Since high sensitivity is a prerequisite for detection of fungal DNA in early disease stages, ITS primers OLG 70/71 were used for quantification of fungal DNA in different resistant OSR cultivars grown in the field. Using our qPCR protocol, fungal DNA was detected at GS 65-67 in stem tissue of all tested cultivars in the absence of visible symptoms. Differences in fungal DNA levels among the tested cultivars were not significant at GS 65-67, but identification of resistant cultivars was possible at GS 80. The presented ITS-based qPCR method enables the detection of *V. longisporum* prior symptom development and classifies genotypes by measurement of biotrophic fungal DNA in pre-matured plant tissue.

References

Debode, J., Van Poucke, K., França, S. C., Maes, M., Höfte, M., Heungens, K. 2011: Detection of multiple Verticillium species in soil using density flotation and real-time polymerase chain reaction. Plant Disease 95: 1571-1580.

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P-19 Colonization of susceptible and resistant hop cultivars following infection by *Verticillium albo-atrum*

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The colonization pattern of *Verticillium albo-atrum* in resistant and susceptible hop plants in a time course experiment was analyzed by quantification of fungal DNA (qRT-PCR) and by histological examination to look for physical responses.

Fungal DNA was quantified by real-time PCR in roots and two stem sections of the infected plants at 3, 6, 10, 15, 20 and 30 dpi. A cyclical pattern of colonization was observed in the roots in the susceptible cultivar, with two peaks of fungal population, at 3 dpi and 10 dpi, followed by a steady decrease of fungus until 30dpi. In the stems, fungal biomass increased through time without any intermittent decrease, indicating a continuous spread up the stem. In the resistant cultivar, root colonization was less extensive than in the susceptible cultivar and was delayed, with a fungal population peak at 15 dpi. Fungal DNA was also detected in stem sections but remained at very low levels throughout the experiment.

Freehand sections from roots and stems of infected plants were examined under a light microscope. Synthesis of cell wall-coating materials, such as callose, lignin or suberin, around infected vessels was detected in the roots and stems of the susceptible cultivar, while this response was less intensive in the resistant plants. Fungal hyphae were found in around 15% of the vessels only in the susceptible cultivar, in both roots and stems, at later stages of infection (15, 20 and 30 dpi), although only a few vessels with spores were observed. Formation of tyloses in xylem vessels was intensive in the roots and stems of both cultivars, but a different dynamic of tylose formation in roots was observed in the two cultivars. In the roots of resistant plants, the highest number of occluded vessels was found at 3dpi (35%), with a decline towards 10dpi (6%), followed by slight increase at 20dpi (19%) and final drop at 30dpi (6%). In the roots of the susceptible cultivar, only a steady increase of vessels with tyloses was found, starting with the lowest number at 3 dpi (9%) and the highest at 30 dpi (65%). In the two stem sections in both cultivars, tyloses occurred at all time points, reaching a peak at 80% and 67% in susceptible and resistant cultivars, respectively, at 20dpi.

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P-20 Differential gene expression during interaction of resistant and susceptible hop cultivars (*Humulus lupulus* L.) with *Verticillium albo-atrum*

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In this study, resistant and susceptible hop cultivars were inoculated with the lethal pathotype of *V.albo-atrum* and stem samples were collected from infected and control plants 10, 20 and 30 days after inoculation. Two differential expression analyses, cDNA-AFLP and GeneSnare, were carried out on the isolated RNA samples. A Blast2go algorithm revealed homology to known genes of 90 TDFs out of a total of 217 TDFs obtained in the two differential analysis and gene ontology categorises them by biological processes to cellular and metabolic processes, biological regulation, response to stimuli, transport and disease resistance. The expression pattern of 34 TDFs with homology to known genes and the additional defence related genes PR-1, PR-2, PR-3 and PR-5 were further analysed by quantitative real-time PCR. The expression pattern in susceptible and resistant cultivars is compared for the genes implicated in disease resistance and defence responses are discussed.

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P-21 HLVe1, a hop (*Humulus lupulus* L.) homolog of the tomato Ve1 gene, recognizes the fungal effector Av1

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The aim of the study was to isolate hop homologs of the tomato Ve1 gene, the role of which in resistance to Verticillium is well established. Through EST database mining and TAIL-PCR based retrieval of genic sequences, the nucleotide sequence of the complete coding region of a hop Ve1 homolog was obtained from the hop wilt resistant cultivar 'Wye Target'. This sequence exhibited tentative polymorphisms within the cultivar. A SNP marker was subsequently developed and its segregation in mapping family showed that HlVe1 marker is The showed linkage with QTL for wilt resistance. Furthermore, co-expression of the HlVe1 variants with *Verticillium dahliae* effector Ave1, which activates Ve1-mediated disease resistance in tomato, in tobacco showed recognition of Ave 1 by HlVe1, hop homolog of the tomato Ve1 gene.

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P-22 Comparative proteomics in interaction of hop and *Verticillium albo-atrum*

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Aggressive strains of *Verticillium albo-atrum* are a serious limitation in hop production. Molecular mechanisms underlying resistance to Verticillium wilt remain poorly understood despite the considerable losses caused by the disease. To characterize the hop – Verticillium interaction, host responses on a proteome level were analysed in roots (infection site) and xylem sap (colonization space) of the susceptible cultivar Celeia and resistant cultivar Wye Target. Proteomic analyses of root and xylem sap samples isolated from control and infected plants of both cultivars were performed with two-dimensional electrophoresis and MALDI-TOF/TOF mass spectrometry. The resistant cultivar showed a very weak response to fungal infection on a protein level. Protein abundance changed significantly after infection in 0% (root) and 7% (xylem sap) of protein spots in Wye Target compared to 27% (root) and 39% (xylem sap) of proteins spots in Celeia. Defence-related proteins chitinase, β-glucanases, thaumatin-like proteins, germin-like proteins and peroxidases accumulated in the roots of infected Celeia plants. Two chitinases and antifungal protein PR-1 were more abundant in xylem sap after infection in both cultivars, with a stronger response in Celeia. These results suggest a constitutive rather than induced resistance mechanism in Wye Target. Additional forms of a lectin that were identified only in the roots of Wye Target indicate a novel role of lectins in Verticillium resistance. Two abundant fungal proteins were also identified in the xylem of susceptible plants; a protein similar to *Fusarium oxysporum* effector Six3 and a lignin-degrading versatile peroxidase.

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P-23 Identification of QTL associated with hop resistance to *Verticillium albo-atrum*

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Outbreaks of the lethal strain of Verticillium wilt infecting hops cause substantial yield losses and can also render fields out of production for several years. Since chemical control is unavailable or hard to apply in field conditions, breeding hop cultivars with genetic resistance in addition to phytosanitary measures is the only way of controlling the disease. In order to elucidate the genetic control of Verticillium wilt resistance in hops, a pseudotestcross population of 144 plants was derived. The mother plant, cultivar 'Wye Target', carries the strongest and predicted genetic resistance while the male breeding line 2/1 is susceptible. Phenotyping of wilting symptoms was performed under control conditions using ten plants for each genotype. A genetic linkage map was constructed using various types of molecular marker but with a high number of codominant SSRs. The genetic linkage map included 203 markers, forming ten major linkage groups of the maternal and paternal maps, covering 552.98 and 441.1 cM, respectively. On a single chromosomal region of LG03 of both parental maps, one significant QTL for Verticillium wilt resistance was detected at LOD 7 for the first time in hop. The QTL accounted for 24.2 to 26.0% of the phenotypic variance. The presented genetic linkage map and identified QTL are an important first step in deciphering the genetic basis of hop Verticillium wilt resistance. This work also opens possibilities of marker development and exploitation in marker assisted selection breeding.

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P-24 *Medicago truncatula* as a model to study Verticillium wilt in legume plants: genetic traits and regulatory mechanisms

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Verticillium wilt is a major threat to alfalfa (*Medicago sativa*) culture in Europe. The model legume plant *Medicago truncatula* was used as a host for studying resistance and susceptibility to *Verticillium albo-atrum*. In addition to presenting well-established genetic resources, this wild plant species enables to investigate biodiversity of the response to the pathogen and putative cross-talk between disease and symbiosis.

Susceptibility levels were assessed by symptom scoring after root-inoculation with an alfalfa strain of *V. albo-atrum* (V31-2) and modeling of disease curves. Resistant and susceptible *M. truncatula* lines were identified and major QTL controlling resistance were detected in three mapping populations. Resistance in line A17 is controlled by a single QTL on linkage group 7, whereas in line DZA 45.5 two QTL were detected on linkage groups 2 and 6. These QTL do not colocalise with putative Ve gene homologs identified in the *M. truncatula* genome sequence. Studies with a GFP-expressing *V. albo-atrum* strain showed that symptoms and colonisation pattern in infected susceptible *M. truncatula* plants were typical of Verticillium wilt. Resistant A17 plants eliminated the fungus from their vessels between 4 and 7 days after inoculation (Ben *et al*., 2013).

The response of a core collection of *M. truncatula* lines to V31-2 inoculation presented a wide range of disease parameters, suggesting highly diverse resistance mechanisms in natural populations. Experiments with additional Verticillium strains isolated from non-legume plants showed that *M. truncatula* was susceptible to some of them (Negahi *et al*., 2013). Four QTL controlling resistance to a non-host strain were identified in a mapping population and were found different from those involved in resistance to V31-2 (Negahi *et al*., in preparation).

To study regulatory mechanisms of the response to V 31-2 we produced libraries of mRNA and small RNA from roots of lines A17 and F83005.5. Sequence analysis of these libraries is under way and will lead to the identification of gene expression patterns and microRNAs associated to resistance. First results will be presented.

Several mutants of the resistant line A17, impaired in different steps of rhizobial symbiosis were affected in their response to *V. albo-atrum*, which suggests that mechanisms involved in the establishment of symbiosis or disease might have some common regulatory control points

References

Ben C., Toueni M., Montanari S., Tardin M.C., Fervel M., Negahi A., Saint-Pierre L., Mathieu G., Gras M.C., Noël D., Prospéri J.M., Pilet-Nayel M.L., Baranger A., Huguet T., Julier B., Rickauer M., Gentzbittel L., 2013. Natural diversity in the model legume Medicago truncatula allows identifying distinct genetic mechanisms conferring partial resistance to Verticillium wilt. J. Exp. Bot 64: 317-332.

Negahi A, Sarrafi A., Ebrahimi A., Maury P., Prospéri J.M., Ben C., Rickauer M. 2013: Genetic variability of tolerance to Verticillium albo-atrum and Verticillium dahliae in Medicago truncatula. Eur. J. Plant Pathol.: accepted

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Poster
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P-25 Development and characterization of microsatellite markers for analysis of genetic diversity in *Verticillium* species

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Verticillium wilt is one of the most important diseases caused by the plant pathogens *Verticillium* spp., infecting the vascular systems of many crop plants. Microsatellite markers, one of the most popular single locus molecular marker systems, can help in studying the ecology and diversity of fungal plant pathogens. In order to examine genetic variability in *Verticillium* spp., we aimed to develop new microsatellite markers from the genomic sequence of the Vams102. The complete genome of Vams102 was searched for the presence of microsatellite tracks and selected loci from either the coding or non-coding part of the genome were used for genetic diversity analysis of *V. albo-atrum* and *V. dahliae* isolates obtained from hop and other crops. Furthermore, an interspecies amplification test of the developed loci was also made within other *Verticillium* specimens: *V. tricorpus*, *V. Longisporium*, *V. lecanii*, *V. nigrescens*, *V. fungicola* and *V. nubilum*. Using twelve polymorphic loci, 54 different alleles were amplified (4.5 average), ranging from 9 to 2 alleles. The allelic polymorphism was used to calculate the Dice coefficient of similarity and to construct a dendrogram. Phenetic analysis separated the isolates into three supported groups representing isolates of *V. albo-atrum*, *V. dahliae* together with *V. longisporium* and alfalfa isolates of *V. albo-atrum*.

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P-26 Comprehensive analysis of the intra- and extracellular metabolome of different *Verticillium* species and *Fusarium oxysporum*

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The genus Verticillium consists of different plant-pathogenic species like *V. dahliae*, *V. albo-atrum* and *V. longisporum*, which are characterized by specific host ranges. In contrast to the other Verticillium species, *Verticillium longisporum* specifically infects plants of the family of the Brassicaceae, which leads to economical losses in oilseed rape cultivation.

Plant infection by fungal pathogens is known to be a highly complex interaction which is based on the exchange of proteins and small molecules of the primary and secondary metabolism. To identify putative metabolites which might be specific for *Verticillium longisporum 43* infection of oilseed rape, we analyzed differences in the metabolome of VL43 and of other Verticillium species and strains (VL32, VdJR2, VD39, Vaa4). Additionally one *Fusarium oxysporum* strain (Fox 5176) was included.

For the analyses fungi were grown in simulated xylem sap medium (SXM). Metabolites of the mycelia (intracellular metabolome) and secreted and converted metabolites in the medium (extracellular metabolome) were analyzed via UPLC-TOF-MS. Afterwards data were processed and differences visualized by the Marvis tool (http://marvis.gobics.de33).

We could detect at least one metabolite-based cluster specific for each fungi species both in the supernatant and mycelium analysis. Compounds of high intensity and prominent distribution between the fungi were verified by MS/MS fragment analysis or coelution with standard substances. Prominent markers detected in all Verticillium species were derived from the anthranilate metabolism. But each Verticillium strain showed a distinct intensity pattern of the different anthranilate derivatives. No anthranilate derivatives were detected in the Fusarium culture, instead and exclusively here a high intensity of the toxin beauvericin was identified.

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P-27 The roles of autophagy in *Verticillium dahliae*

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Verticillium dahliae Kleb. produces, in dead and dying plant tissues, resting structures (microsclerotia) which can survive in the soil for many years in the absence of host plants, are the primary source of disease inoculum, and are difficult to eradicate. To investigate the potential role of autophagy in *V. dahliae* pathogenicity, growth, and survival structure development a *V. dahliae* orthologue of the *Saccharomyces cerevisiae* autophagy gene ATG8 was mutated by transposon mutagenesis, and used to transform *V. dahliae* strain Dvd-T5. Transformants were identified in which the wild-type (WT) VdATG8 had been replaced with the mutant (vdatg8) construct. Analysis of these vdatg8 (null) mutants demonstrated that although they exhibited several developmental defects, those defects did not compromise pathogenicity. Mycelial growth in the mutants was slightly but significantly reduced. Dimorphic growth in a liquid environment was also defective, specifically the transition from mycelial to yeast-like growth (spore production). The most dramatic defect was in microsclerotia (MCS) production. MCS were not formed when vdatg8 cultures were grown on a rich (complete) medium, and their formation was severely reduced during growth on a nitrogen-limited, basal medium. Moreover, during carbon or nitrogen starvation the vdatg8 strains showed only rare development of incompletely melanized MCS. In contrast, mycelial growth and MCS development by the WT strain was maintained under those growth conditions, albeit at a much reduced level. These data provide evidence not only of a role for autophagy in normal MCS development, but also that in WT *V. dahliae* this developmental process is a “default pathway” to survival in times of environmental stress.

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P-28 Extensive chromosomal reshuffling drives evolution of virulence in *Verticillium dahliae*

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Sexual recombination drives genetic diversity in eukaryotic genomes, and fosters adaptation to novel environmental challenges. Although strictly asexual microorganisms are often considered as evolutionary dead ends, they comprise many devastating plant pathogens. Presently, it remains unknown how such asexual pathogens generate the genetic variation that is required for quick adaptation and evolution in the arms race with their hosts. Here we show that extensive chromosomal rearrangements in the strictly asexual plant pathogenic fungus *Verticillium dahliae* establish highly dynamic 'plastic' genomic regions that act as a source for genetic variation to mediate aggressiveness. We show that these plastic regions are greatly enriched for *in planta*-expressed effector genes, encoding secreted proteins that enable host colonization including the previously identified race 1-specific effector Ave1 that activates Ve1-mediated resistance in tomato. The plastic regions occur at the flanks of chromosomal breakpoints and are enriched for repetitive sequence elements, especially retrotransposons. Our results demonstrate that asexual pathogens may evolve by prompting chromosomal rearrangements, enabling rapid development of novel effector genes. Likely, chromosomal reshuffling is a general mechanism for adaptation in asexually propagating organisms.

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P-29 Studying a *Verticillium dahliae* population with a combination of phytopathological and morphological characterization with genetic and molecular profiling

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Understanding the genetic diversity of *V. dahliae* populations and early recording of their pathogenic profile are essential for disease management. A *V. dahliae* population mainly originating from Crete, Greece, was characterized in terms of pathogenicity/virulence, morphology/physiology, vegetative compatibility and mating type. Tomato race 2 has supplanted race 1 and was more virulent on a tomato-susceptible cultivar than race 1. Using a differential host classification system which tests pathogenicity to tomato, eggplant, sweet pepper and turnip, 59 isolates were assigned to tomato, 19 to eggplant, 1 to sweet pepper and 5 to tomato-sweet pepper pathogenicity groups. All isolates from Crete fell into VCG subgroups 2A, 2B and 4B, while a remarkably high incidence of bridging isolates was recorded. The tomato-sweet pepper pathogenicity group was morphologically quite distinct from the others, while conidial length and pigment intensity were discriminatory parameters among VCGs 2A, 2B and 4B. PCR-based molecular marker Tr1/Tr2 was reliable in race prediction among tomato-pathogenic isolates, except for members of VCG 4B, while the application of markers Tm5/Tm7 and 35-1/35-2 was highly successful for tomato pathotype. E10 marker was related to VCG 2B, rather than to pathogenicity groups. A single nucleotide polymorphism in the ITS2 region, and two novel molecular markers, M1 and M2, proved useful for the fast and accurate determination of major VCGs 2A, 2B and 4B, and can be used for high-throughput population analyses in future studies. The mating type was unrelated to VCG classification and probably does not control heterokaryon incompatibility in *V. dahliae*.

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References

Horiuchi, S., Hagiwara, H., Takeuchi, S. 1990: Host specificity of isolates of Verticillium dahliae towards cruciferous and solanaceous plants. In: Biological control of soil-borne pathogens, D. Hornby (Ed), Wallingford, UK: CAB International.

Maruthachalam, K., Atallah, Z. K., Vallad, G. E., Klosterman, S. J., Hayes, R. J., Davis, R. M., *et al*. 2010: Molecular variation among isolates of Verticillium dahliae and polymerase chain reaction-based differentiation of race. Phytopathology 100: 1222-1230.

Papaioannou, I. A., Ligoxigakis, E. K., Vakalounakis, D. J., Markakis, E. A., Typas, M. A. 2013: Phytopathogenic, morphological, genetic and molecular characterization of a Verticillium dahliae population from Crete, Greece. Eur. J. Plant Pathol. doi: 10.1007/s10658-013-0189-4.

Zeise, K., von Tiedemann, A. 2001: Morphological and physiological differentiation among vegetative compatibility groups of Verticillium dahliae in relation to V. longisporum. J. Phytopathol. 149: 469-475.

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P-30 Verticillium species in Tunisia: pathogenic characterization and varietal behavior

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In Tunisia *Verticillium* wilt (VW) is mainly distributed in the coastal regions such as Sousse, Monastir, Mahdia, Nabeul, Tunis and Bizerte and affects many vegetable crops including tomato, potato, eggplant, pepper, melon, squash and artichoke. 155 *Verticillium* isolates were obtained from these hosts and three *Verticillium* species were identified based on morphological, cultural and biometrical characteristics: *V. dahliae* (77%), *V. tricorpus* (17%) and *V. albo-atrum* (6%).

To study the pathogenicity of *Verticillium* isolates, four plant species were used: tomato (cv. Ventura), potato (cv. Spunta), eggplant (cv. Bonica) and melon (cv. Ananas d'Amérique). The majority of *Verticillium* spp. isolates adversely affected the inoculated plants compared to the uninoculated control. A high degree of pathogenic variability among *Verticillium* isolates, and especially those of *V. dahliae* was detected, according to their differential ability to cause wilt and browning of the vascular system together with a reduction on plant height, aerial part weight and tuber yield. Although, *V. dahliae* isolates were the most aggressive toward the different plants tested, some isolates of *V. albo-atrum* and *V. tricorpus* were found to be as aggressive as some *V. dahliae* isolates, according to our experiment conditions. All of the three *Verticillium* species seem contributing, with variable degrees, in the VW complex in Tunisia.

As *V. dahliae* is the most aggressive species in Tunisia, a mixture of isolates is used to test the behavior of potato and melon cultivars towards this pathogen. Based on the severity of foliar damage, all the ten tested potato cultivars exhibited varying degrees of susceptibility to VW ranging from moderate to high. However, cvs. Tango and Elodie were the only cultivars classified as tolerant based on tuber yield reductions.

Under controlled conditions, melon cultivars, Pancha and Ananas d'Amérique, have shown to be moderately susceptible to VW while cvs. Protéo, Maazoul, Mansour and Santom were classified as tolerant. However, under natural infection with *V. dahliae*, a 100% wilt was recorded for the eight melon cultivars tested.

These results highlight the urgent use of effective control measures to reduce VW in many vegetable growing regions in Tunisia.

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P-31 Analysis of the intergenic spacer (IGS) region of the nuclear rDNA of *Verticillium dahliae* for VCG discrimination and phylogenetic studies

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Implementation of effective management strategies for the control of *V. dahliae* has been restricted by our limited understanding of the genetic diversity of the fungus. Vegetative compatibility group (VCG) analysis has been traditionally used for examining fungal population structures. In this study, we performed initially an extended *in silico* analysis of 201 publicly available IGS sequences of *V. dahliae* and determined the most variable IGS sub-region, which was characterized by the presence of several indels of varying length as well as single nucleotide polymorphisms. This sub-region was PCR-amplified from 59 *V. dahliae* isolates covering all VCGs, 4 *V. albo-atrum* and 5 *V. longisporum* isolates, and the amplicons were cloned, sequenced and structurally and phylogenetically analyzed. Variation was largely due to different copy numbers of four classes of short repetitive DNA elements, organized in higher-order repetitive structures or commonly encountered composite blocks. Structural and phylogenetic analyses of sequences of this sub-region were consistent and allowed the identification of two main lineages in *V. dahliae*, i.e., cluster I including VCGs 1A, 1B, 2A, 4B and 3, and cluster II containing VCGs 2B, 4A and 6. IGS sequence analysis is a highly suitable molecular tool for (a) the study of IGS sequence molecular evolution in fungi, (b) rapid inter-specific differentiation, (c) intra-specific discrimination among VCGs of *V. dahliae*, allowing high-throughput VCG confirmation and prediction/profiling, and (d) phylogenetic analysis within and among *V. dahliae* VCGs.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

References:

Collado-Romero, M., Mercado-Blanco, J., Olivares-García, C. & Jiménez-Díaz, R. M. 2008: Phylogenetic analysis of *Verticillium dahliae* vegetative compatibility groups. Phytopathology 98: 1019-1028.

Jiménez-Gasco, M. M., Berbegal, M., Malcolm, G. M., Armengol, J. & Jiménez-Díaz, R. M. 2009: New insights on the phylogenetic relationships among vegetative compatibility groups in *Verticillium dahliae*. In Abstracts of the 10th International Verticillium Symposium, 16-20 November, Corfu, Greece.

Pantou, M. P., Mavridou, A. & Typas, M. A. 2003: IGS sequence variation, group-I introns and the complete nuclear ribosomal DNA of the entomopathogenic fungus *Metarhizium*: excellent tools for isolate detection and phylogenetic analysis. Fungal Genet. Biol. 38: 159-174.

Pramateftaki, P. V., Antoniou, P. P. & Typas, M. A. 2000: The complete DNA sequence of the nuclear RNA gene complex of *Verticillium dahliae*: intraspecific heterogeneity within the intergenic spacer region. Fungal Genet. Biol. 29: 19-27.

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P-32 Molecular characterization and functional analysis of a necrosis- and ethylene-inducing protein encoding gene family from *Verticillium dahliae*

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Verticillium dahliae Kleb. is a hemibiotrophic phytopathogenic fungus that causes wilt disease in a wide range of crops, including cotton. Successful host colonization by hemibiotrophic pathogens requires the induction of plant cell death to provide the saprophytic nutrition for the transition from the biotrophic to the necrotrophic stage. In this study, we identified an NPP1 (necrosis-inducing Phytophthora protein) domain-containing protein family containing nine genes in a virulent, defoliating isolate of *V. dahliae* (V592), named the VdNLP genes. Functional analysis demonstrated that only two of these VdNLP genes, VdNLP1 and VdNLP2, encoded proteins that were capable of inducing necrotic lesions and triggering defense responses in *Nicotiana benthamiana*, Arabidopsis and cotton plants. Both VdNLP1 and VdNLP2 induced the wilting of cotton seedling cotyledons. However, neither VdNLP1 nor VdNLP2 nor both targeted gene deletion mutants affected the pathogenicity of *V. dahliae* V592 in cotton infection. Similar expression and induction patterns were found for seven of the nine VdNLP transcripts. Through a comparison of the conserved amino acid residues of VdNLPs with different necrosis-inducing activities, combined with mutagenesis-based analyses, we identified several novel conserved amino acid residues, in addition to the known conserved heptapeptide GHRHDWE motif and the cysteine residues of the NPP domain-containing protein, that are indispensable for the necrosis-inducing activity of the VdNLP2 protein.

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P-33 Thiazole synthase Thi4 is essential for pathogenicity in the filamentous fungus *Verticillium dahliae*

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Living in the central cylinder of the vascular system, the saprotrophic fungus *Verticillium dahliae* has to survive with low nutrient concentrations supplied by the xylem fluid. Consequently the filamentous fungus has to synthesize all essential nutrients to survive and to develop its full pathogenic potential. This hypothesis was tested by disrupting the essential thiamine pathway. THI4 encodes for the thiazole synthase in the thiamine pathway. Thiamine pyrophosphate is an essential cofactor in all living systems where it plays an important role in amino acid and carbohydrate metabolism. The yeast Thi4p protein was found to be the first protein with a GR2 domain that binds NAD instead of FAD to catalyze the formation of adenylated thiazole (ADT). In *S. cerevisiae*, *A. thaliana* and *F. oxysporum* the highly conserved protein has a dual function. Besides the role as thiazole synthase, it increases the UV-damage tolerance of mitochondrial DNA. In *F. oxysporum* it is involved in the oxidative stress response and the knock-out shows no phenotype during infection.

We could show that in *V. dahliae* the knockout has no phenotype on full medium plates like in *F. oxysporum*. It has also wild type-like phenotype on minimal medium with different carbon sources. In UV-irradiation assays we could show that Thi4 is involved in the repair function of UV lesions like in *S. cerevisiae*. Thi4 knockouts of *F. oxysporum* were not impaired in the infection phenotype in host plants. Infection assays on *S. lycopersicum* with *V. dahliae* ΔTHI4 strain showed a mock-like phenotype. We could show that the fungus still invades the plants through the roots but cannot induce disease symptoms and is therefore apathogenic.

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P-34 The Cpc1 (CpcA/Gcn4) regulator of the cross-pathway control of amino acid biosynthesis is required for plant infection of the vascular pathogen *Verticillium longisporum*

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The plant pathogenic fungus *Verticillium longisporum* is the causal agent of early senescence and ripening in *Brassica napus* (oilseed rape, Canola) and other cruciferous crops. Verticillium wilts have become serious agricultural threats during the last decades. Verticillia infect host-plants through the roots and colonize xylem vessels of the host-plant, which provide an environment with limited carbon sources. *V. longisporum* induces the cross-pathway control in the xylem fluid to cope with an imbalanced amino acid supply.

The transcriptional activator gene VlCPC1 (similar to CpcA/GCN4) was knock-downed via RNA-mediated gene silencing and the expression of the two CPC1 isogenes (VlCPC1-1, VlCPC1-2) in *V. longisporum* could be reduced up to 85%.

The resulting mutants were more sensitive to amino acid starvation induced by 5-methyltryptophane (5-MT). In plant infection assays, the silenced mutant showed significantly less symptoms such as stunting and early senescence. Knockouts of CPC1 in the haploid *V. dahliae* were sensitive to amino acid starvation and strongly reduced in symptom formation in their host *Solanum lycopersicum* (tomato).

The hybrid *V. longisporum* and the haploid *V. dahliae* are the first phytopathogenic fungi, which were shown to require CPC1 for infection and colonization of their respective host plants oilseed rape and tomato.

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P-35 Comprehensive analysis of the intra- and extracellular metabolome of *Verticillium longisporum* 43 grown under different culture conditions

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Infection of rapeseed by the soil-borne, vascular fungal pathogen *Verticillium longisporum* results in severe economic damage worldwide. Till today knowledge on metabolic processes accompanying this interaction is rather scare. The entity of all small molecules of a fungus, the fungal metabolome, is very sensitive to environmental changes. It differs strongly in dependence of nutrient supply, like carbon and nitrogen sources. Therefore we investigated the metabolic situation of *Verticillium longisporum* 43 (VL43) under different nutrient conditions: The fungus was grown in full media (PDB), simulated xylem sap medium (SXM) as well as in xylem sap harvested from rapeseed plants. The cultivation in xylem sap should mimic biotroph, *in planta* condition for fungal growth and metabolism and may allow us to identify metabolites, which the fungus specifically produces in contact to plant material. To get comprehensive metabolome data of VL43 the mycelium (intracellular metabolome) and the culture supernatants, which contain secreted fungal metabolites as well as metabolized components of the medium (extracellular metabolome) was analyzed.

For analysis non-targeted metabolite fingerprinting was performed by UPLC-ESI-TOF-MS measurements. The tool box MarVis has been used for data processing like filtering, adduct correction, automated database search as well as clustering and visualization of the metabolite markers. The intracellular metabolome contains compounds, which accumulate independently from the culture media, like amino acids and small dicarboxylic acids. Clustering analysis also suggests that a huge amount of metabolites are specifically enriched in one of the three conditions. Anthranilate and its derivatives accumulate intra- and extracellular in SXM, but are highly enriched in/by fungus grown in xylem sap. The identity of metabolite markers are currently confirmed by MS/MS analysis, which is the prerequisite for reconstructing pathway specific induced for each condition.

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P-36 Reconstructing lipid metabolism in *Verticillium longisporum*

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Following the technical advances in lipid analysis achieved in the last years, lipids have been attributed in numerous studies crucial roles in the interaction between multicellular organisms and their microbial pathogens. In this study, we have developed methods aiming to characterize the lipidome of the oilseed rape pathogen *Verticillium longisporum*.

Our approach is based in its first stage on the direct infusion of lipid extracts by nanoelectrospray ionization (nanoESI, Advion) into a hybrid triple quadrupole/linear ion trap tandem mass spectrometer (4000 QTRAP®, AB Sciex). In a second stage, the data collected serves to build MS-coupled ultra performance liquid chromatography methods (UPLC®, Waters Corp.) for lipid species confirmation and routine analysis.

The obtained lipid profiles are then combined with genomic and transcriptomic data from the *Verticillium* genome database (VertiBase established by the BioFung consortium) for reconstruction of lipid metabolic pathways. The pathways we have reconstructed so far provide an overview of the lipid metabolism in *V. longisporum* and indicate several features, which might be relevant for its interaction with plant hosts. These features include: i) a particular ergosterol biosynthetic pathway found only in some filamentous fungi, ii) the accumulation of steryl glycosides, iii) the favoured acylation of sterol biosynthetic precursors, iv) the presence of α-hydroxy fatty acyl groups within a characteristic fatty acyl fingerprint, v) the sphingolipid biosynthetic machinery needed to synthesize C9 methylated long chain bases and complex membrane sphingolipids, and vi) a major glycerolipid pathway including the lipid classes PA, PC, PE, PI, PS, DAG and TAG.

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Poster
EPIDEMIOLOGY AND INTEGRATED CONTROL

P-37 Production of microsclerotia of *Verticillium dahliae* to an effective method of inoculation of plants

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Verticillium wilt caused by the fungus *Verticillium dahliae* Kleb. has become a serious threat for the olive tree cultivation in recent years. Microsclerotia (ms) formed by *V. dahliae* are infective and remain viable in the soil for years. At present, the main limitation to study epidemiological aspects and potential means of control of verticillium wilt is the lack of an effective inoculation method that reproduces the natural conditions of infection. The mass production of ms is of crucial importance for the artificial infestation of soil. This work had several objectives, first, to evaluate the ability of ms of 4 isolates of *V. dahliae* (V-004, V-024, V-025, V-117) representative of the fungal collection of the Group of Agroforestry Pathology at the University of Córdoba. To this end, the growth of these isolates was characterized at different temperatures (5, 10, 15, 20, 25, 30 and 35°C). At the same time, the number and size of ms produced in four solid (PDA, PDA diluted to 10%, PDA cellophane and minimal medium) and four liquid culture media, three in continuous agitation (SSN, Czapek-Dox and MSPA), and MSPA without agitation (once elapsed for 30 days of incubation) were determined. The growth of isolates between 0 and 30°C was perfectly adjusted with the model Beta Analytis, demonstrating a significant effect of temperature which was reflected in different growth curves of isolates. The optimum temperature ranged between 20 and 23°C and the maximum growth rate between 1.19 and 1.76 mm/day at 25°C at which major differences between isolates were identified. At 25°C, the sequence of isolates by growth rate was: V117 > V004 = V025 > V024. The production of ms depended on the interaction between isolates and growing medium. The isolate V117 only produced ms in Czapek-Dox (2.2×10^3 ms/ml) and in agitated MSPA, while the three remaining isolates produced them in all the media tested. The most productive and homogeneous medium in terms of size of microsclerotia was agitated MSPA. The latter is being used for the mass production of microsclerotia intended for artificial infestation of soil with different isolates of *V. dahliae*.

References

Bejarano-Alcázar, J., Melero-Vara, J. M., Blanco-López, M. A., Jiménez-Díaz, R. M. 1995: Influence of inoculum density of defoliating and non-defoliating pathotypes of Verticillium dahliae on epidemics of Verticillium wilt of cotton in southern Spain. Phytopathology 85: 1474-1481.

Blanco-López, M. A., Bejarano-Alcázar, J., Melero-Vara, J. M., Jiménez-Díaz, R. M. 1989: Current status of Verticillium wilt of cotton in southern Spain. Pathogen variation and population in soil. In: E. C. Tjamos, C. H. Beckman (Eds), Vascular Wilt Diseases of Plants: Basic Studies and Control. NATO ASI Series H: Cell Biology, Vol. 28: 123-132. Springer-Verlag, New York.

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P-38 Effects of surface drip irrigation on Verticillium wilt of olive in natural environmental conditions

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Verticillium wilt of olive (VWO) caused by *Verticillium dahliae*, is the most serious disease for olive yield in Spain. The expansion of drip-irrigated olive orchards is one of the causes of the current spread and increased severity of VWO attacks. But whether scheduled irrigation affects to the VWO epidemics is unknown. This study was aimed to assess the effects of water content in the soil and drip-irrigation frequency on the progress of VWO and to explore whether the temporal dynamic of fungus in the soil explains the effects before. Pot-experiment under natural environmental conditions was placed at early spring in an experimental field in southern Spain (Córdoba). The potted olive plants of susceptible *cv.* Picual grew in soil infested or not by *V. dahliae* isolate belonging to highly virulent defoliating pathotype (lethal), and were drip-irrigated with two rates of water content in the soil (high and low; HRW and LRW) and three irrigation frequencies (daily, weekly and daily-cum-weekly). Verticillium wilt symptoms were periodically diagnosed and assessed, and soil inoculum level of *V. dahliae* was monitored regularly. Preliminary data collected in the spring to fall period showed that VWO was affected stronger by the irrigation frequency than the rate of water content in the soil. The values of the final disease incidence and the final disease intensity index were significantly lower, and the standardized area under the disease progress curve was lower, in plants daily irrigated than at other irrigation frequencies, regardless of the rate of water content in the soil. Differences in the inoculum levels of *V. dahliae* (ufc.g^{-1}) in the soil between treatments did not explain the development of Verticillium wilt during the experimental period, but the inoculum density was affected stronger by the irrigation frequency than the rate of water content in the soil at late spring and early summer, when the disease incidence and intensity were highest. Research support by project RTA2011-00019-00-00 from INIA and project PP.TRA.TRA2010.10 from IFAPA, both partially funded by European Regional Development Fund (ERDF).

References

Cappaert, M.R., Powelson, M.L., Christensen, N.W., Stevenson, W.R., Rouse, D.I. 1994: Assessment of irrigation as a method of managing potato early dying. Phytopathology 84: 792-800.

Jefferson, P. G., Gossen, B.D. 2002: Irrigation increases Verticillium wilt incidence in a susceptible alfalfa cultivar. Plant Dis. 86: 588-592.

López-Escudero, F.J., Blanco-Lopez, M.A. 2005: Effects of drip irrigation on population of *Verticillium dahliae* in olive orchards. J. Phytopathol. 153: 238-9.

López-Escudero, F.J., Mercado-Blanco, J.M., Roca L., Valverde-Corredor, A., Blanco-Lopez, M.A. 2010: Verticillium wilt of olive in the Guadalquivir Valley (southern Spain): relations with some agronomical factors and spread of *Verticillium dahliae.* Phytopathol. Mediterr. 49: 370-380.

Moraño-Moreno, R., Bejarano-Alcázar, J., Rodríguez-Jurado, D. 2010: Efecto de la dosis y frecuencia de riego sobre los niveles de distintos propágulos de *Verticillium dahliae* en el suelo. XV Congreso de la Sociedad Española de Fitopatología. Vitoria-Gasteiz, España: 284. http://www.sef.es/congresos_sef.php?id_aplic=9&id_area=3

Xiao, C.L., Subbarao, K.V. 2000: Effects of irrigation and *Verticillium dahliae* on cauliflower root and shoot growth dynamics. Phytopathology 90: 995-1004.

Wheeler, T. A., Bordovsky, J. P., Keeling, J. W., Mullinix, B. G., Jr., Woodward, J. E. 2012: Effects of crop rotation, cultivar, and irrigation and nitrogen rate on Verticillium wilt in cotton. Plant Dis. 96: 985-989.

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P-39 Suppression of *Verticillium dahliae* by *Brassica juncea* seed meal amendment

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Biofumigation is considered as a potential control method to *Verticillium dahliae*. The method is defined as incorporation of brassicaceous green manures containing glucosinolates (GSLs). These compounds are hydrolyzed by the enzyme myrosinase to isothiocyanates (ITCs), which have a toxicological effect to microsclerotia. Recent studies have shown that the GSL concentration of the biomass of brassicas and the release efficiency of ITCs is too low to eliminate microsclerotia in natural soils. GSLs are found in all parts of the plant, but the highest concentrations are found in the seed or seed meal that remains after oil extraction.

Therefore *Brassica* seed meal amendments as an alternative biofumigation method can be more effective, because higher ITC concentrations are generated in soil. Seed meals provide additional advantages as their use is not restricted by seasonal production practices as in the case for green manure crops. The use of seed meals allows more rigorous control by increasing the application rates. Also the more uniform distribution of the residue in the soil profile relative to green manure systems can improve the biofumigation effect.

In previous studies the best effect to microsclerotia of *V. dahliae* was achieved by *Brassica juncea* seed meal amendments containing Sinigrin as a single GSL, which is hydrolyzed to 2-propenyl-ITC. The first objective of the present study was to evaluate the efficacy of seed meals of five *B. juncea* cultivars with a bioassay under standardized optimal conditions. Seed meals were mixed with a rate of 0.4 % (vol./vol.) in a sterile quartz sand filled in glass flasks and infested with 200 microsclerotia/g sand. Autoclaved seed meal with a deactivated glucosinolate-myrosinase-system served as a control. Water was added to adjust an optimum moisture level of 60% of maximum water capacity. Flasks were sealed immediately and incubated at 20 °C for 48 h. The sand of each flask was air dried and analyzed for viable microsclerotia using the wet sieving detection method. All tested seed meals showed an efficacy of 100%. Additionally the GSL concentration of the meals was analyzed by HPLC. The Sinigrin contents ranged from 90 to 109 µmol g^{-1}.The effects of the seed meals were compared with the maximum amounts of 2-propenyl-ITC release calculated on the measured Sinigrin concentrations. Considering the LD_{90} values of 2-propenyl-ITC, determined in a similar test system, the impact of seed meals can be explained widely as an ITC-related biofumigation effect.

Additionally *B. juncea* seed meal was tested with different doses in six different natural infested soils using the same bioassay. On base of dose-efficacy curves the LD_{90} values were calculated. In natural infested soils the ITC effect was reduced considering that 2-propenyl-ITC was prone to rapid microbial degradation and sorption to organic matter. According to soil characteristics the calculated doses to achieve an effect of 90% varied between 0.5-2.2% (vol./vol.). Conclusions for seed meal amendments in practice are discussed.

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P-40 Evaluation of organic amendments and microorganisms for the control of *Verticillium dahliae*

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One of the most important challenges of the present olive growing is the control of verticillium wilt caused by the fungus *Verticillium dahliae* Kleb. This fungus has the ability to form structures (microsclerotia) that resist and remain viable in the soil for a long time, which has contributed to the remarkable increase of this disease in recent years. The lack of effective chemicals, both for the treatment of soil and affected plants, motivates the search for alternative control methods. The objective of the present study was to evaluate different organic amendments of green manure (compost and other plant remains), animal (manure and slurry) and industrial waste, as well as several suspensions of microorganisms (mixture of various fungi and bacteria) and extracts from all of them, for the control of *V. dahliae*. To this end, two *in vitro* experiments were carried out. The first evaluated the biofumigant effect on mycelial growth using PDA plates with active growth of 4 isolates of *V. dahliae*. The mycelial growth of the colonies was measured for 10 days. In the second experiment, the effect on microsclerotia of a naturally infested soil with *V. dahliae* (≈55 microsclerotia/g soil) was evaluated. Solid products were mixed with the soil at a 1:1 ratio and then water content was adjusted to field capacity of the soil.

Liquid products were added directly to the ground. Soil samples were incubated at 24°C for 48 h. Subsequently, the inoculum level was analyzed in soil by wet sieving in MSPA media. Application of chicken manure and one of the microorganism mixtures resulted in a reduced mycelial growth of all isolates at rates of 100% and 90%, respectively. These same products, as well as a residue of dairies also reduced the viability of the microsclerotia of the soil up to 100%, while grape compost did up to 90% and composts of alperujo and cork were effective between 40% and 80%. Reduction of the dose of these products down to 10% showed lower effectiveness on the microsclerotia (being higher by the chicken manure), despite increasing the time of incubation to 8, 16 and 21 days.

Currently, selected doses of these organic amendments are being evaluated in applications to 1 litre plastic pots with olive trees of two susceptible cultivars, picual and cornicabra, growing in soil infested with *V. dahliae*.

References

Alfano G., Lustrato G., Lima G., Vitullo D., Ranalli G. 2011: Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. Biol. Control 58: 199-207

López-Escudero F.J., Mercado-Blanco J. 2011: Verticillium wilt of olive: a case study to I implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344: 1-50

Yangui T., Sayadi S., Gargoubi A., Dhouib A. 2010: Fungicidal effect of hydroxytyrosol rich preparations from olive mill wastewater against Verticillium dahliae. Crop Protection 29: 1208-1213

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P-41 Field application of non-pathogenic Verticillium genotypes for regulation of wilt on strawberry plants

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An application of non-pathogenic genotypes of *Verticillium dahliae Kleb.* populations was shown to control wilt symptoms in strawberry plants of the cultivar 'Elsanta' for different climate regions (Schubert *et al*. 2008). In cooperation with a locally based private biotech company, the potential for a marketable product based on the patented technology was investigated by developing production flow, elevating titre samples to a production-oriented scale and the conduction of experiments to prolong shelf life. The result was a soluble biologic compound that could be applied in field testing in three case studies in Brandenburg region in 2011 and 2012.

The objective of this study was to test the biologic compound for its applicability in practice. For further development of the technology, two questions stood at the centre of our research: 1) an assessment of ecological effectiveness of the compound to control wilt symptoms, and 2) an evaluation of practicable inoculation solutions from a farmers' perspective. Research was conducted in close cooperation with local strawberry producers. Inoculation of plants was included into the general workflow of strawberry plantation. Plants were inoculated for 30 min. After plantation, the test rows were treated by the respective farmers as the rest of the plantation.

The results of the field tests showed that inoculation led to a significant reduction of *Verticillium* infestation in the plant as was expected due to the previous experiments. The success rate for controlling wilt was c.a. 50%. However, as a side effect, microbial populations in the plant increased considerably. A strong interference of other pathogens colonising the relieved ecologic niche led to a significant increase of atypically accelerated wilt symptoms seemingly caused by triggering a sequence of diseases involving *Alternaria* and *Phytophtera*. Thus, rating showed no economic benefit for users, contradicting previous experiments in the laboratory and in field tests five years before. The evaluation of practicable solutions showed additive effects related to pre-conditioning of plant stock, previous infestation of *Verticillium* in soils, installation and treatment of the plantation as well as land use measures such as crop rotation. From the perspective of the farmers, economic benefit was due to complementing soil parameters with good quality plant material.

Competitive fruit production in the traditional producing regions in Europe can be ensured by ample yields, good plant quality and an efficient orchestration of supply chains. Regarding further development of an effective wilt control, the authors conceive a gap in the understanding of interrelationships in the delta of plant quality, soil parameters and land use measures. In order to solve basic questions regarding strawberry reproduction, monitoring and wilt control further research will need to address interdependencies in soil-borne diseases in field conditions based on the outcome that regulation of wilt control was successful, but enhanced the pathogen background to a complex course of diseases.

References

Schubert, P., Golldack, J., Schwärzel, H., Lentzsch, P. 2008: Pathogenicity in *Verticillium* on strawberry plants. 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: 138-143. Fördergemeinschaft Ökologischer Obstbau e.V., Weinsberg

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P-42 Detection of *Verticillium dahliae* in soil as a basis for disease risk prediction – 15 years soil analysis in Germany

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Verticillium dahliae causes in Germany severe economic losses in production of strawberry and trees. Reduction of soil inoculum by crop rotation has little effect, because of the wide host range of the fungus and the long viability of microsclerotia. The use of synthetic soil fumigants is restricted and therefore the disease is generally preventive controlled by determining the amount of inoculum in soil with a pre-planting soil analysis as basis for a disease risk assessment of potential planting sites.
15 years ago a soil test was established in practice, which is now widely used by growers in Germany and in neighbouring countries. Microsclerotia in soil are detected with a wet-sieving and plating method according to Harris et al. (1993) and modified by Neubauer and Heitmann (2011) using a semiselective polypectate medium. The method with a detection limit of 0.4 colony forming units/g dried soil is described in detail. The procedure is strongly standardized because small modifications are influencing the detection percentage. Using these standardized method three laboratories from the plant protection services Lower Saxony, Oldenburg, and North Rhine Westphalia, Bonn, as well as the University of Applied Sciences, Osnabrück, analyze more than 800 soil samples per annum.
Previously studies showed significant correlations between inoculum densities assessed by the presented plating method and observed disease incidence in strawberries. Therefore a system with 5 classes of infestation levels was established as a basis for the disease risk prediction. In studies the accuracy of the method was evaluated. The non-random distribution of microsclerotia in soil causes a large variability of results in replicated analysis especially in soils with low inoculum densities near the detection limit. The accuracy of the method is increasing with increasing infestation levels. But relating to the classification of the samples and sites the method shows a satisfactory performance and reproducibility. Using this detection method it is not possible to discriminate between *V. dahliae* and *V. longisporum*. But on base of information about the cropping history of a site the species of the determined population can be estimated.

References:

Harris, D. C., Yang, Y. R. & Ridout, M. S., 1993: The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathology 42, 238-250.
Neubauer C. & Heitmann, B., 2011: Quantitative detection of *Verticillium dahliae* in soil as a basis for selection of planting sites in horticulture. Journal für Kulturpflanzen, 63 (1), 1-8.

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P-43 A comparison of real-time PCR protocols for the quantitative monitoring of asymptomatic olive infections by *Verticillium dahliae* pathotypes

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Early, specific and accurate *in planta* detection and quantification of *Verticillium dahliae* are essential to prevent the spread of Verticillium wilt in olive using certified pathogen-free planting material and development of resistance. We comparatively assessed the accuracy, specificity and efficiency of eight real-time quantitative PCR (qPCR) protocols published since 2002 for the specific detection and quantification of *V. dahliae* in various host plant species and in soil, using a background of DNAs extracted from olive roots, stems and leaves. Results showed that some of those protocols were not specific for *V. dahliae* and/or were inhibited when using backgrounds other than water. Ranking of protocols according to a weighted score system placed protocol TAQ (based on IGS rDNA target gene; Bilodeau *et al*. 2012) and SYBR-4 (based on β-tubulin 2 target gene; Atallah *et al*. 211) first in sensitivity and efficiency for the quantification of *V. dahliae* DNA in small amounts and different types of olive tissues (root and stem) tested. Use of TAQ and SYBR-4 protocol allowed accurate quantification of *V. dahliae* DNA regardless of the background DNA with a detection limit being fixed at a C_T of 36 (approximately 18 fg for SYBR-4 and 15 fg for TAQ) of *V. dahliae*). The amount of DNA from defoliating (D) and nondefoliating (ND) *V. dahliae* pathotypes was monitored in Verticillium wilt-resistant 'Frantoio' olive using the TAQ and SYBR-4 protocols. In the infection bioassay, higher amounts of D-*V. dahliae* DNA were measured in olive stems, whereas the average amount of fungal DNA in roots was higher for ND-infected plants than D-infected ones. Overall, *V. dahliae* DNA amount in all olive tissues tested tended to slightly decrease or remain stable by the end of the experiment (35 days). The SYBR-4 and TAQ protocols further enabled detection of *V. dahliae* in tissues of symptomless plants, suggesting that both techniques can be useful for implementing certification schemes of pathogen-free planting material as well as a helpful tool in breeding resistance to *V. dahliae* in olive.

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References

Atallah, Z. K., Bae, J., Jansky, S. H., Rouse, D. I., Stevenson, W. R. 2007: Multiplex real-time quantitative PCR to detect and quantify Verticillium dahliae colonization in potato lines that differ in response to Verticillium wilt. Phytopathology 97: 865-872.

Bilodeau, G. J., Koike, S. T., Uribe, P., Martin, F. N. 2012: Development of an Assay for Rapid Detection and Quantification of Verticillium dahliae in Soil. Phytopathology 102: 331-343.

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P-44 Optimizing detection of *Verticillium dahliae* in olive tree samples

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Verticillium dahliae is a major cause of olive wilt where crops have been planted in soils contaminated with pathogen inoculum. This is common in many European regions where crops susceptible to *V. dahliae*, particularly cotton, have been grown. A three year EU funded project, VERTIGEEN (), is underway to improve the detection and surveillance of *V. dahliae* in soils and plants from these soils throughout a number of European countries.

In recent years WUR has developed primers for molecular detection of *Verticillium dahliae* (Van Doorn *et al.*, 2009). These primers were validated for specificity using *Verticillium* isolates and other pathogens as targets and were compared with primers developed by Bilodeau (Bilodeau *et al.*, 2012) and FERA. A TaqMan PCR, based on the WUR primers was used to examine olive tree samples for the presence of *V. dahliae*. Leaf and shoot samples from 5-10 branches per tree and 5-10 trees (with and without symptoms) per location were collected from olive plantations affected by Verticillium wilt in Spain, Portugal, Italy and Greece. Soil samples from the same sites were tested for *V. dahliae* by FERA (results presented by Peters *et al.* elsewhere in this Symposium).

V. dahliae was detected in plant samples from all sites and molecular detection proved to be more sensitive than a standard plating assay. However, the percentage of positive samples per tree strongly varied, indicating that for a reliable result several samples from different branches should be tested. In addition, successful detection of *V. dahliae* in symptomatic trees depended on which part of the plant was used. In wood samples the fungus could be detected more successfully in comparison with leaf samples. Also wood samples from last year's part of a shoot proved to be more often positive in TaqMan PCR than this year's part.

These data show that it is possible to use PCR based methods for large-scale screening of standing trees and planting stock for infection by *V. dahliae.* Consequently, it should be possible to use on-site molecular detection methods (such as recent developments in isothermal methods such as LAMP) in order to prevent distribution of this pathogen.

This work was funded by the EU FP7 programme (Vertigeen project).The contributions of the project partners (INOLEO and COTEVISA, Spain; EDOEE, Greece; AAR, Portugal; PELOSI, Italy; FORSITE, UK; LOEWE, Germany) are gratefully acknowledged.

References

Bilodeau, G. J., Koike S. T., Uribe P., Martin F. N. 2012: Development of an assay for rapid detection and quantification of *Verticillium dahliae* in soil. Phytopathology 102: 331-343.

Van Doorn, J., Pham, K. T. K., Hiemstra, J. A. 2009: Molecular detection of *Verticillium dahliae* in soil. 10th International Verticillium Symposium, abstract book: 94.

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P-45 Quantification of *Verticillium dahliae* in different parts of naturally infected olive trees

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Olive (*Olea europaea* L.) is an economically important fruit tree worldwide and particularly in the Mediterranean regions. *Verticillium* wilt is one of the most important disorders in olive orchards throughout the world. Two pathotypes of *V. dahliae* isolates are known as responsible for *Verticillium* wilt, defoliating (D) and nondefoliating (ND). Understanding the distribution pattern of *Verticillium dahliae* within infected plants through the infection process can give us insights into the pathogen attitude in infected plants and help to design effective sampling methods to screen plants for infection. In the present research, QPCR technique has been used to quantify the abundance of the pathogen in different parts of infected olive plants. Samples were collected from diseased olive trees (different cultivars) in infected orchards in Spain, Greece and Portugal, at least 10 trees per location (see Hiemstra *et al.*, elsewhere in this Symposium). From each plant 5-10 branches were sampled to assess the quantity of pathogen in lower, middle and upper parts of the shoot. The pathotype of *Verticillium* was determined by using specific PCR-primers. Our results revealed that the density of the pathogen in the upper and middle parts is mostly higher than in the lower part of the shoots. Also, in some of the plants the colonization of pathogen in the upper part is relatively more than in the middle part, although in some cases it is reverse. What stands out from the results is that the distribution of *V. dahliae* in the olive trees does not follow a particular pattern. In other word, it is highly likely that the distribution process is strongly influenced by a number of factors such as season, plant cultivar, pathotype of the pathogen, the number of initial root infections, the abundance of first inoculum and so on. This approach has provided useful information not only for diagnostic purposes but also for studying the epidemiology of *Verticillium* in olive trees.

This work was partly funded by EU FP7 (Vertigeen project). Also, the contributions of the project partners (INOLEO and COTEVISA, Spain; EDOEE, Greece; AAR, Portugal; PELOSI, Italy; FORSITE, UK; LOEWE, Germany) are gratefully acknowledged.

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P-46 *Verticillium dahliae* - Soil inoculum density and stem necrosis in Bavarian sycamore maple stands

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Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a significant chronic problem in many herbaceous and woody plant species and is widely distributed in sycamore maple (*Acer pseudoplatanus* L.). In the last decade, this soilborne pathogen increasingly caused vascular wilt and, probably in addition with abiotic factors, stem necrosis in maple stands of southern Germany (Siemonsmeier *et al*. 2012). Typical symptoms are a disruptor of the cortex in the lower trunk area with a brown to olive green discoloration of the sapwood.
Verticillium dahliae enters the root system from the soil, where it is present as persistent microsclerotia, mycelia and conidia. In order to understand disease dynamics, the relationship between soil inoculum density and the occurrence of stem necrosis has been studied. In soil, *Verticillium dahliae* populations occur aggregated; therefore various samples were taken per plot and combined to a mixed sample. To investigate the distribution of the highly durable microsclerotia and the infestation level of soil, samples were taken in diseased maple stands and neighbouring forest stands without maple. Samples were analysed by a detection method quantifying microsclerotia according to Harris *et al*. (1993) and Neubauer & Heitmann (2011). Obtained isolates were identified by morphology and PCR with specific primers (Debode *et al*. 2011).

In all investigated forest stands wilt symptoms of sycamore maple were present, and the extend of stem necrosis ranged from 0 to 57%. No correlation between soil inoculum density and the occurrence of stem damages was evident. Colonies of Verticillium were abundant in soil samples of sycamore maple stands as well as in forest stands with spruce or oak. The origin of stem necrosis could be traced back to the dry summer of 2003 for symptom development. Experiments are ongoing to elucidate the possible impact of drought stress on disease development to improve risk analysis for sycamore maple cultivation.

References

Debode, J., Van Poucke, K., França, S.C., Maes, M., Höfte, M., Heungens, K. 2011: Detection of Multiple *Verticillium* Species in Soil Using Density Flotation and Real-Time Polymerase Chain Reaction. Plant Disease 95: 1571-80.

Harris, D.C., Yang, J.R., Ridout, M.S. 1993: The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathology 42: 238-250.

Neubauer, C., Heitmann, B. 2011: Quantitativer Nachweis von *Verticillium dahliae* als Grundlage der Flächen-auswahl im Gartenbau. Journal für Kulturpflanzen 63 (1): 1-8.

Siemonsmeier, A., Nannig, A., Kühn, A., Blaschke, M. 2012: Spatial patterns of microsclerotia from *Verticillium dahliae* Kleb. in soils of Bavarian maple stands. Journal für Kulturpflanzen 64 (9): 348-353.

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P-47 *Botryosphaeria* spp. isolated from olive (*Olea europaea*) sampled for detection of *Verticillium dahliae*

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Verticillium dahliae is a major cause of olive wilt in many European regions where crops susceptible to *V. dahliae*, particularly cotton, have been grown before. To improve the detection and control of *V. dahliae* in soils and in plants from these soils throughout a number of European countries, a three year EU funded project, VERTIGEEN (www.vertigeen.eu), was started recently.

To develop an optimal sampling strategy and efficient sample processing methods for detection of *Verticillium dahliae* (Vd), samples from olive trees in different Mediterranean countries were examined (Hiemstra *et al*., presented elsewhere in this symposium). One group of samples, collected from old olive trees at different locations around the city of Foggia in Italy showed characteristic symptoms of Verticillium wilt. However, when tested with a Vd-specific TaqMan PCR only very few samples were positive for Vd. This result was supported by a standard agar-plating assay. Almost no growth of Vd was observed, whereas several other fungi were isolated frequently. Sequence analysis of the ITS regions of the most commonly isolated types (BLAST search) resulted in the most probable identity of these fungi being *Aspergillus niger*, *Fusarium spp*. and several *Botryosphaeria spp.* with *B. obtusa* and *B. stevensii* being most frequent. Many species of the *Botryosphaeriaceae* are known as common pathogens causing fruit rots or dieback in many woody plants (1) and *B. dothidea* and *B. obtusa* have been reported causing fruit rot and branch canker or dieback in olive (2, 3). Therefore we used generic primers for *Botryosphaeriaceae* species (4) to test all samples once again. This resulted in 66 per cent of the branches from the above mentioned Italian samples being positive for *Botryosphaeria* in contrast to the samples from other countries that all tested negative. These results indicate that at least locally fungi other than *V. dahliae* may contribute to dieback and death of olive branches which should be taken into account when assessing Verticillium wilt incidence. A specific on site test for *V. dahliae* would help to discriminate infection by these fungi from infection by *V. dahliae* as the symptoms might be misleading.

This work was funded by the EU FP7 programme (Vertigeen project).The contributions of the project partners (INOLEO and COTEVISA, Spain; EDOEE, Greece; AAR, Portugal; PELOSI, Italy; FORSITE, UK; LOEWE, Germany) are gratefully acknowledged.

References

Moral J., Luque F., Trapero A. 2008: First Report of *Diplodia seriata*, the Anamorph of "*Botryosphaeria*" *obtusa*, Causing Fruit Rot of Olive in Spain. Plant Dis. 92: 311.

Moral J., Muñoz-díez C., González N., Trapero A., Michailides T. J. 2008: Characterization and pathogenicity of *Botryosphaeriaceae* species collected from olive and other hosts in Spain and California. Phytopathology 100: 1340-51.

Sinclair W. A., Lyon H. H., Johnson W. T. 1987: Diseases of trees and shrubs. Cornell University Press.

Spagnolo A., Marchi G., Peduto F., Phillips A. J. L., Surico G. 2011: Detection of *Botryosphaeriaceae* species within grapevine woody tissues by nested PCR, with particular emphasis on the *Neofusicoccum parvum* / *N. ribis* complex. Eur. J. Plant Pathol. 129: 485-500, 2011.

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P-48 Status of *Verticillium dahliae* race 2 in Tunisia

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Verticillium wilt (VW) caused by *Verticillium albo-atrum* Reinke & Berthold and *V. dahliae* Klebahn, is a widespread disease in many parts of the world causing usually substantial damage. In Tunisia, *V. dahliae* is the predominant species and has been obtained from different plant species. A collection of 91 *V. dahliae* isolates were used for race typing analysis using the root-dipping technique. Classification of isolates to races was attempted by examining their pathogenicity on two differential tomato cultivars cvs. Ventura (lacking the *Ve* resistance gene) and Rio Grande (possessing the *Ve* gene). Of these 91 isolates, 51 obtained from different hosts: tomato (44), potato (2), melon (2), artichoke (2) and olive tree (1) were typed as race 2. The other 37 isolates obtained from tomato (29), potato (4), eggplant (2), artichoke (1) and olive tree (1) were typed as race 1. The remaining 3 isolates from tomato (2) and potato (1) proved non-pathogenic to tomato. Moreover, variation in aggressiveness towards both tomato cultivars was apparent among isolates of *V. dahliae* race 2, via the index of leaf damage and plant stunting. Furthermore, the behavior of tomato cultivars possessing the *Ve* gene, most grown in Tunisia, against selected isolates of *V. dahliae* race 2 was assessed. Under controlled conditions, these cultivars exhibited varying degrees of susceptibility to VW ranging from moderate to high as measured by leaf damage index and plant stunting. When grown in a soil naturally infested by *V. dahliae* race 2, VW incidence was 100% for all the resistant cultivars tested, four months post-planting. The extent of vascular discoloration occasioned by VW reached and even exceeded the half of stem height for some cultivars.

We can conclude that artichoke is considered as a new host of race 2 in the world, whereas potato, melon and olive tree are known hosts of it reported in Tunisia. This suggests that these hosts which are potential reservoirs for both *V. dahliae* races should not be in any case considered in a rotation sequence, especially where tomato follows these crops. Race 2 is now present in three coastal regions (Sousse, Monastir and Nabeul) where vegetable growing is widespread. These results highlight the urgent need of tomato genotypes with useful levels of resistance to *V. dahliae* race 2. The combined use of these genotypes with other control measures such as solarisation, green manures, grafting and biological control may be efficient in controlling this disease.

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P-49 Report of wilt caused by *Verticillium dahliae* on okra (*Albemoschus esculentus*) in Italy

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A widespread mortality of okra (*Abelmoschus esculentus* (L.) Moench; *Malvaceae*) was recently observed in small garden plots near Foggia (Apulia, Southern Italy).
The mortality is due to a disease known as okra wilt, caused by *Verticillium dahliae*. The most evident symptom consists in yellowing of the oldest leaves, stunting, which often develops a burnt appearance, particularly around the margins, followed by wilting of the plant. Additionally, severe brown discoloration of vascular tissues along the stem of infected plants is frequently observed. A fungus was consistently isolated from discoloured vascular tissues on tap-water-agar and on potato-dextrose agar. Preliminary identification of the fungus was based on morphological characteristics of conidia, conidiophores and microsclerotia (Hawksworth and Talboys, 1970) Isolates no. Vd 3919 and Vd 3920 were maintained in the collection of Department of Agricultural and Environmental Science, Chemistry and Plant Protection, University of Foggia. Molecular identification was based on the amplification and sequencing of the ITS1-5.8S-ITS2 region of ribosomal DNA of the fungal isolates and revealed 99-100% similarity with *V. dahliae* sequences (GenBank accession no. EU835817, AY555949, AY 555950, AF 364004). Healthy 50-day old okra plants (cv. Clemson Spineless) were potted in a steam-disinfested soil mix in greenhouse at 24-26°C and used for pathogenicity tests. On June 2010, two colonies of *V. dahliae* grown on PDA were homogenised in sterile tap water to a concentration of $5x10^5$ CFU ml^{-1}. Twenty seedlings were inoculated by root dipping in the inoculum suspension and transplanted in 16-cm diam plastic pots containing a mixture of soil, sand and peat (1:1:1 w/w/w). Ten uninoculated plants served as control.
First symptoms were observed 30 days after inoculation and consisted in wilting, vascular discoloration, stunting, epinasty, moderate defoliation and eventually plant death. *V. dahliae* was re-isolated in August from all the inoculated plants. In contrast, no symptoms were detected on uninoculated control plants.
To our knowledge, this is the first report in Italy of a wilt disease on Okra caused by *V. dahliae*.

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References

Hawksworth D.L., Talboys P.W., Descriptions of Pathogenic Fungi and Bacteria N° 256, C.M.I. 197

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P-50 Effects of experimental warming on the colonization of oilseed rape with *Verticillium longisporum*

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Within the research framework KLIFF (Climate Impact and Adaptation Research in Lower Saxony, Germany), potential effects of rising air and soil temperatures on the life cycle of *Verticillium longisporum*, an important pathogen in oilseed rape, was investigated theoretically (Siebold & Tiedemann 2012a) and experimentally utilizing climate chambers and a field soil warming facility (Siebold & Tiedemann 2012b) in the two consecutive growing seasons 2010/11 and 2011/12. Treatments reflected warming scenarios for Lower Saxony, Germany, by 2050 (mid-term) and 2100 (long-term) as projected by regional climate models. Oilseed rape growth and development responded linearly to increasing temperatures with an average flowering advance of 7 days per 2°C warming. *V. longisporum* colonization, measured by qPCR at different sampling time points, correlated with plant growth stage and was advanced in warmer chambers and plots. In the field experiment 2010/11, plants growing in warmest plots were significantly stronger colonized with *V. longisporum* than plants of all other plots. Warming had much stronger effects on the susceptible cultivar Falcon in enhancing colonization with the pathogen than on the tolerant genotype SEM. This suggests that, besides short crop rotations, warming may be an additional driver for an increased importance of this soil borne pathogen in the future (Siebold & Tiedemann 2013).

References

Siebold, M., Tiedemann, A.v. 2012a: Potential effects of global warming on oilseed rape pathogens in Northern Germany. Fungal Ecol 5 (1): 62-72

Siebold, M., Tiedemann, A.v. 2012b: Application of a robust experimental method to study soil warming effects on oilseed rape. Agr Forest Meteorol 164: 20-28

Siebold, M., Tiedemann, A.v. 2013: Effects of experimental warming on fungal disease progress in oilseed rape. Glob Change Biol, DOI: 10.1111/gcb.12180

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Poster
BIOLOGICAL CONTROL AND MICROBIAL ECOLOGY

P-51 Strategies of the soil bacterium *Streptomyces lividans* to suppress *Verticillium dahliae* and *Verticillium longisporum*

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We found for the first time that the spore-forming soil bacterium Streptomyces lividans strongly reduces the germination of *V. dahliae* and *V. longisporum* conidia, and the subsequent growth of hyphae. Quantification by the use of DNA-intercalating dyes and Calcofluor-staining revealed that during prolonged co-cultivation bacterial hyphae proliferate to a dense network, provoke a poor development of the fungal vegetative hyphae, and an enormous reduction of fungal conidia and microsclerotia. Upon individual application to seeds of the model plant *Arabidopsis thaliana*, either the bacterial spores, or the fungal conidia germinate at or within the mucilage, including its volcano-shaped structures. The extension of hyphae from each individual strain correlates with the reduction of the pectin-containing mucilage-layer. Proliferating hyphae then spread to roots of the emerging seedlings. Plants, which arise in the presence of Verticillium within agar or soil, have damaged root cells, an atrophied stem and root, as well as poorly developed leaves with chlorosis symptoms. In contrast, *S. lividans* hyphae settle in bunches preferentially at the outer layer near tips and alongside roots. Resulting plants have a healthy appearance including an intact root system. *A. thaliana* seeds, which are co-inoculated with Verticillium and *S. lividans*, have preferentially proliferating bacterial hyphae within the mucilage, and at roots of the outgrowing seedlings. As a result, plants have considerably reduced disease-symptoms (Meschke & Schrempf, 2010). Using HPLC and LC-MS analysis of cultures containing *S. lividans* alone or grown together with Verticillium, we found that the prodiginine undecylprodigiosin is highly abundant, and the prodiginine-type streptorubin B is present in smaller amounts. Within co-cultures, the quantity of undecylprodigiosin increased considerably and concentrated at and within fungal hyphae. The addition of purified undecylprodigiosin to growing *V. dahliae* hyphae strongly reduced microsclerotia formation. Undecylprodigiosin was also produced, when *S. lividans* grew on the roots of developing *Arabidopsis thaliana* plants. Furthermore, the presence of the Streptomyces prodiginine producer led to an efficient reduction of Verticillium hyphae and microsclerotia on plant-roots. Based on these novel findings, we deduce that the prodiginines investigated lead to multiple cellular effects, which ultimately impair specific pathways for signal transduction and apoptosis of the fungal plant pathogen in the absence or presence of *Arabidopsis thaliana* (Meschke *et al*., 2012). On-going studies reveal that additional compounds play a role. As spores of the beneficial *S. lividans* strain are obtainable in large quantity, its application for biocontrol is highly attractive.

References

Meschke, H., Schrempf, H. 2010: *Streptomyces lividans* inhibits the proliferation of the fungus *Verticillium dahliae* on seeds and roots of *Arabidopsis thaliana.* Microb. Biotechnol. 3: 428-43.

Meschke H., Walter, S., Schrempf, H. 2012: Characterization and localization of prodiginines from *Streptomyces lividans* suppressing *Verticillium dahliae* in the absence or presence of *Arabidopsis thaliana*. Environ. Microbiol. 14: 940-952.

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P-52 Biological control of the phytopathogenic fungi Verticillia by the rhizobacterium *Pseudomonas fluorescens*

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The filamentous soil-borne fungi *Verticillium longisporum* and *V. dahliae* possess phytopathogenic properties and are responsible for increasing economical losses in the cultivation of oilseed rape (*Brassica napus*)(Vl), and e.g. tomato, olive, lettuce, potato (Vd), respectively. Antagonistic bacteria like *Pseudomonas fluorescens* can be used as biological control agents to reduce the infection intensity of saprophytic fungi in the absence of appropriate fungicides. In this project, we want to study the counteractive potential of *P. fluorescens* on the development of *V. longisporum* and try to understand the processes of interaction between these organisms on a molecular and genetic level.

In plant infection assays with *B. napus* we were able to show the biocontrol potential of *P. fluorescens* towards *V. longisporum*. The plant vitality was significantly increased in the presence of *P. fluorescens* and typical disease symptoms by *V. longisporum* such as formation of necrosis and secondary branching were comparable to mock plants. Regarding the factors plant height and biomass, the biocontrol plants are ranking intermediately between the infected and the mock plants.

We could observe comparable effects in tomato plants that were infected with *V. dahliae*. Symptoms of infected plants that were additionally treated with *P. fluorescens* were strongly reduced and behaved almost like mock plants.

P. fluorescens produces a variety of secondary metabolites that are all controlled by the two-component system GacS/GacA. Mutants of *P. fluorescens* deficient in the production of single secondary metabolites were still able to inhibit the germination of fungal spores. Knockouts of the general regulator *gacA* of *P. fluorescens* lost the ability to inhibit germination of the fungus. The crucial impact of gacA in its antagonistic properties could be confirmed *in planta*.

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P-53 The potential biological control of fluorescent Pseudomonads against *Verticillium dahliae* olive (*Olea europea* L.) wilt pathogen

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Verticillium wilt disease of olive (*Olea europea* L.) incited by *Verticillium dahliae* is an important disease problem, which is difficult to manage and control in Algéria and worldwide. The disease causes general decline and final death of infected trees. Other symptoms may appear as canker, root rot, the dieback, and wilting which consequently result in economic losses to olive tree and their productivity. The diseases are predominantly controlled by chemical pesticides. New restrictions on application of these chemicals and environmental considerations have led to an increased interest in biocontrol agents. Fluorescent pseudomonads were cited as successful potential biocontrol agents. Therefore, the present work was undertaken to search for the indigenous pseudomonads population in Algérian habitat for isolates with potential antagonism against *Verticillium dahliae*. Forty isolates of fluorescent pseudomonads were isolated. Twenty eight (28) isolates were obtained from rhizosphere field soil under healthy and Verticillium infected olive orchards at different location in Algéria. The rest of them (12) isolates of the same bacteria were isolated from vegetable cultivated fields. Their antagonistic activity in vitro against two pathogenic isolates of *V. dahliae* was assayed on three type of culture media namely KB, PDA, and a mixture KB and PDA. Most of those isolates (95%) showed antagonistic effect against *V. dahliae*. Eight isolates caused a total inhibition of mycelium growth and microsclerotia formation on different medium. The antagonistic activity in situ using combination of the pathogen and six bacterial isolates showed that the growth of potato plants was significantly improved in the presence of those bacteria. Disease symptoms were also significantly reduced by 74% in contrast with the control.

References

Kuchta, P., Jpcz, T., Korbin, M. 2008. The suitability of pcr-based techniques for detecting Verticillium dahliae in strawberry plants and soil. Journal of Fruit and Ornamental Plant Research Vol.16: 295-304 Lemanceau, P. 1992. Effets bénéfiques de rhizobactéries sur les plantes: exemple des Pseudomonas spp. fluorescents. Agronomie 12: 413-437.

Prieto, P., Navarro-Raya, C., Valverde-Corredor, A., Amyotte, S.G., Dobinson, K. F., Mercado-Blanco, J. 2009: Colonization process of olive tissues by Verticillium dahliae and its in planta interaction with the biocontrol root endophyte Pseudomonas fluorescens PICF7. Microbial Biotechnology 2(4): 499–511.

Sanei, S. J., Razavi, S. E. 2011: Suppression of Verticillium wilt of Olive by Pseudomonas fluorescens. American Journal of Experimental Agriculture. 1(4): 294-305.

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P-54 Screening of the biocontrol agent *Paenibacilus alvei* K165 as a talk preparation against *Verticillium dahliae*

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The aim of this study was to develop and evaluate the efficacy of easy to apply formulations of an already known biocontrol agent (BCA) of *V. dahliae*. For this purpose, a talk preparation of the BCA *Paenibacilus alvei* K165 was either mixed with potting soil at a ratio of 1%, 5% and 10% and eggplants were grown on this substrate or it was used for seed coating. At the stage of the 3-4th leaf the plants were transplanted to soil infested with 20 *V. dahliae* microsclerotia per gram of soil. It was observed that the ratios 5% and 10% of K165 were the most effective in controlling Verticillium wilt. On the contrary, the seed coating treatment was ineffective. The differences in the efficacy of the various biocontrol preparations to control Verticillium wilt reflect the observed differences in the size of the BCA population between the treatments. Furthermore, it was shown that PR1 and PR4 were over-expressed in the 10% treated plants compared to the seed treated and the inoculated control plants; linking the observed resistance with the induction of plant defense mechanisms.

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P-55 Influence of antagonistic micro-organisms on the growth of strawberry plants in the presence of *Verticillium dahliae*

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Strawberry, an important berry culture in Germany, can be affected by *Verticillium* spp., causing the *Verticillium* wilt disease. Unfortunately in organic and in conventional production no efficient control systems are available. Therefore, a strategy for biological control of *Verticillium* spp. was investigated within a national founded project.

Four micro-organisms (*Trichoderma harzianum* T58, *T. atroviride* P1, *Metarhizium anisopliae* Ma43 and *Bacillus amyloliquefaciens* FZB24) showing an antagonistic potential against the pathogen in *in vitro* screening were selected for *ad planta* experiments. The strawberries used for the trials were Frigo plants cv. Honeoye.

The antagonistic fungi were grown on solid substrate and the conidia were washed off and brought to a concentration of 10^5 conidia mL^{-1}. The bacterium was provided as product (FZB24® fl.) and used as described on the package. A Mixture of the four antagonist suspensions in the same proportion was also tested.

The pathogen (*V. dahliae*) was produced as conidia, used at a concentration of 10^5 conidia mL^{-1}, and as microsclerotia (MS).

In greenhouse and field experiments the antagonists were applied by root dipping before planting and by watering four times after planting. The pathogen was added by pipetting the conidial suspension directly on the roots before covering with soil or MS were mixed into the soil before planting for the greenhouse trials. In the field experiments the pathogen was added at the planting hole as a mixture of MS and sand.

For the greenhouse trials growth and yield parameters were determined and for the field experiments a visual rating was made because in both cases no clear infection symptoms were monitored. The concentration of MS over time was also monitored.

In the greenhouse experiments the growth of the strawberry plants was positively influenced by the antagonist when *V. dahliae* was present. The highest increment in biomass increase, number of runners per plant and root length was achieved by using *T. harzianum*. For plant height and leaf dry mass the highest values were achieved by *M. anisopliae* followed by *T. harzianum*. The highest values for root dry mass and number of plants per runners per plant were achieved by *T. atroviride* and *B. amyloliquefaciens,* respectively. At the end of the trails no dead plants were observed when *T. harzianum* and *M. anisopliae* were applied. In a first field trial *M. anisopliae* had more than 80% of healthy plants whereas the other treatments were about 50% except for the Mixture with less than 30%. In a second field trial the number of dead plants was monitored over time. Six months after planting, 7% of dead plants were observed in the control and in the *T. atroviride* treatment. In the other treatments not more than 3% were monitored. After the winter, the number of dead plants ranged between 20% (*T. harzianum*) and 14% (*M. anisopliae*, *T. atroviride*, Mixture).

The concentration of MS measured in the greenhouse experiments, showed generally a reduction in the proliferation of MS for all antagonists. In the field trials the measurement over time showed no clear tendency.

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P-56 Molecular *in planta* test for the detection of Verticillium species in hops and initial steps towards biological control

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Verticillium wilt, caused by *V. albo-atrum* Reinke & Berthold and *V. dahliae* Klebahn, is a devastating disease in hops causing considerable economic crop losses (Down *et al*. 2007). The soilborne pathogen can survive several years in soil by producing resting structures (Wilhelm 1955; Down *et al*. 2007). Additionally, up to now effective fungicides are not available. Therefore, a fast and accurate detection system as well as alternative control measures to combat the wilt is urgently needed. The biological control is an environmentally friendly plant protection strategy using plant promoting as well as natural antagonistic effects of microorganisms (Berg 2009). The standard cultivation method following by conventional PCR is time-consuming and laborious. Hence, in a cultivation-independent method DNA was isolated directly from the hop bine with a commercially available kit. We established a multiplex real-time PCR assay to detect *V. albo-atrum* and *V. dahliae* simultaneously using TaqMan techniques (Maurer *et al*. accepted). The combination of DNA isolation and the real-time PCR assay is a useful tool for *V. albo-atrum* and *V. dahliae* quantification. The newly developed method is more accurate, sensitive and time-saving compared to the standard PCR technique. This molecular *in planta* test is an application for routine analysis. In addition, it can be helpful for effective plant disease management. Furthermore, we are looking for a control measure to prevent and combat the fungus. Therefore, we selected four bacteria (*Serratia plymuthica*, *Pseudomonas poae*, *Burkholderia terricola* and *Stenotrophomonas rhizophila*) with beneficial and antagonistic properties (Wolf *et al*. 2002; Berg *et al*. 2005; Berg 2009; Gasser *et al*. 2009; Zachow *et al*. 2010). The colonization of the endosphere and rhizosphere of roots was proven successfully by reisolation and microscopic visualization of DsRed-labeled transformants. The analysis with confocal laser scanning microscopy demonstrated the colonization. The bacterial abundances ranged from $\log_{10}$ 3.1 to $\log_{10}$ 4.9 CFU g^{-1} root fresh weight in the rhizosphere and from $\log_{10}$ 3.0 to $\log_{10}$ 6.2 CFU g^{-1} root fresh weight in the endosphere, whereat *B. terricola* showed the highest cell densities.

References

Berg G., Krechel A., Ditz M., Sikora R. A., Ulrich A., Hallmann J. 2005: Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol. Ecol. doi:10.1016/j.femsec.2004.08.006

Berg G. 2009: Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biot. doi:10.1007/s00253-009-2092-7

Down G., Barbara D., Radišek S. 2007: *Verticillium albo-atrum* and *V. dahliae* on hop. Bull. OEPP 37: 528-535.

Gasser I., Cardinale M., Müller H., Heller S., Eberl L., Lindenkamp N., Kaddor C., Steinbüchel A., Berg G. 2011: Analysis of the endophytic lifestyle and plant growth promotion of *Burkholderia terricola* ZR2-12. Plant Soil doi:10.1007/s11104-011-0833-8

Maurer K. A., Radišek S., Berg G., Seefelder S., nd: Real-time PCR assay to detect *Verticillium albo-atrum* and *V. dahliae* in hops: development and comparison with a standard PCR method. J. Plant Dis. Protect. Accepted.

Wilhelm S. 1955: Longevity of the *Verticillium* wilt fungus in the laboratory and in the field. Phytopathology 45: 180-181.

Wolf A., Fritze A., Hagemann M., Berg G. 2002: *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. Int. J. Syst. Evol. Micr. doi:10.1099/ijs.0.02135-0

Zachow C., Fatehi J., Cardinale M., Tilcher R., Berg G. 2010: Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. FEMS Microbiol. Ecol. doi:10.1111/j.1574-6941.2010.00930.x

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P-57 Practical use of endophytic *Verticillium isaacii* for biological control of Verticillium wilt in cauliflower

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Verticillium wilt is one of the most important diseases of cauliflower in Belgium. It is caused by *Verticillium longisporum*. The disease reduces the quality and weight of the cauliflower heads. Previous research in Belgian fields showed that the presence of *Verticillium tricorpus*-like organisms in the soil was negatively correlated with Verticillium wilt of cauliflower. Analysis of these organisms revealed that, although they morphologically resemble *V. tricorpus*, their ITS sequence is 100 % identical to the recently described *Verticillium isaacii* (Inderbitzin *et al.*, 2011). Further work showed that pre-inoculation of the cauliflower seedlings with one of these isolates, *Verticillium* Vt305, could reduce the symptoms and colonization of *V. longisporum* in controlled conditions. These results indicate that *Verticillium* Vt305 is a promising biocontrol agent of Verticillium wilt of cauliflower. However, the challenge is to develop strategies for practical use of *Verticillium* Vt305. The first strategy is the production of cauliflower plantlets inoculated with *Verticillium* Vt305, in order to protect them against *V. longisporum* present in the field. Different inoculation methods were tested: root-dip in spore suspension, mixture of microsclerotia with the substrate and application of microsclerotia suspension directly on the seeds. The amount of *Verticillium* Vt305 DNA in the plantlets was quantified with real-time PCR. All three inoculation methods were successful and *Verticillium* Vt305 was detected in the plants after 6 weeks. Further optimization of the inoculation method is however still required. Through the use of microsclerotia to inoculate the plants with *Verticillium* Vt305, we expect to obtain a better protection against *V. longisporum.* These structures survive for a long time in the soil and resist unfavorable conditions; therefore they are probably more suitable than spores to use as inoculum in practice. The use of non-coated seeds or seeds coated with commercial fungicides did not influence the colonization by *Verticillium* Vt305. Future research will focus on the performance of inoculated plantlets under field conditions. As *V. isaacii* is naturally present in our soils, another strategy relies on the stimulation of the population in the field by rotating cauliflower with host plants of *V. isaacii*. Currently, we are screening several green manure crops as potential host plants of *V. isaacii*. Preliminary results indicate that *Phacelia* and *Solanum sisymbriifolium* are potential crops to stimulate *V. isaacii*. The effect of these crops on the population dynamics of *V. isaacii, V. dahliae* and *V. longisporum* will be studied.

References

Inderbitzin, P., Bostock, R. M., Davis, R. M., Usami, T., Platt, H. W., Subbarao, K. V. 2011: Phylogenetics and Taxonomy of the Fungal Vascular Wilt Pathogen *Verticillium*, with the Description of Five New Species. PLoS ONE 6(12): e28341.

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P-58 Assessing the biofumigation potential of *Brassicaceae* cultivars to *Verticillium dahliae*

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Biofumigation is discussed as an alternative strategy to control *Verticillium dahliae*. The method is defined as incorporation of brassicaceous green manures containing glucosinolates (GSLs). These compounds are hydrolyzed in soil to isothiocyanates (ITCs), which have a toxicological effect to microsclerotia. The concentration of the GSLs varies within and between *Brassica* species, and consequently the concentration and type of ITCs evolved varies. Additionally ITCs differ in their toxicity to *Verticillium*. Therefore the biomasses of different brassicas show different biofumigation effects. Testing the biofumigation potential of a large scale of *Brassica* genotypes in field trials is very extensive. Also it is difficult to provide reproducible results because of many influencing soil factors. Therefore an efficient lab bioassay was developed to assess the biofumigation potential of biomasses of different genotypes under standardized optimal conditions with the aim to select the best one for further field trails.

First of all 19 cultivars of *B. juncea, Sinapis alba* and *Rhaphanus sativus* were grown on the field and biomasses were sampled. Freeze dried, ground shoot tissue of the cultivars were add to a sterile quartz sand filled in glass flasks and infested with 200 microsclerotia/g sand considering the field situation. Water was added to compensate the water loss of the tissue due to freeze drying and to adjust an optimum moisture level of 60% of maximum water capacity. Sand, biomass and water were thoroughly mixed to guarantee a homogenous mixture. Flasks were sealed immediately and incubated at 20 °C for 48 h. The soil of each flask was air dried and analyzed for viable microsclerotia using the wet sieving detection method. Additionally the GSL concentrations of the tissues were analyzed by HPLC.

Under the optimal conditions of the bioassay the tissues of *B. juncea* reduced the number of viable microsclerotia significantly. For the cultivars efficiencies between 69.3-81.3% could be determined. Considering the maximum amounts of 2-propenyl-ITC release calculated on the measured Sinigrin concentrations of the amended tissues as well as the LD_{90} value of 2-propenyl-ITC the effects can be explained widely to the ITC release. In the bioassay freeze dried tissue with a maximum disruption on cell level was used, whereas in practice the plant tissue is incomplete pulverized so that the release efficiency of ITCs is reduced. Also in a natural soil the toxicity of 2-propenyl-ITC is probably much lower, due to the organic matter and microbial activity in the soil. Therefore the biofumigation potential of *B. juncea* green manures must be rated as not adequate.

In comparison to *B. juncea* the amendments with *S. alba* and *R. sativus* had a much lower effect. Mortalities of microsclerotia were determined between 9.8% for the poorest and 37.5% for the best variety. In relation to the GSL contents and the maximum release of benzyl- and 4-methylthiobutenyl-ITC respectively the effects of the tissues were much too low. This indicates that the GSLs were only hydrolyzed to a minor degree because of a low myrosinase activity or other influencing factors. The results show that amendments with shoot tissues of *S. alba* and *R. sativus* are no alternatives to *B. juncea*.

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P-59 The suppression of Verticillium wilt of hop by biofumigation

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Hop (*Humulus lupulus* L.) is a dioecious, perennial climbing plant of the family Cannabaceae, which is native to the temperate Northern Hemisphere. Female plants are cultivated for their inflorescences, termed cones, which are primarily used in the production of beer to provide flavor, bitterness and aroma. One of the major limiting factors in hop production is Verticillium wilt, caused by the soil borne fungus *Verticillium albo-atrum* Reinke & Berthold. Since its first discovery on hops in 1927, the fungus has evolved several highly virulent pathotypes, which induce severe plant symptoms and cause the plants to die (Sewell & Wilson, 1984; Radisek *et al*., 2006). Control of the fungus is particularly difficult due to its production of dark resting mycelium, formed from melanised hyphae, which can persist in the soil for several years. After eradication of infested hop gardens, reduction of the soil borne inoculum is usually based on at least a 4-year crop rotation using non-host plants. However, such lengthy sanitation measurements are incompatible with the demand for quick re-establishment of hop production. Green manures of various crop plants, particularly from the Brassicacea family and genus *Sorghum,* have been shown to reduce the inoculum of some soil-borne diseases and could be effective in suppressing of Verticillium wilts (Davis *et al*., 2012; Larkin *et al*., 2011; Subbarao *et al*., 1999). Both groups of these plants act through a biofumigation effect, which is attributed to the pathogen's toxic volatile compounds released during degradation of plant metabolites, such as glucosinulates and cyanogenic glucosides. In our study, we evaluate the efficacy of various green manure plants in several field trials aimed at the suppression of *V. albo-atrum* and *V. dahliae* in eradicated hop gardens. Measurement of efficacy was based on analysis of fungal inoculum in incorporated nylon meshes before and after treatments. Current trials are focused on studying the effect of intercropping of green manure plants between rows of hop plants in order to reduce fungal soil infection potential and the spread of disease in infected hop gardens.

References

Davis J. R, Huisman O. C, Evereson D. O., Nolte P., Sorensen L. H., Schneider A. T. 2010: Ecological relationship of Verticillium wilt suppression of potato by green manures. Am. J. Pot. Res. 87: 315-326

Larkin R. P., Honeycutt C. W., Olanya O. M. 2011: Management of Verticillium wilt of potato with disease suppressive green manures and as affected by previous cropping history

Radisek S., Jakše J., Javornik B. 2006: Genetic variability and virulence among *Verticilium albo-atrum* isolates from hop. European Journal of Plant Pathology 116: 301-314.

Sewell G. W. F., Wilson J. F. 1984: The nature and distribution of *Verticillium albo-atrum* strains highly pathogenic to the hop. Plant Pathol. 33: 39-51

Subbarao K. V., Hubbard J. C. 1999: Evaluation of Broccoli Residue Incorporation into Field Soil for Verticillium wilt Control in Cauliflower. Plant Dis. 83: 124-129

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P-60 Interaction between *Verticillium dahliae* and *Dickeya solani* on potato

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Verticillium dahliae is the major pathogen of Potato Early Dying (PED) syndrome causing severe yield loss (up to 50% in susceptible cultivars). However, other pathogens such as *Colletotrichum coccodes* or Pectobacterium species may often take part. *Dickeya solani*, a new aggressive soft rot potato bacterium, spread by latent infections in seed tubers in Europe and the Middle East, is causing increasing economic losses. *D. solani* develop at relatively high optimal temperatures, can initiate disease from very low inoculum levels and have a great ability to spread through the plant's vascular tissue. The interaction between *V. dahliae* and *D. solani* was evaluated at different levels with the hypothesis that presence of both pathogens in potato plants might increase disease incidence. At in-vitro tests, fungal growth was inhibited in presence of the bacterium while the formation of microsclerotia was accelerated. Colonization of potato plants by RFP-tagged *D. solani* and YFP-tagged *V. dahliae* was investigated by selective plating, DNA quantification and confocal laser scanning microscopy (CLSM). In co-inoculation with both pathogens, fungal development in roots and stems was delayed as compared to inoculation with *V. dahliae* alone. The levels of fungal colonization forming units were significantly lower in co-inoculation (48h after inoculation) as well as DNA levels determined in RT-PCR reaction. *D. solani* colonization and DNA levels were not influenced by the presence of *V. dahliae*. In conclusion, the findings indicate that *D. solani* and *V. dahliae* are not in a synergistic interaction.

P-61 Comparison of conservation and preservation methods of *Verticillium* spp. cultures

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Collection of Plant Pathogens (Institute of Plant Protection – National Research Institute, Poznań, Poland) includes 190 isolates of Verticillium spp. belonging to 6 species (*Verticillium dahliae, V. albo-atrum, V. tricorpus, V. chlamydosporium, V. longisporum* and *V. fungicola*). They are preserved by means of 4 methods: on PDA medium at 16°C under mineral oil layer, at -80°C in 10% glycerol solution, after lyophilisation and by freezing in liquid nitrogen at -196°C. Forty isolates were selected and recovered from the different preservation methods. Cultures were grown on PDA medium at 24°C. After 5 and 10 days of incubation the cultures were measured, documented and compared with description of the culture done before preservation.

The best method is freezing in liquid nitrogen. Cultures were the most similar to cultures before preservation.

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