cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

ANNUAL MEETING OF WORKING GROUPS 1, 2, 3 AND 4

Management Committe Meeting

13 – 15, September, 2010

Jūrmala, Latvia

Latvijas Valsts
Augļkopības
institūts

Scientific Committee

Charles MANCEAU Marco SCORTICHINI	Working group 1 leaders
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Local organizers:

Silvija RUISA
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Inga MOROČKO-BIČEVSKA

SCIENTIFIC PROGRAM

Monday, 13. September. 2010

WG	Time	Speaker	Presentation
	08:00-09:00		**Registration - Reimbursement Forms**
	9:00	Silvija Ruisa	Welcome Address
	9:10	Maija Bundule	COST National Coordination & Latvian Academy of Sciences
	9:30	Edite Kaufmane	COST Domain Food and Agriculture & Latvia State Institute of Fruit Growing
WG1 Chair: Jaap Janse	**9:50**	Alexander Purcell	**Keynote Lecture:** What do we know and do not know about the invasive potential of *Xylella fastidiosa?*
	10:30	Sandor Sule	Comparison of *Agrobacterium* species with phenotypic, fatty acid analysis (FAME), PCR and CIEF methods
	10:45	Bart Cottyn	Q-BOL Project: DNA barcodes to identify phytobacteria that are subjected to EU quarantine regulations
	11:05	Sopie Cesbron	Genetic diversity of *Xanthomonas arboricola* pv. *juglandis* inferred from multilocus variable-number tandem repeat analysis (MLVA)
	11:25	Wendy Martin	*Xanthomonas arboricola* pv. *pruni* Detection and Monitoring in Holland
	11:45	Davide Giovanarde	STSM Report: An insight in some population features of *Xanthomonas arboricola* pv. *juglandis*
	12:05	Alexandra Craiova & Elena Gavrila	STSM Report: Isolation, identification and quantification of *X. arboricola* pv. *juglandis* populations
	12:25-13:30	Lunch	

WG1 Chair: Joanna Puławska	13:30	Joanna Puławska	Economic significance of crown gall and the diversity of its causal agent – screening of Polish stone fruits nurseries
	13:50	Aida Raio	Stone fruit Agrobacteria and their biocontrol in Southern Italy
	14:10	Tony Campillo	*Agrobacterium tumefaciens:* The At plasmid is essential for growth and survival at high temperatures
	14:30	Sarah McCraw	Metabolic analysis of *Pseudomonas syringae*
	14:50	Alain Bultreys	Diversity, isolate-host relationships, and virulence within populations of *Pseudomonas syringae* from Belgian fruit orchards
	15:10-15:40	Coffee Break	
WG2 Chair: Concepcio Moragrega	15:40	Marcel Wenneker & Jaap Janse	Decline of plum trees caused by *Pseudomonas syringae* pathovars: a serious threat for plum production in the Netherlands
	16:00	Inga Moročko-Bičevska	Characterization of bacterial diseases of stone fruits in Latvia
	16:20	Concepcio Moragrega	Development of a walnut blight forecasting model based on wetness duration and temperature
	16:50-19:00	Poster Session w/ Apero	
	19:15	Social Dinner in Jurmala *(in Baltic Beach Hotel*)	

Tuesday, 14. September. 2010

WG	Time	Speaker	Presentation
WG3 Chair: Mihai Botu	**08:30-08:50**		**Reimbursement Forms - Attendance List**
	9:00	David McNeil	**Keynote Lecture:** Walnut and Walnut Diseases in Australia and China
	9:30	David McNeil	Meeting Report: ISHS Walnut, Australia
	9:45	Mihai Botu	Meeting Report: EUFRIN, Craivo, Romania
	10:00	Gregorio Lopez	Tolerance segregation to *Xanthomonas arboricola* pv. *juglandis* in a progeny of walnut (*Juglans regia* L.)
	10:15-11:30	Coffee break & **Poster Session**	

COST873 Activities & Links Chair: Mihai Botu	11:30	Andjelika Calic	Meeting Report: *Pseudomonas* Training School, Belgrade
	11:45	Sarah McCraw	Meeting Report: *Pseudomonas syringae*and related pathovars; Oxford
	12:00	Joël Pothier	Q-Detect: *Xanthomonas arboricola* pv. *pruni* detection methods for phytosanitary application
	12:15	Jaap Janse	Upcoming Meeting: *Xylella fastidiosa* Training School, Bari, Italy
	12:30	Jaap Janse	Emerging bacterial diseases of fruit trees, not yet occurring in Europe - epidemiology, risks and management
	13:00-14:00	Lunch	
COST873 Planning Discussions Chair: Silvija Ruisa	14:00	Brion Duffy	**COST873 Budgets 2010-2011**: Planing activities in networking, training and research cooperation
	14:10-15:50	WG & STF Leaders	**Break-out Discussions** (Plan 2010-2011 Activities)
	16:00-22:00		Excursion to Riga Old Town & Social Dinner

Wednesday, 15. September. 2010

WG	Time	Speaker	Presentation
WG4 & CoP Chair: Davide Dallai	**9:00**	Pieter De Maayer	The key to ecological success: Comparative genomics reveals key targets for environmental colonisation and plant pathogenesis in the wide host range pathogen *Pantoea ananatis*
	9:20	Emilio Montesinos	Antimicrobial peptides: potential and limitations for plant disease control
	9:50	Emek Aslan	Evaluation of bacterial antagonists and some chemicals to control of bacterial blight of walnut in Turkey
COST873 Planning Future Activities Chair: Joël Pothier	**10:10**	WG & STF Leaders	**Break-out Discussions** (Plan 2010-2012 Activities)
	11:50	WG Leaders & Brion Duffy	**Budget and Research Proposals** (COST873 Activities - 2010-2011)
	12:30	Silvija Ruisa, Edite Kaufmane & Inga Moročko-Bičevska	**Meeting Closure**

	12:45-14:00	*Optional* Lunch	
MCM	**14:00-16:00**	Chair, Brion Duffy	**Management Committee Meeting** [Activities 2010-11; Budget Plan and Grant 2011]

POSTERS

Authors	Title
STSM Report: Hana Matouskova and Jaroslav Horky (CZ) with Alain Bultreys (BE)	Distinguishing of phytopathogenic *Pseudomonas* species
STSM Report: Daiva Burokiene (LT) with Joanna Pulawska (PL)	Differentiation of Lithuanian *Pseudomonas syringae* and *Xanthomonas arboricola* pv. *juglandis* isolate
STSM Report: Senem Akat (TR) with Concepcio Moragrega (ES	Field survey and microorganisms associated with apical necrosis of walnut
Montserrat Roselló and Ana Palacio-Bielsa	First detection of *Xanthomonas arboricola* pv. *pruni* on almond in Europe: detailed symptomology
Tim Kamber et al.	Characterization of the antibacterial peptide Herbicolin I biosynthetic operon in *Pantoea vagans* commercial biocontrol strain C9-1
Brion Duffy et al. (Intl)	FP7 KBBE Project Q-BOL: Quarantine Bar-Coding of Life
Annette Wensing, Esther Moltmann, Wilhelm Jelkmann and Klaus Geider	MALDI-TOF analysis for species identification of plant-associated *Pantoea* and *Brenneria* isolates
Didier Socquet-Juglard, Joël F. Pothier, Danilo Christen, Brion Duffy and Andrea Patocchi	Apricot resistance to *Xanthomonas arboricola* pv. *pruni*: Mapping quantitative trait loci
Hatice Ozaktan, Senem Akat and Lalehan Yolageldi	The two years of experience on etiology of apical necrosis on walnut in Turkey
STSM Report: Senem Akat and Hatice Ozaktan (TR) with Concepcio Moragrega (ES)	Field survey and microorganisms associated with apical necrosis of walnut
Dallai D., Giovanardi D. and Stefani E.	New strategies to control *Xanthomonas arboricola* pv. *pruni* in peach orchards
Anita Solar et al.	Accumulation of phenolic compounds in walnut fruits related to orchard management, phonological stage and presence/absence of *Xanthomonas arboricola* pv. *juglandis*

WORKING GROUP 1

WHAT DO WE KNOW AND WHAT WE NOT KNOW ABOUT THE INVASIVE POTENTIAL OF *XYLELLA FASTIDIOSA*?

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Xylella fastidiosa has an unusually wide range of plant species in which it can multiply and move systemically, as well as a large number of potential insect vector species. Thus the invasive potential of this xylem-restricted bacterium would seem to be large. Yet despite the common occurrence of many known as well as probable host plants and vectors in Europe and Asia (and probably Africa) and centuries of importations of its known systemic hosts from regions where *X. fastidiosa* is endemic, *X. fastidiosa* appears to only occupy the Americas, with the exception of pear leaf scorch in Taiwan.

There is substantial evidence that winter cold severity limits the survival of *X. fastidiosa* in North America, which could explain the absence of the bacterium in northern Europe, but not southern Europe. Unfortunately, we do not know the mechanism by which freezing temperatures eliminate *X. fastidiosa* from plants and cannot describe the climatic requirements for its continuous survival. Some other features of *X. fastidiosa* that could limit its abilities to invade new regions are (i) host ranges of various strains of *X. fastidiosa* are incompatible with establishing endemic populations in Europe, (ii) the lack of vectors that overwinter as adults and can thus infect plants during the early growing season that is critical for the bacterium to survive the subsequent winter, and (iii) microbial antagonists or competitors that limit its occurrence or its induction of disease. With reference to the first of these features (i), the host ranges (and other traits) of various strains of *X. fastidiosa* appear to be influenced by horizontal gene transfer. This could accelerate its evolution of virulence in agricultural settings where new strains would encounter a relatively few clones of host genotypes in grafted fruit trees. Commercial shipments of citrus plants hastened the spread throughout Brazil of the *X. fastidiosa* strain that causes citrus variegated chlorosis disease of orange. The strain causing oleander (*Nerium oleander*) leaf scorch disease in the southwestern United States may have been a recent introduction or the emergence of a new strain due to the introduction of an invasive vector species. The strains causing Pierce's disease of grape have been proposed as having emerged or been introduced in the United States as recently as about 100 years ago. Thus currently unknown or recently adapted strains may be able to establish in new regions. In order to flourish in a given plant community, the host range of *X. fastidiosa* should match the feeding preferences and seasonal abundance patterns of key vectors

and the composition of natural and agricultural plant communities. (ii) In temperate climates in which Pierce's disease is a problem, there is a critical window of opportunity (April-May in northern California) for new infections to establish populations in grape that can successfully overwinter. Later infections do not usually survive the next winter. Although xylem sap-feeders that are the vectors for *X. fastidiosa* are locally common in much of Europe, they seem mostly to overwinter in the egg stage. Thus flying vectors (adults) occur later in the growing season than is the case in comparable regions in the Americas. The invasion of southern California in the 1990s by the glassy-winged sharpshooter (*Homalodisca vitripennis*) demonstrated that the introduction of a new vector can profoundly change the epidemiology of Pierce's disease, in this case from unnoticed to devastating levels. (iii) There is growing interest in biological controls for *X. fastidiosa,* but as yet there is no evidence that microbial antagonists can exclude *X. fastidiosa* from entire areas. *X. fastidiosa*'s cell-cell signaling appears to be a key to regulating its behavior during its colonization of both plants and of insects. In general, low population densities (low concentrations of signal) of *X. fastidiosa* in plants reduce attachment to surfaces to speed systemic movement, whereas high populations (high signal strength) suppress movement and multiplication to avoid killing its host plant. Knock-out mutants of various genes for signal synthesis or utilization profoundly affect this bacterium's population levels, intraplant movement, vector transmission, virulence, and possibly host range. Increased signal added directly to plants as chemical sprays, through genetic modification of plants, or through the introduction of other bacteria that produce high levels of a homologous signal all slow the movement of *X. fastidiosa* and greatly reduce disease symptoms. Other natural bacterial inhabitants of plants produce signals that can interfere with these processes in *X. fastidiosa*.

In summary, the invasive potential *of X. fastidiosa* seems to be heavily influenced by the host range and other genetically controlled characteristics of particular strains and by the available vectors and their seasonal activity. Consequently, quarantine to exclude introductions of *X. fastidiosa* should not concentrate solely on known strains in pathological hosts such as grape, peach, plum, almond and citrus. Preventing insect invasions are difficult to plan, but vectors known to be important in the Americas that thrive in regions climatically similar to European regions should be considered prime threats.

COMPARISON OF *AGROBACTERIUM* SPECIES WITH PHENOTYPING, FATTY ACID ANALYSIS (FAME), PCR AND CIEF METHODS

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Agrobacteria may cause crown-gall or hairy root diseases in many plants including walnut and stonefruits. The most characteristic symptom of the disease caused by *Agrobacterium tumefaciens* is a tumour-like growth on the infected plant, often at the junction between the root and the shoot. Tumors are incited by the conjugative transfer of a DNA segment (T-DNA) from the bacterial tumour-inducing (Ti) plasmid. The closely related species, *A. rhizogenes*, induces root tumors and rarely hairy roots, and carries the distinct Ri (root-inducing) plasmid. *A.vitis* and *A.rubi* are host specialized species causing tumors an grapevine and *Rubus* sp. respectively. Although the taxonomy of *Agrobacterium* is currently under revision (Young et al. 2001, Farrand et al. 2003) it can be generalised that on the basis of biochemical and physiological tests (Keane *et al.* 1970, Kerr and Panagopoulos 1977, Süle 1978) 3 biovars exist within the genus. These biovars subsequently recognized as species, biovar 1 as *A.tumefaciens*, biovar 2 as *A. rhizogenes* and biovar 3 as *A. vitis*.

This division is supported by 16S rRNA and 23S rDNA sequence analysis (Sawada et al. 1992, Willems and Collins 1993, Pulawska *et al.* 2000) and fatty acid analysis (Tighe *et al.* 2000).

Traditionally, detection and enumeration of agrobacteria have been largely based on the use of selective culture and standard biochemical methods. Furthermore, to confirm the pathogenicity of the isolates test plants have to be inoculated and the results can be seen in 2-4 weeks. Such methods suffer from a number of drawbacks. They are time consuming, tedious and low throughput. Recently, the use of PCR has provided highly sensitive detection of pathogenic agrobacteria (Haas *et al.* 1995, Kawaguchi *et al.* 2005, Suzaki *et al.* 2004, Bini *et al.* 2008). More recently, the use of the multiplex polymerase chain reaction (m-PCR) has provided rapid methods for the specific identification of *Agrobacterium* species (Pulawska *et al.* 2006).

Since many important ecological characteristics of bacteria, such as pathogenicity, competitiveness, substrate range, and bioactive molecule production, vary below the species level, additional methods continuously are needed.

In this work we compared the conventional biovar determination with fatty acid analysis, PCR and the new capillary isoelectric focusing (CIEF) methods (Horká *et al.* 2007, 2010). In spite of small differences between strains all species could be clearly determined with all methods. Pathogenicity determination was only possible with test plants and PCR. The advantages of all methods will be discussed.

QBOL, DNA BARCODES TO IDENTIFY PHYTOBACTERIA THAT ARE SUBJECTED TO EU QUARANTINE REGULATIONS

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Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health policies. The EU-FP 7 program QBOL aims to make DNA barcoding available for plant health diagnostics. Within QBOL-WP4, barcode sequences are generated for a selected set of relevant quarantine bacteria that are on the EU Directive and EPPO list, and for which protective measures against introduction into, or spread within the Community need to be taken. DNA barcoding is a molecular technique for species identification that uses short genomic sequences from key gene regions. Knowledge on which gene regions that are indicative for species identification has to be carefully established and validated.

ILVO focuses on the genetic barcoding of a set of quarantine and Q-alert bacteria within the *Xanthomonas* genus, which are mostly classified on the pathovar level. Among the target pathogens are *X. fragariae*, *X. translucens*, the two pathovars of *X. oryzae*, the complex group of pathovars on Citrus, and the range of *X. axonopodis* pathovars. For each target bacterium a working collection was assembled, consisting of the pathotype and other accurately identified strains, and of morphologically and/or taxonomically related strains. Sequence diversity in the core gene *gyrB* proved to provide a phylogenetic framework for the genus *Xanthomonas*. A 530-bp sequence of this gene has been adopted as a first barcode region for *Xanthomonas*. For reliable identification on the pathovar and subspecies level, however, additional barcoding regions are determined to complement the *gyrB* barcode. The DNA barcode sequences plus relevant taxonomic and biological data will be included in a new internet-based database. This database will be made available for plant health diagnostics and for National Plant Protection Organizations (NPPO's).

GENETIC DIVERSITY OF *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS* INFERRED FROM MULTILOCUS VARIABLE-NUMBER TANDEM REPEAT ANALYSIS (MLVA)

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Walnut species (*Juglans* sp.) are important nut producers in temperate regions of Europe, Asia, North America and South America. The Persian (English) walnut (*J. regia* L.) is widely cultivated, the most horticulturally developed and the leading producer of commercial nuts. Bacterial blight is considered as the most important biotic disease in all walnut-growing areas. The causal agent of the disease *is Xanthomonas arboricola* pv. *juglandis* (*X. a.* pv. *juglandis*), also known as *X. campestris* pv. *juglandis* (Pierce) Dye. It causes necrosis on leaves, catkins, twigs and fruits, and can induce important crop losses. Other necrotic syndromes have been observed on walnut which affect fruits and cause brown apical necrosis (BAN). *Fusarium* is the most common genus associated with this complex disease. However, *X. a.* pv. *juglandis* is also associated with the BAN syndrome and could be the true causal agent of the disease. Unusual symptoms were firstly reported in France in 2004. These symptoms are characteristics of a new disease termed vertical oozing canker (VOC). It includes longitudinal deformations of affected trunks, with brown to black exudates staining the bark which appeared mainly in summer months. The final stage of the disease is characterized by a severe distortion of affected trunks. The association of *X. a.* pv. *juglandis* with VOC in France has been reported recently.

An early detection procedure is available for *X. a*. pv *juglandis* (specific, easy to use, rapid, detect viable bacteria). But we need more research for the specific detection of strains that cause VOC. To study genetic diversity in *X. a* pv. juglandis, we used Multilocus Variable-number tandem repeat (VNTR) Analysis (MLVA). MLVA is a fast method that compares the number of tandem repeats (TRs) at multiple VNTR loci, which are areas of the bacterial genome that evolve quickly. Detection of these TR differences can be achieved by PCR using primer pairs designed to anneal to the flanking regions of each TR. It provides a powerful tool for assessing the genetic relationships between bacterial strains of the same species. The genomic sequence of *X. a* pv. *pruni* has been used as genomic ressource to find VNTR useful for MLVA in *X. a* pv. juglandis and other pathovars. 51 VNTR primer pairs have been retained in both coding and non coding regions and tested on 20 strains of different *X. arboricola* pathovars. We chose the 12 most variable loci on 3 strains of *X. a.* pv. *juglandis* to study genetic diversity in 94 isolates of *X. a*. pv *juglandis*. The MLVA dendrogram showed one distinct cluster which corresponded to VOC strains. Our results confirm the suitability of this MLVA method for characterizing X. a. pv *juglandis* isolates. The diversity found may be useful for future epidemiological studies (BAN strains for example).

STSM REPORT: AN INSIGHT IN SOME POPULATION FEATURES OF *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS*

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Xanthomonas arboricola pv. *juglandis* (*Xaj*) is the causal agent of the bacterial blight of walnut, an emerging disease, which the potential to severely affect walnut orchards (Mulrean and Schroth, 1981).

An Italian strain collection of *Xaj*, obtained during the past 3 years from affected orchards in Romagna, was first assayed with conventional PCR with XajF/XajR primer pair developed by Gironde *et al.* (2009) to confirm strain identity. The population structure of the collection of *Xaj* isolates, confirms the presence of different genetic groups identified by rep-PCR (using the REP, BOX and ERIC primers) and by multilocus sequence typing (MLST) and multilocus variable number analysis of tandem repeat (MLVA).

Further on, *Xaj* and *Xaj*-like bacterial isolates from the Italian collection are currently being analysed by MLSA (Multi Locus Sequence Analysis), by using 7 primers for 7 different house-keeping genes, with the purpose to better characterise the Italian isolates for phylotyping.

The study of copper resistance on a wide collection of over 150 *Xaj* strains frequently showed a high resistance (up to 500 ppm Cu^{++}): two strains has been further studied and confirmed the presence of chromosomal genes *copA* and *copB* involed in the general *copABCD* copper resistance structure, as described for *Pseudomonas syringae* (Mellano *et al*, 1988). Sequencing and comparing with other *Xanthomonads* were done.

The elucidation of *Xaj* population structure may help to deeper investigate some more aspects in the molecular epidemiology of the disease, thus allowing a better control strategy in the field.

References

MELLANO M. A. and COOKSEY D. A..1988.Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae pv. tomato. J. Bacteriology* 170: 2879-2883.

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ECONOMIC SIGNIFICANCE OF CROWN GALL AND THE DIVERSITY OF ITS CAUSAL AGENT-SCREENING OF POLISH STONE FRUITS NURSERIES

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Stone fruit and nut production in Europe is limited by increasingly severe losses caused by bacterial diseases. Crown gall caused by tumorigenic *Agrobacterium* spp. is one of the most dangerous diseases for nursery production of these and many other plants. In many countries, tumorigenic agrobacteria are considered as quality pathogens. Bacteria causing tumors create a very heterogeneous group of strains classified to different species and biovars. Additionally several strains which may represent a separate, hitherto unrecognised taxa are described. The diversity of agrobacteria is observed on phenotypic as well as on genetic level. In the later case, high diversity of both chromosomal and plasmid DNA was found.

In years 2007-2009 almost 80 stone fruits nurseries located in different regions of Poland were examined for the presence of crown gall. In half of them, crown gall was observed and galls were sampled for agrobacteria isolation. Out of over 1200 isolates, about 500 were identified as *Agrobacterium* spp. based on 23S rRNA gene - multiplex PCR (Puławska et al., 2006) and PCR with primers complementary to *tms* gene located on Ti plasmid (Puławska and Sobiczewski, 2005). Most of isolated agrobacteria belong to biovar 2 and all of pathogenic isolates posses nopaline type Ti plasmid. Results of preliminary phenotypic and genetic analysis of Polish isolates will be presented.

References

Puławska et al. 2006. *Systematic and Applied Microbiology* 29: 470-479

Puławska & Sobiczewski 2005. *Journal of Applied Microbiology* 98: 710-21

STONE FRUIT AGROBACTERIA AND THEIR BIOCONTROL IN SOUTHERN ITALY

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Control of crown gall by using the *Agrobacterium rhizogenes* strain K84 represents one of the most successful applications of biological control methods. Crown gall incidence of peach rootstock is usually very low (0.01-0,1%) in Italian nurseries that use K84 strain to protect the plants. However, the efficacy of this biocontrol method may be reduced by the selection of pathogenic recombinants that are insensitive to the antagonist. Nine Italian peach nurseries were monitored for three years with the aim of determining whether transconjugant populations may arise following plasmid exchanges between K84 and autochthonous agrobacteria. Biovar 1 and 2 transconjugant strains, harbouring pAgK84 were isolated from a plot of one of the nursery surveyed in this study. PCR-RFLP analysis of the 16 gene and of the intergenic spacer between 16S and 23S genes showed that all transconjugants originated by the transfer of pAgK84 to tumorigenic agrobacteria. Most probably the outbreak of transconjugants in the peach nursery was due to the presence in the soil of an agrocin 84 insensitive agrobacterium population. Further investigations showed that root colonization ability of a transconjugant strain was not influenced by K84, but the root population of the antagonist was drastically reduced in presence of the transconjugant.

Our study regards the sole case of an outbreak in the control of crown gall in an European commercial nursery. K84 strain has been used in many European countries for more than 30 years and during this time the antagonist has shown high efficacy in protecting stone fruit trees and some ornamentals. Some years ago, Italy implemented the Council Directive 2005/25/CE of 14 March 2005 that has set out the uniform principles for evaluation and characterization of plant protection products containing microrganisms and actually in our country, the use of K84 is not allowed. This measure damages the nurseries since the biocontrol strategy represents the only way to prevent crown gall epidemics and reduce economic losses.

METABOLIC ANALYSIS OF *PSEUDOMONAS SYRINGAE*

Sarah L. McCraw, Shyam K. Masakapalli, Arantza Rico, Nicholas J. Kruger, R. George Ratcliffe and Gail M. Preston

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Pseudomonas syringae is a common and economically important plant pathogen, infecting a variety of plants including crops (wheat and barley), solanaceous plants (tomato and tobacco) and stone fruits. *Pseudomonas syringae* grows within its host plant by assimilating metabolites present in leaf exudates and in apoplastic fluid. The metabolic interface between plants and bacterial pathogens is therefore a key determinant in the infection process. In recent years, advances in metabolomics technologies have allowed novel insights into the pathways and fluxes in both plants and pathogens, and in the last couple of years attention has turned to the metabolic interactions between them. Research in this area will provide crucial information for the development of more effective strategies for the control of crop diseases. My work focuses on the interaction between *Pseudomonas syringae* pv. tomato DC3000 (DC3000) and its host, tomato. This model system has been extensively studied and is thought to be representative of interactions between plants and pathogenic bacteria. It is therefore an ideal platform from which to explore metabolomics and begin more general metabolic analyses. There are several approaches which can be employed in studying the metabolic phenotype of bacterial cells and their metabolic interactions. These approaches include examining nutrient assimilation, metabolic profiling and footprinting, and metabolic flux analysis. I will discuss how analysis of nutrient assimilation by DC3000 and apoplastic composition in both healthy and infected plants has shown how DC3000 is adapted for growth in the plant apoplast. I will also demonstrate how the use of metabolic mutants can show the importance of constituents of the apoplast for the growth of this pathogen. Finally, I will present a draft metabolic flux map for DC3000 and prospects for metabolic flux analysis in understanding plant-pathogen interactions.

DIVERSITY, ISOLATE-HOST RELATIONSHIPS AND PATHOGENICITY WITHIN POPULATIONS OF *PSEUDOMONAS SYRINGAE* FROM BELGIAN FRUIT ORCHARDS

Alain Bultreys[1], Valérie Gilbert[1], Frédérique Legros[1], Viviane Planchon[2], Henri Maraite[3] and Isabelle Gheysen[1]

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The objective of this study was to characterize a collection of 356 *Pseudomonas* strains established from diseased organs sampled from fruit orchards in Belgium. Physiological and biochemical tests and REP-PCR, ERIC-PCR, BOX-PCR and IS50-PCR enabled classification of the strains in the pathovars *syringae*, *morsprunorum* race 1 and *morsprunorum* race 2 of *P. syringae*, in *P. syringae* and in *P. viridiflava*. Genetic results confirmed homogeneities in the pathovars *morsprunorum* race 1 and race 2 and the high diversity in the pathovar *syringae*. In the pathovar *syringae*, homogeneous genetic groups consistently found on the same hosts (pear, cherry or plum) were observed. The pathogenicity of 99 selected strains was evaluated in detail by using 17 pathogenicity tests. The *P. syringae* pv. *morsprunorum* strains were pathogenic to stone fruit species. Three groups were defined in the pathovar *syringae*: one group pathogenic in 71.1% of the tests and to lilac included toxic lipodesipeptide producing (TLP+) strains; the second group pathogenic in 26.8% of the tests and non pathogenic to lilac included TLP+ strains: the third group pathogenic in 9.1% of the tests included TLP- strains. The three groups were genetically heterogeneous. Although strain-host relationships were noted within the pathovars *syringae*, *aptata* and *atrofaciens* when considering the strain origins, such relationships were not found in the pathogenicity tests, suggesting that pathogenicity tests could probably not reproduce all the aspects of the host-pathogen interactions. *Pseudomonas syringae* isolates that differed from known fruit pathogens were observed in pear, sour cherry and plum orchards in Belgium.

WORKING GROUP 2

DECLINE OF PLUM TREES CAUSED BY *PSEUDOMONAS SYRINGAE* PASTHOVARS: A SERIOUS THREAT FOR PLUM PRODUCTION IN THE NETHERLANDS

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In the Netherlands, bacterial canker in plum trees (*Prunus domestica*) is a serious and recent problem in plum production. It is caused by *Pseudomonas syringae* pathovars *syringae* and *morsprunorum*. The trunks of the affected plum trees are girdled by bacterial cankers resulting in sudden death of infected trees. A rapid death was observed in several orchards 3-4 years after planting. Disease incidences can be very high, and sometimes complete orchards have to be removed.

Recently, plum cultivation in the Netherlands has changed from a relatively extensive into an intensive cultivation. This was realized by the use of weak rootstocks (e.g. VVA-1 and St-Julien A). Also, some new plum varieties have been developed (e.g. 'Lazoet'). However, due to the risks of losses of trees due to bacterial canker, growers are reluctant to plant new plum orchards. Although several control measures are advised in the Netherlands, e.g. cultivation measures at the planting site, careful pruning with disinfection of pruning tools, and removal of heavily infected trees, in practice no control measures are taken. In general nurseries and fruit growers are not familiar with bacterial diseases and lack knowledge in order to prevent infections. Therefore, control strategies to manage plum decline have to be developed. In 2010 a project is started to study the epidemiology and possible control of plum decline. The project will focus on plum decline in commercial plum orchards and plum nurseries. Factors such as root stocks and cultural practices will be evaluated.

CHARACTERIZATION OF BACTERIAL DISEASES OF STONE FRUITS IN LATVIA

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The commercially grown stone fruit species in Latvia are plums, sweet and sour cherry. Peaches and apricots are grown only in home gardens or in varietal collections. Although fruit growing in Latvia has old traditions, the intensive commercial plantations have been planted during last 15 years. In order to detect pathogens present and to evaluate the most serious treads commercial *Prunus* spp. orchards and varietal collections were surveyed in 2008 and 2009. Samples from trunks, branches, buds and flowers with indicative symptoms of bacterial infection were collected. The possible pathogenic bacteria were isolated from diseased samples by agar plating on *Pseudomonas* semi-selective medium, 5 % sucrose nutrient agar and nutrient dextrose agar. Single colonies with morphology characteristic to pseudomonads and xanthomonads were transfered in pure cultures and preserved. The bacterial isolates were further characterized and identified by LOPAT and GATTa tests. During the surveys most often observed symptoms were different size cankers on trunks and branches resulting in collapse of trees, sunken areas girdling branches with collapsed buds and young leaves. In total 2058 bacteria were isolated in pure cultures and about 700 gram-negative, putative *Pseudomonas* isolates were selected and further characterized by LOPAT and GATTa tests. In Latvia, so far, only fungal diseases have been considered of economic importance in *Prunus* orchards. The preliminary results of current research show that *Pseudomonas syringae* pv. *syringae* is present in sweet cherry and plum orchards in Latvia. The characterization of other bacterial isolates and pathogenicity tests on hosts are currently in progress.

DEVELOPMENT OF A WALNUT BLIGHT FORECASTING MODEL BASED ON WETNESS DURATION AND TEMPERATURE

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Bacterial blight of Persian (English) walnut (*Juglans regia* L.) caused by *Xanthomonas arboricola* pv. *juglandis* is a disease of economic importance in all walnut-producing areas and the severity of outbreaks depends to a great extent on spring weather conditions. The disease is currently an important limiting factor in many walnut orchards and up to 50 % of yield reduction can be produced in highly affected orchards. Although all young walnut tissues are susceptible to infection and necrosis can occur on catkins, female flowers, leaves, fruit and green shoots, economic damage occurs when the developing nuts are infected.

Up to now the walnut blight control is achieved by preventive periodical applications of copper derivatives, but their efficacy is limited. Copper resistant strains of *X. a.* pv. *juglandis* have been isolated from walnut orchards after intensive copper application for disease control. Additionally, intensive applications of copper over many years in commercial orchards have resulted in the accumulation of copper in the soil, with subsequent negative environmental impact and interference in walnut tree metabolism. Consequently, a reduction in copper applications in walnut orchards is needed. A walnut blight forecasting model developed under European weather and inoculum conditions could be a useful tool to help European growers to control the disease and to reduce the bactericide applications.

In this work, a walnut blight forecasting model based on climatic parameters was developed. For this purpose, the effect of wetness period duration and mean air temperature on disease incidence and severity was determined under controlled environment conditions. Walnut potted plants were inoculated with bacterial suspensions and incubated at different temperatures (from 10 to 30 ºC) and wetness period durations (from 0 to 24 hour). The disease levels (incidence and severity) were assessed after incubation at optimal conditions for disease development. The experiment was repeated three times. Data were adjusted to a polynomial equation to establish the relationship between the disease severity and the evaluated climatic parameters. The equation obtained was proposed to be used for prediction of infection risk of *Xanthomonas arboricola* pv. *juglandis* on walnut. The evaluation and validation of the walnut blight prediction model under different climatic conditions are in course.

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EMERGING BACTERIAL DISEASES ON FRUIT TREES, NOT YET OCCURING IN EUROPE AND MEDITERRANEAN BASIN – EPIDEMIOLOGY, RISKS AND MANAGAMENET

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Bacterial diseases of fruit trees are difficult to control (both chemically and biologically), mostly only by preventive measures such as hygiene, healthy planting material, good cultural practices and avoidance of risk planting sites. Moreover bacteria may easily spread by (surface) water, planting material and contaminated implements/ machines and by a-specific or specific insect vectors. Most important risk factors for the introduction of bacterial diseases into Europe are imported infected planting material and (infected) insect vectors. However also plants and plant parts and infected vectors that may be introduced by travellers of any kind (e.g. tourists, pilgrims, military) should be considered. In this contribution the epidemiology, management and main risks of three emerging bacterial diseases of Citrus not yet present, but approaching Europe and the Mediterranean basin, their causal organisms and vectors will be highlighted, especially 1) Citrus huanglongbin or Citrus greening, caused by the heat tolerant *"Candidatus" Liberobacter asiaticus* and and heat sensitive *"Candidatus" L. africanus*, both forms and respective psyllid vectors *Diaphorina citri* and *Trioza erytreae* are present on the Arabian peninsula, with recent reports of huanglongbin occurring in Iran, Mali, Ethiopia and Somalia and *T. erytreae* already present on some Atlantic Ocean Islands. Furthermore in less detail 2) Leaf scorch and leaf scald diseases of grape and diverse fruit and ornamental trees, caused by *Xylella fastidiosa*. For this pathogen, although not yet confirmed from Europe or Mediterranean basin, local possible vectors such as *Cicadella viridis* and *Philaenus spumarius* occur. 3) Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri (X. citri* subsp. *citri)*, the so-called Asiatic, most severe form causing A type cankers, is present already in Irak, Iran, Oman, Somalia, UAE, Saudi-Arabia, Yemen and Reunion. Outbreaks and possible emerging character of some other bacterial pathogens (e.g. *Xanthomonas citri* pv. *mangiferaeindicae* and Phytoplasma phoeniculum on mango and almond resp.) are also mentioned. Since initial management and risk avoiding and initial management measures following an introduction are more or less similar for the three above mentioned pathogens, they will be highlighted for HLB. It has been shown that ornamental and wild hosts may play an important role in spreading of the disease and maintaining the pathogen and its vectors in the environment. These plants should be included in surveys. Rapid and reliable diagnosis remains a key issue, as well as breeding for resistance. It will be argued that the three main diseases addressed in this presentation are emerging threats, with real risks of introduction and in some cases closely approaching the Mediterranean basin.

WORKING GROUPS 3&4

TOLERANCE SEGREGATION TO *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS* IN A PROGENY OF WALNUT (*JUGLANS REGIA* L .)

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The summer conditions in La Alberca, Murcia (ES) usually present high temperatures and low air humidity. In such location, a F_1 progeny of 25 adult genotypes out of walnut cv. *Serr* open pollinated have been cultivated in 2010. No treatments against *Xhanthomonas arboricola* pv. *juglandis* (*Xaj*) had been applied, but aphids and codling moth and other pests were sufficiently controlled. On August 31th, *Xaj* symptoms on leaves, hulls of fruits and wood of the year were recorded according to a scale ranging from 0 to 4, being 0) for no symptoms at all, and 1), 2), 3) and 4) the corresponding categories for <1%, 1-10%, 10-40% and >40% of affected leaf surface respectively. Likewise, some of these genotypes had been grafted on one-year-old seedlings of cv. *Serr* grown in pots.in winter of 2010 and cultured in pots under a shady shelter. In these plants, on August 31th , were also recorded *Xaj* damages as it was done in the open field. An ANOVA of the form $Y_{ij} = \mu + S_i + R_j + SR_{ji}$, with i= 1,2,…, 26 and j=1, 2, 3, being μ the general mean of trial, S the evaluated symptoms and R the replications was calculated, as well as means separation by using the Duncan's test at P<5%.

Genotypes segregation for leaves *Xaj* tolerance in comparison to the parental *Seer* has been pointed out. However, no *Xaj* symptoms were found neither on one-year old sprouts or hulls in the open, nor on wood of the year in pot grafted plants. This last record seems to be in agreement with the absence of *Xaj* damages on wood of the year in plant grown in the open, because the scions of grafted plant of the shelter were harvested out of genotypes placed in the open. In fact, when scions were inoculated with *Xaj* and grown in pot one year latter under shady shelter, secondary infections occurred (Frutos *et al.*, 2008).

A genotype whit less, 8 with equal and 16 with more leaf *Xaj* damages than the parental *Serr* have been recorded, what look like to prove that *Xaj* tolerance segregation can occur even starting from a narrow genetic base. On the other hand, it is well known that *Xaj* tolerance behave strongly dependent of the climatic conditions. In this way, perhaps the more tolerant genotypes in La Alberca could behalf poorly in wetter climatic conditions, more favourable for the disease development. All this lead to believe that

walnut genotypes whit higher *Xaj* tolerance ought to be found in areas of native walnut population with a wide genetic diversity, whit limited or null treatment against *Xaj*, and with optimal climatic conditions for *Xaj* development. For these reasons, perhaps the main candidate areas in Europe holding the prior characteristics for finding *Xaj* tolerance could be the Carpathian and Balkan foothills mountains.

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ANTIMICROBIAL PEPTIDES: POTENTIAL AND LIMITATIONS FOR PLANT DISEASE CONTROL

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Antimicrobial peptides (AMPs) are the first barrier of defense in animals and plants and play a role in antibiosis in microorganisms. AMPs offer great potential as novel products within the new context of regulatory restrictions of pesticides for plant disease protection. However, natural AMPs are produced at low concentrations, may be toxic or have low activity, and are difficult and costly to extract and purify. Synthetic AMPs can be obtained by developing small, truncated compounds, sequence analogs, chimeric constructions, and *de novo* sequences. Compounds can be improved using combinatorial chemistry to optimize leads based on higher activity, and to minimize toxicity and improve stability to digestion by plant tissue proteases. Controlled environment and greenhouse trials with synthetic AMPs have been succesful in controlling plant pathogens in their host plants like several plant pathogenic bacteria and fungi, affecting pome and stonefruit trees. However, the main limitation for its use as plant protection products is the high cost of the chemical synthesis. Several AMPs have been produced by expression in model bacteria and yeast. Also, molecular farming using plants as biofactories is being explored and offer great expectations for a sustainable production. Several examples on development and efficacy will be presented based on a CECMEL11 peptide library and the future trends of this technology in plant protection will be discussed.

EVALUATION OF BACTERIAL ANTAGONISTS AND SOME CHEMICALS TO CONTROL OF BACTERIAL BLIGHT OF WALNUT IN TURKEY

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Bacterial blight of walnut, caused by *Xanthomonas arboricola* pv.*juglandis (Xaj)* is present in all main areas of walnut production in the Western part of Turkey. The goal of this study was to determine whether bacterial antagonists could be used to control of *Xaj*, the causal agent of bacterial blight on walnut. Totally, 35 bacterial antagonists, which 29 bacterial strains of them were isolated as epiphytes from phylloplane of healthy walnut trees, were screened for their in vitro biocontrol activity to *Xaj*. *Pantoae agglomerans* strain C9/1 was also tested as reference biocontrol agent against *Xaj*. Of the 35 fluorescent Pseudomonas strains tested, 18 inhibiting the growth of *Xaj* between 3.0 mm to 13.0 mm on TSA plate were selected and tested for their ability to control of bacterial blight of walnut on the immature nut test. Approximately 39% of the antagonistic bacterial strains tested on immature nut test, significantly reduced bacterial blight of walnut by 73% to 88% compared to the pathogen-alone treatment. Bacterial antagonist strains, which were effectively inhibited the development of *Xaj* on immature nuts were selected for walnut seedling pot test. Prohexadione-Ca (ProCa), which was a plant bioregulator and Acibenzolar-S-methyl were also applied to the walnut seedlings to test the prevention of bacterial blight of walnut. The potted walnut trees were applied with selected antagonistic bacterial suspensions (10^9 cfu/ml) and these chamicals by spraying the leaves, and inoculated with the suspension of *Xaj* strain W7/1 (10^8 cfu/ml) 24h after treatments. According to the in vivo test results on seedlings of different varieties of walnuts (cv.Chandler- susceptible, cv.Franquette-less susceptible, and cv.Şebin-most susceptible), most of the tested bacterial antagonists and chemicals significantly reduced the symptom development on the walnut leaves compared to the pathogen-alone treatment. Bacterial antagonist strains, WH68 and WH48/1A were found to be the most effective for inhibition of *Xaj* in each of three walnut varieties, it was followed by strain WH77/1. The effectiveness of applications of ProCa and Acibenzolar-s-methyl was in a third order for disease prevention and found as effective as Copper based compound.

POSTERS

STSM REPORT: DISTINGUISHING OF PHYTOPATHOGENIC *PSEUDOMONAS* SPECIES

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Stone fruits bacteria isolates, mainly pathovars of phytopathogenic species of genus *Pseudomonas* obtained in the Czech Republic are identified in the State Phytosanitary Administration (SPA) by gas chromatography fatty acid methyl esters analysis with SW Sherlock (MIDI, Inc., USA) and BIOLOG™ System identification with various probability. *Pseudomonas syrigae* pv. *syringae* (Pss) appeared to be the major pathogen causing bacterial canker of stone fruit in the Czech Republic so it is necessary for us to use fast and simple method for identification of Pss.

In 2009 we took part in STSM in Belgium. In the host laboratory at Centre Wallon de recherches agronomiques in Gembleux under heading of Dr. Alain Bultreys we performed lot of new molecular and biochemical analyses for species and pathovars distinguishing:

- visual, spectrophotometrical and HPLC test for pyoverdin production
- PCR and HPLC tests for yersiniabactin production
- biological and PCR detection of toxic lipodepsipeptide
- PCR for coronatine production
- BOX-PCR identification
- rep- and IS-PCR characterisations.

STSM has brought us a new perspective in our field and practical experience in using of new molecular an biochemical methods for the pathovars distinguishing. After this mission we have introduced in our laboratory detection method based on estimation of lipodepsipeptide production on agar media which belongs to the toxin-based identification procedures. Some Czech isolates from SPA collection were succesfully identified with this method. We tried two ways of application of the yeast *Rhodotorula pilimanae* MUCL 3039 – cell suspension of the yeast was sprayed onto the cultures of tested isolate and the second way was spreading the yeast by bacteriological loop around the tested isolate. Production of lipodepsipeptide is indicated by inhibition of yeast growth on peptone-glucose-NaCl agar.

STSM REPORT: DIFFERENTIATION OF LITHUANIAN *PSEUDOMONAS SYRINGAE* AND *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS* ISOLATES

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The genetic characterization is relevant to analyze epidemiology, population, phylogenesis of pathogenic bacteria. There were no detailed investigations on genetic diversity of causal agents isolated from stone fruits and walnuts in Lithuania till now.

The main aim was to apply molecular methods (i.e. rep-PCR, PCR MP, MLST) for characterization of diversity of plant pathogenic bacteria – *Xanthomonas arboricola* pv. *juglandis* (Xaj), *Pseudomonas syringae* pv. *syringae* (Pss), *P. syringae* pv. *morsprunorum* race 1 (Psm1) and race 2 (Psm2).

Twenty one strain of *Pseudomonas syringae* (Pss – 14, Psm1 – 4, Psm2 – 3) and 5 strains of *Xanthomonas arboricola* pv. *juglandis* (Xaj) were investigated using PCR-based techniques. In all tests the reference strains of *Pseudomonas syringae* pv. *syringae*, *P. s.* pv. *morsprunorum* and *Xanthomonas arboricola* pv. *juglandis* were used for comparison.

Characterization of Xaj and *P. syringae* strains using rep-PCR method was performed (Shaad et al., 2001). Xaj strains from Lithuania and Poland produced similar amplification patterns in BOX, ERIC and REP PCRs. Differences between strains of Pss were observed and they were grouped into 5 subgroups. Lithuanian strains of Psm1 and Psm2 were different from reference strains Psm LMG2222 and Psm CFBP3800 based on BOX.

PCR MP analysis of *Pseudomonas syringae* strains using Masny and Płucienniczak (2003) method was performed. DNA was extracted by Aljanabi and Martinez (1997). Differences between Pss strains were estimated and four groups were distinguished. PCR product profiles with Psm2 strains from Lithuania were different from typical strain (CFBP 3800).

Using preliminary results of rep-PCR and MP-PCR, 8 representative strains of Pss, Psm1 and Psm2 for MLST analysis were selected. Four house-keeping genes (*rpo*D, *gyr*B, *gap*A and *glt*A (known as *cts*)) for sequencing were used. All 5 Xaj strains were analysed using three house-keeping genes sequencing (*fyu*A, *gyr*B and *rpo*D). To obtain complete results some analysis should be finalized.

FIRST DETECTION OF *XANTHOMONAS ARBORICOLA* PV. *PRUNI* ON ALMOND IN EUROPE: DETAILED SYMPTOMATOLOGY

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Bacterial leaf spot of stone fruits, caused by *Xanthomonas arboricola* pv. *pruni*, was first detected in Europe in 1920 in Italy. Since then, the bacterium has been identified on several stone fruits and ornamentals as *Prunus laurocerasus* in other European countries, but had not been reported on almond. However, the pathogen was reported on almond in countries of other continents (New Zealand, Australia, India, Pakistan, and unconfirmed in the USA). In Spain, the disease was not detected until 2002 in Extremadura on Japanese plum and between 2006 and 2009, symptoms resembling those of bacterial spot disease were observed on almond in the Comunidad Valenciana and Aragón. As some differences between the symptoms on almond and the described ones on other hosts were observed, the main purpose of this poster is to describe in detail the symptomatology on almond to avoid misdiagnosis. This is especially important, as we are dealing with a quarantine bacterium in the EU.

Symptoms were first noted in spring and were observed until leaf fall. On leaves, initial infections began as small, angular, water-soaked spots, which were surrounded by chlorotic tissue, although chlorosis did not extend more than a few millimetres. The lesions are generally clustered in the areas that remain wet longer (leaf tips, sheltered parts of the leaf blades, along the midrib). Subsequently, the lesions turned light brown, necrotic, and sometimes the necrotic spots fell out. When the lesions coalesced, they produced large necrotic areas. As the lesions dry out, shotholes and leaf tatter result. Sometimes premature leaf drop of infected leaves was observed in severely affected trees. These bacterial spot symptoms can be easily confused with those caused by the fungal disease 'shothole' and also by copper phytotoxicity. Twig lesions have not been observed as commonly as the leaf and fruit symptoms. The twig lesions on current season's wood are dark and elongated along the length of the twig, slightly depressed and often have a shiny, greasy appearance with a water-soaked margin. If the lesion expands it may girdle the twig and dieback will occur. The symptoms on fruits are different from those observed on other stone fruits and look quite specific. Infected fruits initially displayed sunken, corky lesions that oozed gum that may stream or clump. The sunken lesions became raised when the mesocarp dehydrated. Infected fruits either dropped prematurely or remained on trees after harvest. These mummies harbour viable bacteria and can serve as a source of inoculum thereafter.

In all the analyzed almond samples showing the described symptomatology, Xanthomonas-like colonies were isolated on yeast extract peptone glucose agar (YPGA) after incubation at 25 ºC. All strains showed biochemical characteristics and FAME profiling which fit with those described for *X. arboricola* pv. *pruni*. PCR technique with primers Y17CoF/Y17CoR and with a new real-time PCR protocol yielded the amplicons expected. IF and ELISA using commercial polyclonal antibodies proved to be not specific for *X. arboricola* pv. *pruni*. Typical or atypical hypersensitivity reaction was obtained on leaves of tobacco (cv. Xanthi) after one to four days. Pathogenicity was confirmed by inoculation on leaves of potted almond trees. Characteristic symptoms appeared on all inoculated leaves after one week of incubation but not on the negative controls. The original pathogen was reisolated from lesions of inoculated leaves. These results allow us to consider that this is the first detection of this pathogen on almond in Europe.

THE TWO YEARS OF EXPERIENCE ON ETIOLOGY OF APICAL NECROSIS ON WALNUT IN TURKEY

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Apical necrosis (AN) is a disease that causes walnut fruit drop and reduces the yield of walnut in commercial orchards in Marmara Region, Northern west part of Turkey. This brown apical necrosis on walnut fruit, differs in appearence from the black greasy spots of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, or from the blackish sunken necrotic spots of anthracnose, caused by *Gnomonia leptostyla*. The disease causes a premature walnut fruit drop and a yield reduction. Between the years of 2009 and 2010, a study was carried out in a walnut orchard located in Marmara Region of Turkey to substantiate the etiology of AN. In this work the microorganisms associated and symptom evolution, disease progress and cultivar susceptibility have been monitored in a walnut orchard from May to August from external to internal on commercial cultivars. Initial infections were observed in exocarp and endocarp tissues at the end of May. Symptoms were first visible in the first half of June, and the incidence of BAN increased during the growing season, with full expression in the first half of July to the first half of August. Infections in young fruits observed in July affected pericarp tissues and reached kernel whereas early infections (June) and late infections (August) of walnut fruit localized only pericarp tissues. According to our isolation and pathogenicity results from external and internal tissues of walnut fruits of all tested cultivars, the microorganisms associated to AN of walnut included *Xanthomonas arboricola* pv.*juglandis*, which was isolated from all affected tissues of fruits either laying on the ground or still attached to the tree. The Extent of external and internal lesions of the style were not correlated on symptomatic fruit. *Fusarium* spp. and *Alternaria* spp. also seem to be involved in apical necrosis of walnut causing secondary infections or growing as saprophyte on bacterial infected tissues, enhancing the disease symptoms and severity. Cultivar susceptibility has been monitored in a walnut orchard in Turkey from May to August 2009 and 2010. Most commercially cultivars were susceptible to apical necrosis. "Hartley", "Bilecik", "Vina", "Rendede", "Howard", and "Serr" were highly susceptible to apical necrosis, while local Turkish cultivars, " Yalova 1" and "Şebin" were less affected by AN.

NEW STRATEGIES TO CONTROL *XANTHOMONAS ARBORICOLA* PV. *PRUNI* IN PEACH ORCHARDS

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Bacterial leaf/fruit spot and canker of stone fruits, caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*), is a recurrent disease in Italian peach and plum orchards. The pathogen is regulated (Directive 2000/29/EC, Annex A, Part II, Section II) and is inserted in the EPPO A2 list.

An effective control of the disease is based both on the analysis and certification of propagation material and the use of appropriate control strategies in the field. Control of the disease is quite cumbersome and only rely on several copper-based treatments during the whole year, using different compounds and different copper concentration in relationship to the phenological phase of the host. Control strategies based on several copper treatments frequently result in phytotoxicity and enhance the development of copper resistant strains.

In recent years, molecules described as "biostimulants" or "inducers" of systemic resistance, and "foliar fertiliser" (eventually added with a low amount of copper), are being taken into consideration and applied during the growing season in peach and/or plum orchards during periods considered at a high infection risk. We conducted field and glasshouse trials on peach, with the aim to effectively control the disease by using some novel molecules, such as Glucohumates. The organic substance of the such compounds is stabilized and highly humified, thanks to the presence of highly active humic and fulvic acids extracted from Leonardite and Gluconic Acid. Natural plant polyphenols have also been taken into consideration as possible resistance inducers in peach.

Two Glucohumates (one of which with a low ammount of copper) and three different plant polyphenols were tested; six treatments were done in commercial peach orchards, with the presence of a very high natural inoculum, from the end of April to the end of July.

The most remarkable results were obtained with one Glucohumate (with a reduction of the disease by approx. 80%) and one of the plant extracts (with a reduction of the disease by approx. 65%).

Results are very promising and suggest the possibility to implement effective control strategies, where copper compounds and novel molecules are both used in commercial orchards.

In order to study and understand the effect of one of the biomolecules used, untreated and glucohumate treated peach plants were subject to further molecular analyses in order identify possible genes/sequences involved in the induction of disease resistance. A transcriptomic approach was developed in order to detect transcripts present in the plant tissue, after elicitation of an induced protection state. Total RNA was extracted, retro-transcribed and c-DNA-AFLP was done to identify different sequence fingerprints in the protected plant tissue. Discrimination of newly expressed sequences by dHPLC is under way and comparison of transcripts will be done on the complete peach genome database in order to identify the genes or sequences involved in the elicitation of induced resistance.

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