# COST 872 Workshop & MC Meeting

***La Colle-Sur-Loup, May 9-11, 2007***

# PROGRAM

**9th May**
7pm - 8pm: Opening cocktail
8pm – 10pm: Dinner & Discussions (All WGs)

**10th May**
*Morning*
8:30-8:40 Welcome and introduction (John T. JONES)

## WG1: FUNCTIONAL GENOMICS OF PLANT PARASITIC NEMATODES

8:40-9:10 *Invited speaker:* Marcel TIJSTERMAN: **RNAi in *C. elegans*, mechanisms and applications**.

9:10-9:25 **Functional study of proteins secreted by plant-parasitic nematodes.**
Bernard CANNOOT, Godelieve GHEYSEN, Wim GRUNEWALD, Annelies HAEGEMAN, Joachim JACOB, Tina KYNDT, Bartel VANHOLME, and Wouter VAN THUYNE

9:25-9:40 **The identification and characterization of the SPRYSEC-gene family as effecters in *Globodera rostochiensis* plant-parasitism.**
Sajid REHMAN, Hein OVERMARS, Ling QIN, Johannes HELDER, Aska GOVERSE, Jaap BAKKER, and Geert SMANT.

9:40-9:55 **Functional characterisation of nematode chorismate mutases – a role in suppression of plant defence signalling pathways?**
John T. JONES, Mark S. PHILLIPS, Eleanor GILROY, Casey PLAIN, Paul BIRCH, and Vivian BLOK.

9:55-10:10 **A venom allergen-like protein from the potato cyst nematode *Globodera rostochiensis*.**
Jose LOZANO, Ling QIN, Hein OVERMARS, and Geert SMANT.

10:10-10:25 **Transcriptome analysis of the root-knot nematode functions induced during the early steps of parasitism.**
Géraldine DUBREUIL, M. MAGLIANO, E. DELEURY, P. ABAD, and M.N. ROSSO.

10:25-10:50 Coffee break

10:50-11:05 **Does the cuticle of *Meloidogyne artiellia* contain specific pathogen recognition tools?**
Elena FANELLI, Caterina DILEO, Mauro DI VITO, Francesca DE LUCA, and Carla DE GIORGI.

11:05-11:20 **Environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behaviour.**
Rosane H.C. CURTIS, K. MAGUIRE and A. LOVEGROVE.

11:20-11:35 **Turning nematodes on their head - plant parasitic nematodes display neuronal sensitivity to RNAi.**
Aaron G. MAULE, Michael JOHNSTON, Steven MCMASTER, Philip DONNELLY, Johnathan DALZELL, Sue MCKINNEY, Nikki J. MARKS, Michael J. KIMBER, and Colin C. FLEMING.

11:35-11:50 **Manipulating fecundity and lifespan in *Caenorhabditis elegans* using exogenous peptides.**
Keith G. DAVIES and John E. HART.

11:50-12.30 Discussion WG1

12:30-14:00 Lunch

*Afternoon*

## WG 2 : COMPARATIVE GENOMICS OF NEMATODES

14:00-14:30 *Invited speaker:* Mark BLAXTER: **Protein domain and protein family novelty in the Nematoda.**

14:30-14:45 **The *Meloidogyne incognita* genome sequencing initiative.**
Pierre ABAD, Philippe CASTAGNONE, Emeline DELEURY, Marie-Noëlle ROSSO, Laetitia ZURLETTO, Jérôme GOUZY, and Patrick VINCKER.

14:45-15:00 **Annotating the genome of *Meloidogyne incognita.***
Jérôme GOUZY, Emeline DELEURY, Céline NOIROT, Thomas SCHIEX, and Pierre ABAD.

15:00-15:15 **Molecular phylogeny and evolution of plant-parasitism in Tylenchina (Nematoda).**
Wim BERT, Frederik LELIAERT, Andy R. VIERSTRAETE, Jacques R. VANFLETEREN, and Gaetan BORGONIE.

15:15-15:30 **SSU and LSU rDNA-based phylogenetic relationships among nematodes: implications for the evolution of plant parasitism & possibilities for barcode-based detection.**
Martijn HOLTERMAN, Sven van den ELSEN, Hanny van MEGEN, Peter VEENHUIZEN, Roel STAPS, Jaap BAKKER, Geert SMANT, and Johannes HELDER.

15:30-15:45 **Variability and selective constraints of cathepsin L gene occurred during the evolution of plant parasitic nematodes.**
Alain BLANCHARD and Eric GRENIER.

15:45-16:00 **Variability of parasitism genes in golden potato cyst nematode *Globodera rostochiensis.***
Barbara GERIC STARE, Sasa SIRCA, and Gregor UREK.

16.00-16.30 Discussion WG2

16.30-17:00 Coffee break

17:00-18.30 COST 872 MC meeting

17:00-20.00 **Posters**

20:00-21:30 Dinner

**11th May**
*Morning*

## WG3 : FUNCTIONAL GENOMICS OF PLANT RESPONSES

8:30-9:00 *Invited speaker:* Michael G.K. JONES: **Understanding plant responses to nematodes: INPACT technology, an alternative approach to nematode control**

9:00-9:15 **Transcriptome analysis of giant cell three days after infection.**
Marta BARCALA, Keith LINDSEY, Gloria GARCÍA, Roberto SOLANO, Carmen FENOLL, and Carolina ESCOBAR.

9:15-9:30 **The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots.**
Dagmar SZAKASITS, Perta HEINEN, Julia HOFMANN, Krzysztof WIECZOREK, David KREIL, Florian M.W. GRUNDLER, and Holger BOHLMANN.

9:30-9:45 **Molecular characterisation of plant-nematode interactions to aid development of novel resistance.**
P.E. URWIN, M. BAKHETIA, V.L. FULLER, J.M. SMITH, and H.J. ATKINSON.

9:45:10:00 **Expansins and endo-1,4-β-glucanases are involved in cell wall modifications in nematode induced syncytia.**
Krzysztof WIECZOREK, Julia HOFMANN, Andreas BLÖCHL, Dagmar SZAKASITS, Holger BOHLMANN, Daniel J. COSGROVE, David P. KREIL, and Florian M. W. GRUNDLER.

10:00-10:15 **Symplastic and apoplastic nutrient loading of different nematode feeding sites.**
Stefan HOTH, Ulrich HAMMES, and Norbert SAUER.

10:15-10:45 Coffee break

10:45-11:00 **Early events in tomato-*Meloidogyne incognita* interactions.**
Pasqua VERONICO, M. Teresa MELILLO, Paola LEONETTI, and Teresa BLEVE-ZACHEO.

11:00-11:15 **Early molecular responses of coffee (*Coffea arabica*) to root-knot nematodes (*Meloidogyne exigua*) infection.**
Anne-Claire LECOULS, Anne-Sophie PETITOT, and Diana FERNANDEZ.

11:15-11:30 **Comparison of *Hs1*$^{pro-1}$-initiated expression profiles in *Arabidopsis* and sugar beet leads to identification of key genes involved in nematode resistance.**
Wanzhi YE, Jan MENKHAUS, Katrin ASBACH, and Daguang CAI.

11:30-11:45 **Two resistance QTLs display induced and constitutive, as well as additive and epistatic effects, on transcription of defense-related genes.**
Michel CLAVERIE, Véronique DE CONTO, Katell JOLIVET, Marie-Claire KERLAN, Eric GRENIER, Didier MUGNIÉRY, Véronique LEFEBVRE, and Bernard CAROMEL.

11.45-12.15 Discussion WG3

12.15-12.30 Concluding remarks

12:30-14:00 Lunch

14:00-17:00 Group discussions on work program. Attendance not compulsory

**Communications WG1:**

# FUNCTIONAL GENOMICS OF PLANT PARASITIC NEMATODES

*Invited speaker*

## RNAi in *C. elegans*, mechanisms and applications.

Marcel TIJSTERMAN.
*Tijsterman@niob.knaw*

*Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, THE NETHERLANDS.*

The phenomenon of RNA interference (RNAi) occurs in eukaryotic organisms from across the boundaries of taxonomic kingdoms. In all cases, the basic mechanism of RNAi appears to be conserved--an initial trigger [double-stranded RNA (dsRNA) containing perfect homology over at least 19-21/bp with an endogenous gene] is processed into short interfering RNA (siRNA) molecules and these siRNAs stimulate degradation of the homologous mRNA. In the vast majority of species, RNAi can only be initiated following the deliberate introduction of dsRNA into a cell by microinjection, electroporation, or transfection. However, in the nematode worm Caenorhabditis elegans, RNAi can be simply initiated by supplying dsRNA in the surrounding medium or in the diet. Following uptake, this dsRNA triggers a systemic effect, initiating RNAi against the corresponding target gene in tissues that are not in direct contact with the external milieu.
This remarkably phenomenon now allows the systematic analysis of gene function on a genomic scale in an animal system. We are currently using genome-wide RNAi approaches to identify genes and genetic networks that contribute to genome stability and are thus potentially involved in human carcinogenesis.

# Functional study of proteins secreted by plant-parasitic nematodes.

Bernard CANNOOT, Godelieve GHEYSEN, Wim GRUNEWALD, Annelies HAEGEMAN, Joachim JACOB, Tina KYNDT, Bartel VANHOLME, and Wouter VAN THUYNE.
*Bartel.Vanholme@UGent.be*

*Molecular Biotechnology, Ghent University, Coupure links 653 B-9000 Ghent, BELGIUM.*

During the last decade several labs working on plant-parasitic nematodes focussed on the identification of secreted proteins. This effort resulted in many interesting proteins with a putative role in parasitism. Today, the focus shifts from identification towards functional characterization of the many identified putative secreted proteins. For several proteins this process is straightforward. For example, nowadays it is well known that plant-parasitic nematodes secrete cell wall degrading enzymes which macerate the plant tissue and facilitate the migration of the nematode in the plant root. The different genes coding for these enzymes can be expressed in suitable hosts and the produced proteins can be further tested on specific substrates under different experimental conditions. Other secreted proteins are more difficult to link to a specific function in the parasitic lifecycle of the nematode. These are mainly proteins secreted by sedentary nematodes and they are most likely involved in the transformation of plant cells into elaborated feeding structures. Many of these proteins have a putative regulatory function in the plant cell, making experimental design more challenging. The information towards functional characterization of these proteins is gathered in bits and pieces from different independent experiments. In this presentation a short overview will be given on the different proteins our lab is focussing on. In addition we will discuss some of the techniques we use to unravel their putative role in the parasitic process.

# The identification and characterization of the SPRYSEC-gene family as effecters in *Globodera rostochiensis* plant-parasitism.

Sajid REHMAN, Hein OVERMARS, Ling QIN, Johannes HELDER, Aska GOVERSE, Jaap BAKKER, and Geert SMANT.
*geert.smant@wur.nl*

*Wageningen University, laboratory of Nematology Binnehaven 5, 6708 PD Wageningen, THE NETHERLANDS.*

The esophageal gland secretions of plant-parasitic nematode are believed to play an important role in parasitism. We have undertaken a comprehensive transcriptome analysis of all genes expression at the onset of parasitism in *Globodera rostochiensis*. Genes specifically expressed in the esophageal gland cells and encoding proteins with a signal peptide for secretion were selected for further analysis. Seven transcript derived fragments show various degrees of similarity to the SPRY domain in human RanBP9 (RanBPM). Mining the EST database of *G. rostochiensis* with these seven gene fragments resulted in the identification of a large gene family, including at least twenty three members, specifically expressed in the dorsal esophageal gland. Nematodes use the oral stylet to deliver effector molecules into the host cells. The products of at least three family members were shown to be secreted through the oral stylet of the nematode. 3D protein structure modeling of the consensus sequence build from the family members combined with diversity mapping indicates that the majority of the sequence diversity in this gene family is located in one particular surface of the protein. Yeast-two-hybrid analysis using one family member and a tomato root cDNA library pointed at only a few interacting genes, thus shedding light on the potential roles of this gene family in plant-parasitism.

# Functional characterisation of nematode chorismate mutases – a role in suppression of plant defence signalling pathways?

John T. JONES, Mark S. PHILLIPS, Eleanor GILROY, Casey PLAIN, Paul BIRCH, and Vivian BLOK.
*jjones@scri.ac.uk*

*PPI Programme, SCRI, Invergowrie, Dundee, DD2 5DA, SCOTLAND.*

Chorismate mutases (CM) have been identified from cyst and root-knot nematodes. The CM proteins are produced in the oesophageal gland cells and are likely to be secreted into host plants. It has been suggested that CM may deplete precursors of cytoplasmic auxin thus changing auxin levels and triggering the changes associated with early feeding site development. However, these proteins are present in both cyst and root knot nematodes and the feeding sites of these two nematode groups are different in their ontogeny and structure. We are currently investigating the functional role of the nematode chorismate mutases using a variety of approaches. Experiments using CM fusions with green fluorescent protein introduced into plants using virus vectors suggest the proteins are localised to the cytoplasm of plant cells. Knocking out CM expression using RNAi reduced levels of infection compared to plants treated with dsRNA from a non-endogenous gene. The effect was most pronounced when looking at adult females and, since sex is determined by food availability in this nematode, this indicates that one role of CM may be to ensure a healthy and fully functional feeding site is induced, either by inducing the formation of the feeding site or by preventing its breakdown by plant defence systems. Current work is examining a potential role for the nematode CM in suppression of plant basal defences. Preliminary results suggest that expression of the PCN CM in *Pectobacterium atrosepticum* mutants deficient in genes required for normal suppression of basal defences (*hrpW, dspE*) restores the ability of the bacterium to suppress such defence signalling pathways, suggesting that the role of the CM may be to protect the developing feeding site from plant defences rather than inducing the formation of this structure.

# A venom allergen-like protein from the potato cyst nematode *Globodera rostochiensis.*

Jose LOZANO, Ling QIN, Hein OVERMARS, and Geert SMANT.
*jose.lozano@wur.nl*

*Wageningen University, laboratory of Nematology, Binnehaven 5, 6708 PD Wageningen, THE NETHERLANDS.*

Secretory proteins produced in the esophageal gland cells of plant-parasitic nematodes play an important role in parasitism. A venom allergen-like protein from *Globodera rostochiensis* (designated gr-vap-1) was identified, by cDNA-amplified fragment length polymorphism (cDNA-AFLP) approach, as strongly up-regulated in preparasitic J2s. The gr-vap-1 cDNA contained an open reading frame encoding 219 amino acids with the first 26 amino acids being a putative secretion signal. In situ hybridization microscopy showed specific expression of gr-vap1 in the subventral esophageal glands. Temporal expression analysis of gr-vap-1 in different developmental stages revealed expression in pre-parasitic and parasitic J2s and adult males. Protein interaction studies using gr-vap1 and a tomato root cDNA library in a yeast-two-hybrid set-up resulted in specific interactions with various proteins of which some are associated with plant defense mechanisms. The expression and the interaction studies suggest that gr-vap1 may play an important role in the infection of host plants during the motile stages of *G. rostochiensis*.

# Transcriptome analysis of the root-knot nematode functions induced during the early steps of parasitism.

Géraldine DUBREUIL, M. MAGLIANO, E. DELEURY, P. ABAD, and M.N. ROSSO.
*Geraldine.Dubreuil@sophia.inra.fr*

*INRA, UMR 1064 Interactions Plantes Microorganismes et Santé Végétale, 400 Route des Chappes, BP 167, F-06903 Sophia Antipolis, FRANCE.*

Root-knot nematodes, genus *Meloidogyne*, are obligate sedentary root parasites able to infest more than 3000 plant species.
The enzymes secreted by the nematode for the degradation of plant tissues during root invasion are well known. However the proteins involved in the adaptation of the parasite to its host environment and the effectors involved in the modulation of host responses are poorly understood. The aim of this work is to get a global view of nematode genes regulated during the early steps of parasitism.
In this work, we deepen the differences between the transcriptome of pre-parasitic exophyte second stage juveniles and sedentary endophyte third stage juveniles (J3) of *M. incognita* by Subtractive Suppression Hybridization.
From the 89 contigs and 98 unique sequences obtained from the J3-enriched library, 82 contigs overlapped with EST from *M. incognita*. Interestingly, 37 contigs and 68 unique sequences were absent from EST databases. Genes up-regulated in the endophyte stage were validated by macroarrays and real-time quantitative PCR Up-regulation was evidenced for genes involved in detoxification, protein degradation, for a gene encoding a putative secreted protein and genes of unknown function. In particular, the transcripts of a glutathione S-transferase gene, *Mi-gst-1*, were 27 times more abundant in J3 than in J2.
The expression of *Mi-gst-1* in the esophageal secretory glands and the functional analysis by RNA interference suggest that glutathione S-transferases are secreted during parasitism and necessary for the nematode to complete its life cycle in the host.
Secreted glutathione S-transferases could protect the parasite from reactive oxygen species or modulate the plant responses triggered by the pathogen attack.

# Does the cuticle of *Meloidogyne artiellia* contain specific pathogen recognition tools?

Elena FANELLI, Caterina DILEO, Mauro DI VITO, Francesca DE LUCA, and Carla DE GIORGI.
*c.degiorgi@biologia.uniba.it*

*Dipartimento di Biochimica e Biologia Molecolare, Via Amendola 165/A, Bari, ITALY.*

Innate immunity is an evolutionary ancient defense system that enables animals and plants to resist invading microorganisms. This study shows that the over-expression of lysozyme in the plant parasitic nematode *Meloidogyne artiellia* is a defense related mechanism in the inducible defense system triggered by the presence of the Gram-negative bacterium *Serratia marcescens*. By contrast, no regulation of the expression of lysozyme was observed in the presence of *Pasteuria penetrans,* the only known effective biological control agent for *Meloidogyne*. Isolated eggs containing embryos at various developmental stages do not show augmentation of lysozyme expression in the presence of pathogen thus indicating that the pathogen recognition strategy may lie only on the cuticle of emerged juveniles.

# Environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behaviour.

Rosane H.C. CURTIS, K. MAGUIRE, and A. LOVEGROVE.
*rosane.curtis@bbsrc.ac.uk*

*Rothamsted Research, Harpenden, Herts, AL5 2JQ, UNITED KINGDOM.*

This work aims to study some aspects of the early stages of the plant-nematode interactions, which involve host recognition processes in the rhizosphere and the invasion of, and migration within roots as the infective second-stage juveniles move towards their feeding sites. Nematodes can rapidly change their surface composition in response to environmental signals, which may enable animal parasitic nematodes to escape host immune responses and free-living nematodes to escape pathogenic infections[7,8]. Our work has shown that *in vitro*, plant signals present in root exudates, trigger a rapid alteration of the surface cuticle of sedentary plant parasitic nematodes and that the same changes were also induced by phytohormones, such as auxin and cytokinins to *Meloidogyne incognita* but not to *Globodera rostochiensis*[1,2,3,4,6]. Root-knot nematodes invade a large number of plants and not surprisingly, sense and respond to general plant regulators whilst the cuticle of the infective stage of potato cyst nematodes which invades mainly *Solanaceous* plants was not affected in the same way by phytohormones. Plant signals present in root exudates also trigger behavioural responses in the free-living nematode *Caenorhabiditis elegans* and our work shows that this nematode responds to indole-acetic acid (IAA)[4,5], this ability to respond to IAA from plant or bacteria origin could benefit food location In addition, auxin gradient formed in the roots might function as a ***short distance orientation marker*** for *M. incognita* to navigate on the root surface and/or inside the root tissue. RKN invade roots at the zone of elongation where the highest levels of IAA influxes have been determined. We have shown IAA binds to the chemosensory organs of *M. incognita* and we suggest that stimulation of chemosensory receptors by environmental signals may lead to changes in the nematode cuticle and behaviour. Protein sequence using Q-TOF Mass Spectrometry, was obtained from *C. elegans* and *M. incognita* molecules isolated from an IAA affinity chromatography. No reactivity of this protein was obtained with antibodies raised to plant auxin-binding proteins. Further work will concentrate on the functional analysis and localisation of expression of the nematode genes identified as potentially involved in IAA sensing.

**1**. Akhkha *et al.,* 2002. Parasit., 125: 165. **2**. Akhkha *et al.,* 2004. Parasit., 128:533. **3**. Curtis (2007). Nematology, 9(2). *in press*. **4**. Curtis *et al.*, 2006. XXVIII-Symp. European Society Nematologists. **5**. Horiuchi *et al.*, 2005. Planta, 222: 848. **6**. Lopez *et al.*, 2000. Parasitology, 120: 203. **7**. Olsen *et al.*, 2006. Genes, Brain and Behaviour, 1-13. *in press*. **8**. Proudfoot *et al.*, 1993. Parasitology, 107: 559

**Comment [b1]:** You may need to explain here how changes in the sense cell relate to changes in the cuticle. I assume this is through changes in amphidial secretions which form part of the surface coat.

# Turning nematodes on their head - plant parasitic nematodes display neuronal sensitivity to RNAi.

Aaron G. MAULE[1], Michael JOHNSTON[1], Steven MCMASTER[1], Philip DONNELLY[1], Johnathan DALZELL, Sue MCKINNEY[1], Nikki J. MARKS[1], Michael J. KIMBER[2], and Colin C. FLEMING[3,4].
*a.maule@qub.ac.uk*

*[1]Parasitology, School of Biological Sciences, Queen's University Belfast, BT9 7BL, UNITED KINGDOM;*
*[2]School of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA;*
*[3]Agri-Food and Biosciences Institute, Belfast, BT9 5PX, UNITED KINGDOM.*

The susceptibility of plant parasitic nematodes (PPNs) to RNAi is now well established with numerous studies reporting the specific knockdown of target genes in both cyst and root knot nematodes. One feature of these studies has been the need to induce pharyngeal activity in the non-feeding J2s through the application of stimulants to facilitate the uptake of the double-stranded (ds)RNA trigger. More recently, several groups have reported the translation of *in vitro*-triggered gene silencing to *in vivo* silencing through transgenic plant-based strategies for parasite control providing a strong impetus to exploit gene silencing in this context. Once *in planta*, the plant generated dsRNA is believed to be taken in through normal feeding activities of the parasites. We have discovered that simply soaking root knot- or cyst-nematode J2s in dsRNA is enough to trigger the silencing of neuronally expressed genes. Specifically, we have silenced a number of neuropeptide encoding *flp* genes, as well as genes encoding a G-protein coupled neuropeptide receptor, amidating enzyme, acetylcholine receptor subunit and glutamate receptor subunit. In all cases, gene transcript knockdown was observed and distinct and profound phenotypes could be detected, consistent with the disruption of neuronal processes associated with motor function. These observations contrast those made in *C. elegans* wild type where neuronally expressed genes are commonly refractory to RNAi. We propose that these data provide the opportunity to exploit PPNs as a platform for the interrogation of neuronal gene function in nematodes and could have utility in developing strategies for PPN control.

# Manipulating fecundity and lifespan in *Caenorhabditis elegans* using exogenous peptides.

Keith G. DAVIES and John E. HART.
*keith.davies@bbsrc.ac.uk*

*Rothamsted Research, West Common, Harpenden, AL5 2JQ, UNITED KINGDOM.*

We have been able to increase (>40%) and decrease (>60%) the fecundity (number of offspring) of *C. elegans,* plus alter lifespans, by administering synthetic peptides to the aqueous medium in which the nematodes were maintained. Third stage larvae, clearly post-dauer, were used and were fed either pre-killed or live bacteria stain OP50, to avoid a 'two live organisms' protocol. Untreated controls fed live bacteria had significantly more offspring (17 juveniles each vs. 10, $P < 0.05$) than those fed dead bacteria. In our main study, two peptides were compared, these being anagrammatical 14mers, administered at 1 nmol/ml of M9 buffer. The mean number of larvae produced per worm for the three groups of untreated control, EPL001 and EPL030 were respectively 17, 24 (+43%, $P < 0.05$) and 6 (-64%, $P < 0.001$). The period of reproduction was from day 4 to day 21, day 4 to 15 and day 2 to day12 for the EPL001 treated, untreated control and EPL030 treated nematodes respectively and the beginning and end of reproduction was statistically significant between the treatments. The average lifespan of the two groups fed dead and live bacteria were similar, at 11 and 12 days (ns), respectively. There was also no difference in the time for 50% to die (10 days vs. 11, ns) but there was a significant difference in terms of 100% mortality (18 days vs. 28, $P < 0.05$). Average lifespans were not significantly different; at 13, 14 and 13 days (ns), but 100% mortality was increased: 18, 24 and 26 days ($P < 0.05$, test vs. control). The fecundity-enhancing EPL001 concentrated in the genital tract, according to fluorescence localisation studies but the distribution of the fecundity-reducing comparator peptide was less clear-cut.

**Communications WG2:**

# COMPARATIVE GENOMICS OF NEMATODES

*Invited speaker*

## Protein domain and protein family novelty in the Nematoda.

Mark BLAXTER, James WASMUTH, Ralf SCHMID, and Ann HEDLEY.
*mark.blaxter@ed.ac.uk*

*Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3JT, UNITED KINGDOM.*

Using the extensive expressed sequence tag (EST) datasets for nematodes analysed in the NEMBASE database (http://www.nematodes.org/), we have performed a *de novo* analysis of protein domains and protein families across forty species representing four of the five major clades of the phylum. The NEMBASE database contains over 340,000 EST sequences, which define about 120,000 different genes. An additional ~40000 genes derive from the complete genomes of *Caenorhabditis elegans* and *C. briggsae*.

Firstly, we used the prot4EST programme to derive high quality protein sequence predictions for 126,000 EST clusters. The genome-derived *Caenorhabditis* species' proteins were added to this set. These proteins were then compared to each other and clustered using MCLTribe to define protein families. For each protein family, we examined whether it was similar to any non-nematode sequence, or was unique to Nematoda, and also mapped its distribution within Nematoda. Many protein families are unique to Nematoda, and mapping these on the accepted phylogeny based on small subunit ribosomal RNA reveals that new protein families have arisen continuously through nematode evolution. Novel families are particularly frequent within the Tylenchina.

Domains were identified in nematode proteomes by first identifying any sequence segments that could be assigned to known protein domains (such as those defined in Pfam). All remaining sequence was clustered and used to predict novel domains. Again, we have mapped the occurrence of these across the nematodes and in other taxa. In Tylenchina we have identified several domains that are novel, and appear to be restricted to the plant-parasitic species and various species from Viridiplantae. These are further candidates for potential lateral gene transfer events between plant-parasitic nematodes and the organisms with which they share their environments.

# The *Meloidogyne incognita* genome sequencing initiative.

Pierre ABAD[1], Philippe CASTAGNONE[1], Emeline DELEURY[1], Marie-Noëlle ROSSO[1], Laetitia ZURLETTO[1], Jérôme GOUZY[2], and Patrick VINCKER[3].
*Pierre.Abad@sophia.inra.fr*

*1. UMR INRA-UNSA-CNRS, Interactions Plantes-Microorganismes et Santé Végétale, 400 route des Chappes, BP 167, 06903 Sophia Antipolis cedex, FRANCE ;*
*2. Plateforme Bioinformatique du Génopôle Toulouse Midi Pyrénées, BP 52627, 31326 Castanet Cedex, FRANCE ;*
*3. Genoscope, Centre National de Séquençage, 2 rue Gaston Crémieux, CP5706, 91057 Evry cedex, FRANCE.*

Root-knot nematodes (RKN) are obligate parasites of roots able to infest more than 3000 plant species. Among them and because of its ubiquitous distribution, *Meloidogyne incognita* is possibly the most damaging crop pathogen in the world. Although the genome sequences of the free-living species *C. elegans*, and its sister species *C. briggsae*, are available, very little is known about the other members of the phylum Nematoda at the genomic level. Particularly, parasitic nematodes, which constitute half of the earth's nematodes, remain poorly explored. We have successfully submitted to the GENOSCOPE (French National Center for Sequencing) a project for the complete sequencing of the genome of the RKN *M. incognita*. This project has been supported by the scientific community involved in research on RKN worldwide, and a restricted international consortium will perform manual annotation of the genome. The sequencing of the genome of *M. incognita* (~50 Mb) will be achieved this year by shotgun sequencing of genomic DNA (10X coverage), along with the sequencing of both ends of phosmid clones and large collections of EST clones. Progress on the sequencing project will be presented. Because of the sequencing initiative on *M. hapla* genome in the USA, the availability of both phytoparasitic nematode genome sequences will optimize the comparative annotation and facilitate further comparative genomics analyses (e.g. determinants of plant parasitism, mode of reproduction,…) In addition, the complete genome sequences of animal parasite nematodes that will be soon available and of the free- living nematodes will offer an unparalleled opportunity for comparative genomics among nematode genomes.

## Annotating the genome of *Meloidogyne incognita.*

Jérôme GOUZY[2], Emeline DELEURY[1], Céline NOIROT[3], Thomas SCHIEX[3] and Pierre ABAD[1].
*Jerome.Gouzy@toulouse.inra.fr*

*1. UMR INRA-UNSA-CNRS, Interactions Plantes-Microorganismes et Santé Végétale, 400 route des Chappes, BP 167, 06903 Sophia Antipolis cedex, FRANCE ;*
*2. Plateforme Bioinformatique du Génopôle Toulouse Midi Pyrénées, BP 52627, 31326 Castanet Cedex, FRANCE ;*
*3. INRA/CNRS Laboratoire des Interactions Plantes microorganismes (LIPM), BP52627 Chemin de Borde Rouge, 31326 Castanet Cedex, FRANCE.*

Identifying gene structures is not only the first but also an essential step to benefit from the genome sequence of *Meloidogyne incognita*. In the frame of the whole-genome sequencing project, the gene finding we will be performed by using the EuGene annotation platform. EuGene is capable to build gene models by integrating various sources of evidences like similarities with expressed sequences, similarities with protein databases and the exon conservation identified from related genomes. The complete automatic annotation process will be presented.

# Molecular phylogeny and evolution of plant-parasitism in Tylenchina (Nematoda).

Wim BERT, Frederik LELIAERT, Andy R. VIERSTRAETE, Jacques R. VANFLETEREN, and Gaetan BORGONIE.
*Wim.Bert@UGent.be*

*Nematology Section, Department of Biology, Ghent University, Ledeganckstraat 35, 9000 GENT, BELGIUM.*

Nematodes of the suborder Tylenchina *sensu* De Ley & Blaxter 2002 include an ecologically and morphologically diverse array of species that range from soildwelling microbivores to highly specialised plant-parasites. A robust phylogenetic framework is a prerequisite to understand the evolution of parasitism in this group. However, the lack of objective criteria for assessing homology of morphological characters of such diverse array of nematode species has hampered the reconstruction of their phylogeny. Recently, sequences from nuclear ribosomal RNA genes have made a huge contribution to our current knowledge of nematode phylogeny. RNA's transcribed from RNA genes have a complex secondary structure mediated by base pairing between regions of the rRNA molecule. Nevertheless, although several likelihood-based studies have already shown the superiority of considering base pair correlation in RNA stems over methods assuming independent evolution of nucleotides (e.g. Telford et al., 2005), accounting for this secondary structure in the models of evolution used in phylogeny reconstruction is largely ignored (see Subbotin et al., 2007 for exceptions). Here we use base paired models to reconstruct the SSU phylogeny of 93 Tylenchina using a Bayesian search procedure. Feeding type evolution is traced along the trees using parsimony reconstruction implemented in Mesquite v1.11 (Maddison and Maddison, 2006). Evolution from bacterial feeding towards highly specialised sedentary endoparasitism has most likely gone via fungal feeding, root-hair feeding, ecto- and migratory endo-parasitism. Cyst and root-knot nematodes arose independently. Cyst nematodes have a sister-group relationship with the ectoparasitic Hoplolaiminae, and root-knot nematodes have migratory endoparasitic Pratylenchidae as their most recent common ancestor. Present trees can serve to identify nematode clades that include taxa most appropriate for use as comparative model organisms to understand plant-nematode interactions.

# SSU and LSU rDNA-based phylogenetic relationships among nematodes: implications for the evolution of plant parasitism & possibilities for barcode-based detection.

Martijn HOLTERMAN[1], Sven van den ELSEN[1], Hanny van MEGEN[1], Peter VEENHUIZEN[2], Roel STAPS[2], Jaap BAKKER[1], Geert SMANT[1] and Johannes HELDER[1].
*Hans.Helder@wur.nl*

[1]*Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, THE NETHERLANDS ;*
[2] *Blgg b.v. , Nieuwe Kanaal 7F, 6709 PA Wageningen, THE NETHERLANDS.*

Full length small and large subunit (SSU / LSU) ribosomal DNA sequences harbour sufficient signal for the establishment of robust phylogenetic relationships among most major groups of nematodes. Molecular data suggest that plant parasitism arose at least five times independently. The five clusters of plant parasites are found in three different clades; Clade 1 (Triplonchida, 1x), Clade 2 (Dorylaimida, 3x) and Clade 12 (1x) (for clade definition see Holterman et al. 2006). Remarkably, plant parasitism seems to accelerate the evolution of rDNA. This characteristic results in an unexpected high resolution among major impact plant parasites such as root knot and cyst nematodes. We will present detailed relationships among the Meloidogynidae (nested within the Pratylenchidae; Meloidogynidae is strictly spoken an invalid family) and the Heteroderidae (*Heterodera* and *Globodera* spp.), and show how this information can be used for (semi)-quantitative detection. Furthermore, we will try to shed light on the question whether plant parasitism within Clade 12 indeed could be the result of horizontal gene transfer. Endoglucanases have been cloned from a relatively wide range of Tylenchomorpha and we will compare relationships based nematode GH5 sequences with the topology of a tree based on "neutral" SSU / LSU rDNA sequences.

# Variability and selective constraints of cathepsin L gene occurred during the evolution of plant parasitic nematodes.

Alain BLANCHARD[2] and Eric GRENIER[1].
*eric.grenier@rennes.inra.fr*

[1]*INRA UMR BiO3P, Domaine de la motte, Le Rheu, F-35653, FRANCE ;*
[2] *INRA/Univ. Bordeaux 2, UMR Génomique, Développement et Pouvoir Pathogène, Domaine de la Grande Ferrade, 71 avenue Edouard Bourlaux, BP 81, 33883, Villenave-D'Ornon Cedex, FRANCE.*

Cyst (*Globodera* and *Heterodera*) and root-knot (*Meloidogyne*) nematodes of the Heteroderidae family have evolved sophisticated relationships with plant hosts to sustain a sedentary parasitic habit through the elaboration and maintenance of a feeding site. With the increase of our knowledge of the nematode genes involved in the parasitic process, new targets are now available for the development of new control strategies based on artificial resistances. However, before developing such new tool, it is important to study the variability of the target genes and the selection pressure acting on them in order to get insights in the potential range of use and the durability of such resistance. A dataset of more than 30 cathepsin L (parasitism gene) and elongation factor (housekeeping gene) gene sequences from various populations and species of cyst nematodes was compiled to test the hypothesis of particular evolution of genes related to parasitism. Compared to the elongation factor gene, the cathepsin L gene seemed to evolve more rapidly than the housekeeping gene, by accumulating non synonymous substitutions that induced modifications of the corresponding protein. The nature of the selection occurring on these genes was defined through the estimation of the Ka/Ks ratio along the gene sequences and in the various lineages. One particular domain of the mature region of the cathepsin L protein evolved under diversifying selection suggesting an implication of the identified amino acids in molecular interaction with other plant proteins. This provides further informations to functional analyses of the cathepsin L genes with the goal to generate durable artificial resistances based on this gene.

# Variability of parasitism genes in golden potato cyst nematode *Globodera rostochiensis.*

Barbara GERIC STARE, Sasa SIRCA and Gregor UREK.
*barbara.geric@kis.si*

*Agricultural institute of Slovenia, Plant protection department, Hacquetova 17, 1000 Ljubljana, SLOVENIA.*

*Globodera rostochiensis* and *G. pallida* are economically important plant-parasitic cyst nematodes parasitizing on potato, tomato, eggplant and other species of genus *Solanum*. In Slovenia, *G. rostochiensis* has been detected at several locations in recent years while *G. pallida* has not been detected at the fields yet, but was intercepted at the Slovenian border for several times. Potato cyst nematode populations vary in degree of virulence for different potato cultivars. Within *G. rostochiensis* five pathotypes can be distinguished based on the ability to develop on certain cultivars and clones of *Solanum*. We selected the resistant cultivars to be grown in the infested areas by bio-tests on different potato cultivars available in Slovenian seed potato market.
Parasitism is a complex plant-nematode interaction, regulated by expresion of the parasitic factors in the nematode and the defence mechanism in the plant. Parasitic factors secreted by the nematode into plant cells from subventral and dorsal oesophageal gland cells via the stylet are assumed to play key roles during parasitism. Many genes responsible for parasitism of plant-parasitic nematodes were characterized in the last few years; expansin, pectate lyase, cellulase, superoxide dismutase, chorismate mutase, RanBPM etc… However, information on sequence variability of these parasitism factors is scarce. Since different pathotypes affect different potato cultivars, we expected variability in nucleotide sequences of parasitic factors of *G. rostochiensis*. In our study we evaluate genetic variability of two key parasitism genes, expansin and superoxide dismutase, within and between pathotypes of *G. rostochiensis*. Genomic DNA revealed nucleotide sequence variability within exons and introns. Variability within single specimen confirmed existance of gene families for both investigated genes, thus correlation between gene variability and pathotypes is not obvious. Determination of key differences in parasitic genes of different pathotypes could be a basis for development of a novel, molecular based methode for pathotype identification of *G. rostochiensis*.

**Communications WG3:**

**FUNCTIONAL GENOMICS OF PLANT RESPONSE**

*Invited speaker*

## Understanding plant responses to nematodes: INPACT technology, an alternative approach to nematode control.

Michael G.K. JONES, J. FOSU-NYARKO, S. LIU, and Z.-H. WANG.
*m.jones@murdoch.edu.au*

*Western Australian State Agricultural Biotechnology Centre, Murdoch University, South Street, Perth, WA 6150, AUSTRALIA.*

Root-knot nematodes are major plant pathogens in tropical and sub-tropical regions. Study of changes of gene expression in feeding cells (giant cells) of host plants provides information that can be used to develop synthetic host resistance. Such an application will be illustrated in a process called 'INPACT' technology. This is based on knowledge of replication of DNA viruses, and requires two promoters to provide tight control of gene expression. Although originally developed for over-expression of gene products for 'molecular farming', the principle can be applied to develop resistance to plant parasitic nematodes, using information on expression of genes in nematode feeding cells. The principles of this technology will be described, together with progress in its application to control plant parasitic nematodes.

# Transcriptome analysis of giant cell three days after infection.

Marta BARCALA[1], Keith LINDSEY[2], Gloria GARCÍA[3], Roberto SOLANO[3], Carmen FENOLL[1], and Carolina ESCOBAR[1] .
*carolina.escobar@uclm.es*

*[1] Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Campus de la Real Fábrica de Armas, E-45071 Toledo, SPAIN;*
*[2] University of Durham, Department of Biological and Biochemical Science. South Road, DH1 3LE, Durham City, UNITED KINGDOM;*
*[3]Centro Nacional de Biotecnología, Campus de Cantoblanco, Madrid 28049. SPAIN.*

Sedentary endoparasites such as root-knot nematodes (*Meloidogyne spp.*) cause serious economic losses in different crops all over the world. They reprogram specific plant cells, inducing dramatic changes in gene expression and transform them into giant cells. In *Arabidopsis,* a global transcriptome approach from RNA of galls at mid and late-stages of infection (7-21days post inoculation), revealed alterations in the expression of genes with different functions (Jammes et al., 2005). However, further work is needed to determine the localization of the differentially expressed genes in the different gall tissues and especially in the giant cells, as not all these genes will be differentially expressed in giant cells. Our interest is the study of the specific expression changes occurring in the giant cells at the initial stages of development, when a putative differentiation program is selected. Therefore, we micro-dissected giant cells 3 days after infection, a stage when endoreduplication, nuclear divisions and increase of cell size are very active processes (Goverse et al., 2000, Gheysen and Fenoll, 2002). The comparative expression data against normal cells from the vascular cylinder revealed that more than 1000 genes were differentially expressed and strikingly, the vast majority of them were down-regulated. Among the down-regulated genes, those related with defence, stress and secondary metabolism were the most abundant, suggesting a dramatic reprogramming of gene expression. In contrast, genes in the cytoskeleton and cell cycle categories, as well as genes coding for cell wall modifying enzymes are among the groups with a higher number of up-regulated genes.. These results essentially agree and add new data to the previously reported cytoskeleton rearrangements, cell cycle regulation and expansin induction (Reviewed in Gheysen and Fenoll., 2002; De Almeida et al., 2004; Wieczorek et al., 2006). This wide genome approach based in the isolated transcriptome of GC, may help to the understanding of the profound expression changes related to the ontogeny of these specialized cells.

De Almeida Engler J., Van Poucke K., Karimi M., De Groodt R., Gheysen G., and Engler G. 2004. Dynamiccytoskeleton rearrangements in giant cells and syncytia of nematode-infected roots. *Plant J.* 38:12-26.

Gheysen G., and Fenoll C. 2002. Gene expresion in nematode feeding sites. *Annu. Rev. Phytopathol.* 40:191-219.

Goverse A., de Engler J.A., Verhees J., van der Krol S., Helder J.H., and Gheysen G. 2000. Cell cycle activationby plant parasitic nematodes. *Plant Mol. Biol.* 43:747-761.

Jammes F., Lecomte P., de Almeida-Engler J., Bitton F., Martin-Magniette M.L., Renou, J.P., Abad P., and Favery B. 2005. Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. *Plant J.* 44:447-458.

Wieczorek K., Golecki B., Gerdes L., Heinen P., Szakasits D., Durachko D.M., Cosgrove D. J., Kreil D. P., Puzio P. S., Bohlmann H., Grundler F. M. W. 2006. Expansins are involved in the formation of nematode-induced syncytia in roots of Arabidopsis thaliana. *Plant J.* 48:98-112.

# The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots.

Dagmar SZAKASITS, Perta HEINEN, Julia HOFMANN, Krzysztof WIECZOREK, David KREIL, Florian M.W. GRUNDLER, and Holger BOHLMANN.
*holger.bohlmann@boku.ac.at*

*Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, AUSTRIA.*

The cyst nematode *Heterodera schachtii* is a biotrophic plant parasite which can cause significant economic losses on sugar beet but can also complete his life cycle on a variety of other plants including Arabidopsis. Larvae invade the roots of host plants and induce a syncytium on which the nematode feeds throughout his life.

The syncytium develops from a single cell inside the central cylinder through incorporation of neighbouring cells. How the induction and the development of the syncytium is achieved is unknown. We have studied the transcriptome of syncytia induced by *Heterodera schachtii* in Arabidopsis roots. Pure material from syncytia was obtained through microaspiration and used for RNA isolation. This RNA was amplified, labelled, and used for the hybridization of Affymetrix GeneChips. Our results show that a variety of genes is up- or down-regulated in syncytia. Results have been validated for selected genes by real time PCR and *in situ* RT-PCR. In addition, promoter::GUS lines are being prepared. Genes which are strongly upregulated in syncytia are not only of scientific interest but may also be used as targets for applications to engineer nematode-resistant plants.

Closer inspection of the up-regulated genes revealed, that different pathways for genes that are normally specific for certain tissues other than roots, are induced in the syncytium. A principal component analysis of available transcriptome data has shown that syncytia have more in common with seeds and pollen than with roots.

# Molecular characterisation of plant-nematode interactions to aid development of novel resistance.

P.E. URWIN, M. BAKHETIA, V.L. FULLER, J.M. SMITH, and H.J. ATKINSON.
*p.e.urwin@leeds.ac.uk*

*Centre for Plant Sciences, University of Leeds, LS2 9JT, UNITED KINGDOM.*

Characterisation of both the nematode secreted gene products involved in feeding site formation and the plant genes regulated in the interaction can aid development of novel resistance strategies. qPCR analysis has been combined with RNAi to define pharyngeal gland cell genes of *Heterodera glycines* that are required for initial interaction with the host plant. Microarray analysis has identified genes both up- and down-regulated in the feeding sites of *Heterodera schachtii* and *Meloidogyne incognita* parasitising *Arabidopsis* at 21 days post infection. For *H. schachtii* we found 1167 genes changed in expression by >2-fold with 460 showing increased and 707 showing decreased expression in response to nematode infection. Similar numbers of responding genes were identified after infection with *M. incognita*. Those genes specifically induced or up-regulated in the feeding cell may be good targets for RNAi knock-out leading to impaired syncytial function and reduced nematode success. Genes were confirmed as nematode responsive by qPCR and infection of GUS reporter plants. Cluster analysis of the expression profiles of genes expressed in syncytia identified a subset that were characterised by a lack of expression in most organs of uninfected plants. These genes are of particular interest. Work is in progress to determine if the potato homologues are similarly upregulated upon infection with *Globodera pallida*.

# Expansins and endo-1,4-β-glucanases are involved in cell wall modifications in nematode induced syncytia.

Krzysztof WIECZOREK, Julia HOFMANN, Andreas BLÖCHL, Dagmar SZAKASITS, Holger BOHLMANN, Daniel J. COSGROVE, David P. KREIL, and Florian M. W. GRUNDLER.
*krzysztof.wieczorek@boku.ac.at*

*Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, AUSTRIA.*

Root parasitism of the cyst nematode *Heterodera schachtii* is characterised by the formation of syncytial feeding structures. Syncytia are formed by the fusion of root cells and their formation is accompanied by local cell wall degradation, fusion of protoplasts and hypertrophy. Expansins and endo-1,4-β-glucanases are wall-loosening proteins involved in growth and cell wall disassembly. In this study we tested the hypothesis that their expression is up-regulated during syncytium formation in roots of *Arabidopsis thaliana*. Using PCR we screened a specific cDNA library of 5-7-day-old syncytia for all known expansins and endo-1,4-β-glucanases. Expression of *AtEXPA1*, *AtEXPA3*, *AtEXPA4*, *AtEXPA6*, *AtEXPA8*, *AtEXPA10*, *AtEXPA15*, *AtEXPA16*, *AtEXPA20*, and *AtEXPB3* was detected in syncytia. For *AtEXPA1*, *AtEXPA3*, *AtEXPA4*, *AtEXPA6*, *AtEXPA10*, *AtEXPA15*, and *AtEXP16* these results were confirmed with the aid of promoter::GUS lines. Semi-quantitative RT-PCR showed that *AtEXPA3*, *AtEXPA6*, *AtEXPA8*, *AtEXPA10*, and *AtEXPA16* are up-regulated specifically in syncytia and are not transcribed in control roots. Endo-1,4-β-glucanases hydrolyze the 1,4-β-glucosidic linkages between glucose residues. By use of semi-quantitative and quantitative approaches we identified seven genes that are upregulated in syncytia. Two of these genes, coding for secreted AtCel2 and membrane-bound KOR3, are shoot-specific but show high expression in syncytia at different developmental stages. Treatments with sucrose, GA3, and NAA also induced their upregulation in roots, but other hormones resulted in only minor effects. As AtCel2 is related to degradation of the cell wall matrix, we analysed the hemicellulose content in syncytia. The measured values resembled the expression pattern of *AtCel2*. In *kor3* and *cel2* T-DNA mutants an impairment of growth conditions for nematodes could be found. We conclude that syncytium formation involves the specific up-regulation of different expansin and endo-1,4-β-glucanase genes, which likely take part in the cell growth and wall disassembly in syncytia.

# Symplastic and apoplastic nutrient loading of different nematode feeding sites.

Stefan HOTH, Ulrich HAMMES, and Norbert SAUER.
*shoth@biologie.uni-erlangen.de*

*Molecular Plant Physiology / FAU Erlangen, Staudstr. 5, 91058 Erlangen, GERMANY.*

Plant parasitic nematodes induce specialised cellular structures in the host tissue to establish their nutrient supply. Cell fusions result in the formation of syncytia after infestation of plants by cyst forming nematodes, whereas root-knot nematodes induce giant cells that are formed by differentiation of existing cells. In spite of their diverse formation, these feeding structures exhibit functional homology in supplying the essential nutrients for the nematodes. However, the underlying transport mechansims for the nutrient supply are very different. To our surprise, we could previously show that syncytia are connected to the phloem of *Arabidopsis* roots by plasmodesmata, and that cyst nematodes may take advantage of a strongly induced formation of new companion cells and sieve elements to retrieve nutrients symplastically. In contrast, nutrient loading into giant cells induced by root-knot nematodes requires apoplastic transport processes mediated by transport proteins in the plasma membrane.

# Early events in tomato-*Meloidogyne incognita* interactions.

Pasqua VERONICO, M. Teresa MELILLO, Paola LEONETTI, and Teresa BLEVE-ZACHEO.
*p.veronico@ba.ipp.cnr.it*

*IPP-CNR, Via Amendola 165/A, 70126 Bari, ITALY.*

Root-knot nematodes (*Meloidogyne* spp.) are serious pest for many crops. These pathogens have evolved a sophisticated interrelationship with their hosts where they induce a specific type of nurse cells, the multinucleate giant cells. The transformation of the initial feeding cell into nematode feeding site is paralleled by modifications in plant gene expression. The characterisation of several parasitism genes specifically expressed within oesophageal gland cells suggests that their products can influence the host cellular metabolism. In susceptible plants these secreted molecules might serve as virulence factors for successful parasitism. The *Mi* gene, which confers resistance to several species of *Meloidogyne*, is present in many tomato cultivars. Resistance mediated by *Mi* is associated with localized necrosis of injured host tissues and occurs very early after nematode infection. A stress-induced oxidative burst is commonly caused by many abiotic and biotic stresses and is a signature of the HR to pathogen attack. The oxidative burst has several functions in addition to signal transduction and it is required to limit the spread of the pathogen, by favouring, *eg*, the cross-linking of the cell walls. Most importantly, the oxidative burst also sends signals to the attacked and non-attacked cell cytoplasm inducing local cell death. We designed time-course experiments to analyze the production and *in planta* localization of reactive oxygen species (ROS), and the signalling molecule nitric oxide (NO) in compatible and incompatible tomato-*M. incognita* interaction. A great amount of ROS was one of the first signs of incompatibility and the most production was found very early during nematode infection. In parallel, a peak of NO was recorded working together with ROS in triggering HR. These findings indicate that NO cooperates in the regulation of ROS accumulation and modulates the expression of defence-related genes during nematode infection, as reported in other plant-pathogen interactions. Moreover, the involvement of other genes, such as lipoxygenases, was also demonstrated to play a role in these interactions.

# Early molecular responses of coffee (*Coffea arabica*) to root-knot nematodes (*Meloidogyne exigua*) infection.

Anne-Claire LECOULS, Anne-Sophie PETITOT, and Diana FERNANDEZ.
*Diana.Fernandez@mpl.ird.fr*

*IRD - Institut de Recherche pour le Développement, 911, avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, FRANCE.*

Root-knot nematodes (*Meloidogyne* sp.) are major pests damaging the cash crop coffee culture (*Coffea arabica*) in Latin America. Resistance to *M. exigua* is conferred by a single dominant gene (*Mex-1*) and resistant coffee roots exhibit a typical hypersensitive response (HR). To understand physiological and molecular mechanisms underlying coffee resistance responses to *M. exigua*, we undertook a genomic approach based on the construction of substractive (SSH) cDNA libraries. Two different libraries were generated from root tips of resistant *vs.* susceptible and resistant *vs.* control coffee varieties 2-4 days after inoculation with *M. exigua.* A total of 1180 non-redundant ESTs were obtained and these sequences were specific of each library since only 41 (4%) were common to both. Functional annotation of the unigene set showed that 30% of the ESTs encoded putative homologues of known resistance- and defence-related proteins. In addition, half of the ESTs unigene set represented novel coffee genes and 35% of the annoted ESTs did not share significant similarity to plant protein database entries. Real-time quantitative RT-PCR expression analyses of 98 genes from several functional categories were monitored during *M. exigua* infection time-courses of resistant and susceptible coffee varieties. A higher number of genes exhibited expression changes in the susceptible variety than in the resistant variety (60 and 40 %, respectively) and a significant number were activated in the susceptible variety (37%). In HR-exhibiting plants, only 17% of genes were up-regulated, displaying transient regulation over 5-days time course experiments. This study identified novel root-expressed coffee genes and revealed new insights into plant resistance responses to root-knot nematodes.

# Comparison of *Hs1*$^{pro-1}$-initiated expression profiles in *Arabidopsis* and sugar beet leads to identification of key genes involved in nematode resistance.

Wanzhi YE, Jan MENKHAUS, Katrin ASBACH, and Daguang CAI.
*dcai@phytomed.uni-kiel.de*

*Department of Molecular Phytopathology, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str. 9, 24118 Kiel, GERMANY.*

The *Hs1*$^{pro-1}$ locus confers resistance to the beet cyst nematode *Heterodera schachtii* in sugar beet. To identify genes involved in the resistance response, we generated expression profiles of sugar beet by use of suppressive subtractive hybridisation technique (SSH). Root sections of resistant and susceptible beets were harvested 3, 6, and 12 days after nematode infection and RNA isolated from pooled roots. In total, 1560 ESTs from the forward- and 450 from the reverse-library were obtained, which represent 352 und 105 unique genes. From the forward library, 59% of the sequences could be classified into various functional groups whereas about 41% of them remain functionally unknown and unclassified. More than 30 genes from the forward library gave high homology to known genes which are involved in plant defence responses. In addition, we generated transcription profiles of *Hs1*$^{pro-1}$- transgenic Arabidopsis. The transgenic Arabidopsis root sections, 3, 6, and 12 days after nematode infection were used for RNA isolation. Transcription profiling experiments were done with the Affymetrix ATH1 GeneChip in which the vector-transformed Arabidopsis plants served as control. As a result, about 66% of total Arabidopsis transcripts on the ATH1 chip were detected with both samples, from which 711 transcripts were differentially expressed in the Hs1pro-1 transgenic Arabidopsis . Of these, 217 transcripts were up- and 492 transcripts down-regulated. By comparison, we found that an overall match between two expression profiles. Strikingly, 35 of 52 up-regulated defense-related genes with extremely high change fold of >5 revealed by Arabidopsis GeneChips were presented also by the beet forward SSH library, strongly suggesting their possible roles as key genes in the *Hs1*$^{pro-1}$-mediated nematode resistance.

# Two resistance QTLs display induced and constitutive, as well as additive and epistatic effects, on transcription of defense-related genes.

Michel CLAVERIE[1], Véronique DE CONTO[1], Katell JOLIVET[1], Marie-Claire KERLAN[3], Eric GRENIER[2], Didier MUGNIÉRY[2], Véronique LEFEBVRE[1], and Bernard CAROMEL[1].
*Bernard.Caromel@avignon.inra.fr*

*[1] INRA, UR1052 Génétique et Amélioration des Fruits et Légumes, BP 94, F-84143 Montfavet, FRANCE ;*
*[2] INRA UMR BiO3P, Domaine de la motte, Le Rheu, F-35653, FRANCE ;*
*[3] UMR INRA AGROCampus Rennes APBV, Kéraiber, 29260 Ploudaniel, FRANCE.*

Susceptibility to plant pathogens is often considered to involve a too slow or a too weak resistance response as compared to resistance associated with major genes. Quantitative resistance is intermediate between those two states; it may therefore be hypothesized to involve the same mechanisms, but with a quantitative regulation. The quantitative resistance to *G. pallida* from the wild potato *S. sparsipilum* is conferred by one major effect QTL (*GpaVspI*) and one minor (*GpaXIspI*), which, together, but not separately, participate to the induction of a necrotic reaction around the nematode feeding site. Here, we first screened for physiological marker genes witches transcription regulation were associated with resistance. We then studied the link between the transcription of 7 resistance marker genes (the most strongly regulated) and allelic combinations at both QTLs, during a three time-point course, in a sub-sample from the segregating progeny. Surprisingly, most part of gene induction was linked to the smallest effect QTL (*GpaXIspI*). Actually, *GpaVspI* was found to increase constitutive expression of a potato gene homologous to the *A. thaliana* peroxidase A2 gene, and to interact with *GpaXIspI* on constitutive or induced expression of defense-related genes. Our study showed that the differences in levels of resistance result from different transcriptional pattern with quantitative as well as qualitative bases. Furthermore, the functions associated with the genes regulated by the QTLs suggest an important role of protection of root endoderm in quantitative resistance to potato cyst nematode.

**Posters WG1:**

**FUNCTIONAL GENOMICS OF PLANT PARASITIC NEMATODES**

# Parasitic nematode GPCRs as tools for new agrochemical discovery.

Marida BIMONTE[1], Nicole ALYESHMERNI[1], Fabio APONE[1], Paolo BAZZICALUPO[2], and Gabriella COLUCCI[1].
*marida@arterrabio.it*

*[1]Arterra Bioscience Via Comunale Margherita 482, 80145 Napoli, ITALY;*
*[2]Istituto di Genetica e Biofisica - A. Buzzati Traverso, CNR Via P. Castellino 111, 80131 Napoli, ITALY.*

Our goal is the identification of key molecular targets which can be used for the discovery of new agrochemicals with nematocide activity. Since current nematode control in the field depends on highly toxic pesticides, all of which have been classified for restricted use or slated for elimination, it has become necessary to identify new compounds which, besides protecting the plants from parasitic nematode attacks, are safe for the environment.
G protein coupled receptors (GPCRs), the largest family of cell-surface molecules that transduce extracellular signals into cellular physiological responses, have been successfully used for human drug discovery. Many of the most important drugs used in cardiovascular and neuronal diseases, depression and allergies, target human GPCRs. We strongly believe that also chemical agonists or inverse agonists of nematode GPCRs can find very important applications in agriculture to reduce crop losses, and will certainly succeed in the marketplace.
The free living nematode *Caenorhabditis elegans* is a tractable experimental model organism for the study of invertebrate biology, and represents an excellent system to study the functions of parasitic nematode homologous genes. We have identified 6 GPCR genes which are responsible for vital functions in *C.elegans* and cloned the homologous ones in the species *Meloidogyne incognita*, one of the most dangerous pests for economically important crops in Europe. In order to use *M.incognita* GPCRs as targets for novel agrochemicals and to overcome the technical limitations of *M. incognita* as genetic system, we are using *C.elegans* to functionally characterize those GPCRs by RNAi, overexpression and promoter reporter gene expression studies. Moreover, we are carrying out further studies of *M.incognita* GPCRs, using in situ hybridization analysis directly in the parasitic nematode, and by expressing them in heterologous systems.

# Heat shock protein 90 of *Meloidogyne artiellia*: gene structure and expression.

Francesca DE LUCA[1], E. FANELLI[2], A. REYES[3], M. DI VITO[1], and C. DE GIORGI[2].
*f.deluca@ba.ipp.cnr.it*

[1] *Istituto per la Protezione delle Piante, Sezione di Bari, CNR, Via G. Amendola 122/D, 70126 Bari, ITALY;*
[2] *Dipartimento di Biochimica e Biologia Molecolare, Università di Bari, Via Orabona 4/A,70125 Bari, ITALY;*
[3] *MRC-Dunn Human Nutrition Unit, Cambridge, UNITED KINGDOM.*

Heat shock protein 90 (Hsp90) is one of the most abundant and evolutionarily conserved proteins. Hsp90 proteins are important for stress tolerance, for newly synthesised protein folding and for the growth of various organisms. In particular, in the free living nematode *Caenorhabditis elegans* Hsp90 shows the ability to associate with transduction proteins together with its capacity to act as a stress protein, providing a link between various developmental pathways and environmental changes. In this study, we report the molecular analysis of the full-length cDNA of Hsp90 and its corresponding gene isolated from the root-knot nematode *M. artiellia*.On the basis of sequence identity and presence of conserved residues, the Hsp90 analysed in showed the highest amino acid similarity with those of the parasitic nematodes *Brugia pahangi* and *Heterodera glycines*. The expression patterns of the *hsp*90 transcript were investigated among the different life stages. We found that Hsp90 of *M. artiellia* is transcribed constitutivelly in all this study encodes the cytosolic version of the Hsp90. BLAST search indicated that the Hsp90 of *M. artiellia* developmental stages tested, although the levels of transcripts differ between stages. Furthermore, we evaluated if different stimuli (heat shock, presence of compatible host or diapausing conditions) could up- or down-regulate the expression of the *hsp*90 transcript in the infective second stage juveniles.

# High level of resistance to *Meloidogyne incognita* in transgenic *Nicotiana tobacum* plants by knockdown of essential oesophagial pionner genes using RNAi.

Maria Fatima GROSSI-DE-SA, Djair SANTOS LIMA E SOUZA, Eduardo ROMANO, Maira GROSSI-DE-SA, Thales LIMA ROCHA, Aulus BARBOSA, Cezar MARTINS DE SA, Regina CARNEIRO, and Fabiano DE MOURA TEIXEIRA.
*fatimasa@cenargen.embrapa.br ; Brazil.fatimasa@cenargen.embrapa.br*

*Embrapa Recursos Genéticos e Biotecnologia- Final W5 Norte, Brasília DF 70770-900, BRAZIL.*

The *Meloidogyne incognita* is the most harmful phytonematode for worldwide agricultural economy, responsible for 95% of all the infestations caused by root knot species and for crop losses to more than $80 billion/year. Genes encoding oesophagial secretory proteins have been related to the parasitism in all the species of phytonematodes. The nematode secretory proteins including glucanases, pectate lyases, chorismate mutase, calreticulin, transcription factors and many others are actually potential targets to the biotechnological research. In recent works, in vivo expression of dsRNA resulted high level of resistance to *M incognita* in tobacco and arabidopsis plants by the knockdown of a splicing factor, an integrase and the nematode *16D10* gene. In present work, we describe an in vivo RNAi strategy to silence three new genes (Mipsg1, Mipsg7 and Mipsg18) highly and specifically expressed in dorsal oesophagial gland from females of *M.incognita*. Initially ~450-bp sequences were cloned in GATEWAY vectors for expression of RNAi in plants. The constructs were introduced into *N. tabaccum* plants. Fifteen transgenic plants from each construct (PCR was used to confirm the presence of the transgene) were inoculated with 10,000 eggs of *M. incognita*. The number of eggs on the infected roots was analyzed 45 days after inoculation and the Reproduction Factors (RFs) were analysed. Transgenic tobacco plants showed high levels of resistance to *M. incognita* considering the three constructs. The RFs oscilated from 0,0 to 1,6 compared with the vector-transformed line that presented RFs varying from 46-50 times. The Mipsg18 was the most potential target because the primary transformants were in fact, immune to the nematodes. In conclusion, our data demonstrate the potential use of these genes to the development of transgenic crop plants like soybean, cotton and coffee with high level of resistance to *Meloidogyne* species.
Supported by: EMBRAPA, FAPDF, CNPq, CAPES.

# In search of parasitism genes in the burrowing nematode *Radopholus similes.*

Joachim JACOB, Annelies HAEGEMAN, Tina KYNDT, Bartel VANHOLME, and Godelieve GHEYSEN.
*joachim.jacob@UGent.be*

*Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, BELGIUM.*

The technique of RNA interference (RNAi) can be used for unraveling gene function and holds promise for future pest control applications. To exert an effective broad range effect in pest control and at the same time minimizing off-target effects, the choice of suitable target genes is crucial. With plant-parasitic nematodes, a lot of attention has been paid to the identification of parasitism genes and recently, the in-planta generation of dsRNA of such genes has been proven to (effectively) inhibit parasitism in sedentary nematodes. Parasitism genes of sedentary nematodes are genes needed for migration through the plant roots or specifically needed by the sedentary nematode to transform root cells to nematode feeding sites. Migratory species have some parasitism genes in common with the sedentary species, e.g. cell wall degrading enzymes, which could be good targets for RNAi control for migratory species. Besides identification of several β-1,4-endoglucanases in the migratory nematode species *Radopholus similis* by our lab, we are also in search of novel targets for RNAi based pest control. For this purpose we have cloned 4 transthyretin-like genes of *Radopholus similis*, which are conserved nematode-specific proteins with yet unknown function. Our *in situ* analysis shows expression of at least one of them in the ventral nerve ganglia, suggesting a function in the nervous system. This observation indicates them as interesting targets for RNAi experiments in order to unravel their function and for analysing the result of their silencing on the nematode life cycle.

# *C. elegans* as a model system to study neuronal gene expression of *Globodera pallida*.

Joana C. COSTA, Catherine J. LILLEY, Howard J. ATKINSON, and Peter E. URWIN.
*c.j.lilley@leeds.ac.uk*

*Centre for Plant Sciences, University of Leeds, LS2 9JT, UNITED KINGDOM.*

Host location and root invasion by plant parasitic nematodes requires inputs from chemosensory and mechanosensory neurons and their integration into a series of appropriate behavioural responses. Despite the different lifestyles of plant parasitic nematodes and the free-living nematode *Caenorhabditis elegans*, it is likely that they share common neuronal circuitry and neurochemicals, differing principally in the specificity of sensory neurons for particular chemical cues. *C. elegans* has been used as a heterologous host in comparative studies to overcome the refractory nature of plant parasitic nematodes to many genetic approaches. *Globodera pallida* homologues of *C. elegans* genes expressed in a range of sensory and interneurons have been cloned and their promoter regions isolated. Temporal expression analysis shows that genes encoding the FMRFamide-related peptide gp-flp-6, serotonin and dopamine receptors and a vesicular acetylcholine transporter are all most highly expressed in motile stages. In contrast, the *Globodera* homologue of *C. elegans* acetylcholinesterase ACE-2 is expressed most highly in mature females.
GFP reporter constructs controlled by promoter regions of the *Globodera* genes have been introduced into *C. elegans* and the resulting expression patterns analysed. All show expression restricted to neurons with a pattern similar to that reported for *C. elegans*. Functional similarity between the two nematode species is being investigated in experiments to rescue *C. elegans* mutant phenotypes with the corresponding *Globodera* homologues.

# The Nematode effector peptide dgl-3 accumulates in the nucleolus of tobacco cells.

Nikkie van BERS, Hein OVERMARS, Erik SLOOTWEG, Ling QIN, Aska GOVERSE, Jaap BAKKER, and Geert SMANT.
*Hein.Overmars@wur.nl*

*Wageningen University, laboratory of Nematology, Binnehaven 5, 6708 PD Wageningen, THE NETHERLANDS.*

The life cycle of the potato cyst nematode (Globodera rostochiensis) consists of four juvenile stages, followed by an adult stage. After hatching from the egg, the pre-parasitic second stage juvenile (J2) migrates towards and subsequently invades the plant root. The parasitic J2 induces and feeds from a sophisticated cell-complex, the syncytium. A syncytium originates from a partial fusion of neighbouring cells, and it is a metabolically highly active cell, containing multiple enlarged and amoeboid nuclei with prominent nucleoli. Factors produced in the esophageal glands of the nematode are believed to be important in syncytium development. Dgl-3 encodes for a 4.4 kDa peptide from the potato cyst nematode. In this study, we analyse the expression profile of dgl-3 in different life stages of the nematode. Furthermore, we investigate the location of dgl-3 expression in pre-parasitic juveniles and the subcellular in planta localisation of ectopically expressed 35S::GFP-dgl-3 in Nicotiana benthamiana.

# Towards a genomic characterization of *Bursaphelenchus species.*

Janina POLOMSKI and Daniel RIGLING.
*janina.polomski@wsl.ch*

*Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, SWITZERLAND.*

Nematodes in the genus *Bursaphelenchus* are causing wilt diseases in various pine species. Beside the pine wood nematode *B. xylophilus*, a number of other species were found to be pathogenic. *B. xylophilus* is a A1 quarantine nematode that has been recently introduced into Portugal. In Switzerland, the newly described *B. vallesianus* and to a lesser extent *B. mucronatus* are associated with a widespread decline of Scot pines in the Valais. In inoculation trials, both *Bursaphelenchus* species are causing high mortality of young Scot pines, particularly when trees were subjected to water stress. *B. vallesianus* and *B. mucronatus* species are thought to be endemic in Europe. *Bursaphelenchus* species are genetically poorly characterised. In the frame of Cost 872, we propose to (1) identify genes putatively involved in pathogenicity of *B. vallesianus* by using functional genomic toolkits and (2) use candidate genes to assess intra- and interspecific genomic diversity in natural populations.

# Characterization of Root Knot Nematode *Meloidogyne ethiopica.*

Sasa SIRCA, Barbara GERIC STARE, Polona STRAJNAR, and Gregor UREK.
*sasa.sirca@kis.si*

*Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova 17, 1001 Ljubljana, SLOVENIA.*

Root-knot nematodes (*Meloidogyne* spp.) are devastating pathogens of several mono- and dicotyledons. They are considered to be economically one of the most important groups of plant parasitic nematodes. So far, four species of *Meloidogyne* were established in Slovenia: *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood and *M. ethiopica* Withead.

*M. ethiopica* was reported for the first time in Europe. It was isolated in 2003 from heavily infected roots of tomato plants grown in a greenhouse near village of Dornberk, Slovenia. Although this species is a relatively unknown root-knot nematode, *M. ethiopica* parasitizes several economical important crops, such as tomato, cowpea, bean, cabbage, pepper, pumpkin, tobacco, lettuce, and soybean. Infected tomato plants showed severe aboveground symptoms of stunting and wilting while infected roots displayed the largest gall formation when comparing to infestation with other *Meloidogyne* found in Slovenia.

Recently, we have focused our research on the significance of this pest for crop production. The research topics are: determination of the host plants spectre which can be parasitized by the nematode *M. ethiopica*, studies of physiological response of the host plants to the infection, pot trials where the capability of surviving of *M. ethiopica* in our climate conditions is examined and determination of the number of generations which can be produced by *M. ethiopica* with regard to various temperature conditions. In addition, the presence of virulent and avirulent genes, genetic variability of individual

# Functional characterisation of a pectate lyase gene in the plant-parasitic nematode *Heterodera schachtii* using RNA interference.

Wouter VAN THUYNE, Bartel VANHOLME, Bernard CANNOOT, and Godelieve GHEYSEN.
*Wouter.VanThuyne@UGent.be*

*Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, BELGIUM.*

The beet cyst nematode *Heterodera schachtii* is an obligatory sedentary endoparasite. Yield losses of sugar beet caused by this organism are estimated to be 10%, corresponding to a financial annual loss of 90 million Euro.
Cyst nematodes developed an ingenious way to infect plants. After hatching, they migrate intracellularly towards the vascular cylinder of the plant root, using their hollow protrusible stylet to perforate cell walls. Arriving at the vascular cylinder they induce a specific feeding structure, called a syncytium where they stay during the rest of their life cycle.

Recently we discovered two pectate lyase genes in *H. schachtii*. *In situ* hybridisations of these genes show expression in the subventral glands. Several cell wall degrading proteins are secreted by the nematode to enter the plant roots and to migrate through the cell layers. By soaking the preparasitic juveniles in double stranded RNA targeting the pectate lyase gene, a significant reduction of infection was seen after putting them on *Arabidopsis* plants. RT-PCR experiments revealed a reduction of the expression levels of pectate lyase in the treated nematodes.

These infection tests confirm that pectate lyase is an important enzyme that plant-parasitic nematodes need to infect plants.

**Posters WG2:**

**COMPARATIVE GENOMICS OF NEMATODES**

# The *Meloidogyne incognita* genome annotation.

Emeline DELEURY[1,2], Pierre ABAD[1], and Jérôme GOUZY[2].
*Emeline.Deleury@sophia.inra.fr*

*1. UMR INRA-UNSA-CNRS, Interactions Plantes-Microorganismes et Santé Végétale, 400 route des Chappes, BP 167, 06903 Sophia Antipolis cedex, FRANCE ; 2. Plateforme Bioinformatique du Génopôle Toulouse Midi Pyrénées, BP 52627, 31326 Castanet Cedex, FRANCE.*

The whole genome shotgun sequencing of plant-parasitic root-knot nematode *Meloidogyne incognita* is currently in progress at the Genoscope, the french National Sequencing Center. This project will be done in collaboration with INRA (Laboratoire IPMSV, Sophia Antipolis and bioinformatics facility, Toulouse Genopole).
The genome sequence (size estimated at 50 Mb) will be obtained by shotgun sequencing (10x coverage) of chromosomal DNA from Morelos isolate. In order to improve automatic assembly and annotation of the Meloidogyne genome, both ends of BAC library clones (12 500 BAC clones) and large sets of ESTs from various full length cDNA libraries will be sequenced. And the generation of a large numbers of ESTs from various full-length cDNA librairies are produced to facilitate and improve the automatic sequence annotation.
We built a pipeline to automatically annote the genome sequence and manage data and annotations, first in terms of gene structure prediction. Automatic annotations will be made by *EuGene* predictor (Schiex *et al.*, 2001) which needs, first of all, to be paramerized with a great set of full-length and expertised genes for which both genomic and transcript sequences are available. Then, the genes predicted by *EuGene* software will be loaded into a generic *chado* database developped by the Generic Model Organism Database project (GMOD http://www.gmod.org/) and automatic annotations will be accessible by a genome browser - *Apollo* - which provides support to the expert annotation process.

# *C. elegans* as a model system for genetic and molecular dissection of epigenetic mechanisms underlying polynucleotide expansions.

Katherine SCHOUEST[1], Avril COGHLAN[2], and Charles SPILLANE[1].
*c.spillane@ucc.ie*

[1]*Genetics & Biotechnology Lab, Department of Biochemistry & Biosciences Institute, University College Cork, Cork, IRELAND;*
[2]*Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UNITED KINGDOM.*

*Caenorhabditis elegans* has already been proven to be powerful model to study diseases caused by the expansion of nucleotide repeats (such as CAG expansions in Huntington's disease), as well as in revealing potential targets for therapeutic treatment. We are investigating epigenetic phenomena, such as anticipation and parent of origin effects, associated with this type of disorders using *C. elegans* as a model. Anticipation is a clinical phenomenon characterized by an earlier age of onset and/or an increase in disease severity in successive generations of a family, which can be correlated with expansion of nucleotide repeats over generations. This type of expansion often exhibits parent of origin effects, where the sex of the parent transmitting the repeat influences the stability of the repeat in offspring. To identify candidate loci which are prone to polynucleotide repeat expansions, we searched the *C. elegans* genome for tri-nucleotide repeats that have high purity level and are longer than 12 repeat units, as these have been shown most likely to undergo expansion/contraction. We have selected 20 such repeats located in exons of various genes and 17 within intronic regions. A panel of wild isolates of *C. elegans* are being examined for polymorphisms at these repeats. Repeats exhibiting dynamic instability are used in further analysis over successive generations. Such genes will be subjected to further scrutiny to determine if particular expanded tracts have a phenotypic effect, and if so whether it is due to a gain- or loss-of function mechanism. In later stages of the project we plan to conduct genetic screens to identify proteins that affect this mode of inheritance. Our project will facilitate understanding of the epigenetic phenomena controlling polynucleotide expansions.

# A high-throughput screening for harmful plant pathogens in agricultural soils and products.

Peter VEENHUIZEN[1], Roel STAPS[1], Gerrit Karssen[2], and Hans HELDER[3].
*roel.staps@blgg.nl*

[1]*Blgg bv* Nieuwe Kanaal 7F, 6709 PA, Wageningen, *THE NETHERLANDS;*
[2]*Dutch Plant Protection Service, THE NETHERLANDS;*
[3]*Laboratory of Nematology, Department of Plant Sciences, Wageningen University, THE NETHERLANDS.*

Root knot nematodes (RKN) - beyond doubt the most harmful plant parasitic nematodes worldwide - are rather indiscriminate in their taste and attack virtually all ornamentals and vegetables. Two RKN species - *Meloidogyne chitwoodi* and *M. fallax* - are on the European quarantine list. Accurate detection of these plant pathogens is currently highly problematic, causing a serious problem for producers, exporting parties and the Dutch Plant Protection Service (PPS).
One of the deliverables of an ongoing research project at the Laboratory of Nematology (Wageningen University) is an rDNA sequence database of virtually all nematode species found in soil (Holterman *et al*, 2006*).
The company Blgg, together with Wageningen University and the Dutch Plant Protection Service, has in addition identified rDNA sequence signatures for five different RKN species including the quarantine organisms *M. chitwoodi* and *M. fallax*. Because of the economic relevance of these plant pathogens, this knowledge has been patented. Blgg currently develops series of robust and cost-effective molecular tests for the detection of several plant-parasitic nematode species, including the RKN species and potato cyst nematodes. This will enable Blgg to replace current identification product based on microscopic counts by newly developed DNA barcode-based assays.

*Holterman M, van der Wurff A, van den Elsen S, van Megen H, Holovachov O, Bakker J & Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution towards crown clades. *Molecular Biology and Evolution* 23, 1792–1800.

**Posters WG3:**

**FUNCTIONAL GENOMICS OF PLANT RESPONSE**

# A compatible plant-nematode interaction: functional analysis of plant genes involved in nematode feeding cell formation.

Janice de ALMEIDA-ENGLER, Marie-Cécile CAILLAUD, Mohamed Youssef BANORA, Michael QUENTIN, Maria-Cécilia ARIAS, Mathilde CLEMENT, Philippe LECOMTE, Nicolas MARFAING, Laetitia PERFUS-BARBEOCH, Gilbert ENGLER, Pierre ABAD, and Bruno FAVERY.
*Janice.Almeida-Engler@sophia.inra.fr*

*INRA, UMR 1064 Interactions Plantes Microorganismes et Santé Végétale, 400 Route des Chappes, BP 167, F-06903 Sophia Antipolis, FRANCE.*

During a compatible interaction, root-knot nematodes, from the genus Meloidogyne, establish and maintain feeding cells. These permanent and specialized nourishing cells are essential for growth and reproduction of these biotrophic obligate pathogenic nematodes. After root penetration and migration, a juvenile nematode selects five to seven parenchymatic cells that will dedifferentiate into giant feeding cells. These enlarged cells become multinucleate through synchronous nuclear divisions and aborted cytokinesis. A fully differentiated giant cell may contain a large number of polyploid nuclei that most likely have undergone extensive endoreduplication. The hyperplasia of the surrounding cells leads to the formation of a typical root gall. To obtain a more comprehensive view of the molecular mechanisms underlying feeding cell formation, we focused on nematode responsive plant genes and investigated the distribution of the cytoskeleton and its behaviour during feeding site development. The application of a promoter trap strategy in Arabidopsis infected plants, combined with microarray experiments, provide large-scale information about the host response during root-knot nematode infection. We identified more than three thousand genes that display the differential expression between uninfected root tissues and galls at different developmental stages. This genome-wide overview of genes expressed during plant-nematode interaction indicates that essential plant functions are manipulated during pathogenesis. These data are exploited to assess the role of a selection of identified genes by reverse genetics. Approaches such as molecular genetics (mutant, overexpressing lines) and a search for genes encoding interacting proteins is undertaken. In addition, this collection of genes provides a rich new field for cell biologists to investigate gene function. We focus on the functional analysis of genes involved in actin and microtubule cytoskeleton reorganization, genes controlling the cell cycle and genes involved in susceptibility to other pathogens. These approaches will enhance our knowledge on how parasitic nematodes manipulate plants, by modifying root cells into giant cells to successfully lead to disease development.

# Functional Analysis of Germin-like Proteins in the *Hs1$^{pro-1}$*-mediated Nematode Resistance Response.

Katrin ASBACH[1], Yanyan TIAN[2], Jan MENKHAUS[1], Christian JUNG[3], and Daguang CAI[1].
*k.asbach@plantbreeding.uni-kiel.de ; dcai@phytomed.uni-kiel.de*

[1]*Department of Molecular Phytopathology, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str. 9, D-24118 Kiel, GERMANY;*
[2]*Department of Life Science and Engineering, Harbin Institute of Technology, CHINA;*
[3]*Plant Breeding Institute, Christian-Albrechts-University Kiel, Olshausenstr. 40, 24098 Kiel, GERMANY.*

To identify sugar beet genes involved in the *Hs1$^{pro-1}$* mediated resistance response, the cDNA-AFLP technique and ATH1 GeneChips were used for comparative transcriptome analyses. A group of germin-like genes was identified whose expression is specifically upregulated upon nematode infection in both resistant beet and *Hs1$^{pro-1}$* transgenic Arabidopsis, suggesting their role in the *Hs1$^{pro-1}$*-mediated nematode resistance response. To test this, we transformed susceptible beet roots and *Arabidopsis* plants with a full-length germin-like gene *BvGLP-Wag1* by use of *Agrobacterium* mediated transformation. Two gene expression constructs were used for transformation, pAM-BvGLP-Wag1 with 35S-promoter for a constitutive over-expression and pBin-BvGLP-Wag1 with *Hs1*-promoter for a feeding-cell specific expression. Transgenic beet roots and transgenic *Arabidopsis* plants were used for nematode infection experiments in which non-transgenic beet roots and *Arabidopsis* plants served as controls. As a result, a significant reduction in the number of developed female nematodes was observed on both transgenic beet roots and *Arabidopsis* plants, independent on the gene constructs transformed. By comparison, transformation with pAM-BvGLP-Wag1 resulting in a constitutive gene expression in plant cells showed a significantly lower regeneration rate and obvious stagnation of the growth of transgenic beet roots and *Arabidopsis* plants as well. This result strongly suggests that an enhanced expression of germin-like proteins like *BvGLP-Wag1* might be involved in the initiation of stagnation and degeneration of nematode feeding-cells at the early developing stage as observed in the *Hs1$^{pro-1}$*-mediated nematode resistance response. Elucidation of molecular and biochemical nature of the *BvGLP-Wag1* action mode in feeding cells is in progress.

# Plant-nematode interactions: implications for the metabolome.

Tim MILLER[1], Derek STEWART[2], and Vivian BLOK[1].
*vblok@scri.ac.uk*

*[1]Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5JQ, SCOTLAND;*
*[2]Quality, Health and Nutrition Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5JQ, SCOTLAND.*

During the interaction between plant endoparasitic nematodes and their hosts, changes in both localized and systemic gene expression occur leading to either susceptible or resistant interactions. Little is known about how these different interactions affect the host's metabolism and how these relate to changes in gene expression. Metabolite profiling was used to monitor changes in tomato and potato hosts in compatible and incompatible interactions with *Meloidogyne* and *Globodera* to determine if these profiles can be used to differentiate these interactions. An extraction protocol has been established for freeze-dried tomato roots and leaves for analysis with gas chromatography mass spectrometry (GCMS) and high pressure liquid chromatography-photodiode array-mass spectrometry (LC-PDA-MS). This protocol has been tested by comparing metabolite profiles of uninfected tomato with a compatible interaction with *Meloidogyne javanica.* Profiling has also been extended to potato genotypes which differ in susceptibility to the potato cyst nematode *Globodera pallida*. Significant changes in known and/or unknown metabolites in these interactions have been found.

# Characterization of *Arabidopsis* Microtubule-associated proteins (map) involved in feeding cell formation.

Marie-Cécile CAILLAUD, Michael QUENTIN, Philippe LECOMTE, Janice de ALMEIDA-ENGLER, Pierre ABAD and Bruno FAVERY.
*Marie-cecile.Caillaud@sophia.inra.fr*

*INRA-UNSA-CNRS, UMR1064-6192, Interactions Plantes-Microorganismes et Santé Végétale, 400 route des Chappes, F-06903 Sophia Antipolis, FRANCE.*

Among plant pathogens, sedentary endoparasitic nematodes interact with their hosts in a quite unique and intriguing way. During a compatible interaction, these obligate parasites induce the redifferentiation of root cells into multinucleate and hypertrophied feeding cells essential for nematode growth and reproduction. To obtain a more comprehensive view of the molecular mechanisms underlying the feeding cell formation, we focused on the functional analysis of plant genes identified by a promoter trap strategy and the genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis thaliana*. We will present the functional analysis *in planta* of an *Arabidopsis thaliana* Microtubule-Associated Protein, AtMAP65-3. *AtMAP65-3* gene transcriptional activation occurred in developing feeding cell induced by root-knot nematodes and then rapidly faded before fully "giant cell" differentiation. This gene encodes a microtubule-associated protein similar to the tobacco MAP65 known to bind and bundle microtubules *in vitro*. AtMAP65-3 has been shown to be essential for cytokinesis in root cells. We showed that AtMAP65-3 was expressed in shoot and root dividing cells. Phenotypic analyses of *map65-3* mutant revealed multinucleate and hypertrophied cells detectable in root, embryo and shoot. Results obtained upon nematode infection and subcellular localization will be presented. Future perspectives on microtubule reorganization analysis in feeding cells will be discussed.

# Reorganization of the actin cytoskeleton in giant cells induced by root-knot nematodes may requires actin depolymerizing factors.

Mathilde CLÉMENT[1], Andrei SMERTENKO[2], Gilbert ENGLER[1], Bruno FAVERY[1], Pierre ABAD[1], Janice de ALMEIDA-ENGLER[1].
*Mathilde.Clement@inra.sophia.fr*

*[1] UMR "IPMSV" CNRS-INRA-Université de Nice-Sophia-Antipolis, 400 route des Chappes, BP 167, 06903 cedex, Sophia-Antipolis, FRANCE;*
*[2] School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE, ENGLAND.*

Sedentary plant endoparasitic nematodes are pathogens that infect a wide range of plant species. In a compatible interaction, signals from root-knot nematodes induce the formation of specialized cells within the vascular cylinder of plant roots, called "giant cells" or "feeding cells". Root-knot nematodes are able to induce the rearrangement of actin filaments into thick actin cables which are randomly oriented in the cytoplasm and the cortex of giant cells. Based on this observation we questioned which actin binding proteins might play a role in the actin cytoskeleton reorganization. Currently, we investigate the involvement of ADF (Actin Depolymerizing Factors) proteins in the formation of giant cell actin cables. ADF are small actin-binding proteins (10-15 kDa) that can bind G- and F-actins and are known to enhance actin dynamics. Immunocytochemical analyses performed on galls revealed a strong fluorescence of ADF within giant cells. Double immunolocalization experiments suggest a ADF colocalization with actin filaments. Considering that the antibody might cross react with more than one ADF member, a Q-PCR analysis was performed to determine which of the 12 ADFs of *A. thaliana* are specifically expressed in galls. Our experimental data showed that several *AtADF* genes are differentially expressed in giant cells. Thus, more than one actin depolymerising factor seems to be involved in the generation of actin bundles and cables in giant cells. Further analysis of these actin binding proteins will be essential to determine their role in the actin cytoskeleton reorganization in nematode induced feeding cells.

# Characterization of resistance in *Musa* against the burrowing nematode, *Radopholus similis*.

Suganthagunthalam DHAKSHINAMOORTHY, A. ELSEN, and D. DE WAELE.
*Suganthagunthalam.Dhakshinamoorthy@biw.kuleuven.be*

*K.U. Leuven / Division of Crop Biotechnics, Laboratory of Tropical Crop Improvement, Kasteelpark arenberg 13, 3001 Leuven, BELGIUM.*

Within the integrated pest management strategy, host plant resistance appears to be a promising tool to control nematodes in banana. Resistant varieties have the advantages that nematode reproduction is inhibited, no toxic residues are produced, no special application techniques or equipment is required and there is no additional cost to the grower over growing non-resistant varieties. Although a wide range of *Musa* varieties has been screened, only few varieties have been identified as natural sources of resistance against the burrowing nematode. Some of these resistant varieties are currently being used in classical breeding programs world-wide.
Understanding the mechanism of resistance is important as it may enable the breeder to select for a desired characteristic. In addition, knowledge about the resistance mechanisms can provide resistance markers to facilitate screening of *Musa* germplasm. Unfortunately only limited work has been done on the crop resistance against migratory parasitic nematodes and no gene for nematode resistance has yet been identified in banana. Hence, a study will be carried out to characterize the resistance in *Musa* at different levels: varietal, morphological, anatomical, cellular, biochemical and molecular level.

# Characterisation of virulence in *Meloidogyne* spp. and host resistance.

Miguel Angel DÍEZ-ROJO, J.A. LÓPEZ PÉREZ, A. BELLO, L. ROBERTSON.

*Centro de Ciencias Medioambientales, Dept Agroecologia, Calle Serrano 115, DPDO, SPAIN.*

The characterization of virulence in *Meloidogyne* populations is based mainly on the use of biotests, the most widely utilised being the North Carolina differential host test of Hartman & Sasser, 1985. A modification of the biotest, with the inclusion of resistant tomato cv. Euphrates, carrying the *Mi* gene and resistant pepper cv. Atlante containing the *N* gene has allowed us to better characterise the virulence in *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* populations from Spain. A total of 27 biotypes have been found: 20 *M. incognita*, 1 *M. javanica*, 4 *M. arenaria* and 2 *M. hapla*. None of the *M. javanica* populations were found to parasitize susceptible or resistant pepper cultivars tested in Spain. The secretions of plant parasitic nematodes have been implicated in penetration of host plant roots, movement of the nematode througth the plant, protection from active oxygen species, feeding site induction, feeding site maintainance and are thought to contain (a)virulence factors therefore the characterisation of *Meloidogyne* populations and model plant systems are therefore seen as a pre-requisite for future molecular and biochemical studies.

# Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome.

Caroline DJIAN-CAPORALINO[1], Ariane FAZARI[1], Marie-Jeanne ARGUEL[1], Tatiana VERNIE[1], Céline VAN DE CASTEELE[1], Ingrid FAURE[1], Géraldine BRUNOUD[1], Lucette PIJAROWSKI[1], Alain PALLOIX[2], Véronique LEFEBVRE[2], Pierre ABAD[1].
*Caroline.Caporalino@sophia.inra.fr*

*[1)] INRA, UMR 1064 Interactions Plantes Microorganismes et Santé Végétale, 400 Route des Chappes, BP 167, F-06903 Sophia Antipolis, FRANCE ;*
*[2)] INRA, UR1052 Génétique et Amélioration des Fruits et Légumes, BP 94, F-84143 Montfavet, FRANCE.*
*EU-project QLRT-1999-1462 "DREAM",* Website: *www.eu-dream.nl*

In *Capsicum annuum*, resistance to the root-knot nematode (*Meloidogyne* spp.) is controlled by several independent dominant genes—the *Me* genes. Six *Me* genes have previously been shown to be stable at high temperature in three highly resistant and genetically distant accessions: PI 322719, PI 201234, and CM334 (Criollo de Morelos 334). Some genes (*Me4, Mech1*, and *Mech2*) are specific to certain *Meloidogyne* species or populations, whereas others (*Me1, Me3*, and *Me7*) are effective against a wide range of *Meloidogyne* species, including the main species: *M. arenaria, M. javanica*, and *M. incognita*. These genes direct different response patterns in root cells depending on the pepper line and nematode species. Allelism tests and fine mapping using the BSA-AFLP approach showed these genes to be different but linked, with a recombination frequency of 0.02–0.18. Three of the PCR-based markers identified in several genetic backgrounds were common to the six *Me* genes. Comparative mapping with CarthaGene software indicated that these six genes clustered in a single genomic region within a 28 cM interval. Four markers were used to anchor this cluster on the P9 chromosome on an intraspecific reference map for peppers. Other disease resistance factors have earlier been mapped in the vicinity of this cluster. This genomic area is colinear to chromosome T12 of tomato and chromosome XII of potato. Four other nematode resistance genes have earlier been identified in this area, suggesting that these nematode resistance genes are located in orthologous genomic regions in *Solanaceae*. We also analyzed the *Me*-specific PCR-based markers on a set of eight pepper breeding lines. Our preliminary results suggest that they can be used in many susceptible genetic backgrounds. Studies to determine the durability of these *R*-genes (alone or pyramided) in different genetic backgrounds are now underway.

# Identification of *Meloidogyne* and *Heterodera* species by molekuler methods and using MAS for the resistance of wheat varieties.

I. Halil ELEKCIOGLU[1], A. ÖZARSLANDAN[2], Z. DEVRAN[3], N. MUTLU[4], J. NICOL[5], Ö. YORGANCILAR[6], E. SAHIN[1], N. BOLAT[6], and M. CALISKAN[7].
*halile@cu.edu.tr*

*1) University of Cukurova, Faculty of Agriculture, Plant Protection Department, 01330 Adana, TURKEY;*
*2) Adana Plant Protection Research Institute, Adana, TURKEY;*
*3) West Anatolia Agricultural Research Institute, Antalya, TURKEY;*
*4) Alata Horticultural research Institute, mersin, TURKEY;*
*5) CIMMYT (International Maize and Wheat Improvement Centre), Ankara, TURKEY;*
*6) Anadolu Agricultural Research Institute, Eskişehir, TURKEY;*
*7) Central Research Institute For Field Crops, Ankara, TURKEY.*

Identification of plant-parasitic nematodes has traditionally been based on comparative morphology and there are several diagnostic keys. The use of morphometric characteristics to differentiate species is very time consuming and can be unreliable and difficult to use and, more recently, techniques based on protein or DNA differences have been implemented.

Root-knot nematodes cause a great deal of economic losses on many cultivated fruits and vegetables in Turkey. Population of root-knot nematodes were collected from different geographical regions of Turkey. Samples of root-knot nematodes were first homogenized. Then, DNA extraction from egg masses was carried out. rDNA-RFLP and species-specific primers were used to diagnose the important root-knot nematode species. *M. arenaria, M. javanica* and *M. incognit* were determined. At the end of this study, root-knot nematode map of Turkey will be established.

Cereal cyst nematodes are also very important pests in central Anatolia wheat production areas. Protein electrophoresis has successfully been used to distinguish *Heterodera* species, however requires a large number of nematodes and is therefore useful only where pure populations can be obtained. The use of PCR-RFLP overcomes this problem as DNA can be amplified from only one nematode if required, and discrimination of both cyst nematode species has been achieved from 1-10 nematodes. This technique has been successfully implemented in Turkey. These molecular techniques are useful, however given we are still improving our understanding of the complexity of CCN with respect to both the number of species and pathotypes which are representative, further characterization of a greater number of isolates from global collections are needed and PCR probes are needed. It would also be of tremendous value to use to relate this genetic diversity of the pathogen to the source (evolution) of the host genetic resistance which has been found, as most of the identified sources of resistance to *H. avenae* have been found predominantly in wild relatives of wheat in the *Aegilops* genus. Six out of the seven named *Cre* genes for *H. avenae* resistance in wheat as well as *Rkn2* for resistance to both *M. naasi* and *H. avenae* came from four *Aegilops* species and have already been introgressed into hexaploid wheat backgrounds for breeding purposes.

Another important aspect of research is the both the idenfication of gene(s) of resistance against the CCN complex, followed by and understanding of the both the number of genes, location and the identification of a diagnostic maker to the gene of interest. We know to date all genetic resistance identified for CCN in cereals is controlled by a single dominant gene. Molecular technologies have been applied to identify markers for various CCN plant resistance genes using techniques such as RAPD and RFLP, in both barley McIntosh *et al.* (2001) presented information about introgression, substitution and molecular characterization of these resistance sources in cereals. In several Australian cereal breeding programmes, and in CIMMYT International markers for both wheat and barley are being implemented using marker assisted selection (MAS) to pyramid resistance genes against *H. avenae*, pathotype Ha13. However we must not forget the identification and implementation of markers in this way requires sufficient understanding of the biology of the pathogen and genetic control of the resistance.

# Use of model plants to study plant – migratory nematode interactions: advantages and bottlenecks.

Annemie ELSEN, Katrien CARLENS, Nathalie WUYTS, Rony SWENNEN, and Dirk DE WAELE.
*annemie.elsen@biw.kuleuven.be*

*Division of Crop Biotechnics, K.U.Leuven, Kasteelpark Arenberg 13, 3001 Leuven, BELGIUM.*

A more thorough understanding of the interactions between plant-parasitic nematodes and their host plants at the molecular level is needed in order to enhance existing resistance and to develop novel resistance strategies. *Arabidopsis thaliana* has been proposed as a model plant to study plant-nematode interactions. Although studies using *A. thaliana* have led to important findings, *A. thaliana* does have serious limitations as a model, especially to study migratory nematodes. For nematodes like *Radopholus similis* and *Pratylenchus* spp., it is considered a good host, but high variability in nematode reproduction hampers the use of *A. thaliana* as a model plant. In addition, *A. thaliana* does not form symbiotic associations with either rhizobia or arbuscular mycorrhiza.
Therefore, *Nicotiana tabacum*, *Medicago truncatula* and *Lotus japonicus* were suggested as an alternative to *A. thaliana* to compensate for its limitations as a model plant. Their advantages and disadvantages as model plant for plant – migratory nematode interactions will be discussed and compared with *A. thaliana*.

# The *Ma* gene from Myrobalan plum (*Prunus cerasifera* Ehr.) conferring a complete-spectrum resistance to RKN is a member of a TIR-NBS-LRR gene cluster.

Michel CLAVERIE[1,4], Elisabeth DIRLEWANGER[2], Nathalie BOSSELUT[1], Cyril van GHELDER[1], Joël POLIDORI[1], Patrick COSSON[2], Roger VOISIN[1], Marc KLEINHENTZ[2], Bernard LAFARGUE[2], Pierre ABAD[1], Marie-Noëlle ROSSO[1], Boulos CHALHOUB[3], and Daniel ESMENJAUD[1].
*Daniel.Esmenjaud@sophia.inra.fr*

[1]*UMR "Interactions Plantes-Microorganismes et Santé Végétale" (IPMSV), Equipe de Nématologie, INRA, B.P. 167, 06903 Sophia Antipolis, FRANCE ;*
[2]*Unité de Recherche sur les Espèces Fruitières et la Vigne (UREFV), INRA, 33883 Villenave d'Ornon, FRANCE ;*
[3]*Unité de Recherche en Génomique Végétale (URGV), INRA, 91057 Évry, FRANCE ;*
[4]*Present address : Unité de Génétique et Amélioration des Fruits et Légumes (UGAFL), INRA, 84143 Montfavet, FRANCE.*

Sources of resistance to RKN selected for rootstock breeding in *Prunus* spp. exhibit various resistance spectra. Some clonal accessions of Myrobalan plum (*Prunus cerasifera* Ehr.) carry a complete spectrum conferred by the *Ma* gene whereas more restricted spectra have been found in the peach sources Nemared and Shalil (*RMia* gene controlling *M. arenaria* and *M. incognita*) or in the almond source Alnem (*M. javanica*). The completely dominant *Ma* gene, carried by a diploid, near-wild, allogamous plum, confers a high-level resistance (gall-free phenotype) independent of the inoculum pressure and the temperature. *Ma* is heterozygous (*Ma1/ma1*) in the resistant accession P.2175. A high-resolution map, based on BSA/AFLP and on recombination events from several intra- and interspecific segregating crosses totalizing over 1300 individuals, was used to construct the R and S BAC contigs at the *Ma* locus. One 287 kb BAC carrying *Ma1* was detected in the R contig and its sequence analysis revealed a cluster of three TIR-NBS-LRR (TNL) open reading frames (ORFs) lying between candidate ORFs from other multigenic families. New SSR and SCAR markers and 1700 additional plum segregating seedlings reduced the interval spanning the gene to approx. 34 kb with the three TNLs as the sole candidates. One BAC carrying the susceptible haplotype of the gene has recently been sequenced and preliminary comparison of the R and S clusters suggests that TNL1, the longest TNL, is the best candidate to encode *Ma*. Current studies aim at validating TNL1, using hairy roots (in Petri dishes or as composite plants) transformed with the complete gene and its native promoter region.

# Functional analysis of selected tomato genes induced during *Globodera rostochiensis* infection.

Marcin FILIPECKI, Joanna DĄBROWSKA, Magdalena GŁODEK, Sylwia FUDALI, Grzegorz BARTOSZEWSKI, Mirosław SOBCZAK, Władysław GOLINOWSKI, and Stefan MALEPSZY.
*marcin_filipecki@sggw.pl*

*Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warszawa, POLAND.*

abstract

# Functional constraints and evolutionary dynamics of the *Rx1/Gpa2* cluster in potato.

Patrick BUTTERBACH[1], Erik SLOOTWEG[1], Kamila KOROPACKA[1], Laurentiu SPIRIDON[2], Robert DEES[1], Jan ROOSIEN[1], Erin BAKKER[1], Marjon ARENS[1], Andrei PETRESCU[2], Geerts SMANT[1], Jaap BAKKER[1], and Aska GOVERSE[1].
*aska.goverse@wur.nl*

[1]*Plant Sciences Group, Wageningen University and Research Center, Wageningen, THE NETHERLANDS;*
[2] *Institute of Biochemistry, Romanian Acadamy of Sciences, Boekarest, ROMANIA.*

The potato genes *Gpa2* and *Rx1* are highly homologous (~90% sequence identity) *R* genes belonging to the CC-NBS-LRR class, and mediate resistance to the potato cyst nematode *Globodera pallida* and the Potato Virus X, respectively. Both genes are tightly linked and members of a small *R* gene cluster on ChrXII. To date very few examples are known of *R* gene clusters harboring resistances to distinct pathogens with entirely different routes of invasion. The mild inhibition of nematode feeding cell formation could be converted into extreme resistance to Potato Virus X and *vice versa* by exchanging the LRR domains of *Rx1* and *Gpa2.* However, loss-of-nematode resistance was observed when the recombinant protein was expressed under control of the endogenous *Gpa2* promoter instead of the CaMV 35S promoter. Sequence analyses from functional *Rx* alleles from five different *Solanum* species demonstrated that the LRR showed 98% similarity at the amino acid level indicating that the LRR domain is subjected to a high selection pressure to maintain its function. Apparently, a high proportion of amino acids is involved in recognition and/or transduction of the signal leading to resistance. This finding is corroborated by extensive subdomain swapping between parts of the *Rx1* and *Gpa2* LRR region that resulted either in a loss-of-phenotype or effector-independent activation and the analysis of the sequences of about 80 homologs of *Rx/Gpa2* derived from various wild potato species.

## Sugar supply and sugar metabolism of nematode induced syncyti.

Julia HOFMANN, Krzysztof WIECZOREK, Andreas BLÖCHL, Dagmar SZAKASITS, Holger BOHLEMANN, and Florian M.W. GRUNDLER.
*julia.hofmann@boku.ac.at*

*Inst. of Plant Protection, DAPP, Boku, Peter-Jordan Str. 82, 1190 Vienna, AUSTRIA.*

The plant parasitic nematode *Heterodera schachtii* induces syncytial feeding structure in the roots of its host plants that serve as its sole nutrient source. Especially carbohydrates and amino acids have to be taken up by the nematode and therefore need to be supplied from the host plants. In order to understand nematode and therefore syncytium solute supply we studied the apoplasmic and symplasmic nutrient transport pathway into syncytia. Phloem loading experiments showed that during the first days of nematode development syncytia are symplasmically isolated from their surrounding and thus fully dependent on transporter protein activity. At later stages of juvenile development plasmodesmata open to adjacent phloem elements facilitating a symplasmic solute supply. The gene expression of 85 sugar transporters has been studied by gene chip analysis and five of them were chosen for detailed expression studies. Knock-out mutants show the significant importance of At4g21480 and At3g05400 sugar transporter gene expression for nematode development. High levels of sucrose accumulated in syncytia and we further found out that the high sugar levels are buffered by the formation of starch in syncytia, probably to reduce osmotic stress. Infection rates on *Atss1* knock-out mutant result in a significant reduction of nematode development.

# Breeding for resistance in barley against root-lesion nematodes.

Tobias KEIL[1], E. LAUBACH[2], and C. JUNG[1].
*t.keil@plantbreeding.uni-kiel.de*

*[1]Plant Breeding Institute, Christian-Albrechts-University of Kiel, Olshausenstrasse 40, 24098 Kiel, GERMANY;*
*[2]Nordsaat Saatzucht GmbH, Hofweg 8, 23899 Segrahn, GERMANY.*

Root-lesion nematodes are destructive plant-parasitic nematodes, distributed mainly in the temperate zones around the world. In the northern parts of Germany *P. neglectus* and *P. crenatus* cause heavy yield losses in winter barley. Since nematodes cannot be controlled in the field, resistant varieties are the only means to solve this problem. This project aims at selecting barley accessions with resistance to root-lesion nematodes.

In a first step a resistance screening assay has been established in the glasshouse. A total of 600 barley accessions encompassing cultivated (*Hordeum vulgare*) and wild species (*Hordeum spontaneum*) have been screened for resistance against *P. neglectus*. The plants grow in tubes filled with sand. The root infection follows ten days after sowing with 400 nematodes per plant. The nematodes are extracted from roots and sand twelve weeks after the infection with a misting chamber and their number determined under a microscope.

The number of nematodes per plant ranged from 375 for the most resistant accession up to 12000 for the most susceptible accession.

The data will be verified by testing accessions under field conditions. In parallel, resistant lines will be crossed with susceptible ones to introduce resistance into elite material. The future aim is to develop molecular markers closely linked to the resistance genes which can be used as selectable markers during the breeding process.

# Use of genomic information in a program of sustainable resistance to *Globodera pallida*.

Marie-Claire KERLAN[1], L. CHAUVIN[1], E. GRENIER[2], D. MUGNIERY[2], and B. CAROMEL[3].
*Marie-Claire.Kerlan@rennes.inra.fr*

[1] *UMR INRA AGROCampus Rennes APBV, Kéraiber, 29260 Ploudaniel, FRANCE;*
[2] *UMR INRA AGROCampus BiO3P, BP 35327, 35653 Le Rheu cedex, FRANCE;*
[3] *UGAFL, Domaine Saint Maurice, BP 94, 84143 Montfavet cedex, FRANCE.*

*Globodera pallida* is an important potato pest in Western Europe. Our group focuses on sustainability of resistance. For this purpose, we attempt to introduce resistance from different wild species. Until now, close collaborations between geneticists and nematologists allowed to introduce oligogenic resistance from *S. vernei*, *S. sparsipilum* and *S. spegazzinii*, at 2x and 4x levels. Major QTLs were mapped in our group on short arm of chromosome V in *S. sparsipilum* ($GpaV_{spl}$) and *S. spegazzinii* ($GpaV_{spg}$) and explained 76% and 48% of the phenotypic variance respectively. Major QTLs originating from *S. vernei* were also mapped In *S. sparsipilum*, the presence of the major QTL and the minor QTL ($GpaXI_{spl}$) induced a necrosis around the nematode feeding site and enlarged the resistance spectrum whereas, when QTLs were present separately, the nematode developed into male (Caromel *et al*, 2005). Morever, the resistance spectrums were different according to the resistance origin (Caromel, 2004).

We are now investing on different homologues at the *GpaV* locus originating from different wild species. In some of these species, QTL have already been mapped on chromosome V. Do these homologues correspond to the same nucleotidic sequence? If the sequences are different, which amino-acids are involved? Could these modifications be associated to phenotypic variations?
According to the response, a strategy to exploit genetic resources against *G. pallida* will be defined : pyramidization of resistance genes or QTL in a single genotype, combination of resistance factors in space and time. In each case, the nematode virulence will be evaluated.

Caromel *et al* 2005. MPMI 18(11):1186-1194
Caromel, 2004, phD report 133p

# Plant-nematode *in vitro* test systems.

Michael KLEINE and Werner SELIG.
*kleine@planton.de*

*PLANTON GmbH, Kiel-Kanal 44, 24106 Kiel, GERMANY.*

PLANTON is a biotechnology company engaged in plant biotechnology research and product development. PLANTON has developed and applied different test systems to analyse plant-nematode interactions. In vitro test systems have been established for testing the interaction of plant-pathogenic nematodes with different host and non-host plants. A variety of different nematode species was used (e.g. Heterodera schachtii, Globodera rostochiensis, Globodera pallida, Melodoigyne incognita).Therefore, large scale and high throughput assays were performed to screen e.g. in large transgenic and non-transgenic plant populations for specific genotypes. These assays were performed under sterile conditions using nematode populations cultivated in a sterile environment.
In addition, PLANTON uses plant transformation techniques to generate e.g. transgenic hairy root cultures applicable directly for nematode screening. This offers a major speed and cost advantage in testing genes of interest in large quantities with a high statistical safety. Furthermore, in some cases transgenic hairy roots from plant species difficult to regenerate (e.g. soy bean) can be easily generated and tested for plant-nematode interactions.
The screening assays developed by PLANTON are offered as a service to research organisations, plant breeders and plant biotech companies .

# Pathogenicity of the pinewood nematode *Bursaphelenchus xylophilus.*

M. MOTA[1], P. VIEIRA[1], R. COSTA[3], and M.S. PAIS[2].
*mmota@uevora.pt*

*[1]NemaLab-ICAM, Dept. Biologia, Universidade de Évora, 7002-554, Évora, PORTUGAL;*
*[2]Unit of Molecular Biology & Plant Biotechnology – ICAT, FCUL, Campo Grande, 1749-016 Lisboa, PORTUGAL;*
*[3]Departamento de Ecofisiologia e Melhoramento Florestal, Estação Florestal Nacional, Quinta do Marquês, 2780-159 Oeiras – PORTUGAL.*

The presence of *Bursaphelenchus xylophilus*, the pinewood nematode (PWN), an extremely damaging organism and a threat to the territories of the EU, was first reported in Portugal and the EU in May 1999 (Mota et al., 1999). *B. xylophilus* is native to North America, occurring in Canada, the USA and Mexico. It was introduced into Japan, by way of timber trade from North America, where it has spread into China, Taiwan and South Korea. PWN has become the number one pine pest in those countries and causes huge damage in Japan and China (Mamiya, 2004). *B. xylophilus* is now well established throughout the survey area in the Setubal region and constitutes a main concern for national (DGRF, 2006; DGRF-PROLUNP, 2006) and EU government decision bodies. Once inside the tree, *B. xylophilus* becomes pathogenic by feeding on parenchyma cells producing a series of chemicals that may elicit a resistance response, and by causing cavitation in the vascular system, blocking the water movement (Kuroda, 2006, 2004). *B. xylophilus* is referred to as the only pathogen responsible for pine wilt disease, but the mechanism of disease has not been completely elucidated. Furthermore, it has been reported that bacteria associated with the PWN may possibly play a role in pine wilt disease (Zhao and Lin, 2005). According to the most recent findings, it has been postulated that horizontal gene transfer may have occurred from bacteria and fungi to nematodes such as the PWN, which could explain the expression of certain enzymes such as pectate lyase, and cellulase (Jones et al., 2005). However, no report has been made to this date regarding plant (pine) differential expression in the presence of the pinewood nematode. Studies of Portuguese *P.pinaster* population were already performed using AFLPs and cpSSRs markers (Ribeiro, 2001). Similar levels of differentiation were found with both markers. Little or no geographic genetic pattern was found in Portuguese populations of *P.pinaster,* due to human activities during the last century, and to extensive associated gene flow among populations (Ribeiro, 2001). *Pinus pinea* populations were analyzed with SSAP (sequence specific amplification polymorphism) and AFLP (amplified fragment length polymorphism), in order to evaluate genetic variability within and among Portuguese populations. SSAP approach was more efficient to retrieve information than the AFLP one, with the highest number of polymorphic fragments obtained per assay, and higher levels of estimated genetic diversity. The genetic variation was found mainly within populations. (Evaristo et al., 2006). Under natural conditions, *P. pinea* is not a host susceptible of being infectyed by PWN since the vector, *M. galloprovincialis*, is unable to feed and colonize the tree. Recently, preliminary results (Mota et al., unpublished) have demonstrated that PWN is capable of invading, multiplying and infecting *P. pinea* and causing plant mortality, although at a lesser rate than on *P. pinaster*. Until now, and despite studies on the development of disease and resistance factors (Tan et al., 2005; Kuroda, 2004; Matsunaga & Togashi, 2004; Kosaka et al, 2001), no single report has been made on the plant expression (mRNA, proteins) when in the presence of the PWN. This information is essential in order to understand the pathogenicity of PWN on pine species and thus provide information for decision making on re-foresting policies.

# Functional Analysis of Myo-Inositol Oxygenase Genes for Syncytium Induction and Maintainance in *Arabidopsis thaliana.*

Shahid Masood SIDDIQUE, Dagmar SZAKASITS, Julia HOFMANN, Krzysztof WIECZOREK, Florian GRUNDLER, and Holger BOHLMANN.
*shahid.siddique@boku.ac.at*

*Instiute of Plant Protection University of Natural Resources And Applied Life Sciences. 82-Peter Jordan strasse 1080, Vienna, AUSTRIA.*

In plants, UDP-glucuronic acid is synthesized by the oxidation of UDP-glucose by UDP-glucose dehydrogenase. However a second pathway has been described and involves the oxidation of free myo inositol by myo-inositol oxygenase (MIOX). In Arabidopsis, myo-inositol oxygenase is encoded by four genes (MIOX1, MIOX2, MIOX4, MIOX5), MIOX3 being a pseudogene. Transcriptome analysis of syncytia induced by *Heterodera schachtii* in Arabidopsis roots revealed that two MIOX genes are among the most strongly upregulated genes in syncytia(MIOX4 and MIOX5). The other two genes are expressed in control roots and in syncytia. These results have been confirmed with in situ RT-PCR and real-time RT-PCR. We have initiated a project to analyse the role of the MIOX gene family in syncytium development using downregulation of MIOX genes in the syncytium with the help of miRNAs.

# Regulation of stress related genes in *Arabidopsis* giant cells induced by *Meloidogyne* spp.

Ana Belén YUSTE[1], Marta BARCALA[1], Alejandra GARCÍA[1], Janice DE ALMEIDA[2], Keith LINDSEY[3], Carmen FENOLL[1], and Carolina ESCOBAR[1] .
*Anabelen.yuste@uclm.es ; carolina.escobar@uclm.es*

*[1] Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Campus de la Real Fábrica de Armas, E-45071 Toledo, SPAIN;*
*[2] INRA, UMR Interactions Plantes-Microorganismes et Sante Vegetale, 400 Route des Chappes, 06903 Sophia-Antipolis, FRANCE ;*
*[3] University of Durham, Department of Biological and Biochemical Science. South Road, DH1 3LE, Durham City, UNITED KINGDOM.*

Root-knot nematodes induce dramatic metabolic and morphological changes in a group of cells differentiated from the xylem parenchima, producing a group of cells that they use to feed (giant cells). Molecular changes such as up and down regulation of gene expression in galls from several plant species have been extensively described (Reviewed in Gheysen and Fenoll, 2002). Recent genome-wide expression profiling of *Meloidogyne spp.*-Arabidopsis responses indicated that a high proportion of defence related genes were down-regulated, suggesting that successful establishment of root-knot nematodes is associated with a suppression of plant defence mechanisms (Jammes et al., 2005). Further analysis on the localization of the particular transcripts in the different gall tissues will inform about the expression of this gene category in giant cells. Using laser capture microdissection, we have isolated RNA from giant cells 3 days after infection that allowed us to perform a genome-wide expression profiling with microarray technology. A partial analysis of genes related to biotic and abiotic stress, including genes from the ascorbate-glutathione cycle, is reported. Most of the stress-related genes in giant cells, including genes typically expressed during biotic interactions, were either not altered or down-regulated as compared to control uninfected tissues. These results suggest that in initial stages of giant cell development, expression of genes related to defence and stress is shut-off. Surprisingly, among the very few genes up-regulated in this category, are genes related to the heat-shock response (a heat-shock transcription factor and two small heat-shock proteins) and a key gene from the ascorbate-glutathion cycle. Accordingly to these results, the promoter of a small heat-shock protein from sunflower has been previously reported to be active in giant cells (Escobar et al., 2003). Small heat-shock proteins are important for protein protection not only under stress conditions; they are also important for proper function of many proteins that act in a wide variety of cellular processes, such as transcription, cell signalling and assembly/disassembly of cytoskeletal components (Basha *et al.,* 2004), all of them processes described to be altered in giant cells (Gheysen and Fenoll, 2002; de Almeida Engler *et al.*, 2004).

Basha, E., Lee, G.J., Breci, L.A., Hausrath, A.C., Buan, N.R., Giese, K.C., and Vierling, E. 2004. The Identity of Proteins Associated with a Small Heat Shock Protein during Heat Stress in Vivo Indicates That These Chaperones Protect a Wide Range of Cellular Functions. J. Biol. Chem. 279:7566-7575.

De Almeida Engler, J., Van Poucke, K., Karimi, M., De Groodt, R., Gheysen, G., and Engler, G. 2004. Dynamic cytoskeleton rearrangements in giant cells and syncytia of nematode-infected roots. Plant J. 38:12-26.

Escobar, C., Barcala, M., Portillo, M., Almoguera, C., Jordano, J., and Fenoll, C. 2003. Induction of the Hahsp17.7G4 promoter by root-knot nematodes: involvement of heat-shock elements in promoter activity in giant cells. Mol. Plant-Microbe Interact. 16:1062-1068.

Gheysen, G., and Fenoll, C. 2002. Gene expresion in nematode feeding sites. Annu. Rev. Phytopathol. 40:191-219.

Jammes, F., Lecomte, P., de Almeida-Engler, J., Bitton, F., Martin-Magniette, M.L., Renou, J.P., Abad, P., and Favery, B. 2005. Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. Plant J. 44:447-458.

**LIST OF PARTICIPANTS**

## AUSTRALIA

Michael JONES
*m.jones@murdoch.edu.au*
Western Australian State Agricultural Biotechnology Centre, Murdoch University, South Street, Perth, WA 6150, Australia

## AUSTRIA

Holger BOHLMANN
*holger.bohlmann@boku.ac.at*
Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, Austria

Abdel Nasser ELASHRY
*nasserelashry@hotmail.com*
University of Natural Resources and Applied Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Peter Jordan Str. 82, 1190 Vienna, Austria

Michaela GRIESSER
*michaela.griesser@boku.ac.at*
Institut for Plant Protection, DAPP, Boku Vienna, Peter Jordan Straße 82, Austria

Florian GRUNDLER
*florian.grundler@boku.ac.at*
Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, Austria

Julia HOFMANN
*julia.hofmann@boku.ac.at*
Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, Austria

Shahid Masood SIDDIQUE
*shahid.siddique@boku.ac.at*
Instiute of Plant Protection University of Natural Resources And Applied Life Sciences. 82-Peter Jordan strasse 1080, Vienna, Austria

Krzysztof WIECZOREK
*krzysztof.wieczorek@boku.ac.at*
Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Peter-Jordan Str. 82, A-1190, Vienna, Austria

## BELGIUM

Wim BERT
*Wim.Bert@UGent.be*
Nematology Section, Department of Biology, Ghent University, Ledeganckstraat 35, 9000 GENT, Belgium

Suganthagunthalam DHAKSHINAMOORTHY
*Suganthagunthalam.Dhakshinamoorthy@biw.kuleuven.be*
K.U. Leuven / Division of Crop Biotechnics, Laboratory of Tropical Crop Improvement, Kasteelpark arenberg 13, 3001 Leuven, Belgium

Annemie ELSEN
*annemie.elsen@biw.kuleuven.be*
Division of Crop Biotechnics, K.U.Leuven, Kasteelpark Arenberg 13, 3001 Leuven, Belgium

Godelieve GHEYSEN
*Godelieve.Gheysen@UGent.be*
Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium

Joachim JACOBS
*Joachim.Jacob@UGent.be*
Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium

Tina KYNDT
*Tina.Kyndt@UGent.be*
Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium

Bartel VANHOLME
*Bartel.Vanholme@UGent.be*
Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium

Wouter VAN THUYNE
*Wouter.VanThuyne@UGent.be*
Molecular Biotechnology, Ghent University, Coupure links 653 B-9000 Ghent, Belgium

John WILLIAMS
*COST office, Belgium*
COST office Food & Agriculture, 147 avenue Louise, 1050 Brussels, Belgium

## BRAZIL

Maria Fatima GROSSI DE SA
*fatimasa@cenargen.embrapa.br*
Embrapa Recursos Genéticos e Biotecnologia- Final W5 Norte, Brasília DF 70770-900, Brazil

## FRANCE

Pierre ABAD
*Pierre.Abad@sophia.inra.fr*
Janice de ALMEIDA ENGLER
*Janice.Almeida-Engler@sophia.inra.fr*
Maria Cecilia ARIAS
*Maria-Cecilia.Arias@sophia.inra.fr*
Mylène ARISTE ZELISE
*Mylène.Ariste.Zelise@sophia.inra.fr*
Fabien BALDACCI
*Fabien.Baldacci@sophia.inra.fr*
Mohamed Youssef BANORA
*Youssef-Mohamed.Banora@sophia.inra.fr*
Michel BONGIOVANI
*Michel.Bongiovani@sophia.inra.fr*
Nathalie BOSSELUT
*Nathalie.Bosselut@sophia.inra.fr*
Matthieu CABARET
*Matthieu.Cabaret@sophia.inra.fr*
Marie-Cécile CAILLAUD
*Marie-Cécile.Caillaud@sophia.inra.fr*
Chantal CASTAGNONE
*Chantal.Castagnone@sophia.inra.fr*
Mathilde CLEMENT
*Mathilde.Clement@sophia.inra.fr*
Carine CROZAT
*Carine.Crozat@sophia.inra.fr*
Florence DEAU
*Florence.Deau@sophia.inra.fr*
Emeline DELEURY
*Emeline.Deleury@sophia.inra.fr*
Caroline DJIAN-CAPORALINO
*Caroline.Caporalino@sophia.inra.fr*
Géraldine DUBREUIL
*Géraldine.Dubreuil@sophia.inra.fr*
INRA, UMR 1064 Interactions Plantes Microorganismes et Santé Végétale, 400 Route des Chappes, BP 167, F-06903 Sophia Antipolis, France

Daniel ESMENJAUD
*Daniel.Esmenjaud@sophia.inra.fr*
Bruno FAVERY
*Bruno.Favery@sophia.inra.fr*
Ariane FAZARI
*Ariane.Fazari@sophia.inra.fr*
Philippe LECOMTE
*Philippe.Lecomte@sophia.inra.fr*
Marc MAGLIANO
*Marc.Magliano@sophia.inra.fr*
Nathalie MARTEU
*Nathalie.Marteu@sophia.inra.fr*
Maria Belen MONTERO PALMERO
*Maria-Belen.Montero@sophia.inra.fr*
Karine MULET
*Karine.Mulet@sophia.inra.fr*
Amel OUASSA
*Amel.Ouassa@sophia.inra.fr*
Michaël QUENTIN
*Michael.Quentin@sophia.inra.fr*
Marie-Noëlle ROSSO
*Marie-Noelle.Rosso@sophia.inra.fr*
Cyril VAN GHELDER
*Cyril.Van-Ghelder@sophia.inra.fr*
Roger VOISIN
*Roger.Voisin@sophia.inra.fr*
Laetitia ZURLETTO
*Laetitia.Zurletto@sophia.inra.fr*
INRA, UMR 1064 Interactions Plantes Microorganismes et Santé Végétale, 400 Route des Chappes, BP 167, F-06903 Sophia Antipolis, France

Bernard CAROMEL
*Bernard.Caromel@avignon.inra.fr*
INRA, UR1052 Génétique et Amélioration des Fruits et Légumes, BP 94, F-84143 Montfavet, France

Diana FERNANDEZ
*fernande@mpl.ird.fr*
IRD - Institut de Recherche pour le Développement, 911, avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France

Jérôme GOUZY
*jerome.gouzy@toulouse.inra.fr*
Plateforme Bioinformatique du Génopôle Toulouse Midi Pyrénées, BP 52627, 31326 Castanet Cedex, France

Eric GRENIER
*Eric.Grenier@rennes.inra.fr*
INRA UMR BiO3P, Domaine de la motte, Le Rheu, F-35653, France

Marie-Claire KERLAN
*Marie-Claire.Kerlan@rennes.inra.fr*
UMR INRA AGROCampus Rennes APBV, Kéraiber, 29260 Ploudaniel, France

Eric PETIT
*eric.petit@univ-rennes1.fr*
UMR INRA-ENSAR Bio3P, DOMAINE de la MOTTE, 35653 Le Rheu Cedex France

## GERMANY

Katrin ASBACH
*k.asbach@plantbreeding.uni-kiel.de*
Department of Molecular Phytopathology, Christian-Albrechts-University Kiel, Hermann Rodewald Str. 9. D-24118 Kiel

Daguang CAI
*dcai@plantbreeding.uni-kiel.de*
Department of Molecular Phytopathology, Christian-Albrechts-University Kiel, Hermann-Rodewald-Str. 9, 24118 Kiel, Germany

Keith DAVIES
*keith.davies@bbsrc.ac.uk*
Hermann-Rodewald-Str. 9, 24118 Kiel, Germany

Stefan HOTH
*shoth@biologie.uni-erlangen.de*
Molecular Plant Physiology / FAU Erlangen, Staudstr. 5, 91058 Erlangen, Germany

Tobias KEIL
*t.keil@plantbreeding.uni-kiel.de*
Plant Breeding Institute, Christian-Albrechts-University of Kiel, Olshausenstrasse 40, 24098 Kiel, Germany

Michael KLEINE
*kleine@planton.de*
PLANTON GmbH, Kiel-Kanal 44, 24106 Kiel, Germany

## IRELAND

Charles SPILLANE
*C.Spillane@ucc.ie*
Genetics & Biotechnology Lab, Department of Biochemistry & Biosciences Institute, University College Cork, Cork, Ireland

## ITALY

Marida BIMONTE
*marida@arterrabio.it*
Arterra Bioscience Via Comunale Margherita 482, 80145 Napoli, Italy

Teresa BLEVE ZACCHEO
*t.bleve@ba.ipp.cnr.it*
IPP-CNR, Via Amendola 165/A, 70126 Bari, Italy

Gabriella COLUCCI
*gcolucci@arterrabio.it*
Arterra Bioscience Via Comunale Margherita 482, 80145 Napoli, Italy

Carla DE GIORGI
*c.degiorgi@biologia.uniba.it*
Dipartimento di Biochimica e Biologia Molecolare, Via Amendola 165/A, Bari, Italy

Francesca DE LUCA
*f.deluca@ba.ipp.cnr.it*
Dipartimento di Biochimica e Biologia Molecolare, Via Amendola 165/A, Bari, Italy

Caterina DILEO
*c.dileo@biologia.uniba.it*
Dipartimento di Biochimica e Biologia Molecolare, Via Amendola 165/A, Bari, Italy

Francisca FERNÁNDEZ DEL CAMPO
*francisca.delcampo@uam.es*
Istituto Protezione delle Piante-CNR, Via Amendola 122/D, Bari, Italy

Pasqua (Orietta) VERONICO
*p.veronico@ba.ipp.cnr.it*
IPP-CNR, Via Amendola 165/A, 70126 Bari, Italy

## NORWAY

Ricardo HOLGADO
*ricardo.holgado@bioforsk.no*
Norwegian Institute for Agricultural & Environmental Research, Plant Health & Plant Protection Division, Department of Entomology & Nematology., Høgskoleveien 7 N-1432 Ås, Norway

Christer MAGNUSSON
*christer.magnusson@bioforsk.no*
Norwegian Institute for Agricultural & Environmental Research, Plant Health & Plant Protection Division, Department of Entomology & Nematology, Høgskoleveien 7 N-1432 Ås, Norway

## POLAND

Marcin FILIPECKI
*marcin_filipecki@sggw.pl*
Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warszawa, Poland

Sylwia FUDALI
*sylwia_fudali@sggw.pl*
Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warszawa, Poland

Wladyslaw GOLINOWSKI
*wladyslaw_golinowski@sggw.pl*
Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warszawa, Poland

Slawomir JANAKOWSKI
*slawomir_janakowski@sggw.pl*
Warsaw Agricultural University, Department of Botany, Nowoursynowska 150, Building 37, 02776 Warsaw, Poland

Daniel KOLENDA
*danielkolenda@tlen.pl*
Warsaw Agriculture University, Department of Botany, Nowoursynowska 159, 02-776 Warsaw, Poland

Wojciech KUREK
*wojciech_kurek@sggw.pl*
Department of Botany, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland

Miroslaw SOBCZAK
*sobczak@delta.sggw.waw.pl*
Department of Plant Genetics, Breeding and Biotechnology, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warszawa, Poland

## PORTUGAL

Manuel MOTA
*mmota@uevora.pt*
NemaLab-ICAM, Dept. Biologia, Universidade de Évora, 7002-554, Évora, Portugal

Maria Salomé PAIS
*mariapais2004@yahoo.co.uk*
NemaLab-ICAM, Dept. Biologia, Universidade de Évora, 7002-554, Évora, Portugal

Paulo VIERA
*pvieira@uevora.pt*
NemaLab-ICAM, Dept. Biologia, Universidade de Évora, 7002-554, Évora, Portugal

## SCOTLAND

Vivian BLOK
*vblok@scri.ac.uk*
Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5JQ, Scotland

John JONES
*jjones@scri.ac.uk*
PPI Programme, SCRI, Invergowrie, Dundee, DD2 5DA, Scotland

## SLOVENIA

Barbara GERIC STARE
*barbara.geric@kis.si*
Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova 17, 1001 Ljubljana, Slovenia

Saša ŠIRCA
*sasa.sirca@kis.si*
Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova 17, 1001 Ljubljana, Slovenia

Polona STRAJNAR
*polona.strajnar@kis.si*
Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova 17, 1001 Ljubljana, Slovenia

Gregor UREK
*gregor.urek@kis.si*
Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova 17, 1001 Ljubljana, Slovenia

## SPAIN

Miguel Angel DIEZ-ROJO
*diez.rojo@ccma.csic.es*
Centro de Ciencias Medioambientales, Dept Agroecologia, Calle Serrano 115, DPDO, Spain

Carolina ESCOBAR
*Carolina.escobar@uclm.es*
Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Campus de la Real Fábrica de Armas, E-45071 Toledo, Spain

Maria Fé ANDRÉS
*mafay@ccma.csic.es*
Instituto de Ciencias Agrarias, Dpto Protección vegetal, C/ Serrano 115 dpdo, 28006 Madrid, Spain

Carmen FENOLL
*Carmen.Fenoll@uclm.es*
Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Campus de la Real Fábrica de Armas, E-45071 Toledo, Spain

Lee ROBERTSON
*lee.r@ccma.csic.es*
Centro de Ciencias Medioambientales, Dept Agroecologia, Calle Serrano 115, DPDO, Spain

Soledad VERDEJO
*soledad.verdejo@irta.es*
IRTA, Crop Protection, Crta de Cabrils, Km2. 08348 Cabrils, Barcelona, Spain

Ana Belen YUSTE MARTINEZ
*AnaBelen.Yuste@uclm.es*
Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Campus de la Real Fábrica de Armas, E-45071 Toledo, Spain

## SWITZERLAND

Sebastian KIEWNICK
*sebastian.kiewnick@acw.admin.ch*
Agroscope Changins-Waedenswil ACW, Research Station ACW, Plant Protection, Ecotoxicology and Soil Zoology, Schoss P.O. Box 185, CH-8820 Waedenswil, Switzerland

Janina POLOMSKI
*janina.polomski@wsl.ch*
Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland

## THE NETHERLANDS

Aska GOVERSE
*aska.goverse@wur.nl*
Wageningen University, laboratory of Nematology Binnehaven 5, 6708 PD Wageningen, The Netherlands

Hans HELDER
*hans.helder@wur.nl*
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands

Feddo JURRISEN
*feddo.jurrisen@wur.nl*
Wageningen University, laboratory of Nematology, Binnehaven 5, 6708 PD Wageningen, The Netherlands

Jose LOZANO
*jose.lozano@wur.nl*
Wageningen University, laboratory of Nematology, Binnehaven 5, 6708 PD Wageningen, The Netherlands

Hein OVERMARS
*hein.overmars@wur.nl*
Wageningen University, laboratory of Nematology Binnehaven 5, 6708 PD Wageningen, The Netherlands

Geert SMANT
*Geert.Smant@wur.nl*
Wageningen University, laboratory of Nematology Binnehaven 5, 6708 PD Wageningen, The Netherlands

Roel STAPS
*roel.staps@blgg.nl*
Blgg bv Nieuwe Kanaal 7F, 6709 PA, Wageningen, The Netherlands

Marcel TIJSTERMAN
*marcel.tijsterman@niob.knaw.nl*
Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Hans VAN DEN HEUVEL
*hans.vandenheuvel@deruiterseeds.com*
De Ruiter Seeds / Corporate Research, P.O. Box 1050, 2660 BB Bergschenhoek, The Netherlands

Peter VEENHUIZEN
*peter.veenhuizen@blgg.nl*
Blgg bv Nieuwe Kanaal 7F, 6709 PA, Wageningen, The Netherlands

## TURKEY

I. Halil ELEKCIOGLU
*halile@cukurova.edu.tr*
Plant Protection Department of the Univ. of Cukurova, University of Cukurova Faculty of Agriculture Plant Protection Department, 01330 adana, Turkey

## UNITED KINGDOM

Mark BLAXTER
*mark.blaxter@ed.ac.uk*
Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom

Rosane CURTIS
*rosane.curtis@bbsrc.ac.uk*
Rothamsted Research, Harpenden, Herts, AL5 2JQ, United Kingdom

Catherine LILLEY
*C.J.Lilley@leeds.ac.uk*
Centre for Plant Sciences, University of Leeds, LS2 9JT, United Kingdom

Aaron MAULE
*a.maule@qub.ac.uk*
Parasitology, School of Biological Sciences, Queen's University Belfast, BT9 7BL, United Kingdom

Peter URWIN
*p.e.urwin@leeds.ac.uk*
Centre for Plant Sciences, University of Leeds, LS2 9JT, United Kingdom

Mark VINEY
*Mark.Viney@bristol.ac.uk*
School of Biological Sciences, University of Bristol, United Kingdom