COST ACTION FA1206

Strigolactones: biological roles and applications

WG1, WG2, WG4 MEETINGS

WG1 Strigolactones as Plant Hormones

WG2 SLs as Signals for Parasitic Plants

WG 4 SLs Chemistry and Biochemistry

15th -18th September 2015

Bucharest, Romania

ORGANIZING COMMITTEE

Cost-European Cooperation in Science and Technology Action FA1206
National COST Office Romania

Prof. Cristina Prandi (IT) – Chair of the Action FA1206
Dr. Hinanit Koltai (IL) – Vice Chair of the Action FA1206 and of WG1 Co-leader
Prof. BinneZwanenburg(NL) – WG4 leader
Prof. Francisco Macias (ES) – WG4 co-leader
Dr. Maurizio Vurro (IT) – WG2 leader
Prof. Tatiana Eugenia Sesan – Local Manager and Organiser for Romania
Dr. Florin Oancea – COST national net, Scientific Director ICECHIM Bucharest – Local Organiser
Elena Dinu – COST Country Representative for Romania

SCIENTIFIC COMMITTEE

Dr. Hinanit Koltai (IL) – Vice Chair of the Action FA1206 and of WG1 Co-leader
Prof. BinneZwanenburg (NL) – WG4 leader
Prof. Francisco Macias (ES) – WG4 co-leader
Dr. Maurizio Vurro (IT) – WG2 leader
Prof. Tatiana Eugenia Sesan – Local Manager and Organiser for Romania
Dr. Florin Oancea – COST national net, Scientific Director ICECHIM Bucharest – Local Organiser

LOCAL ORGANIZERS

National COST Office
National Research-Development Institute for Chemistry and Petrochemistry (ICECHIM) Bucharest
University of Bucharest – Biology Faculty

Prof. Tatiana Eugenia Şesan- Local Manager and Organiser for Romania
Dr. Florin Oancea – COST national net, Scientific Director ICECHIM Bucharest – Local Organiser
Dr. Eng.SandaVelea
Mat. Octavian Frangu
Ec. Constantin Toader
Prof. Elena Delian
Prof. Beatrice Iacomi

PhD Students

Iuliana Răut

PRACTICAL INFORMATION

Hotel

IBIS GARA DE NORD, CaleaGrivitei nr.143, district 1, Bucharest, www.ibishotels.ro

Transportation from Bucharest airport to the hotel

Bus 780 from Bucharest Otopeni till Gara de Nord (North Railway Station). Then you should follow Grivitei street for 150m to the South (if you look to the South, thus with the North station on the back, Grivitei street is on the left, from Grivitei street you should see IBIS logo on the top of the hotel). Buses are each 30 min on Sundays. The magnetic card for 2 travels cost 7 RON (1.6 EUR) and are available near the bus station.

Transportation from the hotel to ICECHIM

From the Ibis hotel go to the South (near Hello hotels) IntrareaLuncani till the trolleybus station MinisterulTransporturilor (100 m). Take trolleybus 62,93,96 from this station till Pod Cotroceni / Cotroceni bridge (Gara de Nord, Witing, Plevnei, Pod Cotroceni). From the station you should go 50m to the bridge and then turn right on SplaiulIndependentei. Follow SplaiulIndependentei and on 150m you will arrive to ICECHIM gate, ICECHIM building being on the back, on a small hill with stairs. Indicators and posters will be visible.

Meeting Venue

The conference will take place at the National Research-Development Institute for Chemistry and Petrochemistry (ICECHIM) in Bucharest, 202 SplaiulIndependenţei Sector 6, Bucharest, Romania.

Registration/Help-Desk

Ibis Hotel Staff will be available for assistance Monday 15th September 2015 from 17.00 to 19.00 andWednesday-Friday 16th-18thSeptember 2015 from 8.30at the National Research-Development Institute (ICECHIM).

Badges

Badges and conference information will be handed out to all delegates upon registration. Name badges are required for entry to all meeting events including coffee breaks and social events.

Coffee Breaks

Refreshments will be served in the hall by the conference room, 1stfloor at ICECHIM.

Meals

- ✓ Breakfast is included in the price of the room. Serving will start at 7 a.m.
- ✓ Lunch will be available on 16thand 18th September in the hall of ICECHIM - near the conference room.
- ✓ Social dinner will take place on 16th September (20.00-22.00) at the Restaurant Caru cu Bere, in the historical centre of Bucharest - http://www.carucubere.ro/en/

Professional Tour

Thefirst part of the tour will start at 9.00 at the Ibis Hotel - until lunch time –we will visit the Experimental Field of the National Research-Development Institute for Agriculture Fundulea.

The second part will be a touristic tour, travelling along Prahova Valley to the Peleş Royale Castle in Sinaia, and it will include the visit of the castle in the afternoon.

The tour will end at 8 p.m.

AGENDA

	Tuesday 15th September	Wednesday 16th September	Thursday 17th September	Friday 18th September
09:00-09:15		**WG4 Opening remarks**		**WG2 Opening remarks**
09:15-10:00		**Christopher McErlean** Synthesis of Strigolactone Mimics		**David Sands** The Toothpick Method of Striga Biocontrol: Deployable and Inexpensive for Smallholder Farmers
10:00-10:40		**Francisco Macias** New Multifunctional Parasitic Weed Stimulants		**Maurizio Vurro** Goodness of microbes to biodegrade SLs
10:40-10:50		**Petr Tarkowski** LC-MS studies of strigolactones		**Peter Toth** What are the similarities in strigolactone requirements of various broomrapes during the germination?
10:50-11:00		**Miroslav Strnad** Recent progress in hormonomics of strigolactone mutants and strigolactone biological chemistry		
11:00-11:30		**Coffee break/poster**	**Visit to the agricultural experimental station**	**Coffee break/poster**
11:30-11:50		**Stefano Parisotto** Strigolactones mechanism of action and distribution in living organisms: a chemical contribution		**DiegoRubiales** Strigolactone content in crops: how can breeders make direct use of this to select for Orobanche resistance or trap crops in large segregating populations?
11:50-12:10		**Binne Zwanenburg** New Convenient Route to Solanacol		**Poster Flash presentations**
12:10-12:30		**Piermichele Kobauri** Insights in the computer-aided design of new Strigolactones analogues with a ligand-based pharmacophore approach		
12:30-12.45		**Melissa Van Overtveldt** The development of iso-indole based fluorescent Strigolactones		**Poster awards and closing remarks**
12:45-14:30		***Lunch/poster***	***Lunch***	***Lunch/poster***

14:30-14:40		***WG1 Opening remarks***	***Excursion***	***MC meeting***
14:40-15:20		***Andrea Chini*** *Pim1-kinase inhibitors modulate JA and auxin responses*		
15:20-15:40	***Bucharest tour***	***Kebert Marko*** *Phytohormone profiles as indicators of clone-dependent responses to excess copper in poplar*		
15:40-16:00		***Struck Sylvia*** *Molecular insight into strigolactone signaling to control root architecture*		
16:00-16:30		***Coffee break***		
16:30-16:50		***Dor Eugenia*** *Do strigolactones play a role in tomato somatic embryogenesis?*		***Departures***
16.50-17:30		***Francesca Baroccio*** - *Conceivable registration procedures for Strigolactones*		
17:30-18:30		***WG1, WG2 and WG4 meeting (parallel)***		
20:00-22:00	***Welcome***	***Social Dinner***		

*Posters available for all the meetings

PROGRAM

Wednesday, September 16, 2015

9.00-9.15 – Opening of COST FA1206 meeting; Welcome by local organizers and opening remarks

WG 4 Session *Strigolactones Chemistry and Biochemistry*
Chairpersons: Binne Zwanenburg, Francisco Macias

9.15 – Opening Session WG4; opening remarks
9.15 – 10.00 **– Chris Mc Erlean –** Synthesis of Strigolactone mimics: Outcomes and Opportunities
10.00 – 10.40 – **Francisco Macias –** New Multifunctional Parasitic Weed Stimulants
10.40 – 10.50 – **Petr Tarkowski –** LC-MS studies of strigolactones
10.50 – 11.00 – **Miroslav Strnad –** Recent progress in hormonomics of strigolactone mutants and strigolactone biological chemistry

11.00 – 11.30 – Coffee break (30 min)

11.30 – 11.50 – **Stefano Parisotto –** Strigolactones mechanism of action and distribution in living organisms: a chemical contribution
11.50 – 12.10 – **Binne Zwanenburg –** New convenient route to Solanacol
12.10 – 12.30 – **Piermichele Kobauri –** Development of a ligand-based pharmacophore model for predicting strigolactones activity in parasitic weed germination as well as the hormone roles in plants
12.30 – 12.45 – **Melissa Van Overtveldt –** Th +e development of isoindole based fluorescent Strigolactones

12.45 – 14.30 – Lunch / poster

WG 1 Session *Strigolactones as Plant Hormones*
Chairpersons: Hinanit Koltai, Florin Oancea / Tatiana Eugenia Sesan

14.30 – 14.40 – WG1 Opening remarks
14.40 – 15.20 – **Andrea Chini -** Pim1-kinase inhibitors modulate JA and auxin responses (keynotes)
15.20 – 15.40 – **Marko Kebert -** Phytohormone profiles as indicators of clone-dependent responses to excess copper in poplar
15.40 – 16.00 – **Sylwia Struck -** Molecular insight into strigolactone signaling to control root architecture

16.00 – 16.30 – Coffee break (30 min)

16.30 – 16.50 – **Eugenia Dor -** Do strigolactones play a role in tomato somatic embryogenesis
16.50 – 17.30 – **Francesca Baroccio** - Conceivable registration procedures for Strigolactones
17.30 – 18.30 – **WG1, WG2 and WG4 meeting (parallel)**
20.00 – 22.00 – Social Dinner at the Restaurant Caru cu Bere (Old City)

Thursday, September 16, 2015

9.00 – 12.45 – Visit to the experimental field at the National Research-Development Insitute for Agriculture Fundulea

12.45 – 14.30 – Lunch

14.30 – 18.30 (20.00) – Excursion to Sinaia, visit at Peleş Castle, back to Bucharest, Hotel Ibis

Friday, September 18, 2015

WG 2 Session *Strigolactones as Signals for Parasitic Plants*
Chairpersons: Maurizio Vurro, Diego Rubiales

9.00 – 9.15 – Opening Session WG2; opening remarks
9.15 – 10.00 **– David Sands** - The Toothpick method of *Striga* biocontrol: Deployable and Inexpensive for Smallholder Farmers
10.00 – 10.40 – **Maurizio Vurro -** Goodness of microbes to biodegrade SLs
10.40 – 11.00 – **Peter Toth -** What are the similarities in Strigolactone requirements of various broomrapes during the germination?

11.00 – 11.30 – Coffee break / poster (30 min)

11.30 – 11.50 – **Diego Rubiales -** Strigolactone content in crops: how can breeders make direct use of this to select for *Orobanche* resistance or trap crops in large segregating populations?
12.10 – 12.30 – Poster Flash presentations
12.30 – 12.45 – Poster awards and closing remarks

12.45 – 14.30 – Lunch / poster

14.30 – 16.00 – MC Meeting
16.00 – Departures

Abstracts*

* Abstracts included in this book have the form sent by authors.

WORKING GROUP 1
STRIGOLACTONES AS PLANT HORMONES

Lectures

Pim1-kinase inhibitors modulate JA and auxin responses

Andrea Chini[1], Isabel Monte[1], Marta Boter[1], Glenn Hicks[2], Natasha Raikhel[2], Roberto Solano[1]

[1]Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología-CSIC. Campus Universidad Autónoma, 28049 Madrid, Spain; [2]Center for Plant Cell Biology/Department of Botany and Plant Sciences, University of California, Riverside, CA; achini@cnb.csic.es

The phytohormone jasmonoyl-L-isoleucine (JA-Ile) regulates many developmental and stress responses in plants. A co-receptor complex formed by the F-box protein COI1 and a JAZ repressor perceives the hormone, activating specific signalling pathways. JA-Ile antagonists would represent invaluable tools to explore the role of JA-Ile in specific tissues or developmental stages and also to identify novel regulatory processes of the signalling pathway. Two complementary chemical screens identified four compounds exhibiting a robust inhibitory effect on the hormone-mediated COI-JAZ interaction and preventing the degradation of JAZ1 and JAZ9 *in vivo*. One molecule, J4, also restrains specific JA-induced responses *in planta*, such as JA-mediated gene expression, growth inhibition, chlorophyll degradation and anthocyanin accumulation. Besides JA signalling, this molecule also prevents several auxin responses *in planta* including hormone-mediated formation of the perception complex, gene expression and gravitropic response. J4 specifically acts on JA- and auxin-mediated responses without affecting other hormonal pathways. The J4 compound was described as a Ser/Thr protein kinase inhibitor in mammalian cells; here, we show that J4 and additional Pim1 kinase inhibitor reduce the phosphorilation activity of the Ser/Thr kinase AtPim1 *in planta.* The activity of J4 as Ser/Thr-kinases inhibitor highlights phosphorylation as an important post-transcriptional regulatory process in JA and auxin signalling.

Phytohormone profiles as indicators of clone-dependent responses to excess copper in poplar

Marko Kebert[1], Francesca Rapparini[2], Luisa Neri[2], Giampaolo Bertazza[2], Sasa Orlović[1], Stefania Biondi[3]

[1] Institute of Lowland Forestry and Environment (ILFE), 21000 Novi Sad, Serbia; [2] Institute of Biometeorology (IBIMET), National Research Council (CNR), 40129 Bologna, Italy; [3] Dept. of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, 40126 Bologna, Italy; kebertmarko@gmail.com

Auxin, abscisic acid (ABA) and polyamines (PAs) appear to play a role in stress adaptation. Their involvement in plants exposed to high levels of copper (Cu) has, however, received scant attention. Poplars are good candidates for phytoremediation of metal-polluted soils but their hormonal responses to abiotic stress have been insufficiently investigated. In the present work, three poplar clones (M1, PE19/66, and B229) were comparatively analyzed in a pot experiment for their responses to 300 mg/kg $Cu(NO_3)_2$. After four months, Cu accumulation in plant organs, shoot growth, leaf gas-exchanges and photosynthetic pigments were evaluated. Although strong accumulation of Cu occurred in the roots, plants did not display evident toxicity symptoms and no growth inhibition. Two protective mechanisms, enhanced guaiacol peroxidase activity and proline accumulation, were activated in Cu-treated clones B229 and PE19/66. After Cu treatment, free putrescine (Put) increased in leaves of clones M1 and B229, and free Spd accumulated markedly in roots of the same clones. Conjugated Put also increased markedly in both leaves and roots of Cu-treated clones M1 and B229, while conjugated Spd and Spm accumulated only in roots of clone M1. In clone PE19/66 (leaves and roots), strong accumulation of ABA was observed, whereas in B229 auxin was significantly affected by Cu treatment (only in roots). Overall, the three clones exhibited good tolerance to excess Cu, but their physiological responses and hormonal profiles were differentially affected, suggesting that specific genotype-dependent tolerance mechanisms were activated.

Molecular insight into strigolactone signaling to control root architecture

Sylwia Struk[1,2], Alan Walton[1,2] , Carolien De Cuyper[1,2], Cedrick Matthys[1,2], Annick De Keyser[1,2] and Sofie Goormachtig[1,2]

[1]Department of Plant Systems Biology, Flanders Institute for Biotechnology, Technologiepark 927, 9052 Ghent, Belgium; [2] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium; systr@psb.vib-ugent.be

Strigolactones (SLs), as plant hormones control various developmental processes such as germination, photomorphogenesis, and root and shoot architecture. Optimal root growth is essential for plant development and it is generally known that root growth is greatly influenced by both abiotic and biotic interactions. SLs influence many aspects of root architecture, including primary root length, lateral root formation, adventitious rooting and root hair elongation.

In Arabidopsis, responses to SLs require the F-box protein MAX2 and the α/β-hydrolase D14. Perception of SLs by D14 triggers MAX2-mediated degradation of the target proteins activating physiological responses. In rice, DWARF53 was shown to be an interactor of D3 (MAX2 rice orthologue) and D14. Genetic analysis in Arabidopsis revealed a homologous protein, SMAX1 to be involved in SL dependent germination.

We want to further unravel the SL signaling in the root of *Arabidopsis thaliana*. The tandem affinity purification (TAP) technique is a unique approach, which allows identifying protein complexes in conditions that are close to physiological ones. We use TAP to isolate the protein complexes to which known SL signaling components MAX2, D14 and SMAX1 homologues belong in root tissue treated and untreated with GR24. Preliminary results have identified, similarly to what was found in petunia and rice, MAX2 -D14 in the same protein complex. On the other hand, the interaction between MAX2 and SMAX1-like protein or any other proposed interactor was not detected in any conditions (with/without GR24). Confirmation analyses for other interactors are ongoing and interesting candidates will be further analyzed in more detail at the molecular and physiological level.

Overall, newly identified interactor proteins will help to improve our knowledge of the SL signaling cascade in *Arabidopsis thaliana*.

Do strigolactones play a role in tomato somatic embryogenesis

E. Dor, Y. L. Wu and J. Hershenhorn

Department of Weed Research, ARO, Newe Ya'ar Research Center, P. O. Box 1021, Ramat Yishay 30095, Israel;
evgeniad@volcani.agri.gov.il

Embryogenesis is one of the three common pathways of plant tissue culture regeneration. Compared with propagation from pre-existing meristem and organogenesis, somatic embryos have a single-cell origin thus, reduces the development of chimeras. Somatic embryogenesis requires the reprogramming of gene expression patterns: the genes functioning in differentiated somatic cells are suppressed while the genes necessary for embryogenesis are activated. During this process, differentiated somatic cells acquire embryogenic competence and proliferate as embryogenic cells, followed by the development of somatic embryos. The acquisition of embryogenic competence is restricted to certain cells that are responsive to chemical and physical stimuli. Plant growth regulators play a central role in mediating the signal transduction cascade leading to the reprogramming of gene expression patterns. Somatic embryogenesis of M82 tomato and it strigolactone lacking mutant *SL-ORT1,* and the influence of synthetic strigolactone GR24 on this process were studied in the project. GR24 influenced somatic embryogenesis of M82 and SL-ORT with different way, indicating that strigolactones influenced cytokinin - auxin ratio in tomato somatic embryogenesis.

Conceivable registration procedures for strigolactones

Francesca Baroccio

Ministry of Agriculture Food and Forestry (Mipaaf)
Central Inspectorate for quality control and antifraud of foodstuff and agricultural products (ICQRF)
Laboratory of Rome, f.baroccio@politicheagricole.it

Strigolactones are a novel class of plant hormones, known to be involved in the interaction between plants and their environment.

The discovery of new natural molecules to be used in agriculture generates new scientific, methodological and regulatory issues.

The first step to understand in which categories these substances may fall is to identify what are their characteristics. From a survey intending to know the properties attributed to these substances, in order to understand how in the future they can be regulated, different scientific studies indicates a number of properties and functions. On the basis of their so far proved agronomic properties and mechanisms of action, these chemicals, in view of a future regulation, may be configured as biostimulants, plant strengtheners, or finally as active substances to be used in plant protection products.

The present work will illustrate what is the current European regulation related to these different classes of products and which are the regulatory procedures for their inclusion in the legislation.

POSTERS WG1

INHOSPITABLE signaling in *Lotus japonicus*

Samy Carbonnel[1], Salar Torabi[1], Maximilian Griesmann[1], Trevor Wang[2], Yuhong Tang[3], Michael Udvardi[3], Caroline Gutjahr[3]

[1]Faculty of Biology, Genetics, University of Munich (LMU), Biocenter Martinsried, Großhaderner Str. 2-4, 82152 Martinsried, Germany. [2]John Innes Center, Norwich Research Park, Colney Lane, Norwich, UK. 3Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401 USA; salar.torabi@biologie.uni-muenchen.de

More than 80 % of land plant species live in a symbiosis with Glomeromycota fungi, called arbuscular mycorrhiza (AM) that is based on the mutual exchange of nutrients between both partners. AM colonization is initiated by a molecular dialogue involving plant phytohormones and fungal chitin-derived signals called Myc factors. After a successful recognition, AM fungi form a hyphopodium at the root surface and penetrate into the root. Then they develop intercellular hyphae along the root axis and form branched arbuscules inside cortex cells. It has been reported that the AM colonization in rice and pea is dependent on the F-box protein MAX2, which is involved in the perception of strigolactones and karrikin.

Furthermore the rice *inhospitable* mutant was found being unable to respond to the AM fungus. The *Inhospitable* gene encodes an α/β-fold hydrolase, that has the potential to recognize a small molecule and most probably acts as a receptor or in a receptor complex. We study the role of MAX2 and INHOSPITABLE signalling in *Lotus japonicus.* Phenotypic analysis of several *max2* alleles in *Lotus japonicus,* revealed a reduction of AM colonization, and proved a conserved role of *MAX2* in AM symbiosis. Progress in understanding the role of MAX2 and INHOSPITABLE in AM symbiosis using genetic and biochemical approaches in *Lotus japonicus* will be presented.

Strigolactone signalling in moss: a transcriptomic study

Mauricio Lopez-Obando[1,2], Beate Hoffmann[1,2], Sandrine Balzergue[4,5], François-Didier Boyer[1,2,3], Catherine Rameau[1,2] and Sandrine Bonhomme[1,2*]

[1]INRA, Institut Jean-Pierre Bourgin, UMR 1318, ERL CNRS 3559, Saclay Plant Sciences, RD10, F-78026 Versailles, France ; [2]AgroParisTech, Institut Jean-Pierre Bourgin, UMR 1318, ERL CNRS 3559, Saclay Plant Sciences, RD10, F-78026 Versailles, France ; [3]Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, UPR2301 CNRS, 1 avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France; [4] Unité de Recherche en Génomique Végétale, INRA/CNRS, CP5708, 91057 Evry Cedex, France ; [5] present address : INRA, Groupe EPICENTER, Institut de recherche en Horticulture et Semences UMR 1345-IRHS, 49071 Beaucouzé ;
sandrine.bonhomme@versailles.inra.fr

Bryophytes are non vascular plants and represent the extant-living descendants of the earliest plant species that colonized land 470 million years ago. Strigolactones (SLs), as hormones, may have played a role in plant terrestrialization. Most genes involved in SL biosynthesis and signalling pathways are found in the genome of the bryophyte model *Physcomitrella patens* (*P. patens*), e.g. homologues for both *CAROTENOID CLEAVAGE DIOXYGENASE 7*(*CCD7)*, and *CCD8* SL biosynthesis genes. Previously, we demonstrated that the *CCD8* homologue in moss is implicated in SL biosynthesis as in vascular plants. Analysis of the SL-deficient KO mutant in *PpCCD8* showed that in *P. patens*, SLs control branching of protonema filaments (Proust et al, 2011) and gametophore (Coudert et al, 2015) but also plant extension (Hoffmann et al, 2014). Still, the SL signalling pathway in moss is unknown.

A complementary approach to mutant analysis for deciphering SL-signalling in moss is to exploit transcriptomic data obtained following SL treatments. In this poster, we present the results of RNA-seq and microarray analysis of the *Ppccd8* mutant in response to SL (natural SLs exported in the medium by wild type plants and racemic mixture of (±)-GR24) in both dark and light conditions, along with the confirmation of differentially expressed genes by RT-qPCR. Analysis of transcript response in the putative SL receptors of PpKAI2-LIKE gene familly using the (+)-GR24 and (-)-GR24 enantiomers will also be presented and compared to previous data using (±)-GR24.

Involvement of Sorghum *MAX1* Genes in Biosynthesis of Strigolactones and Strigolactone-like Compounds

Mahdere Z. Schimels[1], Kristýna Floková[1], Carolien P. Ruyter-Spira[1,2], Patrick J. Rich[3], Gebisa Ejeta[3], Harro J. Bouwmeester[1,4,*]

[1]Laboratory of Plant Physiology, Wageningen University, 6708 PB, Wageningen, The Netherlands [2]Bioscience, Plant Research International, 6708 PB, Wageningen, The Netherlands; [3]Department of Agronomy, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47907-2054, USA; [4]Centre for Biosystems Genomics, 6700 AB, Wageningen, The Netherlands; harro.bouwmeester@wur.nl

Strigolactones (SLs) are root-exuded plant signaling molecules of carotenoid origin that also act as endogenous plant hormones. De novo biosynthesis appears to play a crucial role in the control of the level of SLs and was observed to be enhanced especially in response to reduced availability of inorganic phosphate. Several genes (*D27*, *CCD7*, *CCD8*, and *MAX1*) have been described to act in the biosynthetic pathway of SLs in various plant species. The product of β-carotene cleavage by *CCD8* – carlactone – is considered as the first intermediate in which the typical strigolactone D-ring is present. Further carlactone conversion to SLs requires involvement of cytochrome (CYP) P450 More Axillary Growth 1 (MAX1).

The aim of this study is to elucidate the role of the four sorghum *MAX 1* homologues (Sb04g007880, Sb10g022310, Sb03g032210 and Sb03g0322200) in SL biosynthesis and/or - diversification. Agrobacterium-mediated transient expression of rice strigolactone biosynthetic genes (*D27*, *CCD7*, *CCD8*) together with different sorghum *MAX1* combinations in leaves of *Nicotiana benthamiana* was performed to investigate the accumulation of potential SLs/SL-like compounds.

WORKING GROUP 2
STRIGOLACTONES AS SIGNALS FOR PARASITIC PLANTS

Lectures

The Toothpick method of *Striga* biocontrol: Deployable and inexpensive for smallholder farmers

David C. Sands, Henry Sila Nzioki, Florence Oyosi, Cindy E. Morris, Eylul Kaya, Claire S. Baker

Montana State University, KALRO Kenya, Liberty Initiators Network, INRA-Avignon; davidsands41@yahoo.com

Striga hermonthica (witchweed) is a parasitic weed that attacks and dramatically reduces the yield of sorghum, millet, sugarcane and maize crops throughout sub-Saharan Africa. Striga control includes herbicide resistant maize, Striga-resistant maize, crop rotation, conventional biocontrol, "push-pull" and weeding. To date, none of these strategies have been highly effective and researchers continue to seek for more efficient, affordable and deployable control methods for the smallholder farmer. While wild type strains of *Fusarium oxysporum* have been reported to reduce the incidence of Striga infection, none are effective enough to restore crop yield or to significantly reduce the soil seed bank. Extreme hypervirulence is a rare characteristic in plant pathogens that depend on their host for existence. We have successfully developed effective biocontrol agents, including strains of *Fusarium oxysporum* for control of noxious weeds by selecting for natural hypervirulent variants of fusaria. For Striga biocontrol, used variants that excrete leucine, tyrosine and methionine, interfering with the Striga's amino acid biosynthesis. These host-specific yet enhanced strains can kill their target host more rapidly, reducing the amount of inoculum needed, thus being more affordable. Three strains of the fungus *Fusarium oxysporum f.sp. Strigae* (Foxy T14) were coated onto toothpicks and given to 500 smallholder farmers (85% women) for trials in western Kenya in 2014. They made fresh Foxy T14 inoculum by placing the toothpicks in boiled/cooled grain three days prior to planting. Then this inoculum was co-planted with maize seeds, resulting in decreased Striga counts and an average yield increase of 43 - 56% (short and long rainy seasons, 2014). This is now a validated low-input and sustainable approach appropriate for smallholder weed control.

Goodness of microbes to biodegrade SLs

Rocío Pineda-Martos[1], Angela Boari[2], Biancamaria Ciasca[2], Veronica M.T. Lattanzio[2], Maurizio Vurro[2,*]

[1]Agricultural and Plant Biochemistry and Proteomics Research Group, Department of Biochemistry and Molecular Biology, University of Córdoba, Severo Ochoa Building, Campus Universitario de Rabanales, Ctra. Madrid-Córdoba km 396, 14071 Córdoba, Spain; [2]Institute of Sciences of Food Production, C.N.R., via Amendola 122/O, 70126 Bari, Italy; maurizio.vurro@ispa.cnr.it

Seed germination is a key phase of the parasitic plant life cycle, which begins with the recognition as germination stimulants of the secondary metabolites (mainly the carotenoid-derived strigolactones - SLs) secreted by the host roots. Interventions during this stage would be particularly suitable for parasitic weed management practices since seedlings of the parasite cannot persist in the absence of a host root.

Considering that SLs play several other roles in nature , both in the plant and in the biotic environment surrounding it, we hypothesized that microbes could detect and biotransform SLs, preventing germination of parasitic weed seeds, and thus they could be used as biocontrol agents, acting as a "physiological" barrier against parasitic weeds.

To prove this hypothesis, fungi with different ecological functions were considered for their possible capability to metabolize SLs, i.e.: one strain of *Fusarium oxysporum* having potential as biological agent of *Phelipanche ramosa*; one strain of *Trichoderma harzianum* (a promising biopesticide); and one strain of *Botrytis cinerea* (a necrotrophic pathogen).

Numerous experiments were carried out by adding four different natural or synthetic SLs (i.e.: GR24, strigol, 5-deoxystrigol and 4-deoxyorobanchol) to fungal cultures, followed by: chemical extraction, determination of the SL content by LC-MS/MS, and biological assays. Differences were observed among microorganisms, treatments and compounds used.

What are the similarities in Strigolactone requirements of various broomrapes during the germination?

Peter Toth ??????

Strigolactone content in crops: how can breeders make direct use of this to select for *Orobanche* resistance or trap crops in large segregating populations?

Diego Rubiales[1,*], Mónica Fernández-Aparicio[2]

[1]Institute for Sustainable Agriculture, CSIC, Córdoba, Spain; [2] INRA-UMR 1347 Agroécologie, 17 Rue Sully, F-21000 Dijon, France; diego.rubiales@ias.csic.es

Crop resistance against broomrape weeds is a multicomponent phenomenon, being the result of a battery of avoidance factors and (or) resistance mechanisms acting at different levels of the infection process. Pre-attachment mechanisms of resistance act prior the parasite makes contact with the host root. Among them, low induction of seed germination has been identified in several crop species. Tomato, faba bean and pea accessions that do not induce broomrape seed germination are now available. In pea and tomato, there is a range of strigolactone (SL) deficient mutants that could be exploited in breeding. In faba bean, a number of well adapted breeding lines have been produced whose resistance is associated with low SL exudation. However, in most instances the mechanisms underlying remains largely unidentified and insufficiently been exploited in resistance breeding due to the technical difficulties in its identification.

An alternative management strategy to resistance breeding could be exploitation of suicidal germination by synthesizing and directly applying SLs to the field. In addition, breeding for high SL exudation levels could result in more effective trap crops. Another potential application is intercropping. Inhibitory activity of these accompanying inhibitory crops can be increased by selecting for increased production and exudation of such alellopathic metabolites.

Any of these breeding approaches is possible by simple selection of existing variation or by various biotechnological approaches. In any case, a closer interaction of breeders with chemists and biochemists is needed. Recent developments in screening and analytical protocols allow a better understanding of the underlying mechanisms. However, they are still time consuming and therefore, unaffordable for breeders that ought to handle massive amounts of segregating populations involving thousands of plants in order to discard most of them and to retain only the really interesting ones. Therefore, faster although sufficiently reliable throughput screenings methods are still needed.

WORKING GROUP 4
STRIGOLACTONES CHEMISTRY AND BIOCHEMISTRY

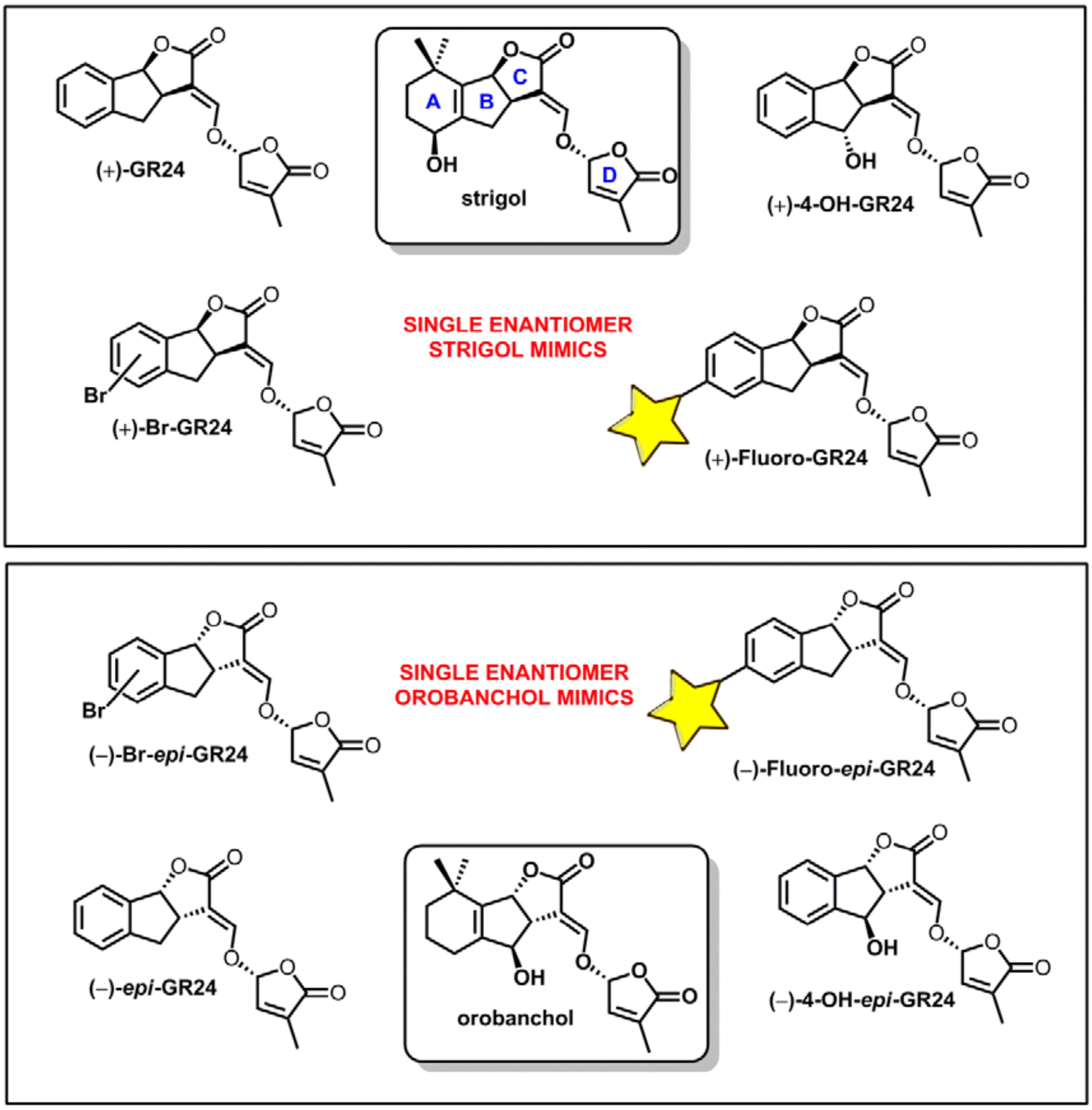
Lectures

Synthesis of Strigolactone Mimics

Liam J. Bromhead, Joanne C. Morris, Christopher S. P. McErlean*

School of Chemistry, the University of Sydney, Sydney, NSW 2006, Australia
christopher.mcerlean@sydney.edu.au

This presentation will detail our recent synthetic work on the rapid, enantioselective synthesis of strigolactone mimics and analogues based on the GR24 scaffold. Access to these compounds in enantiomerically pure form is vital to understanding the multi-faceted biological effects that these small molecules exhibit *in planta*.



New Multifunctional Parasitic Weed Stimulants

Antonio Cala[1], Rondinelle Gomes Pereira[1], Monica Fernandez-Aparicio[2], Jose M.G. Molinillo[1], Diego Rubiales[3], Francisco A. Macias[1,*]

[1]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), School of Sciences, University of Cadiz, C/República Saharaui, n 7, 11510 Puerto Real, Cádiz, Spain; [2]INRA-UMR 1347 Agroécologie, 17 Rue Sully, F-21000 Dijon, France ; [3]Institute for Sustainable Agriculture, Avda. Menendez Pidal, s/n, Campus Alameda del Obispo, 14004 Córdoba, Spain; famacias@uca.es

N-substituted phthalimides exhibit different biological activities depending on the substitution. Such as, anticancer activity and germination stimulant. The stimulatory activity represents a similar behavior as gibberellins.

Gibberellins are terpenic plant hormones with germination and stimulatory activity towards different growth steps in different plant species. In the same manner, strigolactones are plant hormones and natural promotors of parasitic weeds germination. They are formed with a typical ring structure sequence ABCD. The D ring is a lactone and has been found to be essential for their biological activity.

Recently, we introduced a lactone moiety to phthalimides and performed the germination of parasitic weeds with a family of compounds we called phthalimide-lactones. A lactone ring have been found previously partly responsible for the cytotoxic and anti-flammatory activity of sesquiterpene lactones. Lastly, we have introduced different modifications in gibberellic acid, including a lactone ring, to obtain new active compounds in a new strategy.

The aim of this project is to obtain multifunctional compounds that stimulate parasitic weeds. The preliminary results are promising, we have phthalimide-lactones that are stimulants of different species of parasitic weeds at high levels. Our current results on phthalimides and gibberellin derivatives are presented and discussed.

LC-MS studies of strigolactones

Petr Tarkowski[1,2,*], Rostislav Halouzka[2], Sanja Ćavar Zeljković[2], Jakub Kořistka[2]

[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Šlechtitelů 29, 78371 Olomouc, Czech Republic; [2]Centre of the Region Haná for Biotechnological and Agricultural Research, Central Laboratories and Research Support Faculty of Science, Palacky University, Šlechtitelů 27, 78371 Olomouc, Czech Republic; tarkowski@genobanka.cz

Chemical analysis of plant hormones is an integral part of the studies of plant development. The information about phytohormone profile is often very valuable for researchers dealing with any kind of hormone-regulated processes. Plant tissue represents a complex multi-component mixture that contains phytohormones in minute quantities (fmol to pmol per gram of fresh weight) along with many other compounds with similar chemical structure and/or physico-chemical properties. Therefore, the analysis demands rapid, sensitive and sufficiently selective analytical tools that should be applicable to compounds of varying polarity and functional groups.

Here, we present comparison of three different solid-phase extraction (SPE) approaches for isolation of strigolactones from plant extracts including silica, silica-based hydrophobic and polymer SPE-sorbents. Moreover, we tested UHPLC-MS/MS conditions for the simultaneous determination of GR24 and required abstract attached.four naturally occurring strigolactones. An influence of mobile phase composition on RP chromatographic separation and mass spectrometric signal character and intensity are discussed.

Recent progress in hormonomics of strigolactone mutants and strigolactone biological chemistry

Miroslav Strnad*, Ondřej Novák,Danuše Tarkowská, Tomáš Pospíšil, Katerina Barošová, Tomáš Dostál, Ondřej Kováč, Lukáš Spíchal and Binne Zwanenburg

Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnolgical and Agricultural Research, Palacky University & Institute of Experimental Botany AS CR, Šlechtitelů 27, Olomouc, CZ-783 71, Czech Republic; miroslav.strnad@upol.cz

High-resolution measurements of phytohormones are necessary for physiological studies of strigolactone mode of action. Application of targeted metabolomics is an optimal method for the phytohormonal screening. Our work is focused on the development of high-throughput miniaturized purification methods for minute amounts of plant tissue (1-10 mg). We have also developed several fast chromatographic separations (UHPLC) and highly sensitive tandem mass spectrometry (MS/MS) methods for simultaneous profiling of almost all phytohormone metabolites. Moreover, we applied fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with a highly sensitive LC-MS method for analysis of phytohormonal biosynthesis and homeostasis at cellular resolution. Strigolactones (SLs) as new plant hormones function as germination stimulant of parasitic weeds. Since, natural SLs have too complex structure for simple routine synthesis the needs for simple molecule with a good stimulatory activity is high. There are two groups of SL derivatives: SL analogues - all contain the D-ring connected with an enone moiety through an enol ether unit and SL mimics which only have the D-ring with an appropriate leaving group at C-5. Recently prepared SLs mimics and their structure-activity relationship will be also presented.

Strigolactones mechanism of action and distribution in living organisms: a chemical contribution

Stefano Parisotto, Cristina Prandi

Department of Chemistry, University of Turin, via P. Giuria 7 10125 Turin, Italy; cristina.prandi@unito.it

Stereochemistry plays a crucial role in Strigolactones Structure-activity relationship as compounds with unnatural configuration at the D-ring may induce biological effects unrelated to Strigolactones. An assessment of their stereochemistry is therefore mandatory if we wish to avoid the activation of these responses and therefore misinterpretation of our results.

We have synthesized a series of Strigolactones analogues, both fluorescent and not, whose absolute configuration of has been elucidated on the base of X-ray and CD spectra and put in relation with their biological activity, thus confirming the high specificity of the response.

Moreover, aiming to the synthesis of fluorescent tagged analogues suitable for confocal microscopy investigations, a new strategy has been developed for the modification of unsymmetrical azadipyrromethenes by exploiting the Heck reaction. The obtained aza-BODIPY probes carry different functionalities suitable to hook small active molecules and thus allow the synthesis of red fluorescent tagged bioactive molecules. Their use could boost the *in vivo* visualizations thanks to the minimal background interference from biomolecules auto-fluorescence and to the possibility of use them in combination with GFP tagged receptors.

New Convenient Route to Solanacol

Sanja Ćavar Zeljković[1]*, Binne Zwanenburg[2]*

[1]Palacky University, Faculty of Science, Centre of Region Hana for Biotechnological and Agricultural Research, Central Laboratories and Research Support, Slechtitelu 27, 78371 Olomouc, Czech Republic; [2]Radboud University Nijmegen, Institute for Molecules and Materials, Department of Organic Chemistry, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands; B.Zwanenburg@science.ru.nl

The novel synthetic route of strigolactone solanacol has been described. This synthesis is shorter and more efficient than those described in the literature [1]. The precursor ABC scaffold without a C4-OH was prepared as described earlier [2] by a Stobbe condensation of 3,4-dimethylbenzaldehyde with dimethyl succinate, subsequent intramolecular Friedel-Crafts acylation, followed by reduction with sodium borohydride and acid-catalyzed lactonization. Oxidation at C4 of this precursor was accomplished using *tert*-butylhydroperoxide in the presence of manganese (III) acetate, giving the ABC scaffold with a C4 keto group. Attachment of the D-ring was performed by formylation of the ABC scaffold with the C4-keto group and subsequent treatment with bromo butenolide to give the keto derivative of solanacol. Selective Luche reduction of the oxo group gave the C4 syn alcohol, which is inverted into the C4 anti epimer by a Mitsunobu procedure to give the desired (racemic) solanacol in a good overall yield. Moreover, this strategy will be used for synthesis of orobancohol and other natural strigolactones with hydroxy group.

[1]Tetrahedron 66, 7198 (2010);

[2]Chem. Eur. J. 16, 13941 (2010).

Development of a *ligand-based pharmacophore* model for predicting the Strigolactones activity in parasitic weed germination as well as the hormonal roles in plants

P. Kobauri,[1] S. Mensa[1], C. Prandi[2] and M. L. Lolli[1]

[1] MEDSynth, Department of Drug Science and Technology, University of Turin, via Pietro Giuria, 9, 10125 Torino, Italy; [2] Department of Chemistry, University of Turin, via Pietro Giuria, 7, 10125 Torino, Italy; marco.lolli@unito.it

Characterized by a peculiar structure able to suggest a promising role as antitumoral agents, Strigolactones (ST) have plenty of roles inside the plant life circle. They are active in parasitic weed germination, hyphal branching in AM Fungi, hormonal regulation. At the moment, selective activation of the mechanism behind these activities are still under investigation. While the mode of action of ST in plants is better knowledge, the target structure of the parasitic is still unknown. For moving ahead the understanding of these mechanisms, it is urgent to move ahead from the ST intrinsic structure by design non-strigolactonic active molecules able to activate or inhibite the ST target.

In this occasion, we built up a *ligand-based pharmacophore model*, able to extract the structural ST requisite responsible of the activity. This powerful tool is able to both support following synthetic guidelines for optimized STs as well as to perform *virtual screening* of chemical libraries, in order to find new entities to be tested and, possibly, improved with a hit-to-lead optimization.

The preliminary results on the models for parasitic weed germination and hormonal roles in plants are presented and discussed, along with further scenarios that can arise from their improving and consequent application in the close future.

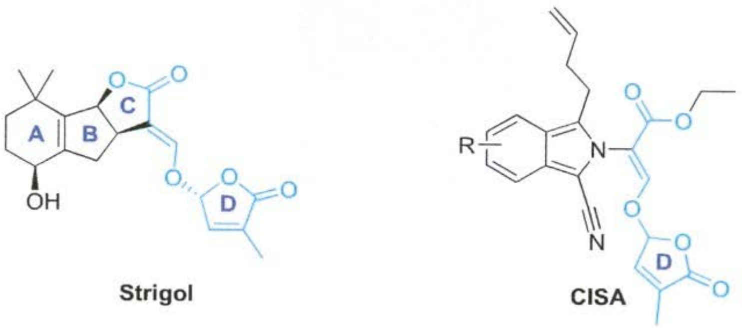
The Development of Isoindole Based Fluorescent Strigolactones

Melissa Van Overtveldt, Thomas Heugebaert, Christian V. Stevens

SynBioC Research Group, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium;
Melissa.VanOvertveldt@UGent.be

Natural strigolactones typically consist of a tricyclic lactone (A-, B-, C-rings), connected via an enol ether bridge to an α, β-unsaturated furanone moiety (D-ring). Whereas the lactone D-ring is generally regarded as the bioactiphore, the ABC part can be significantly modified without affecting the activity as plant hormone or germination stimulant. This allowed us to replace this tricyclic system with a fluorescent 1-cyanoisoindole based backbone, leading to the synthesis of a series of labeled analogues. This class of analogues can be used as fluorescent probes for *in vivo* transportation and receptor localization studies.

In total, 7 **C**yano**I**soindole **S**trigolactone **A**nalogues (CISA) have been synthesized. The method contains five major steps and includes a 5-*exo-dig* acid-catalyzed cyclization, followed by a Claisen type rearrangement. All compounds were assessed for their fluorescent properties and excitation and emission spectra were acquired. CISA-1 was also evaluated for a broad range of know strigolactone activities, and its stability and potency were compared to the commercially available GR24.



References

A. Rasmussen, T. Heugebaert, C. Matthys, R. Van Deun, F.-D. Boyer, S. Goormachtig, C. Stevens, D. Geelen, *Mol. Plant* **2013**, *6*, 100-112.

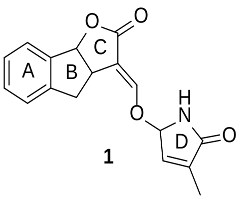
POSTERS WG4

Inhibitors of Strigolactones perception: synthesis and first drug design through a ligand based pharmacophore model of the SL receptor

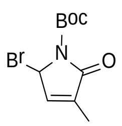
Emma Artuso[1], Piermichele Kobauri[2] , Giulia Molinaro[1], Marco Lucio Lolli[2], Chiara Lombardi[1], Stefano Parisotto[1] and Cristina Prandi[1]

[1]Department of Organic Chemistry, University of Turin, via Pietro Giuria 7, 10125, Turin, Italy;
[2] Department of Science and Drug Technology, University of Turin, via Pietro Giuria 9, 10125, Turin, Italy
cristina.prandi@unito.it

Strigolactones (SLs) are multifunctional molecules that function as phytohormones, inhibiting shoot branching and also functioning in the rhizospheric communication with symbiotic fungi and parasitic weeds. Therefore, chemicals that regulate the functions of SLs will find large applications in agricultural field.
SLs biosynthetic pathway as well as their receptor site are not fully clarified. To date, the differences observed in the response of parasitic weeds, fungi and plants for the hormonal activity suggest that distinct perception systems are involved. Recently DWARF14 (D14) has been shown to be a receptor for SLs in plants, and the D-ring moiety of SL is essential for its recognition by D14.
The use of specific inhibitors is an alternative and valuable way to determine the physiological functions of SLs would be a useful tool both for functional studies and for the assessment of the effect of SLs in plants. Moreover, SL inhibitors can be applicable for the regulation of germination and infestation of root parasitic weeds as well as for the control of plant physiology and architecture.
Tebuconazole derivatives TS13 and TS108[1] (TIS series) have been identified as potent SL biosynthesis inhibitors acting on cytochrome P450 monooxygenases. However, there has been no report of any antagonists of SLs perception able to compete with active SLs in the receptor binding pocket.
Ligand-based pharmacophore modeling is playing a key role for the identification of ligand features for specific targets. In order to design the best suitable inhibitors of perception we have exploited a ligand based pharmacophore model of the SLs receptor using Ligand Scout. The pharmacophoric model helped us to design a family of potential inhibitors of SLs perception having a lattame-D ring **1**. We synthesized the Bromo-Boc-lattame **2** through a four-step procedure where the key step is a ring-closing metathesis. Then we attached the lattame **2** on the tricyclic framework of GR24 to get the GR24-like inhibitor **1**.



1



2

[1]Nakamura, H.; Asami, T. *Front Plant Sci.* **2014**, 5:623, and ref. therein.

Inuloxins: Plant Sesquiterpene Lactones Inhibiting Parasitic Plant Seed Germination

Marco Evidente[1], Ernesto Santoro[2], Alessio Cimmino[1], Marco Masi[1], Ana G. Petrovic[3], Stefano Superchi[2], Nina Berova[4], Antonio Evidente[1,*]

[1]Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Napoli, Italy; [2]Dipartimento di Chimica, Università della Basilicata, via dell'Ateneo Lucano, 10, 85100, Potenza, Italy; [3]Department of Life Sciences, New York Institute of Technology (NYIT), 1855 Broadway, New York, NY, 10023, USA; [4]Department of Chemistry, Columbia University, 3000 Broadway, 3114, New York, New York 10027 USA; evidente@unina.it

Broomrapes (*Orobanche* spp.) and dodders (*Cuscuta* spp.), belonging to Orobanchaceae and Convolvulaceae families, respectively, are parasitic weeds attacking a large number of important crops and causing severe yield losses. Heavy infestations lead not only to a complete crop failure, but also have a great negative impact over many years, because seeds can survive in soil for a long period of time, preventing the reasonable production of legumes and vegetables in the infested fields.

Although a large number of methods to control parasitic weeds have been proposed, their effective management is very difficult to be reached because of their physiological traits and life cycle. Considering that seed germination is a key phase for parasitic plants development and infestation, a further approach proposed for the management of these weeds was the use of natural metabolites produced by plants as seed germination inhibitors. Indeed, plants may compete by suppressing the growth of neighbouring plants through the production and release of allelopathic compounds.

In an early attempt to find novel bioactive metabolites to be used as natural and safe herbicides for this management, the organic extracts of 10 Mediterranean plants were evaluated for the inhibitory effects to seeds of field dodder (*Cuscuta campestris* Yuncker) and crenate broomrape (*Orobanche crenata* Forsk.). Among them, the extract obtained from *Inula viscosa* (L.) Aiton, Asteraceae, was one of the most active and then deeper investigations were carried out in order to purify and identify the bioactive compound(s).

Despite the number of biological activities reported for the extracts of this plant, no studies were carried out for their herbicidal properties, and neither for parasitic weed management.

Four new phytotoxic bi- and tri-cyclic sesquiterpene lactones, named inuloxins A-D, were isolated together with the known α-costic acid, from the aerial parts of *Inula viscosa* (family Asteraceae). The structures of inuloxins A-D were established by spectroscopic and chemical methods and determined as: (4*E*,7*R**,8*R**,10*S**)-3-oxo-germacra-4,11(13)-dien-8β-12-olide (A), its 11,13-dihydro analogue (B), (5*R**,7*R**,8*R**,10*R**)-1,15-methylene-5β-hydroxy-eudesm-1(15),11(13)-dien-8β-12-olide (C), and (7R*,8*R**)-1,4-dimethyl-4-hydroxy-secoeudesm-5(10),11(13)-dien-8β-12-olide (D). The *S* absolute stereochemistry at C-5 of 5-hydroxyhexan-2-yl side chain of inuloxin D was assigned applying an advanced Mosher's method. The phytotoxic activity of inuloxins A-D, of the diazo and monoacetyl derivatives (of inuloxin A and C, respectively), as well as of α-costic acid was evaluated against two parasitic plant species, i.e.: crenate broomrape (*Orobanche crenata*) and field dodder (*Cuscuta campestris*). Inuloxins A, C and D were the most active on both parasites and caused up to 100% inhibition of the seed germination. Inuloxin B was less activite on *Cuscuta* and completely inactive against *Orobanche*.

More recently, the absolute configuration (AC) (7*R*,8*R*,10*S*), close related to the biological activity, of inuloxin A (**1**), was determined by combined use of three chiroptical techniques, optical rotation, dispersion electronic circular dichroism and vibrational circular dichroism (ORD, ECD and VCD). Work is in progress to assign the AC to inuloxins B and C using the same chiroptical and computational methods.

New strigolactone analogs as potential plant hormones

Emilian Georgescu [1], Florentina Georgescu [2], Alina Nicolescu [3,4], Florin Oancea[5], Sanda Velea[5], Lucian Vlădulescu [2], Marius-Constantin Vlădulescu [2], Călin Deleanu [3,4]

[1]Oltchim Research Center, Str. Uzinei 1, 240050 Ramnicu Valcea, Romania; [2]Research Dept., Teso Spec S.R.L., Str. Muncii 53, 915200 Fundulea, Calarasi, Romania; [3] "C. D. Nenitzescu" Centre of Organic Chemistry, Romanian Academy, Spl. Independentei 202-B, 060023 Bucharest, Romania; [4]"Petru Poni" Institute of Macromolecular Chemistry, Romanian Academy, Aleea Grigore Ghica Voda 41-A, 700487 Iasi, Romania; [5]National Research & Development Institute for Chemistry & Petrochemistry–ICECHIM, Spl. Independentei 202, 060021, Bucharest, Romania;

Strigolactones are known as rhizosphere communication signal molecules between plants and root of parasitic weeds, and between plants and arbuscular mycorrhizal (AM) fungi which helps plants to adapt to environmental changes. Strigolactones are considered also a novel class of plant hormones that control shoot branching outgrowth and root development. The molecular mechanisms for their various activities as well as protein receptors involved in arbuscular mycorrhizal fungi and root parasitic plant seeds activities have not yet been fully revealed. They are mainly biosynthesized in the lower parts of the stem and roots. Usually, natural strigolactones can only be obtained from plants roots or by long multistep syntheses in minute amounts. The importance of strigolactones in plant biology and the difficulties to obtain natural strigolactones due to long multistep syntheses prompted us to obtain new strigolactone synthetic analogs. The objective of the present work was to obtain strigolactone synthetic analogs easily accessible in sizeable quantities and useful to control plant architectures without favoring the development of parasitic plants.

At least 19 naturally occurring strigolactones have been identified in root exudates of different mono- and dicotyledonous plant species and have been structurally characterized. The new synthesized strigolactone analogs contain an aromatic or heteroaromatic ring, present in various bioactive molecules, connected by an enol ether link to a furan-2-one moiety. The structures of all new synthesized strigolactone analogs were assigned on the basis of chemical and spectral analysis (GC-MS, IR, ^{1}H and ^{13}C NMR spectra) and their bioactivity has been tested.

"This work was supported by the Ministry of National Education – Research Activity, CNDI– UEFISCDI, project number 159/2014 - CERES, Programme PN2 P4 Partnership PCCA 2013"

Quantitative Structure-Activity Relationship Analysis of Some Strigolactone Analogues with Fluorescent Properties – A Preliminary Study

Strahinja Kovačević[1,*], Sanja Podunavac-Kuzmanović[1], Lidija Jevrić[1], Cristina Prandi[2]

[1]University of Novi Sad, Faculty of Technology Novi Sad, Department of Applied and Engineering Chemistry, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia (*strahko@uns.ac.rs); [2]Università degli Studi di Torino, Dipartimento di Chimica Generale e Chimica Organica, via P. Giuria 7, 1025 Torino, Italy

This preliminary study deals with the estimation of the correlations between the biological activity of a class of synthetic strigolactone (SL) analogues (GR24, PLO65, PLN655, PLC655 and PLS65) and their molecular structure coded by *in silico* molecular descriptors. Biological activity was determined as *in vitro* germination activity for seeds of holoparasite plant *Orobanche aegyptiaca* [1]. The set of physico-chemical, lipophilicity, quantum-chemical and absorption, distribution, metabolism, excretion (ADME) molecular descriptors was calculated after optimization of 3D structure with Molecular Mechanics (MM2) method by using a suitable software for molecular design. Quantitative Structure-Activity Relationship (QSAR) approach with appropriate descriptors-selection methods could offer significant guidelines for syntheses of new biologically active compounds [2]. In this case, QSAR analysis resulted in linear and polynomial regression models which successfully correlate plasma protein binding (PPB), lowest unoccupied molecular orbital (LUMO) energy, blood brain barrier penetration (BBB) ability and protease inhibition (PI) with germination percentages (%G). There are strong linear correlations between PPB and %G (R = -0.9495; R^2_{adj} = 0.8687; SD = 2.88; F = 27.5), LUMO and %G (R = -0.9737; R^2_{adj} = 0.9308; SD = 1.73; F = 54.8) and BBB and %G (R = 0.9510; R^2_{adj} = 0.8725; SD = 5.35; F = 28.4). The outstanding second-degree polynomial dependence was obtained between PI and %G (R = 0.9950; R^2_{adj} = 0.9802; SD = 2.11; F = 99.9). The selected descriptors describe the complex behavior of compounds in biological system. The obtained results should be treated as preliminary ones, since the QSAR models were based on a small set of molecules. Therefore, despite good statistical parameters, the prediction ability of these QSAR models is limited. Nevertheless, this study reveals which molecular descriptors could be used further for QSAR modeling of the germination activity of the extended set of SL derivatives toward *Orobanche aegyptiaca* seeds.

Acknowledgement: The authors gratefully acknowledge the financial support from COST Action FA1206.

References:

[1] Bhattacharya C, Bonfante P, Deagostino A, Kapulnik Y, Larini P, Occhiato EG, Prandi C, Venturello P, A new class of conjugated strigolactone analogues with fluorescent properties: synthesis and biological activity. *Org. Biomol. Chem.* 2009, 7, 3413-3420.

[2] Kovačević SZ, Podunavac-Kuzmanović SO, Jevrić LR, Djurendić EA, Ajduković JJ, Non-linear assessment of anticancer activity of 17-picolyl and 17-picolinylidene androstane derivatives – Chemometric guidelines for further syntheses. *Eur. J. Pharm. Sci.* 2014, 62, 258-266.

Strigolactone analogues in medical field: new anti-cancer formulations and biotinylated derivatives

Lombardi Chiara[1], Artuso Emma[1], Trotta Francesco[1], Fabrizio Caldera[1],Cavalli Roberta[2] and Prandi Cristina[1]
[1]Department of Chemistry, University of Turin, via P. Giuria 7, 10125 Turin (Italy); [2]Department of Drug Science and Technology, via P. Giuria 9, 10125 Turin (Italy); chlombar@unito.it

As other plant derived compounds, it has been demonstrated that SLs can inhibit cancer cell proliferation and induce apoptosis, unveiling another significant effect of this challenging class of molecules. In order to improve their activity we have started working on new anti-cancer formulations with improved delivery systems.

The design of new nanocarriers as a strategy for the delivery of anti-cancer drugs offers a potential platform to improve their activity, for example by achieving targeted release into tumor tissues.

Cyclodextrin-based nanosponges are a novel nanosized delivery system composed of hyper-cross-linked cyclodextrins connected in a three-dimensional network which already proved to be excellent carriers for anti-cancer drugs, such as paclitaxel.

A study has been carried out in order to evaluate the possibility of using carbonate nanosponges as SL analogs carriers.

Moreover, isolation and identification of SL-protein receptor/s, whether for plant and fungi or cancer cells, could be realized attaching biotinylated species to immobilized avidin or streptavidin. For this purpose we are synthesizing biotin labelled indole-derived analogues which can be used for affinity chromatography.

Raut et al. ICECHIM

Iuliana Răut[1, 2], Tatiana Eugenia Şesan [1, 2], Florin Oancea[1], Melania Liliana Arsene[1], Mihaela Badea-Doni[1], Gelu Vasilescu[1], Luiza Jecu[1]

[1] INCDCP-ICECHIM, Spl. Independentei 202, 060021, Bucharest, Romania; [2]University Bucharest, Faculty of Biology, Spl. Independentei 91-95, Bucharest, Romania;

"This work was supported by the Ministry of National Education – Research Activity, CNDI–UEFISCDI, project number 159/2014 - CERES, 160/2014 – MAIA, Programme PN2 P4 Partnership PCCA 2013"

Searching for compounds with strigolactone–like biological activities in algae

Iva Smýkalová[1,*], Jiří Kopecký[3], Eliška Ondráčková[1], Radoslava Matúšová[2]

[1] Agritec Plant Research Ltd., Zemědělská 16, Šumperk, 787 01, Czech Republic Allelopathy; [2] Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademicka 2, 950 07 Nitra, Slovak Republic; [3]Department of phototrophic microorganisms, Institute of microbiology, Academy of Sciences of the Czech Republic, 739 01-Třeboň, Czech Republic; smykalova@agritec.cz

Microalgae are on the evolutionary lower developmental stage than higher plants. An application of microalgae in agricultural systems is currently at the begining, but results indicate several perspectives in their use. Currently the most commonly marine species of macroalgae as biostimulants or biofertilizers are used, but microalgae as source of bioactive molecules in agriculture are rarely practically applied. The aim of this work is to carry out a study on the compounds with strigolactone-like activities in microalgae, which might be used in plant protection against parasitic weeds such as *Phelipanche*, *Orobanche* and *Striga* spp.

We used highly sensitive germination bioassay of parasitic weed seeds (*Striga hermonthica* and *Phelipanche aegyptiaca*) to look for new compounds with strigolactone-like activities. Crude extracts from microalgae, macroalgae and several higher plants were tested on preconditioned *S. hermonthica* and *P. aegyptiaca* seeds. First results confirmed the presence of compounds with germination stimulant activities in all groups of organisms studied. Up to now, we tested 43 different crude extracts from microalgae, macroalagae and weeds of higher plants and we found 14 samples with the germination stimulation activity on seeds of *Phelipanche aegyptiaca*. Among the samples tested, three candidates belong to microalgae group. Further studies will focus on the determination and characterisation of these molecules.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract No. SK-CZ-2013-0121 and by the Ministry of Education, Youth and Sports, COST Action1206, no. LD14101.