The 8th International Symposium on the Plant Hormone Ethylene

21st to 25th June 2009

Cornell University

Ithaca, New York, USA.

Web Site: http://www.hort.cornell.edu/ethylene/index.html

E-Mail: ethylene@cornell.edu

Local Organizing Committee:

- Peter Davies, Cornell University, Convener
- James Giovannoni, USDA-ARS/Boyce Thompson Institute, Cornell University
- William Miller, Cornell University
- Chris Watkins, Cornell University

Symposium Committee:

- Peter Davies, Cornell University, Chair
- David Clark, University of Florida
- Mark Dahmer, AgroFresh Inc.
- James Giovannoni, USDA-ARS/Boyce Thompson Institute, Cornell University
- Harry Klee, University of Florida
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- Angelo Ramina, Università di Padova, Italy
- Sara Patterson, University of Wisconsin, Madison
- Chris Watkins, Cornell University

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- Craig Cramer, Department of Horticulture, Cornell University

Abstract booklet assembled by Caroline von Dahl, Boyce Thompson Institute, Cornell University

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Program

Session Locations:
All talks will be delivered in **Room 233 Plant Science Building**.
In the event of construction in the Plant Science building talks will be held in room B45 Warren Hall (across the quadrangle).
Poster sessions will be held in **rooms 143 and 141** Plant Science Building.

Sunday, June 21st

PLENARY SESSION

Moderator
Peter Davies, Cornell University, USA

5:00 - 5:15 **Welcome and announcements**

5:15 - 6:15 **Donald Grierson**, University of Nottingham, UK
Ethylene, a simple molecule with a complex lifestyle

The Shang Fa Yang Memorial Lecture

6:15 - 6:45 **Christopher Watkins**, Cornell University, USA
Overview of applied ethylene biology

Evening reception with buffet dinner: 7pm hors d'oeuvres; dinner at 7:30
Biotech Building lower level (across road from Plant Science Building)

Monday, June 22nd

ETHYLENE SIGNALING AND HORMONE INTERACTIONS

Moderator
Jim Giovannoni, USDA-ARS/Boyce Thompson Institute, Cornell University, USA

8.30 - 9.00 **Jose M. Alonso**, North Carolina State University, USA
The ethylene-auxin connection: a model for signal interaction and integration

9.00 - 9.15 **Georg Groth**, Heinrich-Heine University, Germany
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9.30 - 9.45 **Yusuke Kamiyoshihara**, Nagoya University, Japan
Analysis of Protein Phosphatase Involved in the Post-translational Regulatory Mechanism of ACC Synthase

9.45 - 10.00 **Nigel Gapper**, Boyce Thompson Institute for Plant Research, USA
Functional characterization of SlFBOX1, a tomato FBOX protein: A novel regulator of ethylene signaling?

10.00 - 10.30 *Break*
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12.00 - 2.00 *Lunch: All lunches are at Northstar dining room, Apel commons, North Campus*

Moderator
Chris Watkins, Cornell University, USA

2.00 - 2.30 **Gloria K. Muday**, Wake Forest University, USA
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RPN10-silenced tomatoes have prolonged flower longevity and reduced C2H4 production

2.45 - 3.00 **Elizabeth Fox**, USDA-ARS and Boyce Thompson Institute, USA
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3.00 - 3.15 **Je Min Lee**, Boyce Thompson Institute for Plant Research, USA
Integrated analysis of transcriptome and metabolite profiles in tomato wild species introgression lines to identify candidate regulatory genes.

3.15 - 3.45 *Break*

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Control of ethylene responses by the GREEN-RIPE gene family

4.15 - 4.30 **Chi-Kuang Wen**, Chinese Academy of Science, Shanghai, China
A Study of ETR1 and ERS1 signaling reveals functional divergence and cooperativity of Arabidopsis ethylene receptors

4.30 - 4.45 **Sacco te Lintel Hekkert**, Sensor Sense BV, The Netherlands
Highly sensitive ethylene detector for online measurements on biological samples

4.45 - 5.00 **Hitoshi Mori**, Nagoya University, Japan
The ethylene response factors Snorkel1 and Snorkel2 allow rice to adapt to deep water.

5.00 - 6.45
Poster Session

Free Evening

Tuesday, June 23nd

ABSCISSION, SENESCENCE, FRUIT RIPENING

Moderator
Sara Patterson, University of Wisconsin, USA

8.30 - 9.00 **Sara Patterson**, University of Wisconsin, Madison, USA
Molecular analysis of ethylene responses and recovery in Dianthus floral senescence

9.00 - 9.15 **Cai-Zhong Jiang**, USDA-ARS, USA
Molecular analysis of the interaction of ethylene and auxin during flower abscission

9.15 - 9.30 **Catharina Merchante**, Universidad de Málaga, Spain
Investigating the role of ethylene in strawberry fruits

9.30 - 9.45 **Vera Hershkovitz**, The Volcani Center, Israel
Seed Involvement in Ethylene Perception during Avocado Ripening and Senescence

9.45 - 10.00 **Bram van de Poel,** Katholieke Universiteit Leuven, Belgium
The role of s-adenosyl-L-methionine during climacteric ripening of tomato

10.00 - 10.30 *Break*

10.30 - 11.00 **Kenichi Shibuya**, NARO, Japan
Programmed cell death during flower senescence

11.00 - 11.15 **Robert Schaffer**, Institute of Plant and Food Research, New Zealand
Taking ethylene out of the fruit ripening equation

11.15 - 11.30 **Jun Song**, Atlantic Food and Horticulture Research Centre, Canada
Proteomic analysis of differentially expressed proteins in apple fruit during ripening and senescence

11.30 - 11.45 **Wendy C. Schotsmans**, IRTA, Spain
Temperature dependent ethylene metabolism during storage of 'Rich Lady' peach

11.45 - 12.00 **Haya Friedman**, The Volcani Center, Israel
Expression of MaMADS2 and its interactions with ethylene suggest that it acts upstream to ethylene production

12.00 - 2.00 *Lunch*

Moderator
Angelo Ramina, Università di Padova, Italy

2.00 - 2.30 **Coralie C. Lashbrook**, Iowa State University, USA
Modeling cell wall structural dynamics in Arabidopsis abscission zones

2.30 - 2.45 **Sofia G. Foukaraki**, Cranfield University, UK
Effect of transition between ethylene and air storage on two potato varieties

2.45 - 3.00 **Manuela Donetti**, Cranfield University, UK
Influence of season and origin on ripening of imported avocado cv. Hass fruit.

3.00 - 3.15 **Livio Trainotti**, Università di Padova, Italy
Interactions between ethylene and auxin during peach fruit ripening.

3.15 - 3.45 *Break*

3.45 - 4.15 **Pietro Tonutti**, Scuola Superiore Sant'Anna, Italy
Ethylene and postharvest physiology in climacteric and nonclimacteric fruit in the genomics era

4.15 - 4.30 **Nurit Katzir**, Newe Ya'ar Research Center, Israel
Melon fruit development and quality: climacteric vs. non-climacteric ripening

4.30 – 4.45 **Giovanni Giuliano**, Casaccia Research Center, Italy
Altered ripening characteristics of "Golden" tomato fruits

4.45 - 5.00 **Max Villalobos**, University of California, USA
Modulating 1-MCP effect in 'Bartlett' pears with maturity, ethylene exposure, and cold storage

5.00 - 6.00 **Poster Session**

Evening: *Conference Banquet at Statler Hotel*

6.30 - 7.30 Predinner drinks, Statler Hotel ballroom foyer, first floor.

7.30 - 9.30 Banquet: Statler Hotel ballroom, first floor.

Speaker: Professor James Reveal
The Lewis and Clark expedition to the American West

Jim Reveal is a Professor Emeritus in the department of Plant Biology. He is a national expert on the botanical discoveries made by Lewis and Clark during the first American overland expedition to the Pacific coast (1803-1806), and also on the life of Meriwether Lewis.

Wednesday, June 24th

ETHYLENE RECEPTORS – FROM THE RECEPTOR TO APPLICATIONS

Moderator
Mark Dahmer, AgroFresh Inc, Centennial, Colorado

8.00 - 8.30 **Raphael Goren**, The Hebrew University of Jerusalem, Israel
The effect of both (i) volatile and (ii) water soluble cyclopropene as antagonists of ethylene action

8.30 - 9.00 **James Mattheis**, USDA-ARS, USA
Processes of Temperate Fruit Development Regulated by Ethylene Action

9.00 - 9.15 **Maria Angeles Chiriboga**, IRTA, Spain
Ethylene metabolism does not entirely explain softening during storage of 1-MCP treated 'Conference' pears

9.15 - 9.30 **Jennifer R. DeEll**, OMAFRA, Canada
Ethylene Inhibition Influences Physiological Disorders in Apples

9.30 - 10.00 **Eric Schaller**, Dartmouth College, USA
Regulation of Ethylene Receptor Signal Output

10.00 - 10.30 *Break*

10.30 - 11.00 **Donald Huber**, University of Florida, USA
Factors Influencing the Responsiveness of Climacteric Fruits to 1-MCP.

11.00 - 11.15 **Brad M. Binder**, University of Tennessee, USA
ETR1 Receptor Domains Involved in Ethylene-Stimulated Nutational Bending

11.15 - 11.30 **John K. Fellman**, Washington State University, USA
Employment of a marker-based technique to detect MCP use and effects in apple

Optional tour to wineries and Taughannock State Park, with lunch at Wagner Winery.
Tickets required
11.30 - 11.45 *Load busses for tour in front of the Plant Science building*
Return at 6pm

Dinner: on your own

Evening Moderator
David Clark, University of Florida, USA

7.30 - 8.00 **Caren Chang**, University of Maryland, USA
Analyses in Ethylene Signal Transduction using Molecular Genetics and Proteomics

8.00 - 8.15 **Qian Liu**, Chinese Academy of Science, China
Functional analysis of plant RTH genes in the regulation of ethylene response and possible roles of ethylene in the rice growth and development

8.15 - 8.30 **Jin-Song Zhang**, Chinese Academy of Sciences, China
Rice ethylene receptor OsETR2 delays floral transition and affects starch accumulation

8.30 - 8.45 **David Clark**, University of Florida, USA
Ethylene regulation of a specialized CHORISMATE MUTASE in petunia flowers

8:45 - 10.00 **Poster Session**
Drinks in tent

Thursday, June 25th

STRESS BIOLOGY

Moderator
Bill Miller, Cornell University, USA

8.30 - 9.00 **Fred E. Below**, University of Illinois, USA
Ethylene control for achieving high crop yields

9.00 - 9.15 **Etti Or**, Volcani Center ARO, Israel
Indications for Ethylene:ABA interplay in response to bud dormancy release stimuli

9.15 - 9.30 **W. Roland Leatherwood**, Cornell University, USA
Long term low concentration ethylene exposure affects growth and development of 28 ornamental taxa

9.30 - 9.45 **Michelle L. Jones**, Ohio State University, USA
Ethylene regulation of phosphorus remobilization during leaf and petal senescence

9.45 - 10.00 **P.V. Vara Prasad**, Kansas State University, USA
Effect of 1-methylcyclopropene on soybean flower and pod abortion under heat stress

10.00 - 10.30 *Break*

10.30 - 11.00 **Bruce Bugbee**, Utah State University, USA
Ethylene synthesis and sensitivity: whole plant studies in controlled environments

11.00 - 11.15 **Imene Rajhi**, University of Tokyo, Japan
Identification of genes involved in aerenchyma formation induced by ethylene in maize

11.15 - 11.30 **Rashmi Sasidharan**, Institute of Environmental Biology, The Netherlands
The role of group VII ethylene response factor (ERF) genes in the contrasting flooding responses of two Rumex species.

11.30 - 11.45 **Pascal Montoro**, UMR DAP, CIRAD, France
Regulation of the expression of ethylene biosynthesis genes in Hevea brasiliensis

11.45 - 12.00 **Francisco J. Romera**, Córdoba University, Spain
Iron deficiency up-regulates genes involved in both ethylene synthesis and signaling

12.00 - 2.00 *Lunch*

Moderator
Peter Davies, Cornell University, USA

2.00 - 2.30 **Ian T. Baldwin**, Max Planck Institute for Chemical Ecology, Germany
Asking the plant about "stress"

2.30 - 2.45 **F. Paul Silverman**, Valent Biosciences Corporation, USA
Ethylene biosynthesis inhibition by strobilurin fungicides

2.45 - 3.00 **Daniel R. Gallie**, University of California, USA
Ethylene regulates photosynthesis through alterations in non-photochemical quenching

3.00 - 3.15 **Michael T. McManus**, Massey University, New Zealand
Ethylene interacts with auxin in response to phosphate deficiency in white clover

3.15 – 3.45 *Break*

3.45 - 4.15 **Ronald Pierik**, Utrecht University, The Netherlands
Struggling for light: Regulation of plant-plant interactions

4.15 - 4.30 **Caroline C. von Dahl**, Boyce Thompson Institute, USA
Herbivore-induced ethylene primes a direct defense in ethylene-deficient neighbors.

4.30 – 5.00 *Business meeting*

5pm: *Departing reception in tent on Agriculture quadrangle, with snacks and drinks.*

Dinner on your own.

Farewell 'til we met again!

The Shang Fa Yang Memorial Lecture

Ethylene, a simple molecule with a complex lifestyle

Don Grierson, Zhefeng Lin and Silin Zhong

Division of Plant & Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom.

e-mail: donald.grierson@nottingham.ac.uk

Shang-Fa Yang was a pioneer in studying the biosynthesis and function of ethylene in plants. In this lecture I shall discuss some of his contributions to the study of ethylene biosynthesis and function, and discuss new results and unanswered questions.

The simple chemical nature of ethylene contrasts with the regulatory complexity of its biosynthesis and action. This is illustrated by the multiplicity of genes encoding key ethylene biosynthesis enzymes ACC synthase and ACC oxidase, multiple ethylene receptors and signal transduction components, and the complexity of regulatory steps involving signalling relays and control of mRNA and protein synthesis and turnover. *Arabidopsis* has provided the key to unlocking the ethylene black box, but important information about flower and fruit development and ripening has come from other species and there are still many unresolved aspects of the network. Firstly, at present we know about two transcriptional regulators for ACS and ACO genes (LeHB-1 and RIN) but there must surely be many more to discover that control the 15 or more members of these two gene families expressed in different tissues and organs and in response to various environmental and hormonal signals. Secondly, many details of the precise functions and interactions of the different ethylene receptors, and the multiple CTRs in species such as tomato, remain to be elucidated. Thirdly, it is clear that ethylene regulates flower organ development. A potential regulatory network involving ethylene and homeotic proteins, including HD-Zip (such as LeHB-1 and TM1) and MADS box proteins (such as RIN and TAG1), may regulate this fundamental developmental process.

Overview of Applied Ethylene Biology

Chris Watkins

Presenter email: cbw3@cornell.edu

Department of Horticulture, Cornell University, Ithaca, NY 14853, USA

The role of ethylene in plant growth, development and storage of horticultural products is well appreciated, both from its beneficial and detrimental aspects. Traditionally the beneficial roles of ethylene have been exploited where color development, uniform and/or enhance ripening was desired, whereas detrimental effects have been reduced by avoidance, ventilation and other handling and storage strategies. However, the breakthroughs in understanding of ethylene biosynthesis and action by Yang, Lieberman, Burg, Sisler and others have led to the development of exciting chemical technologies that control ethylene responses of many horticultural products. The technologies include aminoethoxyvinylglycine (AVG), known commercially as ReTain, 1-methyl-cyclopropene (1-MCP) known as Ethylbloc and SmartFresh for use on horticultural products, and the new preharvest 1-MCP formulations of Harvista and Invinsa. The uptake of AVG and 1-MCP by horticultural industries has been impressive and these products illustrate the commercialization of knowledge arising from addressing fundamental research questions. In this presentation, applied ethylene biology will be discussed, with special focus on these new technologies, as well as the insights that they provide into management of ripening and senescence.

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The ethylene response factors Snorkel1 and Snorkel2 allow rice to adapt to deep water.

5.00 - 6.45
Poster Session

Free Evening

The ethylene-auxin connection: a model for signal interaction and integration

Jose M. Alonso

Presenter email: jmalonso@ncsu.edu

Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614 USA

Survival of plants largely dependents on their ability to coordinate internal programs, such as growth and development, with the external conditions of the ever-changing environment. Central to this integration process are plant hormones that act as executers of both internally and externally generated signals. It is remarkable that a limited set of plant hormones is capable of triggering a large number of tissue-specific developmental stage-dependent changes in response to particular environmental conditions. Evidently, interactions between hormones and other spatial and temporal information rich signals are critical for achieving this wide diversity of plant responses. Our recent studies shed light on a crucial role of auxin biosynthesis in modulating several ethylene responses in Arabidopsis. Taking advantage of the strict relationship between ethylene and auxin and using a combination of genetic, cellular, and molecular approaches, several new ethylene mutants have been identified. Among the genes identified are *WEI8/TAA1* and *TAR*s, a new family of auxin biosynthetic genes. A detailed characterization of these genes not only confirms their role in the interaction between ethylene and auxin, but also supports the idea that local auxin production may play a key role during development as well as in response to environmental cues.

Recent identification of several additional ethylene and auxin mutants is uncovering multiple modes of crosstalk between these two hormones. Our results suggest existence of a complex molecular network of ethylene-auxin interactions that underlies the phenotypic plasticity of the ethylene response in plants.

Molecular characterization of EIN2, a central element in plant hormone signaling

Silke Allekotte[1], Jan Voet van Vormizeele[1], Nicole Voet van Vormizeele[1] and Georg Groth[1]

Presenter email: Georg.Groth@uni-duesseldorf.de

[1]Department of Biology, Plant Biochemistry, Heinrich-Heine University, Universitätststr.1, 40225 Düsseldorf, Germany

Reverse genetics has identified the membrane protein Ethylene Insensitive 2 (EIN2) as a central component of ethylene signalling in *Arabidopsis* [1]. Sequence analysis suggests a bipartite structure of EIN2 consisting of a hydrophobic amino-terminal domain (amino acids 1-461) which was shown to constitutively activate ethylene responses in mutant plants and a predominately hydrophilic carboxyl-terminal region (amino acids 462-1294) which does not show homology to any known protein. Similarity of the amino-terminal domain of EIN2 to a mammalian family of metal-ion transporters (NRAMP) suggested that EIN2 might function as sensor or even as transporter of divalent cations [1]. However, neither of these functions nor the underlying molecular mechanism of signal transfer to or from EIN2 have been resolved yet. We have cloned and expressed the carboxyl-terminal part of EIN2 from *Arabidopsis thaliana* in the enterobacterium *E. coli*. Over-expressed recombinant EIN2 was purified to homogeneity from extracts of the *E. coli* cells by metal-chelate affinity chromatography and gel filtration. A single band on SDS-PAGE at an apparent molecular weight of 98 kDa confirmed purity and homogeneity of the recombinant protein. The purified EIN2 was characterized by biochemical and biophysical techniques. Fluorescence spectroscopy, taking advantage of the endogenous tryptophan residues in the recombinant EIN2 and isothermal titration calorimetry, revealed that EIN2 binds 3-4 calcium ions in its carboxyl-terminal domain with dissociation constants of 7 µM and 410-860 µM, respectively. CD spectroscopy revealed that binding of the divalent ion is associated with a substantial conformational change in the EIN2 protein. Our results imply a specific interaction of the carboxyl-terminal domain of EIN2 with calcium and suggest that Ca2+ might play a role in signaling to and/or from EIN2.

[1] Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker, JR (1999). EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science 284: 2148–52.

Structural features and distinctive expression patterns of tomato ERFs are the main components underlying specificity and diversity of ethylene responses.

Julien Pirrello[1], B. C. Narasimha Prasad[1], Isabelle Mila[1], Masaru Ohme-Takagi[2], Alain Latché[1], Jean Claude Pech[1], Farid Regad[1], Mondher Bouzayen[1]

Presenter email: j.pirrello@ensat.fr

(1) Université de Toulouse,UMR990 INRA / INP-ENSA Toulouse Génomique et Biotechnologie des Fruits, Avenue de l'Agrobiopole BP 32607, Castanet-Tolosan F-31326, France. (2) Gene Function Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Central 4, Tsukuba 305-8562, Japan

Our current knowledge of the ethylene transduction pathway cannot explain by which means the hormone select its target genes and how this signalling cascade is channelled to specific responses. Ethylene Response Factors (ERF) represent the second largest family of plant transcription factor and are know to modulate the expression of ethylene-regulated genes. To gain better insight on the specific function of ERF genes and to uncover the mechanism by which these transcriptional regulators may contribute to the specificity and diversity of ethylene responses, we isolated 28 tomato ERFs and performed their structural and functional analysis. Phylogenetic analysis demonstrated that tomato ERFs fall into 8 subclasses characterised by distinct motifs. The ability of ERFs to control the transcription activity of synthetic and native GCC-containing promoters was assessed by transient expression assays revealing a clear relationship between structure and function. Moreover, ERFs can be also active on target promoters lacking the canonical GCC box. Gel shift assays showed that nucleotide sequences in the vicinity of the GCC box strongly impacts the affinity of ERF proteins to this *cis*-element. Expression studies indicated that members of the ERF genes family display distinctive organ-specific patterns and that while most ERF genes are ethylene-responsive, some are insensitive to ethylene. Our study shows that expression of some ERF genes is also regulated by auxin suggesting that ERFs can play a role in the interplay between different hormone signalings. These data represent an important step towards understanding the molecular mechanisms by which the plant hormone ethylene can regulate a wide range of physiological processes in a highly specific and coordinated manner.

Analysis of Protein Phosphatase Involved in the Post-translational Regulatory Mechanism of ACC Synthase

Yusuke Kamiyoshihara, Hitoshi Mori

Presenter email: kamiyoshihara.yusuke@b.mbox.nagoya-u.ac.jp

Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

ACC synthase (ACS) is the rate-limiting enzyme in the ethylene biosynthesis pathway. We previously reported that LeACS2, a wound-inducible ACS in tomato, is phosphorylated at Ser-460 by CDPK. This Ser residue is conserved among almost all ACS isozymes. On the other hand, a subset of ACS isozymes possesses additional Ser residues that are phosphorylated by MAPK. Inhibitors of protein kinase and phosphatase alter ACS turnover. Furthermore, analysis by phosphate affinity SDS-PAGE revealed that ACS is phosphorylated immediately after translation. These findings suggest that the dephosphorylation step is the rate-limiting step of ACS turnover. Because ACS turnover is notably delayed by treatment with okadaic acid, which is a potent inhibitor of PPP family phosphatases including PP1 and PP2A, such protein phosphatases may dephosphorylate ACS. Therefore, to elucidate the regulation of the dephosphorylation step of ACS, here we focused on the Arabidopsis mutant rcn1, in which a scaffold subunit of PP2A is disrupted. It was reported that dark-grown seedlings of rcn1 produce elevated levels of ethylene compared to wild-type. Furthermore, we revealed that after auxin treatment, ACS activity and ACC contents were higher in rcn1 than in wild-type, although mRNA levels of each ACS isozyme were the same between rcn1 and wild-type, suggesting that PP2A containing RCN1 is involved in the dephosphorylation of ACS. PP2A consists of three subunits (catalytic, scaffold, and regulatory) and each subunit is encoded by a multi-gene family. To identify the regulatory subunit involved in the recognition of ACS, we purified PP2A holoenzymes from rcn1 and wild-type using a Microcystin affinity column and analyzed the subunit composition by mass spectrometry. Some subunits were identified only in wild-type, suggesting that these are the regulatory subunits involved in the recognition of ACS.

Functional characterization of SlFBOX1, a tomato FBOX protein: A novel regulator of ethylene signaling?

Nigel Gapper[1], Rob Alba[1,2] and Jim Giovannoni[1]

Presenter email: neg29@cornell.edu

[1]Boyce Thompson Institute for Plant Research, Tower Road, Cornell University, Ithaca, NY, 14850, USA.
[2]Current address: Crop Composition Team, Monsanto Company, Lindbergh Blvd. St. Louis, MO 63167, USA.

Ripening and senescence culminate the last stages of fruit development. Tomato has been used as a model system to study this pre-programmed developmental event in part due to the availability of the ripening mutants *nor* (non-ripening) and *rin* (ripening inhibitor). These are mutations to transcription factors which act as global regulators of the ripening process in tomato. While the characterization of these two mutants have led to great understanding of the process of ripening, other genes also involved in the developmental process are of great interest. We have used a genomics approach mining microarray data to identify candidate genes that are involved in ripening. To functionally characterize candidate ripening regulatory genes, we have made RNAi knock outs of these candidates. Here we report preliminary data for one of our constructs, a knockout in an FBOX protein, designated SlFBOX1. Transgenic plants exhibit delayed fruit ripening as measured by color change during the ripening process. In addition, maximum ethylene evolution occurred two days earlier during ripening development than in control lines. Further, transgenic seedlings were hypersensitive to ACC in the triple response assay. Other FBOX proteins target either EIN3 (EBF1 and EBF2) or EIN2 (ETP1 and ETP2) for protein degradation in Arabidopsis. The sequence of SlFBOX1 is quite distinct from either of the EBF or ETP tomato homologs, so we hypothesize SlFBOX1 regulates a novel mechanism for ethylene signaling in tomato. Our results will be reported and discussed.

Genetic regulation of fruit ripening and ethylene response in tomato.

James Giovannoni[1,2], Cornelius Barry[1,2], MiYoung Chung[1,2], Julia Vrebalov[1,2] and JeMin Lee[1,2]

Presenter email: jjg33@cornell.edu

[1]Boyce Thompson Institute for Plant Research, Ithaca, NY 14853 USA
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The ripening and development of fleshy fruits is regulated by environmental, hormonal and developmental cues. Ethylene is the key ripening hormone of climacteric fruits and can influence ripening in many non-climacteric fruits. Our laboratory uses tomato as a model system to understand ripening regulation and has identified a number of necessary ripening genes via positional cloning of loci underlying ripening mutations and transcriptional profiling studies of ripening associated gene expression. To date we have identified six transcription factors that we have shown to be necessary for tomato fruit ripening via transgenic studies including two MADS-box, two NAC domain, an Ethylene Response Factor (ERF) and an APETALA2 gene homolog. One of the MADS-box genes, *TAGL1*, is especially intriguing in that it suggests a molecular link between fleshy fruit development and eventual ripening via a single gene product. A summary of these gene activities in the context of other reported regulatory and ethylene response genes will be presented.

Visiting ethylene/auxin cross-talk through uncovering the role of tomato Aux/IAA3 and *HOOKLESS* genes in differential growth

Mondher Bouzayen, Salma Chaabouni, Brian Jones, Corinne Delalande, Alain Latché, Jean Claude Pech

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The growth of plant organs is dependent on an intricate orchestration of hormonal and non-hormonal signals and identifying the central actors of the interplay between different signaling pathways is critical to understanding the complex mechanisms underlying the control of plant growth and development. Interplay between ethylene and auxin is among the most frequently addressed and these two phytohormones are known to regulate agonistly or antagonistly many processes of plant development but yet the key integrating molecular players remain largely undiscovered or uncharacterized. We report here on the identification and characterization of molecular actors that take part in the auxin/ethylene cross-talk associated with differential growth and show that a member of the tomato auxin/indole-3-acetic acid (*Aux/IAA*) gene family, Sl-*IAA3,* intersects the auxin and ethylene signal transduction pathways. The Sl-*IAA3* gene encodes a nuclear-targeted protein that can repress transcription from auxin-responsive promoters. Sl-*IAA3* expression is auxin and ethylene dependent and associated with tissues undergoing differential growth such as in epinastic petioles and apical hook. Down-regulation of Sl-*IAA3* in tomato results in auxin and ethylene-related phenotypes, including altered apical dominance, lower auxin sensitivity, exaggerated apical hook curvature in the dark and reduced petiole epinasty in the light. These data clearly position the Sl-IAA3 protein at the crossroads of auxin and ethylene signalling. Loss-of-function mutation in the Arabidopsis *HLS1* gene results in the absence of hook even in the presence of exogenous ethylene. Extendeding our investigation of the Arabidopsis *hls1* mutant phenotypes uncovered the importance of *HLS* gene in the integration of multiple signalling pathways. Two functional tomato hookless genes were isolated and the expression of Sl-*HLS2* in the hook is restricted to the outer face, opposite to Sl-*IAA3* whose expression is localized in the inner face of the hook curvature. The data suggest that Sl-HLS2 and Sl-IAA3 exert antagonist control of cell elongation in the inner and outer part of the apical hook.

Arabidopsis HSP40 regulates ethylene biosynthesis by altering ACS protein stability

Gyeong Mee Yoon[1], Hyun Sook Chae[1], Koji Tanase[2], Maureen Hansen[1] and Joseph J. Kieber[1]

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The phytohormone ethylene influences numerous plant growth and developmental processes and its biosynthesis is highly regulated by a wide variety of developmental, hormonal and environmental factors. The first committed and generally rate-limiting step in ethylene biosynthesis is catalyzed by ACC synthase (ACS), which is encoded by a multigene family that is regulated at both the level of transcription and protein stability. Using a yeast two-hybrid screen, we identified Arabidopsis orthologs of DnaJ/HSP40 (ATJ2/ATJ3) as proteins that interacted directly with ACS5. The ATJ3 interaction was confirmed *in vitro* and *in planta* and found to be specific for type-2 ACS proteins. While HSP40 has been extensively characterized in *E. coli*, much less is known regarding its function in eukaryotic cells, particularly in plants. Disruption of the two closely related *ATJ2* and *ATJ3* genes blocks the stabilization of ACS5 protein that occurs following treatment of etiolated seedlings with cytokinin and light. The a*tj2*/*atj3* double mutants were altered in other aspects of plant growth, including flowering time and fertility, the latter of which showed a temperature-dependent phenotype. These studies reveal a novel role for HSP40 in higher eukaryotes and add to our understanding of the regulation of protein turnover in plants.

Identification of *Solanum habrochaites* QTL that affect ethylene emissions in tomato fruit

Valeriano Dal Cin[1], Brian Kevany[1], Zhangjun Fei[2] and Harry J. Klee[1]

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In recent years the wild relatives of plants such as tomato have been studied to introduce valuable traits into commercial varieties and to study basic processes. Studies on ethylene have always occupied a central place in research because of its importance to many processes related to agriculture, such as tomato fruit ripening. While many of the genes responsible for ethylene synthesis and perception have been identified, the regulatory network controlling autocatalytic climacteric ethylene synthesis is not well understood. We have exploited a near-isogenic population of *S. habrochaites* introgression lines to identify quantitative trait loci (QTLs) affecting ethylene emissions during ripening to better understand the regulation of ripening-associated ethylene. *S. habrochaites* fruits produce at least 7-fold more ethylene during ripening yet ripen 20 days later than do cultivated *S. lycopersicum* fruits. A total of 17 QTLs altered in ethylene emission were identified; three had emissions more than twice the level of the tomato parent, eleven had less than a two-fold increase and three had significantly reduced emissions at one or more ripening stages. While several of these QTLs co-segregate with known ethylene-related genes, many do not correspond to known genes. Since ethylene affects time to ripening, in the same population we also critically examined time to ripening. We monitored time to flowering, days from pollination to breaker and other fruit and plant physiological traits. These studies indicated that in this population there is a high variability for these traits which can be studied for a basic and applied purpose.

Ethylene and auxin cross-talk at veraison in grape

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Grape is a non-climacteric fruit, in which ripening is ethylene-independent. However, it has been shown that the hormone is capable of affecting grape berry maturation either by itself or through the interaction with other hormones. Transcription profiling carried out by using an oligo-based microarray (AROS Grape V1.0) on Cabernet sauvignon berries pointed out that exogenous ethylene applied at veraison enhanced expression of genes involved in the regulation of cell water status (aquaporins) and cell wall metabolism (Chervin et al. 2008, Physiologia Plantarum 134: 534-546). This up-regulating effect has been related to larger size of berries at harvest and to an acceleration of ripening. An opposite effect has been reported for auxin applied at same development stage. To further assess the relationship between ethylene and auxin the same microarray platform have been used in experiment designed to screen the gene pool affected by naphtalenacetic acid (NAA, 200mg/l) sprayed at the veraison in Merlot berries. The results showed that 393 of 14 562 genes of microarray slides were significantly modulated by the treatment. This was paralleled by a retardation of berry weight increases, color development, and hexose accumulation, leading to a delay in the onset of ripening approximately of three week. The expression analysis pointed out a delayed pattern of expression of genes encoding aquaporins, cell wall hydrolases, malic enzyme, pyruvate decarboxylase, alcohol dehydrogenase, sucrose synthase, an acid invertase and sugar transporters, as well as genes encoding ACC oxidase, catalyzing the last step of ethylene biosynthesis, and AUX/IAA and ERF5 involved in the auxin and ethylene action, respectively. Based on this information a counteracting action of ethylene and IAA in the onset of grape ripening might be hypothesized.

Ethylene modulates auxin transport and root development in Arabidopsis and tomato

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Auxin stimulates lateral root formation, but the role of ethylene in this process has not been well studied. We utilized Arabidopsis and tomato mutants altered in ethylene signaling and synthesis to explore the role of ethylene in root branching. In Arabidopsis, enhanced ethylene synthesis or signaling, through the *eto1-1* and *ctr1-1* mutations, or ACC application, negatively impacts lateral root formation. In contrast, mutations that block ethylene responses, *etr1-3* and *ein2-5* enhance lateral root formation and render it insensitive to ACC. Surprisingly, ACC treatments or the *eto1-1* mutation significantly enhance radiolabeled IAA transport in the primary roots, as well as increased DR5-GUS expression in the central cylinder of the root apex. *ein2-5* and *etr1-3,* have less root acropetal IAA transport and it is no longer regulated by ACC. Similarly, in tomato, root growth and auxin transport show the same ethylene dependence. The ethylene insensitive mutants, *Never-ripe*, *green-ripe*, *ripening inhibitor*, and *non-ripening* mutants all have enhanced root formation, while the *epi* mutant, with enhanced ethylene synthesis and signaling, has fewer lateral roots. *aux1-7*, *lax3*, *pin3* and *pin7*, which have mutations in IAA influx and efflux proteins, respectively, are insensitive to the ethylene inhibition of lateral root formation. *aux1* is also insensitive to the effect of ethylene on IAA transport and DR5-GUS expression. *AUX1* gene expression is enhanced by ACC treatment in an *etr1-3* dependent fashion, as judged by quantitative real time PCR. The expression domain and fluorescence intensity of an AUX1::YFP transgene also increases in response to ethylene treatment. These results are consistent with ethylene enhanced gene expression of transport proteins redirecting auxin into the primary root polar transport stream and away from developing lateral roots. Together, these experiments indicate a negative role for ethylene in root formation in Arabidopsis by modulation of auxin transport through both auxin influx and efflux carriers.

RPN10-silenced tomatoes have prolonged flower longevity and reduced C2H4 production

Simona Cristescu[1], Lisette Nitsch[2], Mieke Wolters-Arts[2], Celestina Mariani[2], Frans Harren[1], Wim H. Vriezen[2]

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Fruits develop from ovaries and this development is triggered by pollination or fertilization. The elucidation of hormones function during ovary development is a challenge task (Vriezen *et al*, 2008). We have investigated the hormonal interactions between ethylene (C2H4), auxin and abscisic acid (ABA) leading to flowers senescence using ABA- hypersensitive tomato plants. These transgenic plants were obtained by applying an RNAi approach to silence the tomato homologue *Arabidopsis RPN10*. In *RPN10*-silenced tomato plants flower development until the mature flower-stage seemed unaffected. However, the unpollinated flowers of the transgenic tomato lines displayed delayed senescence compared to unpollinated wild-type flowers. Ethylene production from a single detached tomato flower was measured in continuous flow using a sensitive laser-based detector (type ETD-300; Sensor Sense BV, Nijmegen, NL) in combination with a gas handling system. The ETD-300 is a state-of-the art ethylene detector based on laser photoacoustic spectroscopy (Cristescu *et al*., 2008) that is able to detect on-line about 300 pptv (parts-per-trillion volume, $1{:}10^{12}$) of ethylene within a 5-s time scale. The gas handling was performed by a valve control box (Sensor Sense) that allowed automated sampling of ethylene production at a flow rate of 1 l /h and its transport to the ETD-300 alternately, in succession for 10 min for each cuvette flower. The typical ethylene emission rates from a single flower measured in continuous flow were below 400 pptv. The ethylene production in the transgenic plants was lower as compared to the wild-type flowers throughout the period of flower opening and the whole full bloom stage, which might be the cause of reduced senescence. Possible cross-talk between auxin, ABA and ethylene signalling during flower senescence is discussed.

W.H. Vriezen, R. Feron, F. Maretto, J. Keijman, C. Mariani. Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. New Phytologist 177: 60-76 (2008)
S.M. Cristescu, S.T. Persijn, S. te Lintel Hekkert, F.J.M. Harren. Laser-based systems for trace gas detection in life sciences. Appl. Phys. B 92: 343-349 (2008)

nei (*nonripe ethylene insensitive)*, a novel ripening mutant in tomato

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The phenotype of ripe fruit is the result of numerous biochemical and physiological changes occurring at the final stage of fruit development rendering the organ edible and attractive to seed dispersing animals and valuable to humans as an important food source and an agricultural commodity. In addition, fruits contribute a large portion of vitamins, antioxidants, minerals and fiber to the human diet. Ripening changes of these fruit, although variable among species, generally include modification of cell wall ultrastructure and texture, conversion of starch to sugars, increased susceptibility to post-harvest pathogens, modifications in pigment biosynthesis and accumulation, and heightened levels of flavor and aroma volatiles. *nei* (*nonripe ethylene insensitive*) is a recessive tomato mutation resulting from EMS mutagenesis and manifests in ripening inhibition and maintenance of nutritional profiles reminiscent of green unripe fruit. Along with the ripening phenotype, the mutant exhibits ethylene insensitivity in other tested tissues. Seedlings of *nei* treated with the ethylene precursor, ACC, were found to be inhibited in the triple response. In addition, whole plants treated with exogenous ethylene exhibited a decrease in petiole epinasty when compared to its wild-type near isoline M82. Adventitious root formation is also reduced in the mutant. As the fruit do not respond to exogenous ethylene it appears the mutant is likely involved in the ethylene signal transduction pathway. Preliminary mapping of *nei* placed the gene responsible for the mutant on chromosome 9. Fine mapping is in progress and at least one candidate gene has been identified and is being tested for possible mutations. Progeny testing with some ripening mutants and map positions of others suggests that *nei* is not allelic to any known ripening mutants.

Integrated analysis of transcriptome and metabolite profiles in tomato wild species introgression lines to identify candidate regulatory genes.

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Phenotypic variation of diverse metabolites including vitamins, minerals, and antioxidants in tomato fruit has and will continue to be utilized for crop improvement. Comparative analysis of metabolic and gene expression profiling data from isogenic wild species introgression lines provides a unique opportunity for exploring the phenotypic outcomes of defined areas of the genome and for generating candidate genes for regulation of complex biochemical traits. Analysis of a series of tomato introgression lines (ILs) containing chromosomal segments of *Solanum pennellii* (LA716) in a *S. lycopersicum* (cv M82) background allowed us to identify a subset of ILs with significant variation in carotenoid accumulation. Gene expression profiling has been conducted using the tomato long-oligo array 'TOM2', which represents approximately 12,000 tomato unigenes. Through a combination of transcriptome and targeted metabolite analysis in the ILs, we seek to identify regulatory elements impacting the carotenoid biosynthesis pathway as a component to a larger effort toward elucidating the regulatory mechanisms of additional nutrient, aroma, and flavor pathways. This effort has resulted in identification of a ERF transcription factor which is inversely correlated with carotenoid levels. Details regarding functional characterization of this gene will be presented.

Control of ethylene responses by the *GREEN-RIPE* gene family

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To achieve full development of the ripe phenotype, climacteric fruits, such as tomato, apple and banana, require synthesis, perception and signal transduction of the plant hormone ethylene. The non-ripening phenotype of the dominant *Green-ripe* (*Gr*) mutant of tomato is the result of reduced ethylene responsiveness in fruit tissues. In addition, a subset of ethylene responses associated with floral senescence, abscission and root elongation are also impacted in mutant plants, but to a lesser extent (Barry et al., 2005). The *Gr* locus was identified via positional cloning and *GR* encodes a tomato homolog of the Arabidopsis gene, *REVERSION TO ETHYLENE SENSITIVITY 1* (*RTE1*). A *CaMV35:GR* transgene recreates the *Gr* mutant phenotype but does not lead to a whole plant reduction in ethylene responsiveness suggesting tissue-specific modulation of ethylene responses by *GR* in tomato (Barry et al., 2006). Transgenic complementation and over-expression analysis of *GR* and *RTE1* in tomato and Arabidopsis indicate that these genes are not functionally equivalent. However, a second tomato gene that we have designated *GREEN-RIPE LIKE 1* (*GRL1*), is able to complement the Arabidopsis *rte1-3* loss of function mutant allele but does not appear to influence fruit ripening. Current research efforts are focused on defining the functional differences between *GR* and *GRL1* and how these differences contribute to mediating distinct subsets of ethylene responses in tomato.

Barry CS, McQuinn R, Thompson AJ, Seymour GB, Grierson D, Giovannoni JJ (2005) Ethylene insensitivity conferred by the *Green-ripe* (*Gr*) and *Never-ripe 2* (*Nr-2*) mutants of tomato. Plant Physiology 138: 267-275.
Barry CS, Giovannoni JJ. (2006) Ripening in the tomato *Green-ripe* mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci USA. 103: 7923-7928.

A Study of ETR1 and ERS1 Signaling Reveals Functional Divergence and Cooperativity of Arabidopsis Ethylene Receptors

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It is challenging to study individual ethylene receptor subtypes in Arabidopsis due to genetic redundancy. In this work, we examined signaling of ETR1 and ERS1 in receptor-defective mutants. We show that the loss of ETR1 led to hyper-responsiveness to ethylene while the loss of ERS1 led to hypo-responsiveness, implying that ERS1 may conditionally repress total receptor signal output. We next show that ERS1 overexpression substantially weakened the ETR1 signal output, providing an experimental evidence to support our hypothesis. Notably, the receptor combination, rather than the number of receptor subtype, is relevant to total receptor signal strength. We further examined signal output conveyed by constitutively active receptor isoforms. AVG, which eliminates endogenous ethylene and indirectly facilitates the ethylene-free receptor formation, failed to restore the seedling hypocotyl elongation in those receptor-defective mutants, implying that constitutive receptor signaling is dependent on other wild-type subtypes. On the other hand, Ag(I)-mediated ethylene insensitivity was largely ETR1 dependent. In addition to pharmaceutical treatments, constitutive receptor signaling of etr1-1 and ers1-1 was next examined in quadruple and quintuple receptor mutants. ers1-1 alone was unable to convey receptor signal in mutants lacking wild-type receptors. These results indicate that receptor isoforms with constitutive signaling activity cannot function alone, lending a support to our hypothesis that receptors function cooperatively. Our study suggests that specific cooperativity among receptor subtypes may determine the final signal strength, explaining why plants would have multiple receptor subtypes and how a plant would be able to respond to a wide range of ethylene concentrations. ERS1 is inhibitory to the ETR signaling while ETR1 plays a major role in response to Ag(I). Under ethylene treatment, wild-type receptor subtypes are in fact not functionally inactivated while they are essential to the signaling of constitutively active isoforms.

Highly sensitive ethylene detector for online measurements on biological samples

Sacco te Lintel Hekkert[1], Marko Kamp[1], Abir Salman[2], Heloisa Filgueiras[2], Amanda J. Lloyd[3], Luis A. J. Mur[3]

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Biological research on the plant hormone ethylene is often hampered by the detection limit of the available instrumentation. Preconcentration steps had to be included in the measuring scheme, making the traditional methods (gas chromatography, gas chromatography combined with mass spectrometry, or dispersive IR absorption techniques) time-consuming and often not very specific. Time resolved measurements are in many cases impossible, and operation by specialists is often required.
In comparison to the conventional detection methods, the optical detection techniques using lasers in combination with modern spectroscopic techniques are an excellent option for sensitive monitoring of ethylene. Sensor Sense developed a highly sensitive on-line laser-based ethylene detector (type ETD-300) that is two orders of magnitude more sensitive than other commercial available detectors. With its detection limit of 300 pptv (1 ppbv = 1 part per trillion volume = 1:1012) and time resolution of 5 seconds it is unique in the world. Many dynamic processes in single plant or plant organ can be now revealed in real time without incubation periods. For many biological applications in which averaging over a one minute time scale is no problem, detection limits below the 100 pptv can be easily accessed opening new fields of investigation. In combination with a gas handling system, the ETD-300 is currently used in several research areas including plant physiology (seed germination, flower senescence, interaction with other hormone, programmed cell death, abiotic stress), microbiology (nitrogen fixation by cyanobacteria), post-harvest research (fruit ripening, plant-pathogen interaction), and environmental science. To demonstrate the ETD-300 performances, applications in monitoring ethylene emission involved in a) wound–induced red discoloration in fresh-cut endive, b) the signaling pathway during the heat stress in *Arabidopsis* and c) defense response upon pathogen attack in *Arabidopsis* are presented.

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The ethylene response factors *Snorkel1* and *Snorkel2* allow rice to adapt to deep water.

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Habitat expansion is one of the most important strategies for living organisms to survive and thrive. Organisms have developed many different biological functions to overcome severe environmental conditions and have succeeded in adapting to many unfavorable environments on Earth. In contrast to motile organisms, plants are sedentary and cannot rapidly remove themselves from sudden environmental change; therefore, plants must face the environmental alteration directly. Water is essential for all life, and plants that occupy habitats around swamps and rivers have an advantage in terms of water availability, but such plants are frequently at risk because of heavy rain and sudden flooding. Deepwater rice evolved and adapted to flooding by gaining the ability to significantly elongate its internodes that act like a snorkel to allow gas exchange with the atmosphere and thus avoid drowning. We identified the genes that trigger the deep-water response, *Snorkel1* and *Snorkel2*, which encode ethylene response factors involved in the signaling of the gaseous phytohormone ethylene. Under deep-water, ethylene accumulates in the node, triggering *Snorkels* expression that causes remarkable internode elongation. A comparison of the genomic regions of *Snorkel1* and *Snorkel2* in ordinary rice and some wild rice species indicated that these genes were obtained before or early in rice divergence and were then lost during domestication. We also demonstrated that the introduction of three deepwater rice QTLs into non-deepwater rice enables to become deepwater rice. This suggests that breeding using the QTLs can help resolve serious problem in rice yields in flood-prone areas.

Tuesday, June 23nd

ABSCISSION, SENESCENCE, FRUIT RIPENING

Moderator
Sara Patterson, University of Wisconsin, USA

8.30 - 9.00 **Sara Patterson**, University of Wisconsin, Madison, USA
Molecular analysis of ethylene responses and recovery in Dianthus floral senescence

9.00 - 9.15 **Cai-Zhong Jiang**, USDA-ARS, USA
Molecular analysis of the interaction of ethylene and auxin during flower abscission

9.15 - 9.30 **Catharina Merchante**, Universidad de Málaga, Spain
Investigating the role of ethylene in strawberry fruits

9.30 - 9.45 **Vera Hershkovitz**, The Volcani Center, Israel
Seed Involvement in Ethylene Perception during Avocado Ripening and Senescence

9.45 - 10.00 **Bram van de Poel,** Katholieke Universiteit Leuven, Belgium
The role of s-adenosyl-L-methionine during climacteric ripening of tomato

10.00 - 10.30 *Break*

10.30 - 11.00 **Kenichi Shibuya**, NARO, Japan
Programmed cell death during flower senescence

11.00 - 11.15 **Robert Schaffer**, Institute of Plant and Food Research, New Zealand
Taking ethylene out of the fruit ripening equation

11.15 - 11.30 **Jun Song**, Atlantic Food and Horticulture Research Centre, Canada
Proteomic analysis of differentially expressed proteins in apple fruit during ripening and senescence

11.30 - 11.45 **Wendy C. Schotsmans**, IRTA, Spain
Temperature dependent ethylene metabolism during storage of 'Rich Lady' peach

11.45 - 12.00 **Haya Friedman**, The Volcani Center, Israel
Expression of MaMADS2 and its interactions with ethylene suggest that it acts upstream to ethylene production

12.00 - 2.00 *Lunch*

Moderator
Angelo Ramina, Università di Padova, Italy

2.00 - 2.30 **Coralie C. Lashbrook**, Iowa State University, USA
Modeling cell wall structural dynamics in Arabidopsis abscission zones

2.30 - 2.45 **Sofia G. Foukaraki**, Cranfield University, UK
Effect of transition between ethylene and air storage on two potato varieties

2.45 - 3.00 **Manuela Donetti**, Cranfield University, UK
Influence of season and origin on ripening of imported avocado cv. Hass fruit.

3.00 - 3.15 **Livio Trainotti**, Università di Padova, Italy
Interactions between ethylene and auxin during peach fruit ripening.

3.15 - 3.45 *Break*

3.45 - 4.15 **Pietro Tonutti**, Scuola Superiore Sant'Anna, Italy
Ethylene and postharvest physiology in climacteric and nonclimacteric fruit in the genomics era

4.15 - 4.30 **Nurit Katzir**, Newe Ya'ar Research Center, Israel
Melon fruit development and quality: climacteric vs. non-climacteric ripening

4.30 – 4.45 **Giovanni Giuliano**, Casaccia Research Center, Italy
Altered ripening characteristics of "Golden" tomato fruits

4.45 - 5.00 **Max Villalobos**, University of California, USA
Modulating 1-MCP effect in 'Bartlett' pears with maturity, ethylene exposure, and cold storage

5.00 - 6.00 **Poster Session**

Evening: *Conference Banquet at Statler Hotel*

6.30 - 7.30 Predinner drinks, Statler Hotel ballroom foyer, first floor.

7.30 - 9.30 Banquet: Statler Hotel ballroom, first floor.

Speaker: Professor James Reveal
The Lewis and Clark expedition to the American West

Jim Reveal is a Professor Emeritus in the department of Plant Biology. He is a national expert on the botanical discoveries made by Lewis and Clark during the first American overland expedition to the Pacific coast (1803-1806), and also on the life of Meriwether Lewis.

Molecular analysis of ethylene responses and recovery in Dianthus floral senescence

Sara Patterson

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Department of Horticulture; University of Wisconsin-Madison; Madison, WI, USA 53706

Ethylene regulates myriad aspects of plant growth and development, including fruit ripening, flower senescence, and floral organ abscission. Much of what is known about the molecular mechanisms of ethylene action comes from studies in *Arabidopsis*. In *Arabidopsis*, ethylene perception by a family of five receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) inactivates the immediate downstream MAP kinase cascade, thereby repressing the negative regulation that these receptors enforce at the cell membrane. Ethylene action can be suppressed by ethylene antagonists such as 1-methylcyclopropene (1-MCP). 1-MCP acts by binding to members of the ethylene receptor family and represses ethylene responses, such as floral senescence and petal abscission. Despite its antagonistic propensity, plants treated singly with 1-MCP often regain sensitivity to ethylene (i.e., recover) several days post-treatment. However, plants that undergo successive 1-MCP treatments prior to their recovery of ethylene sensitivity reveal prolonged repression of floral senescence. To explain these observations, we hypothesize that ethylene receptors are synthesized *de novo* post-treatment with 1-MCP, and that this accounts for the ability of plants to regain ethylene sensitivity. We are exploring this hypothesis in carnation (*Dianthus caryophyllus*), a tractable system because of its high sensitivity and rapid response to gaseous regulators such as ethylene and 1-MCP. Using a combination of physiology, histology and gene expression analyses over developmental time, we aim to determine some details of when and where flowers regain ethylene sensitivity following 1-MCP treatment. Currently, we are surveying the expression patterns of *DcETR1*, *DcERS1* and *DcERS2* ethylene receptor genes across defined late-stages of floral development in plants treated with either ethylene or 1-MCP compared to non-treated plants. Results from our analyses will be presented.

Molecular analysis of the interaction of ethylene and auxin during flower abscission

Shimon Meir[1], Cai-Zhong Jiang[2], Amnon Lers[1], Sonia Philosoph-Hadas[1], Shaul Burd[1], Srivignesh Sundaresan[1], K.S. Vijay Selvaraj[1], Bettina Kochanek[1], Andrew J. MacNish[3] and Michael S. Reid[3]

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Abscission, the separation of organs from the parent plant, results in postharvest quality loss in many ornamentals and other fresh produce. The process is initiated by changes in the auxin gradient across the abscission zone (AZ), is triggered by ethylene, and may be accelerated by postharvest stress. Although changes in gene expression have been correlated with the ethylene-mediated *execution* of abscission, there is almost no information on the molecular and biochemical basis of the increase in sensitivity of the AZ to ethylene. We examined transcriptome changes in the tomato (*Solanum lycopersicum Mill.*) flower AZ during the rapid acquisition of ethylene sensitivity following flower removal, which depletes the AZ from auxin. Microarray analysis using the Affymetrix Tomato GeneChip revealed changes in expression, occurring prior to and during pedicel abscission, of many genes with possible regulatory functions. They included a range of auxin- and ethylene-related transcription factors (TFs), other TFs that are transiently induced just after flower removal, and a set of novel AZ-specific genes. To facilitate functional studies we implemented an efficient Virus-Induced Gene Silencing (VIGS) system in tomato using tobacco rattle virus (TRV) and an anthocyanin regulatory gene (*Lc*) as a silencing reporter. A phenotype showing a significant delay in pedicel abscission in response to auxin depletion (obtained by flower removal) was observed when we silenced several novel AZ-specific genes. These results shed light on the mechanism of increased sensitivity of the AZ to ethylene, and further expand our knowledge of auxin-ethylene cross talk during the abscission process.

Investigating the role of ethylene in strawberry fruits

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Strawberry fruit has typically been catalogued as non-climacteric but there is still some controversy about the role that ethylene may play in the ripening of this fruit. In an attempt to clarify such a role, we have generated ethylene insensitive strawberry (*Fragaria x ananassa* cv. Chandler) plants by ectopically expressing *etr1-1,* a dominant allele of the *Arabidopsis* ethylene receptor ETR1. *In vitro* strawberry plants expressing *etr1-1* both regenerated and multiplied faster than the non-transformed control plants reaching a larger size indicative of ethylene insensitivity. This ethylene insensitivity was verified by the culture of the *in vitr*o plants in medium supplemented with ACC and also by the molecular analysis of three ethylene responsive genes (*FaERF1-3*). Interestingly, the transgenic lines did not show any apparent phenotypic difference in relation to the control plants during their later vegetative growth or during fruit ripening in the first harvest, suggesting that ethylene might not be essential in the strawberry fruit ripening process.

We also analyzed the expression of the genes *FaERF1-3* during the development and ripening of the strawberry fruit and in vegetative tissues. Both *FaERF1* and *FaERF3* showed maximum expression levels in fruits, whereas the expression of *FaERF2* was highest in the root with minor changes during fruit development and ripening. It is noteworthy that both *FaERF1* and *FaERF3* show highest expression in the achenes (the true fruits) suggesting the attractive possibility that ethylene may play an important role in achene development.

A more detailed phenotypical and molecular analysis of fruit ripening in the transgenic lines in this second season is under investigation.

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Seed Involvement in Ethylene Perception during Avocado Ripening and Senescence

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To elucidate the role of seed in avocado ripening and senescence, the differential expression of two ethylene receptor *PaETR* and *PaERS1* and signal transduction element *PaCTR1* in seeded and seedless mature avocado fruit (*Persea americana* cv. Arad) was investigated. Ethylene components were studied in response to exogenous application of ethylene (10 µl l^{-1}, 18 h at 20ºC) or 1-methylcyclopropene (1-MCP) (150 nl l^{-1}, 18 h at 20ºC). The expression of *PaETR, PaERS1* and *PaCTR1* increased in parallel to the onset of climacteric ethylene and CO2 peak and decreased after climacteric both in seeded and seedless fruit. However, in seedless fruit the levels of these genes were significantly higher. Moreover, seedless mature fruit had a shorter preclimacteric lag period compared to mature seeded fruit, suggesting that the seed delays the climacteric burst of ethylene in mature fruit. In addition, seedless ethylene-treated fruit attained their climacteric peak within 7 d at 20°C compared to 11 d in seeded ethylene-treated fruit. On the other hand, application of 1-MCP equally and effectively delayed the climacteric peak and down-regulated expression of *PaETR, PaERS1* and *PaCTR1* genes in seeded and seedless fruit. All together our data demonstrate that seed is involved in regulation of ethylene responsiveness during ripening and senescence, and act to delay climacteric in mature seeded fruit.

The role of s-adenosyl-L-methionine during climacteric ripening of tomato

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S-adenosyl-L-methionine (SAM) plays an important role in both eukaryotic and prokaryotic cells. It is the main methyl-donor in all living organs and it is involved in polyamine biosynthesis. In plants, SAM is also a substrate for the production of ethylene. SAM is converted to ACC (1-aminocyclopropane-1-carboxylic acid) by ACC-synthase (ACS) with the cleavage of 5'-methylthioadenosine. The role of SAM during climacteric ripening, besides being a substrate for ACS, is not yet fully elaborated on. SAM has not yet been quantified in fruit tissue in relation to climacteric ripening and other processes like e.g. methylation activity.
Our current research project aims to develop a kinetic model that describes ethylene production during climacteric ripening of tomato. Within this framework we collect systematic quantitative data on the ethylene biosynthesis pathway. To routinely quantify SAM in tomato tissue we developed a capillary electrophoresis technique. Our results surprisingly show that the SAM-pool is not constant during tomato fruit development and ripening. Initially, the SAM content is high (8.5 ± 1.7 nmol/g FW) measured shortly after anthesis and decreases as the fruit further develops. SAM-content is more or less constant (5.9 ± 0.6 nmol/g FW) when the fruit is fully developed but still preclimacteric. At the onset of climacteric ripening, SAM content increases and exceeds preclimacteric values (up to 10.6 ± 0.9 nmol/g FW). During post-harvest storage, SAM content will decrease again (until 4.7 ± 0.6 nmol/g FW). The SAM-profile matches exactly the ethylene production profile. These results suggest that SAM content is regulated during fruit development, climacteric ripening and postharvest storage. Further research is needed to elaborate on the underlying control mechanism. Is there an increased SAM synthesis, or a reduced SAM uptake by other pathways, during climacteric ripening of tomato?

Programmed cell death during flower senescence

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The onset and progression of petal senescence, which is a type of programmed cell death (PCD), are highly regulated developmental processes. Japanese morning glory (*Ipomoea nil*) has ephemeral flowers that open in the morning and generally show visible petal senescence symptoms within the same day. To identify the genes regulating PCD in petal senescence (PSRs: petal senescence-related genes), we isolated genes showing changes in expression during petal senescence in Japanese morning glory. In a recent functional study, we produced transgenic plants with reduced expression of PSRs and found that a putative membrane protein, InPSR26, regulates progression of PCD during petal senescence. *InPSR26* is dominantly expressed in petal limbs and its transcript level increases prior to visible senescence symptoms. Reduced *InPSR26* expression in transgenic plants (PSR26r lines) resulted in accelerated petal wilting with hastened development of PCD symptoms, as well as reduced transcript levels of autophagy-related genes in the petals. Autophagy visualized by monodansylcadaverine staining indicated reduced autophagic activity in the PSR26r plants. These results suggest that InPSR26 acts to delay the progression of PCD during petal senescence, possibly through regulation of the autophagic process. We will discuss regulatory mechanisms underlying petal senescence focusing on autophagy and further discuss regulation of autophagy by ethylene.

Taking ethylene out of the fruit ripening equation

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Suppressing the *MdACO1* gene in apples stops all ripening related ethylene biosynthesis. *MdACO1* suppressed apples that have been treated with the inhibitor of ethylene action 1-MCP, have identical ripening characters such as colour change, starch clearance, volatile production and flesh softening to *MdACO1* suppressed apples that have not been treated. These *MdACO1* suppressed apples have been used as a tool to understand the role of ethylene in apple fruit ripening. Using different concentrations of ethylene to induce ripening established that ripening characters displayed different sensitivities to ethylene with fruit softening and aroma volatiles being less sensitive to ethylene compared to starch and skin colour. A genomics approach has identified genes involved in volatile and fruit softening. The characterisation of one ethylene induced cell wall gene *MdPOLYGALACTURONASE1* (*MdPG1*) suggests a role of cold in the regulation of this gene that is independent to ethylene. Using transient assays we have characterised the role of both *CBF*-like and *EIN3*-like genes in the regulation of *MdPG1*. Overall this research has given a greater understanding in the role of ethylene in apple fruit ripening.

Proteomic analysis of differentially expressed proteins in apple fruit during ripening and senescence

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Apple fruit (*Malus domestica* Barkh) is well known as climacteric fruit and a good model to study fruit ripening and senescence. Despite the intensive biochemical and physiological studies on apples with additional genomic and molecular investigations, very few data available at proteomic level and fruit ripening and senescence at the gene and protein levels have not been fully understood. In this study, apple fruit harvested prior to ripening at pre-climactic stage were allowed to naturally ripe and stimulated with treatment of exogenous ethylene. Postharvest physiological quality indices such as respiration, ethylene production and chlorophyll fluorescence were characterized during fruit ripening and senescence. Proteomic approaches using two dimensional electrophoresis (2DE) technique was employed to separate the total proteins from fruit at seven different stages of ripening and senescence. After imaging analysis and corresponding statistical analysis including ANOVA and principle component analysis (PCA) on 2053 spots, 189 spots were found to be significantly changed which is about 9% protein of total protein population were changed. The significantly changed spots comprised of the group of proteins present only at certain fruit stages and those were significantly changed during whole study. Among 189 candidate spots, 102 spots were further selected and identified by applying nano-spray liquid chromatography and mass spectrometry analysis with sequence and express sequence tag (EST) data searching. Analysis and identification of proteins revealed that apple fruit ripening and senescence is associated with many groups of protein functions such as carbohydrate metabolisms, antioxidant, energy, ethylene biosynthesis and signal transduction as well as stress resistance including allergens. In addition, ethylene treatment induced a group of unique proteins that were not present during normal fruit ripening. This study demonstrated the complexity and dynamic changes of protein profiles of apple fruit during ripening and in response to exogenous ethylene treatment. The understanding of identified proteins and their function may help to explore the mechanism ripening and senescence of climacteric fruit.

Temperature dependent ethylene metabolism during storage of 'Rich Lady' peach

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Peach fruit ripen very quickly at ambient temperatures and storage at very low temperatures often leads to chilling injury symptoms. The objective of our research was to quantitatively follow the ethylene metabolism intermediates and enzymes of fruit during storage at different temperatures. Peach fruit was harvested in 2008 at the optimal harvest date and was stored at 4 different temperatures (20°C, 10°C, 4°C and -1°C). The measurement interval depended on the temperature with 9 to 15 time points for each temperature until no further firmness changes were noted. The content of 1-aminocyclopropane-1-carboxylic acid (ACC) and its conjugated form 1-(malonyl) aminocyclopropane-1-carboxylic acid (MACC) were measured as well as ACC synthase (ACS) and ACC oxidase (ACO). These changes were linked with the firmness changes followed during the same time period. Temperature affected ACC production more compared with MACC production, with complete inhibition of ACC production at -1°C whereas some MACC was produced at this temperature. At higher temperatures the production of both ACC and MACC was also higher. The lower ACC production at lower temperatures was mainly due to an inhibition of ACS activity. At 4°C there was ACS activity which increased with storage time, but at -1°C there was no ACS activity at all. ACO activity was minimal for both -1°C and 4°C with no significant difference between the two. The complete inhibition of ACC production at -1°C concurs with the total lack of softening in fruit stored at -1°C. At 4°C the fruit started to soften after 15-20 days which coincided with the increase in ACS activity and ACC content. The point at which fast softening started for fruit kept at 10°C and 20°C also coincided with the increase in ACC production. These results show an enzyme dependent effect of temperature.

Expression of *MaMADS2* and its interactions with ethylene suggest that it acts upstream to ethylene production

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Several members of the *MADS-box* gene family were cloned from banana cultivar Grand Nain (*MaMADS1-6*), however only *MaMADS2*, based on its expression patterns and interactions with ethylene is suggested to be a candidate master regulator of ripening in banana. Expression of *MaMADS2* is higher in the pulp than in the peel and the increase in expression of *MaMADS2*, together with *MaMADS3, 4* and *5* preceded ethylene production, but coincided with the CO2 respiration peak in the pulp. On the other hand, in the peel, the expression of the genes *MaMADS2* together with *MaMADS1, 3* and *4* coincided with increased ethylene production. Ethylene applied early after harvest and 1-MCP applied at the onset of increase in ethylene production, did not affect the levels of *MaMADS2*, examined immediately after treatment, although these treatments affected the expression levels of the other *MaMADS-box* genes either in peel or in pulp. On the other hand, application of high levels of carbon dioxide immediately after harvest, reduced the levels of *MaMADS2,* as well as a number of the other *MaMADS-box* genes. These suggest that *MaMADS2* expression is not dependent on ethylene, but possibly on mitochondrial function. Although the gene showed only low homology to tomato *RIN*, we suggest that *MaMADS2* may function similarly to RIN, acting first in the pulp possibly with other MaMADS-box proteins to activate the ripening program. Transgenic banana plants downregulated in *MaMADS2* and *MaMADS1* were created and should bear fruit shortly.

Modeling cell wall structural dynamics in *Arabidopsis* abscission zones

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Shedding of many plant organs occurs at specialized sites called abscission zones (AZs). We have coupled laser capture microdissection of *Arabidopsis* AZ cells to global gene profiling to reveal the AZ transcriptome of floral stamens shedding post-pollination. Cell wall remodeling functions are greatly overrepresented in transcript populations regulated at high statistical significance over five stages of stamen development spanning pre-pollination to organ shed. Hierarchical clustering of gene expression data corresponding to cell wall-related transcripts reveals that the disassembly of AZ cell walls by glycosyl hydrolases and other wall modifying proteins takes place in a complex backdrop of glycosyltransferase expression. The apparent maintenance of many cellulose and hemicellulose building activities in tissues destined for detachment suggests the need to refine previous abscission models emphasizing cell wall catabolism.

Effect of transition between ethylene and air storage on two potato varieties

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Exposure to ethylene (10 μL L-1) extends potato storage life, but also affects taste and texture. Most research has been conducted on potato cv. Russett Burbank tubers, and there is a paucity of work concerning UK-grown potato cultivars. In addition, the transition between ethylene and air during storage has not been investigated.
In this study, the effect of the transition between ethylene (10 μL L-1) and air (and vice versa) on potato cv. Maris Piper and potato variety Mayan Gold, in terms of sugars composition, was assessed. After harvest, potatoes were transported to Sutton Bridge Experimental Unit (Lincs., UK) and initially stored at 15°C, then slowly cooled to 6°C over two weeks. Tubers were then stored in the presence or absence of continuous ethylene (10 μL L-1) at 6°C. When tubers showed first indication of sprouting (eye movement), they were transferred to/from ethylene.
Significant differences were shown between cultivars regarding their sugar content. Sucrose, glucose and fructose concentration in tubers of both cultivars increased during storage. Higher sucrose, glucose and fructose concentrations were recorded in ethylene-treated vs. untreated tubers at the time of tuber transition for cv. Maris Piper, but not for Mayan Gold. However, under continuous ethylene treatment (10 μL L-1) sugar content increased between time of eye movement and four weeks later in Mayan Gold but not in Maris Piper tubers. The results herein suggest that both cultivars responded differently to ethylene. The combination of ethylene and air treatments at different storage timings could prolong storage life while suppressing the increase in sugars during storage.

Influence of season and origin on ripening of imported avocado cv. Hass fruit.

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Quality of avocado fruit can be affected by country of origin. In addition, fruit maturity at harvest plays a role in determining subsequent ripening behaviour. Generally late season fruit show faster mesocarp softening than fruit from earlier harvests. The present study aimed to further investigate the relationship between endogenous ethylene production and mesocarp softening on imported fruit from three different seasons (*viz*. early, middle and late) originating from Spain (ca. 9 days transit) vs. South African (SA)(ca. 25 days transit). Indicators of fruit ripening such as ethylene, respiration rate, firmness and objective colour were recorded during shelf life (18°C) at days 0, 1, 2, 4 and 7. Temporal change in sugars was also measured as these are known to be possible biomarkers of ethylene-induced ripening.
Significant differences were observed during the climacteric peak in the amount of ethylene and carbon dioxide produced by fruits from different seasons and origins. Spanish fruit from early and late seasons followed similar patterns, whereas middle season fruit showed almost 2-fold higher levels of ethylene and lower CO2 production. Although ethylene production was lower than middle season, late season fruit softened significantly faster than fruit from earlier seasons. In contrast, early season fruit from SA showed a higher but delayed climacteric peak compared to their respective Spanish counterparts. The respiration rate was slightly increasing in the first two days of shelf life in all SA fruits. This was accompanied by a significant decrease in firmness, with slower softening observed in early season fruit. Hue angle (H°) decreased faster in middle Spanish and late SA fruit. Moreover sugar analysis showed differences in temporal change in D-mannoheptulose and perseitol as affected by origin and season. Data suggests that ripening physiology is closely related to ethylene production during ripening yet is also influenced by maturity and origin.

Interactions between ethylene and auxin during peach fruit ripening.

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Peach is a climacteric fruit. A complex network of signals is involved in the regulation of its ripening with ethylene being regarded as the main player. However, concomitant with climacteric ethylene production, increases in free auxin can be measured in peach mesocarp. A genomic approach with the μPEACH 1.0 microarray has been used to shed some light on the signals leading to the transition from preclimacteric to climacteric fruit. Treating preclimacteric fruit with ethylene, with an auxin analogue (naphthalene acetic acid, NAA), and with 1-methylcyclopropene (1-MCP), known to block ethylene receptors, confirmed the importance of ethylene for the regulation of ripening and highlighted the independent role played by auxin. Many genes encoding proteins involved in auxin biosynthesis, transport and signalling (IAA amidohydrolase, PIN, TIR1-like receptors, Auxin Response Factors and Aux/IAAs) are induced during climacteric, thus strengthening the idea that this hormone is actively involved in the ripening process. Furthermore, it resulted that genes belonging to the ethylene domain, such as 1-aminocyclopropane-1-carboxylate synthase 1 (ACS1) and an ethylene response factor (ERF) are induced by auxin, while genes belonging to the auxin domain, such as a PIN efflux facilitator, are induced by ethylene.
Many of the ethylene-regulated genes (either induced or repressed) respond in the opposite way to 1-MCP (either down- or up-regulated, respectively), thus confirming the ethylene responsiveness. But several auxin-induced genes are up-regulated also by 1-MCP, leading us to hypothesize a rise in free IAA. This increase is in agreement with the induction of a GH3 gene, whose expression is widely used as a marker for free auxin, and of an IAA amidohydrolase, that releases IAA from the conjugated forms. Being ACS1 induced by auxin, it resulted to be upregulated also by 1-MCP, thus strengthening the idea of the existence of an important cross-talk between the two hormones.

Ethylene and postharvest physiology in climacteric and nonclimacteric fruit in the genomics era

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Genomics tools such as microarrays are increasingly used for elucidating and studying transcript profile changes and gene regulation in relation to specific fruit developmental stages (e.g. ripening) and following different postharvest conditions, practices and treatments aimed at prolonging storage- and taste-life and/or affecting composition in both climacteric and non-climacteric fruit. Microarray hybridizations have been in particular useful to describe the role of ethylene by means of exogenous treatments with the hormone and the use of its inhibitor 1-MCP. Analyses, performed on both melting flesh varieties and ripening mutants such as Stony Hard (SH), allowed to identify possible mechanisms regulating ripening responses and processes including cross-talks between ethylene and other hormones (in particular auxins). Although the burst of ethylene is not present at ripening in non-climacteric fruit, ethylene-related genes appear to be differentially expressed and, possibly, play a role in the transition from immature to mature fruit and in fruit composition at ripening as observed in olives, using a cDNA library subtractive hybridization approach to identify differentially expressed genes throughout development, and in wine grapes where the treatment with exogenous ethylene after harvest dramatically changed must composition that resulted enriched (up to 30-40%) in terms of phenol compounds content. Hybridizations with the latest 30k Combimatrix chip pointed out that this is the result of both an increased expression, in skins, of genes involved (structurally and with a regulatory role as Transcription factors) in the phenylpropanoid biosynthetic pathway and genes with a role in membrane permeability changes and cell wall metabolism leading to an increased extractability during maceration.

Melon fruit development and quality: climacteric *vs*. non-climacteric ripening

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Melon (*Cucumis melo* L.) is a highly polymorphic species comprising a wide range of wild and cultivated genotypes. These genotypes vary with respect to fruit traits, including ripening physiology, sugar and organic acid contents, color, taste and aroma. Melon fruit ripening is typically climacteric and associated with increased CO2 evolution, an ethylene burst and fruit abscission. However, there are melon types that neither emit elevated quantities of ethylene during ripening, nor abscise, and are therefore considered non-climacteric. The non-climacteric melons were suggested to be defective in their ethylene synthesis or response. Climacteric melons exhibit typically strong aroma compared with the non-climacteric types and, in addition, climacteric ripening is associated with shorter shelf life.
A number of readily measured factors, such as sugar concentration, aroma volatiles, carotenoid pigments and organic acid content contribute to melon quality. Recently, a genomic and genetic infrastructure has been developed in order to better understand the genetic mechanisms underlying these traits. The resources developed include marker-merged linkage maps, QTLs mapping populations, melon-fruit EST collections constructed through various approaches and a database presenting these resources to the public (http://www.ICUGI.org). This infrastructure has enabled insight into the processes associated with melon fruit development and ripening, with special emphasis on climacteric versus non-climacteric melons.

Altered ripening characteristics of "Golden" tomato fruits

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Spoilage is one of the major causes for post-harvest fruit loss, and also negatively impacts fruit nutritional characteristics. Strategies for the simultaneous improvement of the nutritional value and of the shelf-life of fruits will greatly benefit consumers and producers. During ripening, tomato fruits undergo softening and accumulate the red carotenoid, lycopene. Transgenic overexpression of lycopene beta-cyclase (LCY-b) results in a "Golden" phenotype due to the accumulation of beta-carotene (provitamin A). Unexpectedly, "Golden" fruits also show decreased ethylene production and ACO activity, reduced water loss, increased pericarp and cuticle thickness, and increased fruit firmness and shelf-life. These phenotypic modifications are associated with widespread transcriptome and metabolome remodelling. Similar phenotypes are observed in fruits of the *BETA* mutant, which overexpress a different lycopene beta-cyclase. These observations suggest that lycopene beta-cyclases or their product, beta-carotene, have hitherto unsuspected roles in the control of tomato fruit maturation, and that their genetic manipulation may interfere with ethylene production/signaling.

Modulating 1-MCP Effect in 'Bartlett' Pears with Maturity, Ethylene Exposure, and Cold Storage

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'Bartlett' pears were treated with 0.3 µL L-1 1-MCP (1-methylcyclopropene) for 12 hr at 20C immediately after harvest during distinct seasons, maturities, ethylene treatments prior to 1-MCP, and storage times after 1-MCP. 1-MCP decreased softening rate, ethylene production, respiration rate, yellow color development, controlled or reduced scald (superficial and senescent) and internal breakdown. Ripening recovery in 1-MCP treated fruit was partially induced by cold storage which stimulated most of the ethylene biosynthesis machinery as evaluated by ethylene production, 1-aminocyclopropene carboxylic acid synthase (ACS) activity, 1-aminocyclopropene carboxylic acid oxidase (ACO) activity and transcript levels of genes associated with these enzymes. 1-MCP treatment of the most mature fruit at harvest stimulated partial to moderate recovery of ethylene production, ACS and ACO activity and fruit softening in one season, but not in another season. Ethylene exposure prior to 1-MCP treatment resulted in full ripening recovery, which suggests this might be used to modulate the 1-MCP response in 'Bartlett' pears. We demonstrated that 1-MCP can be used to control or reduce scald and internal breakdown in 'Bartlett' pears, but it is challenging to fully induce the capacity for ripening, even after extended periods of cold storage; resulting in crunchy pears. However, partial ethylene treatments prior to the 1-MCP application might be used to modulate fruit response and allow recovery of ripening capacity.

Wednesday, June 24th

ETHYLENE RECEPTORS – FROM THE RECEPTOR TO APPLICATIONS

Moderator
Mark Dahmer, AgroFresh Inc, Centennial, Colorado

8.00 - 8.30 **Raphael Goren**, The Hebrew University of Jerusalem, Israel
The effect of both (i) volatile and (ii) water soluble cyclopropene as antagonists of ethylene action

8.30 - 9.00 **James Mattheis**, USDA-ARS, USA
Processes of Temperate Fruit Development Regulated by Ethylene Action

9.00 - 9.15 **Maria Angeles Chiriboga**, IRTA, Spain
Ethylene metabolism does not entirely explain softening during storage of 1-MCP treated 'Conference' pears

9.15 - 9.30 **Jennifer R. DeEll**, OMAFRA, Canada
Ethylene Inhibition Influences Physiological Disorders in Apples

9.30 - 10.00 **Eric Schaller**, Dartmouth College, USA
Regulation of Ethylene Receptor Signal Output

10.00 - 10.30 *Break*

10.30 - 11.00 **Donald Huber**, University of Florida, USA
Factors Influencing the Responsiveness of Climacteric Fruits to 1-MCP.

11.00 - 11.15 **Brad M. Binder**, University of Tennessee, USA
ETR1 Receptor Domains Involved in Ethylene-Stimulated Nutational Bending

11.15 - 11.30 **John K. Fellman**, Washington State University, USA
Employment of a marker-based technique to detect MCP use and effects in apple

Optional tour to wineries and Taughannock State Park, with lunch at Wagner Winery.
Tickets required
11.30 - 11.45 *Load busses for tour in front of the Plant Science building*
Return at 6pm

Dinner: on your own

Evening Moderator
David Clark, University of Florida, USA

7.30 - 8.00 **Caren Chang**, University of Maryland, USA
Analyses in Ethylene Signal Transduction using Molecular Genetics and Proteomics

8.00 - 8.15 **Qian Liu**, Chinese Academy of Science, China
Functional analysis of plant RTH genes in the regulation of ethylene response and possible roles of ethylene in the rice growth and development

8.15 - 8.30 **Jin-Song Zhang**, Chinese Academy of Sciences, China
Rice ethylene receptor OsETR2 delays floral transition and affects starch accumulation

8.30 - 8.45 **David Clark**, University of Florida, USA
Ethylene regulation of a specialized CHORISMATE MUTASE in petunia flowers

8:45 - 10.00 **Poster Session**
Drinks in tent

The effect of both (i) volatile and (ii) water soluble cyclopropene as antagonists of ethylene action

Raphael Goren[1], Edward C. Sisler[2] and Moshe Huberman[1]

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[1]Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel, [2]Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC 27695-7622, USA

The potency to antagonize ethylene-induced action of twelve new volatile cyclopropene derivatives, synthesized by E.C. Sisler, was assessed by pre-treatment of avocado fruit, etiolated pea seedlings and abscission of citrus leaf explants. In some cases the volatile derivatives were found to be slightly more effective than 1-MCP, and in other systems less effective. 3-cyclopropyl-1-enyl-propanoic acid was selected from the above derivatives to synthesize 3-cyclopropyl-1-enyl-propanoic acid, sodium salt (CPAS, Patent Application No.: PCT/IL2008/000995; US Application No. 61/144758.). CAPS, by itself, delayed peach softening. Pre-treatment of CPAS considerably antagonized ethylene-induced delay of peel color change in 'Hass' avocado and banana fruit and delayed, to a lesser extent, their softening. Addition of surfactants improved the effect of CAPS on delaying fruit softening. Senescence of cut flowers was markedly delayed following a short loading period of the inhibitor. Pre treatment spray of aqueous solution of CPAS significantly reversed ethylene-induced leaf epinasty of young tomato and young and mature cotton seedlings. It also concomitantly antagonized ethylene-induced chlorophyll degradation in these leaves, as well as in young and mature tobacco leaves. CPAS also antagonized ethylene-induced petioles abscission of young and mature cotton leaves. Pre-spraying of 6 months old wheat plants, growing under controlled conditions, after spike heading at "milky" stage I with CAPS antagonized flag leaf epinasty in response to the following spray of ethephon, and also reduced significantly its effect on the decrease of chlorophyll content, mainly in the flag leaf. CPAS also antagonized ethephon-induced decrease of grain fresh weight in both "milky" stags I, and II (7 months old), which even resulted by an increase of grain yield in the range of 5% to 16%, respectively. At harvest time, wheat plants that were grown under controlled conditions and that were sprayed with CPAS at "milky" stage I, their total dry weight grain yield increased, under this condition, by 15% to 18%. When treated at stage II, one month later, the yield increased by18% to 20%.

Processes of Temperate Fruit Development Regulated by Ethylene Action

James Mattheis

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US Department of Agriculture, Agricultural Research Service, Tree Fruit Research Laboratory, Wenatchee, WA 98801, USA

Inhibition of ethylene action by 1-MCP during climacteric tree fruit ripening has confirmed a number of ripening and senescence processes are regulated by ethylene. For apple, respiration and acid loss, softening, volatile production, and chlorophyll metabolism are slowed in the absence of ethylene action resulting in delayed ripening and a greatly extended marketing period. While similar responses are inducible with 1-MCP in pears, a lack of predictable ripening currently limits wide scale commercialization. Inhibition of ethylene action via postharvest 1-MCP exposure can also prevent development of a number of physiological disorders including superficial scald, and senescent core flush, however, other disorders including peel and cortex CO2 injury can be exacerbated following treatment with 1-MCP. The physiological mechanisms by which most of these disorders proceed are poorly understood and remain to be elucidated.

Ethylene metabolism does not entirely explain softening during storage of 1-MCP treated 'Conference' pears

Maria Angeles Chiriboga; Wendy Schotsmans; Inmaculada Recasens; Christian Larrigaudière

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Institute for Food and Agricultural Research and Technology, Av. Alcalde Rovira Roure, 191, 25198 Lleida, Spain

Application of 1-methycyclopropene (1-MCP) in 'Conference' pears is problematic since they do not always recover their ability to soften after a period of storage and thus do not become ready for consumption. The physiological aspects of this phenomenon were explored through a study of the ethylene metabolism. Fruit were collected at the optimal harvest date (O), 7 days before (O-7) and 7 days after (O+7) and were treated with 300 ppb of 1-MCP. Control and treated fruit were stored in air at 0ºC during 3.5 months. After cold storage, fruit were kept for a shelf life period of 11 days at 20°C during which firmness and the ethylene metabolism were followed, including the production of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene as well as the activity of ACC synthase (ACS) and ACC oxidase (ACO). During shelf life, control fruit softened whereas treated fruit did not, irrespective of harvest date, with some softening seen after 11 days for O+7. The difference between control and treated fruit was also clear in the ethylene production with normal climacteric ethylene production for all control fruit and total inhibition for treated fruit for the first 9 days after which there was minor recuperation of ethylene production for O and O+7. ACS activity and ACC levels were higher for the control fruit but there was no difference in ACC levels between harvest dates. ACS activity increased slightly for the fruit from O+7 whereas ACO activity increased slightly for O, which could account for the increase seen in ethylene production. The inhibition of ethylene production in treated fruit concurs with the lack of softening of these fruit compared with controls. However, the softening seen after 11 days for the last harvest was not reflected in the ethylene metabolism and is likely due to another trigger in this cultivar.

Ethylene Inhibition Influences Physiological Disorders in Apples

Jennifer R. DeEll[1], Behrouz Ehsani-Moghaddam[1], and Chris B. Watkins[2]

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[2]Department of Horticulture, 127 Plant Science Building, Cornell University, Ithaca, NY 14853, USA

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, delays the ripening and senescence of many climacteric fruits, including apple. As such, 1-MCP reduces respiration and ethylene production, and slows softening and deterioration of apple fruit.
The objective of this work was to examine the effects of 1-MCP on common physiological and storage disorders in apples. 'Empire', 'Honeycrisp', and 'McIntosh' apples were harvested in multiple years and cooled over night to ~3ºC. Half of the fruit were then treated with 1-MCP (1 ppm) for 24 hours. All apples were stored in either ambient air (0 or 3ºC) for 3-4 months or in controlled atmosphere (CA) for 6-10 months. CA regimes ranging 2-2.5% O2 + 2% CO2 at 1-3ºC for 'Empire', 1.7-2.5% O2 + 1-2.5% CO2 at 3ºC for 'Honeycrisp', and 2.5% O2 + 2.5-4.5% CO2 at 3ºC for 'McIntosh' were utilized. Senescent-related disorders, such as senescent breakdown and peel greasiness, were substantially reduced in apples treated with 1-MCP. Superficial scald and core browning in air-stored 'McIntosh' were also reduced with 1-MCP. Conversely, disorders related to CA stress tended to be exacerbated by 1-MCP. The incidence of external and/or internal CO2 injury was greatly increased in all cultivars treated with 1-MCP.
Flesh browning (i.e. low temperature breakdown, vascular breakdown, and/or internal browning) incidence and severity also increased in 1-MCP-treated 'Empire' stored in CA for 9 or 10 months. The role of ethylene and 1-MCP in the development of stress-related physiological disorders in apples needs to be investigated further.

Regulation of Ethylene Receptor Signal Output

Brenda P. Hall, Derek M. Thibault, Zhiyong Gao, Yi-Feng Chen, Matthew M. Bombyk, and G. Eric Schaller

Presenter email: george.e.schaller@dartmouth.edu

Department of Biological Sciences, Dartmouth College, Hanover, NH 03755 USA

The gaseous hormone ethylene is perceived by a five-member receptor family in Arabidopsis. Here we report on three aspects of ethylene receptor signaling. First, we address the role of histidine-kinase activity found in the subfamily-1 receptors ETR1 and ERS1, but not in the subfamily-2 receptors ETR2, ERS1, and EIN4. Our recent molecular genetic studies indicate that the kinase activity of ETR1, although not essential to signaling, plays a role in modulating output from the receptor. Second, we address the importance of protein-protein interactions for signaling, including physical interactions among the receptors themselves. Our data indicate that receptors may exist in plants as clusters in a manner potentially analogous to that found with the histidine kinase-linked chemoreceptors of bacteria. Clustering of receptors can help explain the broad range of ethylene responsiveness found in plants as well as the dominant nature of some ethylene insensitive receptor mutations. Third, we address the possibility that signal output from the receptors is negatively regulated by a small family of ethylene-induced proteins (the EFMs; Ethylene Feedback Mediators). These proteins could serve as a molecular switch to recalibrate signal output after the initial ethylene perception. Taken together, these data suggest that the tremendous range of ethylene receptor signaling capacity is achieved in part by incorporating a variety of regulatory mechanisms.

Factors Influencing the Responsiveness of Climacteric Fruits to 1-MCP

Donald Huber[1], Sun Tay Choi[2], Brandon Hurr[1], and Zhengke Zhang[1]

Presenter email: djhuber@ufl.edu

[1]Horticultural Sciences Dept., IFAS, University of Florida, Gainesville, FL, USA, [2]National Horticultural Research Institute, Rural Development Administration, 475 Imok-Dong, Jangan-Gu, Suwon 440-706, Republic of Korea

A number of climacteric fruits show incomplete ripening if treated with 1-methylcyclopropene (1-MCP, SmartFreshSM Quality System) prior to the onset of ripening. Global recovery of ripening features and attainment of optimum quality are best achieved if 1-MCP is applied after ripening initiation. While this approach works well for fruits including tomato, papaya and cantaloupe, other climacteric fruits including avocado and banana become progressively less sensitive to the ethylene antagonist when applied within a few days after ripening initiation. We have been investigating factors that may contribute to these response differences. We have identified a number of cellular components that exhibit sorption of 1-MCP, and plantain, avocado and apple show exceptionally high sorption compared with tomato and cantaloupe fruits. Highly esterified pectin and lignin represent particularly strong sorption targets. Using tomato fruit as a model system, we have examined the influence of internal ethylene levels on 1-MCP responsiveness. Breaker-turning tomato fruit are completely insensitive to saturating levels (500 nL L^{-1}) of 1-MCP applied in the presence of 100 µL L^{-1} ethylene. When treated with ethylene and 1-MCP in succession, fruit remain insensitive to subsequent 1-MCP treatment in the short term with full responsiveness recovering over 6 h, during which time internal ethylene levels decline to pretreatment values. As a third factor contributing to 1-MCP responses in general, we have determined that plant tissues are capable of metabolizing 1-MCP, possibly through oxidative reactions. Cell free extracts of apple tissue show rapid consumption of headspace 1-MCP in a reaction that occurs optimally over the pH range of 4 to 6 and is inhibited by heating (100%), anoxia (50%), and ascorbate (100%). Apple shows much higher 1-MCP catabolism than tomato fruit. We propose that the response intensity of climacteric fruits and other commodities to 1-MCP is influenced by non-specific sorption, internal ethylene levels, and 1-MCP metabolism.

ETR1 Receptor Domains Involved in Ethylene-Stimulated Nutational Bending

Brad M. Binder[1], Wuyi Wang[2], J. Brett Case[1], Christina L. Schmitt[1], and M. Blaine Stalans[1]

Presenter email: bbinder@utk.edu

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Ethylene affects many aspects of plant development including seedling growth. We recently found that ethylene stimulates nutational bending of dark-grown Arabidopsis seedlings. Paradoxically, loss of ETR1 abolishes this phenotype while loss of other receptor isoforms leads to constitutive nutations. Since the phenotype exhibited by *etr1* null plants is opposite to current models of receptor function, we have initiated experiments to determine the underlying mechanism for this. We find that ETR1 histidine kinase activity and phosphotransfer through receptor receiver domains are not required for ethylene-stimulated nutations. However, truncated ETR1 receptors lacking the receiver domain fail to rescue the nutation phenotype in *etr1* null plants. These transgenes are functional since they still rescue reduced growth. We explored this requirement for various receptor domains by generating chimeric receptors with domains swapped from the ERS1 and EIN4 receptors into ETR1. Our previous work also demonstrated that ethylene-stimulated nutations are likely to involve alterations in auxin signaling or transport. We have started a mutational approach to better define the links between ethylene signaling and alterations in auxin biology that lead to this phenotype.

Employment of a Marker-Based Technique to Detect MCP Use and Effects in Apple

Paul G. Levesque, Suresh Iyer, John K. Fellman

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Postharvest Physiology Laboratory, Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414 USA

1-methylcyclopropene (1-MCP) is a safe and effective ethylene action inhibitor that works by binding the initial ethylene receptor in fruits, preventing biochemical signal transduction. 1-MCP formulations used commercially by the Global fruit industry are used to maintain postharvest quality by preventing or delaying ripening and reducing incidence of ethylene-related disorders. Once 1-MCP is applied to fruit, it is difficult to ascertain treatment status or effectiveness. In order to determine the presence or absence of 1-MCP in presumably treated fruit, we report development and use of a reverse-transcription polymerase chain reaction (RT-PCR) based method to examine expression of ethylene-sensitive genes known to exist in apple (*Malus domestica* Borkh.). Expression of transcripts from the genes *Md-PG* and *Md-ACS1*, respectively coding for an apple endopolygalacturonase and 1-aminocyclopropane 1-carboxylic acid synthase, were employed as molecular markers to determine 1-MCP application in various apple cultivars at harvest and after storage. Storage and ethylene treatment did not appreciably induce marker expression once ethylene action was blocked by 1-MCP. Use of the method effectively confirmed presence or absence of 1-MCP treatment regardless of formulation or application method. Our newlydeveloped method has proven workable in all apple cultivars tested thus far.

Analyses in Ethylene Signal Transduction using Molecular Genetics and Proteomics

Caren Chang, Maximo Rivarola, Josephine Resnick, Chunhai Dong, Jianhong Chang, Chris McClellan, and Ruiqiang Chen

Presenter email: carenc@umd.edu

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742 USA

In the past 15 years, great progress has been made in identifying key players and regulatory mechanisms in the ethylene-signaling pathway, from the perception of ethylene to gene expression. We are continuing to dissect the ethylene-response pathway in Arabidopsis using molecular genetic approaches and by exploring proteomic approaches. Genetic screens for ethylene-response mutants have yielded several genes of interest. One in particular is *REVERSION-TO-ETHYLENE SENSITIVITY* (*RTE1*), which was identified by the ability of the *rte1* mutation to suppress ethylene insensitivity of the *etr1-2* dominant mutant. *RTE1* encodes a novel integral membrane protein conserved in plants, animals and some protists. Genetic data indicate that RTE1 promotes signaling specifically in the ETR1 ethylene receptor, most likely through the amino-terminal ethylene-binding domain of ETR1. Interestingly, there are two classes of *etr1* dominant mutant alleles – those that are dependent on *RTE1* for ethylene insensitivity and those that are *RTE1*-independent. Dominant *etr1* mutations that require *RTE1* to confer ethylene insensitivity are unexpectedly silent when transferred to identical conserved positions in other Arabidopsis ethylene receptor genes. Thus, ETR1 is clearly distinct from the other ethylene receptors despite being highly conserved. We are currently investigating the cellular role of RTE1 and how it regulates ETR1.

We are also starting to investigate ethylene responses at the protein level based on posttranslational modifications upon ethylene treatment. One approach is to identify proteins that are rapidly modified in response to ethylene using 2-dimensional difference gel electrophoresis (2-D DIGE) coupled with mass spectrometry. 2-D DIGE has the potential to uncover additional components in ethylene signaling while also revealing ethylene-induced changes in protein levels, activity and localization. A second approach is the identification of ethylene-regulated protein phosphorylation using multidimensional protein identification technology (MudPIT). Such approaches may help to dissect cellular responses that do not necessarily involve transcriptional responses and/or are involved in crosstalk.

Functional Analysis of Plant *RTH* Genes in the Regulation of Ethylene Response and Possible Roles of Ethylene in the Rice Growth and Development

Wei Zhang[1], Xin Zhou[1,2], Ai-Bei Xu[1], Shu-Miaw Chaw[3], Chi-Kuang Wen[1] and Qian Liu[1]

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The Arabidopsis *RTE1* gene encodes a Golgi-associated protein and specifically acts through the N-terminal half of the ETR1 receptor to repress ethylene response. *RTH* (*RTE1 Homologue*) genes are prevalently found in higher eukaryotes while their functions remain elusive. In this study, we investigated if function of any *RTH* genes, including lower and higher plant species, is functionally conserved in Arabidopsis. *RTHs* of *Physcomitrella patens* (*PpRTH1* and *PpRTH2*), *Selaginella moellendorffii*, (*SmRTH*), and *Oryza sativa* (*OsRTH1*, *OsRTH2*, and *OsRTH3*) were each expressed in Arabidopsis. We show that expression of *35S::OsRTH1* conferred ethylene insensitivity in *ETR1*- dependent manner. In addition, *35S::OsRTH1* was able to restore ethylene insensitivity in *etr1-2 rte1-2*. Moreover, the GFP-OsRTH1 protein is also localized to the Golgi. These results imply that *OsRTH1* is an orthologue of Arabidopsis *RTE1*. To reciprocally examine if *OsRTH1* would regulate ethylene response, *OsRTH1* was overexpressed in the rice plant. *OsRTH1ox* lines were subjected to leaf senescence test; they exhibited insensitivity to ethylene and the leaves did not turn yellow while untransformed rice leaves senesced. This result is consistent with 1-MCP treatment, which blocks ethylene binding to the receptors, that leaves of 1-MCP-treated rice and *OsRTHox* lines had similar chlorophyll content. We next exploited *OsRTH1ox* lines to study possible effects of ethylene on the rice growth and development. Our data show that ethylene insensitivity caused reduction in the rice grain yield but not on grain weight. *OsRTHox* lines exhibited a shorter shoot, likely due to inhibition in cell elongation. Possible effects of ethylene insensitivity on submergence tolerance were further evaluated and discussed.

Rice ethylene receptor OsETR2 delays floral transition and affects starch accumulation

Hada Wuriyanghan[1], Bo Zhang[1], Wan-Hong Cao[1, 2], Biao Ma, Gang Lei, Yun-Feng Liu, Wei Wei, Li-Jun Chen, Hao-Wei Chen, Yang-Rong Cao, Si-Jie He, Shou-Yi Chen, Jin-Song Zhang

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Plant Gene Research Center, National Key Lab of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; [1]These authors contribute equally to this work. [2]Current address: Syngenta Biotechnology China, Beijing

Ethylene regulates multiple aspects of plant growth and development, however, its roles in monocotyledonous plants are less known. Ethylene receptor is the first component of ethylene signaling. In this study, we characterized a subfamily II ethylene receptor OsETR2 in rice. The OsETR2 has autophosphorylation ability, is a Ser/Thr kinase but not a His kinase, and can phosphorylate its receiver domain. Mutation of the N-box of the kinase domain abolished the kinase activity of the OsETR2, suggesting that the N-box is essential for phosphorylation.
Overexpression of the *OsETR2* in transgenic rice plants reduced ethylene sensitivity and delayed floral transition whereas RNAi plants exhibited early flowering. Conversely, the *OsETR2* T-DNA insertion mutant *osetr2* showed enhanced ethylene sensitivity and early flowering. The ratio of effective panicles and seed-setting rate was reduced in the *OsETR2*-overexpressing plants, while thousand-seed weight was substantially enhanced in both the *OsETR2*-RNAi plants and the *osetr2* mutant compared to controls. Starch granules accumulated in internodes of the *OsETR2*-overexpressing plants but not in the *osetr2* mutant. The *OsGI* and *RCN1* that cause delayed flowering were upregulated in *OsETR2*-overexpressing plants but downregulated in *osetr2* mutant. Conversely, α-amylase gene *RAmy3D* was suppressed in *OsETR2*-overexpressing plants but enhanced in *osetr2* mutant. These results suggest that OsETR2 may delay flowering and cause starch accumulation in stems through regulation of downstream genes. These studies provide important clues for improvement of yield-related traits through manipulation of ethylene signaling.

Ethylene Regulation of a Specialized CHORISMATE MUTASE in Petunia Flowers

Thomas Colquhoun[1], Bernardus Schimmel[1], Jooyoung Kim[1], Didier Reinhardt[2] and David Clark[1]

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In *Petunia* x *hybrida* 'Mitchell Diploid' (MD), floral fragrance is comprised of 13 volatile benzenoids/phenylpropanoids that are derived from phenylalanine. The metabolic cost for floral volatile benzenoid/phenylpropanoid (FVBP) production is extremely high, with up to 0.15 mg gfw-1 hr-1 of FVBPs emitted by flowers during peak production at night. Several genes involved in the direct synthesis of FVBPs have been isolated and characterized in Petunia through reverse genetic and biochemical approaches. In an effort to understand the regulation of "upstream" components of FVBP biosynthesis, we cloned and characterized two CHORISMATE MUTASE (PhCM1 and PhCM2) cDNAs from petunia. *PhCM1* has a specialized transcript accumulation profile: 1) it accumulates at the onset of anthesis and is highly expressed until corolla senescence, 2) it is specific to floral organs, predominantly the corolla, 3) it follows a diurnal pattern with highest accumulation prior to peak emission at night, and 4) it is down-regulated rapidly by pollination and exogenous ethylene treatment. *PhCM2* followed a constitutive transcript accumulation profile. Recombinant PhCM1 is allosterically regulated by tryptophan, but phenylalanine and tyrosine show no allosteric effects. *PhCM1* RNAi knockdown plants have flowers with reduced total FVBP emission by approximately 60- 70%, and are reduced in total CM activity by 80-85% compared to MD. These results demonstrate that PhCM1 is the principle CM protein responsible for the coupling of metabolites from the shikimate pathwat to the synthesis of FVBPs in the MD flower.

Thursday, June 25th

STRESS BIOLOGY

Moderator
Bill Miller, Cornell University, USA

8.30 - 9.00 **Fred E. Below**, University of Illinois, USA
Ethylene control for achieving high crop yields

9.00 - 9.15 **Etti Or**, Volcani Center ARO, Israel
Indications for Ethylene:ABA interplay in response to bud dormancy release stimuli

9.15 - 9.30 **W. Roland Leatherwood**, Cornell University, USA
Long term low concentration ethylene exposure affects growth and development of 28 ornamental taxa

9.30 - 9.45 **Michelle L. Jones**, Ohio State University, USA
Ethylene regulation of phosphorus remobilization during leaf and petal senescence

9.45 - 10.00 **P.V. Vara Prasad**, Kansas State University, USA
Effect of 1-methylcyclopropene on soybean flower and pod abortion under heat stress

10.00 - 10.30 *Break*

10.30 - 11.00 **Bruce Bugbee**, Utah State University, USA
Ethylene synthesis and sensitivity: whole plant studies in controlled environments

11.00 - 11.15 **Imene Rajhi**, University of Tokyo, Japan
Identification of genes involved in aerenchyma formation induced by ethylene in maize

11.15 - 11.30 **Rashmi Sasidharan**, Institute of Environmental Biology, The Netherlands
The role of group VII ethylene response factor (ERF) genes in the contrasting flooding responses of two Rumex species.

11.30 - 11.45 **Pascal Montoro**, UMR DAP, CIRAD, France
Regulation of the expression of ethylene biosynthesis genes in Hevea brasiliensis

11.45 - 12.00 **Francisco J. Romera**, Córdoba University, Spain
Iron deficiency up-regulates genes involved in both ethylene synthesis and signaling

12.00 - 2.00 *Lunch*

Moderator
Peter Davies, Cornell University, USA

2.00 - 2.30 **Ian T. Baldwin**, Max Planck Institute for Chemical Ecology, Germany
Asking the plant about "stress"

2.30 - 2.45 **F. Paul Silverman**, Valent Biosciences Corporation, USA
Ethylene biosynthesis inhibition by strobilurin fungicides

2.45 - 3.00 **Daniel R. Gallie**, University of California, USA
Ethylene regulates photosynthesis through alterations in non-photochemical quenching

3.00 - 3.15 **Michael T. McManus**, Massey University, New Zealand
Ethylene interacts with auxin in response to phosphate deficiency in white clover

3.15 – 3.45 *Break*

3.45 - 4.15 **Ronald Pierik**, Utrecht University, The Netherlands
Struggling for light: Regulation of plant-plant interactions

4.15 - 4.30 **Caroline C. von Dahl**, Boyce Thompson Institute, USA
Herbivore-induced ethylene primes a direct defense in ethylene-deficient neighbors.

4.30 – 5.00 *Business meeting*

5pm: *Departing reception in tent on Agriculture quadrangle, with snacks and drinks.*

Dinner on your own.

Farewell 'til we met again!

Ethylene Control for Achieving High Crop Yields

Fred E. Below and Martin Uribelarrea

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Department of Crop Sciences, University of Illinois, Urbana, IL 61801 USA

Crops are continually subject to environmental and biological stresses that chip away at their yield potential. Many of the physiological and plant growth responses to these stresses are modulated by the natural plant hormone ethylene. The biosynthesis of ethylene is enhanced under stress, which may result in measurable yield losses. Ethylene is unique among plant growth regulators in that it is a gas, which allows it to be released by stressed plants, and perceived by adjacent plants. Increases in ethylene level can mimic many of the symptoms of plant stress, or can induce acclimation processes which aid in plant tolerance and survival to stress. Thus, in a way plants talk to each other about stresses via ethylene. Ethylene is known to play a role in shade avoidance at high plant populations, in mediating the time course of leaf senescence, and in plant responses to a number of abiotic stresses like high temperature. We are investigating some new and existing technologies as a way of protecting plants from stress, by either altering the level of plant ethylene, or alternatively, by decreasing the plant's sensitivity to ethylene. We have found that the competitive ethylene inhibitor 1-MCP, which decreases the plant's sensitivity to ethylene, minimizes the negative impacts of supra-optimal plant populations, and decreases yield reductions due to high temperature stress. Conversely, increasing the level of plant ethylene with the slow-release ethylene formulation, ethephon enhances the negative effects of high plant population and makes the plants noticeably shorter. We also believe that the late-season 'greening effect' associated with strobilurin-based fungicides is associated with a decrease in ethylene biosynthesis, which acts as a long-term signal to alter the senescence trajectory of the leaves. Tools to manage plant ethylene are clearly one way to mitigate plant stress resulting in higher crop productivity.

Indications for Ethylene:ABA interplay in response to bud dormancy release stimuli

Etti Or, Tamar Halaly, Xuequn Pang, Ron Ophir, Chuanlin Zhang

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Department of Fruit Tree Sciences, Institute of Plant Sciences, Volcani Center ARO, Bet Dagan 50250, Israel.

Hydrogen cyanamide (HC) provides controlled, synchronized and relatively rapid induction of grape bud dormancy release within a well-characterized time frame, thereby creating a traceable and reliable system that facilitate the identification of various biochemical components possibly involved in mechanism of grape bud dormancy release. Using this system, we previously demonstrated changes in the expression of various genes that suggested the development of oxidative and respiratory stress in the bud. A comparative approach was than adopted and showed that the same genes are similarly induced by another dormancy release stimulus, heat shock (HS), also in different timing and intensity. These findings, which suggest that similar mechanisms are triggered by different stimuli, led to a large-scale transcriptomic analysis of bud response to HC and HS in an attempt to expose the factors and pathways involved in dormancy release. Based on the outcome of this analysis, a surprising similarity is exposed of bud response to that of submerged plants. This similarity led to a new link in the field of bud dormancy release between sub lethal stress, perturbation of mitochondrial activity, development of hypoxic metabolism, ethylene:ABA interplay and cell enlargement. A working model will be presented for the cascade that lead from the stimuli application to dormancy release, and involvement of ethylene biosynthesis and signaling will be presented.

Long term low concentration ethylene exposure affects growth and development of 28 ornamental taxa

W. Roland Leatherwood[1] and Neil S. Mattson[1]

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Five plants each of 28 ornamental taxa were grown in equivalent greenhouse environments for two weeks after transplanting. Ethylene was released daily for six more weeks in each house from 4 p.m. to 9 a.m. to achieve the treatment concentration of 0, 0.01 or 0.05 $\mu L \cdot L^{-1}$ ethylene. Growth and development responses were measured by number of abscised organs, flower counts, plant height, diameter, fresh and dry weight.

Increased growth of *Ocimum basilicum* L., *Solenostemon scutellarioides* (L.) Codd, and *Dahlia* Cav. was positively correlated with ethylene concentration. *Cuphea hyssopifolia* Kunth, *Lobelia erinus* L., and *Osteospermum ecklonis* (DC.) Norl. grew larger at 0.01 $\mu L \cdot L^{-1}$ ethylene than at either 0, or 0.05 $\mu L \cdot L^{-1}$ ethylene. Increased growth was negatively correlated to ethylene concentration for *Gerbera jamesonii* Bolus ex Hook. f., *Capsicum annuum* L., *Solanum lycopersicum* L., and *Petunia integrifolia* (Hook.) Schinz & Thell. While ethylene inhibited lateral branch growth of *Fuchsia hybrida* hort. ex Siebold & Voss, *Calibrachoa* Llave & Lex. and *Portulaca oleracea* L., it promoted the same for *Ocimum basilicum*, *Lobelia erinus* and *Cuphea hyssopifolia*.

Flowering was variably inhibited by any ethylene amount for most taxa though some partially recovered from the effect over time. Where flowering did occur in ethylene's presence, flower size was reduced and flower senescence was more rapid compared to control. Interestingly, 0.01 $\mu L \cdot L^{-1}$ ethylene inhibited flowering of *Antirrhinum majus* L. yet 0.05 $\mu L \cdot L^{-1}$ did not.

Clearly, a given ethylene concentration can be a growth inhibitor for some species while for others it encourages growth. Additionally, ethylene may encourage a specific response at one concentration while suppressing that same response at another concentration. These results lend support to the hypothesis that ethylene response, whether growth promotion or inhibition, is incorporated into a single concentration dependent biphasic response model.

Ethylene regulation of phosphorus remobilization during leaf and petal senescence

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The programmed degradation of macromolecules during senescence allows the plant to remobilize nutrients from dying to developing tissues. These studies investigated ethylene's role in nutrient remobilization during petal and leaf senescence in petunia. Only nitrogen and phosphorus levels were found to change significantly from petal opening to the advanced stages of senescence in both pollinated and unpollinated *Petunia x hybrida* 'Mitchell Diploid' (MD) flowers. The largest senescence-related changes in the nutrient content of petals were consistently observed with phosphorus. To further investigate the mechanisms of P remobilization during petal senescence the expression of five high-affinity phosphate transporters was investigated. Only one phosphate transporter (*PhPT1*) was found to be induced during petal senescence. Relative abundance of *PhPT1* in petals increased following treatment with 0.1 μl l^{-1} ethylene for 2 hours. The P and N content of petals was determined in ethylene sensitive MD petunias and transgenic petunias with reduced sensitivity to ethylene (35S::*etr1-1*). When compared to the total P content of corollas on the day of flower opening, P in MD corollas had decreased 74% by the late stage of senescence. In contrast, P levels were only reduced by an average of 32% during *etr1-1* corolla senescence. The N content decreased by 60% in MD and 45% in *etr1-1* corollas. *PhPT1* transcript abundance increased in senescing MD corollas and much smaller increases were detected in *etr1-1* corollas. *PhPT1* transcripts were also detected in petunia roots and leaves and mRNA abundance increased in both organs following P deprivation. Phosphorus deprivation resulted in the induction of *PhPT1* gene expression in the leaves of MD but not *etr1-1* petunias. These experiments indicate that expression of the high-affinity phosphate transporter *PhPT1* is regulated by ethylene in both petals and leaves and *PhPT1* appears to be involved in Pi reallocation during senescence.

Effect of 1-Methylcyclopropene on Soybean Flower and Pod Abortion under Heat Stress

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In soybean (*Glycine max* L. Merr.) high temperature (heat) stress causes early senescence of leaves and abortion of flower, and pods through enhanced ethylene level. Thus, we hypothesize that foliar application of ethylene inhibitor (1-MCP) can alter leaf senescence and flower and pod abortion. The objectives of this research was to determine the effect of ethylene inhibitor on physiological and biochemical traits associated with leaf senescence, flower and pod abortion under optimum temperature and heat stress conditions. Soybean cultivars K03-2897 and LS03-4993 were grown up to full bloom at optimum temperature (28/18ºC). At full bloom one half of plants in each genotype were exposed to 1000 ppb of 1-MCP gas for 5 hours. Each set of treated and untreated plants were exposed to optimum or heat stress (38/28ºC). Heat stress treatment was imposed for 14 d after exposure to 1-MCP. During the stress and recovery data on physiological and biochemical parameters, flower abscission and pod set were recorded. Exposure to heat stress decreased photosynthetic rate, stomatal conductance, chlorophylls content and Fv/Fm ratio by about 19, 12, 20, 5 and 7%, respectively, compared to optimum temperature. Heat stress conditions significantly increased flower and pod abscission and decreased pod-set (by 22%) when compared to optimum temperatures. However, foliar spray of 1-MCP improved gas exchange, chlorophylls content and Fv/Fm ratio and decreased pod abortion compared to unsprayed control under heat stress conditions. These preliminary results suggest that application of 1-MCP can decrease rate of leaf senescence and increase pod-set under heat stress conditions in soybean.

Ethylene Synthesis and Sensitivity: Whole Plant Studies in Controlled Environments

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Sealed plant growth chambers have long been used in plant physiological studies to quantify photosynthesis and transpiration via gas exchange. We used extremely well sealed chambers, coupled with thermal desorption and GC analysis of ethylene, to continuously quantify ethylene synthesis and sensitivity of intact plants. Our findings indicate that ethylene is 10,000 times more toxic to plants than carbon monoxide is to people. In spite of the extensive literature on ethylene, we still have a limited understanding of the synthesis rates throughout the plant life cycle. We also have a poor understanding of the sensitivity of intact, rapidly growing plants to atmospheric ethylene. We know ethylene synthesis and sensitivity are influenced by biotic and abiotic stresses but whole plant responses have not been accurately quantified. The per-plant ethylene synthesis rate ranged from 0.1 to 80 pmol $plant^{-1}$ s^{-1}. However, when normalized to net photosynthetic rate, this range was 1 to 4 μmol of ethylene synthesis per mol of CO2 uptake. There was a twenty fold increase in ethylene synthesis near the end of the photoperiod for several species. Surprisingly, ethylene synthesis was reduced during drought stress. Blocking the perception of ethylene with 1-MCP did not increase ethylene synthesis.

Identification of genes involved in aerenchyma formation induced by ethylene in maize

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Ethylene is a plant stress hormone, and is rapidly synthesized as a signal to stresses such as flooding. Aerenchyma is a tissue that contains intercellular spaces which enhance flooding tolerance in some plant species by facililating gas diffusion between roots and shoots. In maize roots, aerenchyma is formed by the programmed death of cells in the mid cortex. It is initiated specifically by ethylene, whether produced endogenously or applied exogenously. In the present study we used a microarray to identify genes that are involved in aerenchyma formation and that are induced by ethylene. To identify the appropriate time to harvest RNA, we grew 3-day-old aerobically grown maize (*Zea mays*, inbred line B73) seedlings under four conditions: under aerobic conditions with and without ethylene treatment, and under waterlogged conditions with and without pretreatment with 1-MCP, an inhibitor of ethylene perception. Cross sections of roots were prepared after 0, 6, 12, 18 and 24 hours of growth, and aerenchyma formation was measured using the software ImageJ. Under aerobic conditions aerenchyma formation started 6 hours after application of ethylene. On the other hand, aerenchyma formation started 18 hours after the start of waterlogging treatment, but not when the seedlings were pretreated with 1-MCP. Accordingly, for the microarray experiment, roots of 3-day-old aerobically-grown seedlings were grown for 6 hours under waterlogged conditions with and without the pre-treatment of 1-MCP. RNA was prepared from root cortex tissues by laser microdissection, amplified and used to prepare a 15K cDNA microarray. Thirty-six genes were identified as potentially involved in aerenchyma formation and are currently being characterized.

The role of group VII ethylene response factor (ERF) genes in the contrasting flooding responses of two *Rumex* species.

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Floods are an inherent natural event in many ecosystems worldwide, but flooding frequencies are predicted to increase in the near future. Since complete submergence of plants severely impairs their energy acquisition, plants have developed adaptive strategies to cope with submergence and these can be classified into two syndromes. The first is a submergence escape syndrome where rapid stem and leaf elongation towards the water surface helps a plant escape submerged conditions. The second syndrome is a quiescence strategy which involves reductions in growth and metabolism. Recent studies in rice have characterized two master regulators, called *SUB1A* and *SUB1C*, which together determine the adaptive strategy followed by rice during submergence. These genes are members of subgroup VII of the Ethylene Responsive Factor (ERF) family of transcription factors. *SUB1C* enhances elongation growth and starch breakdown for carbohydrate mobilisation, whereas *SUB1A* - which is unique to a subset of rice cultivars - inhibits this escape strategy by reducing *SUB1C* expression. We have identified genes from the same subgroup VII *ERF* family in the dicot species (*Rumex palustris, Rumex acetosa*) that are models for ecophysiological variation in submergence responses. While *R. palustris* responds to flooding with the escape strategy, *R. acetosa* maintains a quiescent state during submergence. We hypothesize that subgroup VII *ERFs* act as a master switch to determine the adaptive strategy that is followed during submergence. However, the current state of knowledge on *SUB1* regulation is entirely based on domesticated rice. We have investigated the differential regulation of these SUB1-like genes in the two species of *Rumex*. This yields some insight into how natural selection has acted on an evolutionarily conserved member of the ERF transcription factor family to control submergence response.

Regulation of the expression of ethylene biosynthesis genes in *Hevea brasiliensis*

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Ethephon, an ethylene generator, is applied to the bark of rubber trees to increase natural rubber production by stimulating both latex flow and regeneration. A good command of ethephon concentrations and its frequency of application is required to avoid cell an oxidative burst resulting in tapping panel dryness (TPD) and leading to a loss of production. Although a little is known about the molecular response to ethylene stimulation further studies on ethylene biosynthesis and its regulation were needed to gain a better understanding of the mechanisms involved in latex production. Several genes involved in the ethylene biosynthesis were previously isolated. In this study, we have monitored in bark tissues of juvenile budded plants of three *Hevea* clones with contrasting metabolism the expression of eight genes by real-time RT-PCR. *SAMS, ACS1, ACS-F3* and *ACS-F10* transcripts were dramatically accumulated after ethylene application in clone PB 260 when clones RRIM 600 and PB 217 did not respond to the stimulation. This effect is transient for *ACS* genes. The expression of *ACO1*, *ACO2*, *ACO3* and β*CAS* genes was very slightly modified upon ethylene treatment whatever the clone. The clone PB 260 is known to be susceptible to ethephon and leads to TPD. Our data suggests that an exogenous supply of ethylene or ethephon triggers endogenous ethylene production *via* autocatalytic reactions in this clone, and response to ethylene may provoke early senescence in latex cells.

Iron deficiency up-regulates genes involved in both ethylene synthesis and signaling

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In *Arabidopsis* (Strategy I plant), Fe deficiency up-regulates several genes involved in Fe acquisition, like the ferric reductase gene (*FRO2*), the Fe(II) transporter gene (*IRT1*) and the FIT transcription factor gene (*FIT*), which is necessary for the activation of both *FRO2* and *IRT1*. Several years ago, Romera *et al.* (1999; Ann. Bot. 83:51) found enhanced ethylene production by roots of Fe-deficient Strategy I plants. More recently, Lucena *et al.* (2006; J. Exp. Bot. 57: 4145) presented evidence suggesting a role for ethylene in the up-regulation of *FIT* and, consequently, of *FRO2* and *IRT1*. In this work we have studied whether or not Fe deficiency affects genes involved in ethylene synthesis (*ACS4*, *ACS6*, *ACS9*, *ACO1* and *ACO2*) and signaling (*ETR1*, *CTR1*, *EIN2* and *EIN3*). For this, *Arabidopsis thaliana* cv Columbia plants were grown in nutrient solution with or without Fe, and roots were collected to later analyse gene expression by RT-PCR. In additional experiments, some Fe-sufficient plants were treated with ethylene in their roots and then collected to analyse gene expression. The results obtained show that Fe deficiency up-regulates the expression of genes involved in ethylene synthesis (*ACS4*, *ACS6*, *ACS9*, *ACO1* and *ACO2*) and signaling (*ETR1*, *CTR1*, *EIN2* and *EIN3*) in the roots. On the other hand, the application of ethylene to Fe-sufficient plants also enhanced the expression of the signaling genes above mentioned. The results obtained on the up-regulation of ethylene synthesis genes could explain the higher ethylene production of Fe-deficient roots showed by Romera *et al.* (1999). In addition, the results obtained uncover new possibilities in ethylene signaling: the responses of Fe-deficient roots to ethylene would not rely only on ethylene concentration but also on modifications of the number of ethylene signaling proteins, partly caused by ethylene itself.

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Asking the plant about "stress"

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Stress, like beauty, is often "in the eye of the beholder", and yet plants, as beholders, are rarely asked by biologists what is stressful (or beautiful) and what is not. This talk will address the subjectivity of defining stress and place stress signaling in the context of phenotypic plasticity and "priming" and emphasize the many markers of stress (from phytohormones to transcripts that are elicited by biotic and abiotic challenges) that can be used to "ask the plant" about a definition of stress. The talk will also emphasize what we have learned from asking *Nicotiana attenuata* what is stressful in its environment.

Ethylene biosynthesis inhibition by strobilurin fungicides

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Strobilurin fungicides have been reported to inhibit ethylene biosynthesis in some plants. However, the nature of the observed inhibition is still not clear: the molecular target has not been conclusively identified, and published studies have focused on just two fungicides: kresoxim-methyl and pyraclostrobin. We have determined the relative ethylene biosynthetic inhibition of several representative stobilurins and have found that they differ widely in their ability to inhibit ethylene production. In the mung bean hypocotyl assay, inhibition of ethylene biosynthesis was greatest with kresoxim-methyl. Kresoxim-methyl inhibited auxin-induced ethylene with an IC50 of 45 μM, while aminoethoxyvinylglycine had an IC50 of 3 μM. Moreover, our data show that strobilurins preferentially inhibit ACC oxidase, the terminal enzyme in ethylene biosynthesis, not ACC synthase, as Grossmann suggested (Pest. Sci. 50:11). In the cotton cotyledon assay, both azoxystrobin and kresoxim-methyl inhibited conversion of ACC to ethylene with an IC50 of about 60 μM. The structure-activity relationship of strobilurins with regard to ethylene may be important, considering the recent interest in strobilurins for plant health promotion.

Ethylene regulates photosynthesis through alterations in nonphotochemical quenching

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When the capacity of photosynthesis to use absorbed light energy is exceeded, the excess absorbed light energy can result in the production of reactive oxygen species (ROS). ROS can damage photosystem II (PSII), resulting in damage to the reaction centers and inhibition of mechanisms to repair them. Non-photochemical quenching (NPQ) is induced in response to excess light to reduce the generation of ROS. NPQ processes include thermal dissipation of excitation energy as mediated through the Xanthophyll cycle (qE), photoinhibition (qI), and state transitions (qT). Under normal growth conditions, qE predominates, dissipating excess excitation energy as heat to prevent photoinhibition and damage to the photosynthetic apparatus. Thus, NPQ plays a key role in regulating light harvesting and photosynthetic performance. Given the competitive nature of NPQ and photochemistry, the induction of NPQ results in a reduction in photosynthetic activity. We have examined the role of ethylene in regulating photosynthesis. Arabidopsis mutants *eto1* (increased ethylene production) and *ctr1* (constitutive ethylene signaling) exhibit reduced levels of photochemistry but elevated levels of NPQ. The over induction of NPQ in the *eto1* mutant can be corrected through treatment with 1-MCP, a potent inhibitor of ethylene perception. Despite an elevated induction of NPQ, functioning of the Xanthophyll cycle was substantially impaired in the *ctr1* mutant, specifically in the expression of enzymes required for the cycle. Consequently, the *ctr1* mutant is unable to generate zeaxanthin that is required for the induction of the qE component of NPQ. *ctr1* plants are sensitive to high light stress and experience an elevated level of photoinhibition which likely accounts for the high level of NPQ observed in plants with elevated ethylene signaling. Our results reveal for the first time that ethylene regulates a specific and integral process involved in controlling photosynthetic activity.

Ethylene interacts with auxin in response to phosphate deficiency in white clover

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Phosphate (Pi) supply is one of the major limiting factors to plant productivity, since it affects growth and development directly. In response to Pi deficiency, plants have been shown to alter root system architecture, such as adjusting primary root (PR) lengths, lateral root (LR) lengths and LR density. In this project, therefore, the responses of roots of the agronomically important legume, white clover (*Trifolium repens* L.), are examined in response to Pi deficiency, particularly with respect to the interaction of the plant hormones, auxin and ethylene. In response to Pi deficiency, white clover roots slightly increased the length of the PR, the production of LRs with a proposed increase in the sensitivity to ethylene. Ethylene was shown to be one of the major determinants of white clover root growth because a low concentration (100 nM) of 1-aminocyclopropane-1-carboxylic acid (ACC) stimulated the root growth whereas 300 ppm 1-methylcyclopropene (1-MCP) inhibited root growth. For auxin, a low concentration (5 nM) of 1-naphthylacetic acid (1-NAA) was stimulatory and 100 nM 1-N-naphthylphthalamic acid (1- PA) was inhibitory to root growth. To support the role of ethylene and auxin, a GUS reporter gene driven by an auxin responsive promoter (DR5::GUS), was transformed into white clover and it was observed that both Pi deficiency and ACC treatment caused an increase in DR5::GUS expression and thus an increase in auxin responses. In terms of ethylene biosynthesis in response to Pi-deficiency, an initial response is the induction of a specific isoform of ACC oxidase, designated *Trifolium repens ACC oxidase 1 (TR-ACO1),* with *TR-ACO1p::GFP* transformants displaying expression in the developing lateral root primordia in response to Pi deficiency.

Struggling for light: Regulation of plant-plant interactions

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Plants growing in dense vegetations interact in various ways and compete with proximate neighbors for light. They can ensure growth and survival through an escape syndrome known as shade avoidance. Upon perception of neighbors plants elongate their shoots and move their leaves upwards. Neighbor detection can occur through spectral changes in the light reflected from or transmitted through neighboring vegetation. Red light (R) is absorbed for photosynthesis whereas far-red light (FR) is reflected, thus lowering the R:FR ratio which can be sensed by the phytochrome photoreceptors. We showed recently that plant neighbor detection is, however, more complex and involves several other signals. Among these appeared to be a chemical one: the volatile plant hormone ethylene. Ethylene was shown to accumulate within the atmosphere of dense, greenhouse-grown tobacco stands. The levels found in these stands were sufficiently elevated to induce shade avoidance responses in isolated plants. Furthermore, transgenic ethylene insensitive genotypes showed delayed shade avoidance responses to neighbors and performed inferior to wild type in competition trials. Subsequent studies on Arabidopsis have unraveled more of the regulatory pathways through which ethylene acts during light-mediated shade avoidance responses, including interactions with auxin and gibberellins. The complexity of above-ground plant neighbor detection as well as the mechanisms regulating the adaptive responses will be discussed.

Herbivore-induced ethylene primes a direct defense in ethylene-deficient neighbors.

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Interplant communication via volatile organic compounds (VOCs) has been shown to induce or prime defense responses against pathogens and insects in the information receiving plant. Until now the observation of this phenomenon is variable and its relevance questionable. Characterization of the VOC perception and the identification of the specific VOC receptors are necessary to provide the molecular tools to test the ecological relevance of plant-plant communication. Ethylene, for which the perception and signal transduction are well known, is a small VOC that has been implicated to function in plant-plant communication. We used genetically transformed *Nicotiana attenuata* plants silenced for their ethylene biosynthesis (ir-*aco*) to investigate ethylene's potential in herbivore-related plant-plant signaling. In *N. attenuata* plants intra-species plant-to-plant priming has not been demonstrated, although *N. attenuata* is capable of priming its defense responses when transplanted adjacent to clipped *Artemisia tridentata*. Interestingly, ir-*aco* plants downwind of herbivore-induced conspecific wild type (WT) plants responded by priming of their proteinase inhibitor (PI) activity. This priming response could neither be observed in ir-*aco* plants exposed to herbivore-induced volatiles of ir-*aco*, nor in WT plants exposed to volatiles originating from herbivore-induced WT plants. Further results suggest that ir-*aco* plants are more sensitive to ethylene than WT plants. In other words, *N. attenuata* plants adjust their ethylene sensitivity according to their own endogenous ethylene concentrations. As constitutive and herbivore-induced VOC emissions are influenced by a variety of abiotic factors, such as nutrient availability, temperature, wind, radiation, and ozone exposure, we hypothesize that plants only respond to external VOCs if the compounds concentration is well above their internal levels and thus, that the environmental condition of a plant may determine whether or not the plant will be receptive to VOC from its neighbors.

#1 Christopher B. Cerveny, Cornell University, USA
Ethylene Inhibits Root Growth and Promotes Flower Abortion in Hydroponic Tulips

#2 Jianhong Chang, University of Maryland, USA
Investigation of RTE1 molecular function in Arabidopsis

#3 Stephen Deslauriers, University of California, USA
A receptor like kinaseis a regulator of the ethylene signaling pathway.

#4 Tang Dongqin, Shanghai JiaoTong University, China
Cloning and Characterization Of a new ACO gene LlACO1 From Lilium longiflorum

#5 Antonio Ferrante, University of Pisa, Italy
Role of ethylene in the lignification of stem in gerbera flowers

#6 Jamil Harb, Birzeit University, Palestine
Understanding how 'Honeycrisp' apples maintain crispness by elucidating molecular mechanisms involved in softening and ethylene production

#7 Maria. S. Hernández, Universidad Nacional de Colombia, Colombia
Physiological behavior and quality during growth of purple passion fruit

#8 Maria. S. Hernández, Universidad Nacional de Colombia, Colombia
Copoazu fruit postharvest behaviour during low temperature storage

#9 Ray J. Hoobler, Purfresh, Inc., USA
Ethylene mitigation by controlled ozone injection: kinetic modeling and applications

#10 Hannah James, Cornell University, USA
Flesh browning and 1-Methylcyclopropene (1-MCP) in the 'Empire' apple

#11 Manuel Jamilena, Universidad de Almería, Spain
Two CTR1-like genes in Cucurbita pepo are differentially regulated during female and male flower development and in response to ethylene

#12 Manuel Jamilena, Universidad de Almería, Spain
Regulation of ethylene-related genes during flower development in two Cucurbita pepo lines differing in sexual expression and ethylene sensitivity

#13 Seok-Kyu Jung, Cornell University, USA
1-Methylcyclopropene (1-MCP) effects on cluster tomatoes

#14 Y. Kubo, Okayama University, Japan
Functional screen for the secretomes associated with fruit softening in pear

#15 D. Mbéguié-A-Mbéguié, CIRAD, UMR QUALITROP, France
Transcriptional regulation of Banana EIN3-like genes expression in fruit

#16 Eric G. Mworia, Okayama University, Japan
Characterization of ethylene regulation and softening during fruit ripening in kiwifruit, Actinidia chinensis 'Sanuki Gold'

#17 Sangeeta Negi, Wake Forest University, USA
Ethylene negatively regulates lateral root formation in tomato.

#18 Aluh Nikmatullah, Massey University, New Zealand
Differential expression of TR-ACO1 and TR-ACO2 during water deficit in the leaves of white clover (Trifolium repens L.)

#19 L.M. Robles, University of California, USA
Characterization of the enhanced ethylene response 6 mutant in Arabidopsis.

#20 David Rudell, USDA-ARS, USA
Ethylene insensitivity alters ripening-associated metabolomic changes in apple peel

#21 Imanishi Shunsuke, National Institute of Vegetable and Tea Science, Japan
Monitoring the Expression of Ethylene Related Genes in Tomato Mutant by Microarray

#22 Harpaz-Saad Smadar, University of North Carolina, USA
Post-translational regulation of the turn-over of different types of ACC Synthase.

#23 Gerardo J. Suazo Jiménez, Cornell University, USA
Ethylene production by Fusarium oxysporum f.sp. tulipae in 42 tulip cultivars

#24 Poornima Sukumar, Wake Forest University, USA
Ethylene-auxin cross talk regulates adventitious root formation in Arabidopsis and Tomato

#25 Derek M. Thibault, Dartmouth College, USA
Characterization of the ETHYLENE FEEDBACK MEDIATOR (EFM) Gene Family of Arabidopsis

#26 Trivellini A, Università degli Studi di Pisa, Italy
Spatial temporal gene expression of ethylene biosynthesis enzymes and receptors in Hibiscus rosa-sinensis L. flowers

#27 Bruce D. Whitaker, USDA, USA
Ethylene up-regulates α-farnesene synthase gene AFS1 in scald-susceptible pome fruits

#28 Tianbao Yang, USDA-ARS, USA
Perspective of utilizing ethylene-responsive SR/CAMTA for postharvest improvement

Ethylene Inhibits Root Growth and Promotes Flower Abortion in Hydroponic Tulips

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Ethylene effects were investigated on two hydroponically grown tulip cultivars, Markant and Carreria. Pre-cooled bulbs were treated with ethylene (flow-through) at nominal 0, 0.1, 1.0, or 10 $\mu L \cdot L^{-1}$ in a modified hydroponic system. Tulips were exposed to ethylene for one week and then were either destructively harvested for root measurements or forced in a greenhouse for flower measurements. Ethylene exposure as low as 1 $\mu L \cdot L^{-1}$ in the earliest stages of growth reduced shoot and root elongation and subsequently increased flower bud abortion. At 10 $\mu L \cdot L^{-1}$, root growth was essentially eliminated. When bulbs were treated with 1-MCP prior to a one week ethylene treatment at 1.0 $\mu L \cdot L^{-1}$, the harmful effects associated with ethylene exposure were eliminated. This study further illustrates the deleterious effects of ethylene exposure on tulip, and demonstrates a potential benefit to treating bulbs with 1-MCP prior to planting.

Investigation of RTE1 molecular function in Arabidopsis

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In Arabidopsis, ethylene is perceived by a family of five receptors, one of which is ETR1. The Arabidopsis *REVERSION-TO-ETHYLENE SENSITIVITY1* (*RTE1*) gene is a positive regulator of *ETR1* that was identified in a genetic screen for suppressors of the dominant ethylene-insensitive mutation *etr1-2*. *RTE1* encodes a novel integral membrane protein of unknown function in plants, animals and some protists. The RTE1 protein co-localizes with ETR1 at the Golgi apparatus and the endoplasmic reticulum in Arabidopsis. The molecular mechanism by which RTE1 regulates ETR1 remains unknown. We propose that RTE1 may affect the conformation of the ETR1 ethylene binding domain (EBD), resulting in the promotion or stabilization of the ETR1 signaling "on" state. RTE1 may play a direct role in the folding of the ETR1 EBD or affect the ETR1 EBD through regulating the membrane environment of the ETR1 receptor. We took different approaches to gain some insights into the molecular function of RTE1 and the basis for the specificity for regulating ETR1. By screening for RTE1 interacting proteins in a yeast split-ubiquitin assay, we identified a non-specific lipid transfer protein (ns-LTP) and an ER-localized cytochrome *b5* isoform (AtCb5-3). The ns-LTP and AtCb5-3 proteins possibly affect membrane composition and fluidity respectively, which would be compatible with our hypothesis that RTE1 may play a role in conformational changes of the ETR1 N-terminal transmembrane region that regulate C-terminal signaling output. Recently, we found that human RTE1 is able to bind heme in a hemin-agarose affinity chromatography assay. It is worth noting that the GAF domain in phytochromes binds a tetrapyrrole chromophore, suggesting that the ethylene receptor GAF domain might similarly bind a tetrapyrrole molecule, e.g. heme. The role of heme in RTE1 and/or ETR1 function is currently under investigation.

A receptor like kinaseis a regulator of the ethylene signaling pathway.

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Ethylene signaling plays many crucial roles in plant development and defense, yet questions remain about how the signal is transduced including how the pathway is reset following a signaling event. In order to gain a better understanding of the pathway, mutagenized Arabidopsis seeds were screened for lines that displayed an *enhanced ethylene response* (*eer*) phenotype in relation to the seedling triple response. This has led to the isolation of *eer7*, which displays a profoundly heightened sensitivity to ethylene compared to Col-0 wt. In support of this, Northern analysis has revealed differential expression of ethylene-inducible genes when comparing Col-0 wt and *eer7*, including enhanced expression of both *basic chitinase* and *PDF1.2* in ethylene treated *eer7* leaves. Utilizing a map-based cloning approach, the *eer7* mutation was localized to a gene encoding a receptor-like kinase. An allelic mutation as well as functional complementation of *eer7* confirmed that mutation of this kinase results in the observed *eer* phenotype. Gene expression analysis also revealed that *EER7* expression is induced by ethylene. Double and triple mutant analyses suggest that EER7 acts at the same level or after CTR1 in the pathway, and is partially independent of EIN2. Work is underway regarding analysis of EIN2 protein levels following ethylene treatment to determine the effect of *eer7* on EIN2 stability. Additionally, work with an *ein3-1;eer7* double mutant, which has a phenotype nearly identical to *eer7* indicates that the *eer7* mutant phenotype is largely independent of EIN3 function. Based on our results, we propose that EER7 is a key factor in opposing ethylene response presumably through regulation of either transcription of a subset of ethylene inducible genes required for resetting this pathway or turnover of an existing unidentified group of protein factors responsible for manifestation of ethylene response.

Cloning and Characterization Of a new ACO gene *LlACO1* From *Lilium longiflorum*

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A new gene *LlACO1* (GenBank Acc. No. EU249333) encoding 1-aminocyclopropane-1-carboxylate oxidase was isolated from *Lilium longiflorum* Thunb. 'Snow Queen' by rapid amplification of cDNA ends (RACE). The full-length cDNA of *LlACO1* was 1,067 bp and contained a 954 bp open reading frame (ORF) encoding 317 amino acids. Sequence analysis indicated that the putative amino acid sequence had high identity with some other ACO proteins. On the protein level, LlACO1 was 86%, 74%, 73% and 72% identical to ACOs from *Tulipa gesneriana* (BAE20198), *Pelargonium hortorum* (AAC48977.1), *Petunia* × *hybrida* (QO8507) and *Fagus sylvatica* (CAD21844.1) respectively. Multi-alignment analysis showed that the deduced LlACO1 protein had several conserved fragments with other ACO proteins. The distorted double-stranded β helix (DSBH), which was close to the active site of ACO protein, was existed in LlACO1. The tertiary structure of LlACO1 also showed that the homology domain of LlACO1, being a functional domain as an oxygenase, was located in the core area and most of β strand formed the DSBH, implying that LlACO1 belonged to the super family of oxygenases and oxidases. Tissue specific expression analysis indicated that *LlACO1* expressed in all the tested tissues including leaves, stems and roots.

Role of ethylene in the lignification of stem in gerbera flowers

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The vase life of cut gerbera flowers is often limited by the stem break that occurs within 10 cm below the capitulum. During vase life the stem with progressive weight losses is not able to hold the flower head and bends until to break. The ethylene and its precursor may be involved in stem lignification through the phenylpropanoid pathway. Cut gerbera flowers were treated with ethylene precursors and lignin biosynthesis was studied. Treatments were applied for 24 h using 10, 100 and 1000 μM ACC. Stem elongation and lignification were monitored in control and treatments. Lignin content was measured in the last 10 cm below the capitulum. Phenylalanine ammonia lyase enzyme (PAL) activity and gene expression were monitored and stem break occurrence was determined.

Understanding how 'Honeycrisp' apples maintain crispness by elucidating molecular mechanisms involved in softening and ethylene production

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The 'Honeycrisp' (HC) apple is new cultivar that has become very popular due to its flavor and beneficial texture characteristics. A unique feature of the apple is that it maintains firmness over extended periods during postharvest storage, unlike other cultivars that soften rapidly. Our objective is to elucidate the biochemical changes and molecular mechanisms that are responsible for the slow softening of HC compared to a rapidly softening cultivar, 'McIntosh' (MC). Fruits from both cultivars were picked during the normal harvest period and stored at 20°C for two weeks. Internal ethylene concentrations (IECs) and flesh firmness of 5 fruit were measured every 3 days and cortical tissue frozen in liquid nitrogen for further analysis. HC fruit had lower IECs, both at harvest and during storage at 20°C than MC fruit. Further, MC fruit lost more than 33% of their initial firmness values, compared to HC fruit which remained almost as firm as at harvest. Quantitative real time RT-PCR (qPCR) was carried out using primers designed for genes involved in ethylene biosynthesis, perception, and signal transduction (ACC synthase (*MdACS*), ACC oxidase (*MdACO*), ethylene receptor (*MdETR* and *MdERS*), Ethylene response factor (*MdERF*)), as well as cell wall degradation (polygalacturonase (*MdPG*), xyloglucan endotransglucosylase (*MdXTH*), expansin (*MdEXP*), β-galactosidase (*MdBGal*), and arabinofuranosidase (*MdAFase*)), The expression of *MdACO1*, *MdACO2*, *MdACS1*, *MdACS3*, *MdERS1*, *MdETR1*, *MdETR1-2*, *MdERF1*, *MdERF2*, and *MdBGal* genes in HC fruit was either higher than or equivalent to MC at all sampling times . The expression of *MdACO* and *MdACS* genes at harvest and early during storage was much higher in HC than in MC fruits. However, the expression of *MdAFase*, *MdPG*, and *MdEXP3* genes was significantly higher at most sampling times, in MC than in HC fruits. The levels of the ethylene precursor ACC, as well as free uronic acids are currently being investigated in this sample set. Our results will be presented and discussed.

Physiological behavior and quality during growth of purple passion fruit

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Purple passion fruit (*Passiflora edulis* flavicarpa Sims.)is a south American drupe from the *Passifloraceae* family. widely grown in tropical and subtropical climates. Fruit harvest maturity is a very important issue the fruit is a promissory fruit to be exported from Colombia. This work was carried out in order to establish some growth pattern and indexes to assist fruit harvesting. The period between fruit set and full ripening of purple passion fruit grown in the area of production of Colombia was 90±5 days. Growth pattern exhibited a classical sigmoid curve with three stages. The longitudinal and equatorial traits of the fruit fitted a logistic model that were identified at tissue level as follows: S1, involving cellular division and expansion during the first 30 days of growth; S2, maximum fruit growth, during which cellular expansion took place (56 days more), and a final S3 state of 7days more to reach physiological maturity. After this time, the fruit can be harvested when fruit reached 20% of purple skin color. The climacteric respiratory pattern at 20ºC of purple passion´ during development was characterized by high respiration rates after detaching the fruit (3824 nmol kg^{-1} s^{-1} of CO2) concomitant with a peak of ethylene production (14,40 $nmol.kg^{-1}.s^{-1}$.). A total soluble solids value of 17 oBrix, matched with an increase in sugars and ascorbic acid content, and a decrease in titratable acidity. Citric acid was the main organic acid in the edible pulp and ascorbic acid was present in a moderate concentration (11 g per 100 g fresh pulp). The days after fruit set combined with skin color and sugar content can be recommended as harvest indices for purple passion fruit.

Copoazu fruit postharvest behaviour during low temperature storage

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Copoazú is an ellipsoid drupe from the Amazonian region with high ascorbic acid content. As many other tropical fruits such as arazá and guava, copoazú suffers quality losses particularly during low temperature storage due to chilling injury and associated decay. To test the chilling sensitivity of copoazu fruit, they were harvested in ripe stage and stored at 6ºC or 12ºC (90% RH in both cases) and 20ºC and 75% RH as reference. Copoazú fruit showed a climacteric ripening pattern at 20ºC accompanied by a peak of ethylene production, with high levels of both activities. Weight loss and decay were the most limiting quality traits for copoazú fruit during storage particularly in treatments with high levels and early development of the typical climacteric peak of ethylene production. Copoazú developed chilling injury symptoms during storage such as flesh uneven ripening (e.g. no flesh color changes), severe flesh softening and decreased levels in ascorbic acid and sugars, particularly at 6ºC. Decay in the post-storage shelf
life periods was particularly noticeable after storage at 6ºC in copoazú fruits. The storage of copoazú fruit at 12ºC is recommended because this temperature kept better fruit quality by preventing chilling injury and delaying ripening .The storage at 12ºC also reduced ascorbic acid losses and allowed normal fruit ripening during a poststorage shelf-life at 20ºC.

Ethylene mitigation by controlled ozone injection: kinetic modeling and applications

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In the commercial produce industry, ethylene mitigation is primarily accomplished via cold storage where reduced temperatures reduce fruit respiration and ethylene production. The cascade effect of the ethylene hormone increases ripening and decreases the time available for shipment and storage of climacteric fruit. The use of ethylene inhibitors such as 1-MCP for climacteric fruit can further reduce ethylene production; however, there are reports that these negatively impact fruit taste and aroma. Also, some cultivars are not compatible with standard treatments and synthetic chemical inhibitors are not an option for organic food production. The use of ethylene absorbers, such as potassium permanganate and ionic air scrubbers in theory is effective but in practice is limited by the number of air exchanges possible in large commercial facilities; they also require replacement/disposal when spent and degradation of the absorber material may occur over time. We have implemented a controlled ozone injection system to maintain low ethylene levels for both shipping containers and storage facilities. A kinetic reaction and air exchange model has been developed to analyze ozone demand requirements and provide guidance in developing appropriate ozone control systems. Methodology has been developed to measure ethylene levels in actual shipping containers and commercial cold storage facilities as low as 10 ppb. Laboratory and large scale testing show that controlled ozone injection systems can maintain lower ethylene levels and thus retard the ripening of climacteric fruit.

Flesh browning and 1-Methylcyclopropene (1-MCP) in the 'Empire' apple

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The effects of several factors on the development of flesh browning (FB) in 'Empire' apples (*Malus x domestica* Borkh.) with and without 1-MCP application were investigated. These factors included harvest maturity, pre-harvest application of ReTain™ (aminoethoxyvinylglycine) or Harvista™ (1-MCP), CO2 partial pressure, storage temperature, DPA and calcium application, and delays between harvest and establishment of controlled atmosphere (CA) and application of 1-MCP. When fruit were stored at 0.5 ºC in air or at 1.6 ºC in 2 kPa O2 with 1, 2.5 or 5 kPa CO2, the incidence of FB was not consistently different between control, ReTain™ or Harvista™, with or without 1-MCP application. Treatments that were designed to reduce the incidence of FB during storage were not successful if the fruit had been treated with 1-MCP. Fruit stored at 3.3 ºC had a significantly lower incidence of FB (3.0%) than those stored at 0.5 (23.8%) or 1.6 ºC (22.6%). However, this effect was lost with the application of 1-MCP where the incidence of FB at 3.3 ºC increased to 51.6%. Similarly, delaying the application of CA saw a significantly lower incidence of FB (7.6%) than no delay (50.0%). However, when fruit were treated with 1-MCP, there was no significant difference in the incidence of FB between delay CA (52.3%) and control fruit (45.3%). The use of 1-MCP was found to increase the incidence of FB when fruit were treated with DPA, calcium and a combination of DPA and calcium. Delaying the application of 1-MCP to four (47.4%) or eight (26.1%) months reduced the incidence of FB compared to those treated at harvest (55.2%). The data from this study indicate that the beneficial effects of storing 'Empire' apples at 3.3 oC, delaying the establishment of CA and treating the fruit with DPA and/or calcium on reducing the incidence of FB are lost when 1-MCP is applied at harvest.

Two *CTR1*-like genes in *Cucurbita pepo* are differentially regulated during female and male flower development and in response to ethylene

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Ethylene is an essential regulator of flower development in *Cucurbita pepo*. It participates in the differential development of male and female flowers, and in the maturation, senescence and abscission of floral organs. We have isolated two *CTR1*-like genes from the genome of *C. pepo*, *CpCTR1* and *CpCTR2*, and analysed their expression patterns during female and male flower development and in response to ethylene. The proteins encoded by *CpCTR1* and *CpCTR2* share the highest homology with CTR1-like protein kinases in *Arabidopsis* and other species, but they are quite different from other related protein kinases such as *Arabidopsis* EDR1 and tomato LeCTR2. The C-terminal ends of both CpCTR1 and CpCTR2 conserve all the motifs of CTR1-like ser/thr kinase domains, which suggest that they could both function as regulators of ethylene signalling. In accordance with this general function, the transcripts of both genes were detected in different organs of the plant, including roots, leaves, shoots and shoot apices, but were mostly accumulated in mature flowers. A detailed gene expression analysis during female and male flower development has indicated that *CpCTR1* and *CpCTR2* could regulate different ethylene responses. The expression of *CpCTR2* is induced in mature male and female flowers, when ethylene production is also higher. This suggests that this gene must not participate in the sexual differentiation of male and female flowers, a process that has to be initiated much earlier in floral buds, but it could be involved in the maturation and abscission of floral organs. The expression of *CpCTR1*, however, is more constitutive, accumulating its transcript in all floral organs from the earliest stages of flower development. Given that the transcription of *CpCTR1* is up-regulated by ethylene in male, but not in female flower buds, it is likely that the gene participates as a negative regulator of ethylene action during the sexual differentiation of male and female flowers.

Regulation of ethylene-related genes during flower development in two *Cucurbita pepo* lines differing in sexual expression and ethylene sensitivity

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Ethylene is the main regulator of sexual determination in *Cucurbita pepo*. We have identified two inbred lines, *Bolognesse* (*Bog*) and *Vegetable spaghetti* (*Veg*), whose contrasting sexual expression is associated with differences in ethylene sensitivity. In *Bog*, which is very sensitive to ethylene, the production of female flowers is very early and high, while in *Veg*, with a very low sensitivity to ethylene, the production of female flowers is very delayed and reduced. In this paper we compare the production of ethylene as well as the expression of genes involved in the biosynthesis, perception and signalling of ethylene throughout the development of male and female flowers in the two contrasting lines. Although the production of ethylene in seedling and leaves of *Bog* is similar or even less than in *Veg*, throughout the development of female flowers *Bog* produces much more ethylene than *Veg*. Concomitant with this ethylene production, the transcription level of the ethylene biosynthesis genes *CpACS* and *CpACO* is much higher in *Bog* than in *Veg* female flowers, but does not differ between the male flowers of the two lines. The transcription of the three ethylene receptor genes studied, *CpETR1*, *CpETR2* and *CpERS1*, and the two signalling genes, *CpCTR1* and *CpCTR2*, is also up-regulated in female flowers of the *Bog* line in comparison with those of the *Veg* line, particularly in the reproductive organs (pistil and ovary). In comparison with the *Veg* line, therefore, the earlier and higher production of female flowers in *Bog* is not only associated with a higher expression of ethylene biosynthesis genes *CpACS* and *CpACO* but also with an up-regulation of ethylene receptor and *CTR1*-like genes during the later stages of female flower development. Given that *Bog* is more sensitive to ethylene than *Veg*, these data indicate that the expression levels of ethylene receptor and *CTR1*-like genes, as negative regulators of ethylene response, are not inversely correlated with ethylene sensitivity, but seem to be up-regulated by ethylene during female flower maturation.

1-Methylcyclopropene (1-MCP) effects on cluster tomatoes

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The effects of the 1-methylcyclopropene (1-MCP) on inhibition of tomato fruit ripening has been well studied, but little is known about its effects on cluster, or tomato-on-thevine (TOV), cultivars. The fruit are inherently more variable in terms of maturity at the time of harvest and treatment, and the stem is an additional quality factor of importance. We have studied the effects of 1-MCP on tomato quality where fruit were harvested when the least mature fruit on the cluster was mature green, breaker or light red. 1-MCP at 500 ppb and 1000 ppb was applied for 4, 8 and 12 hour exposure periods. The results show that the most important factor for 1-MCP treatment was the maturity of the tomato cluster at the time of treatment, and the rates and exposure time were less critical. 1-MCP treatment is not recommended for mature green and breaker TOV clusters. However, the quality of light red clusters after 1-MCP treatment was better than that of untreated clusters as over-ripening was prevented for up to 18 days at 12 ºC. Thus, riper, more uniform fruit can be harvested and marketed successfully with 1-MCP treatment. The most significant effect of 1-MCP treatment, however, is the maintenance of vine tissue quality, and this benefit is related to its effect of reducing loss of water content of the vine.

Functional screen for the secretomes associated with fruit softening in pear

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Fruit softening is one of the biochemical processes that take place during fruit ripening and is a major factor that influences the shelf-life and fruit quality. The full complement of individual key determinants of fruit softening have not yet been identified, although it is recognized that cell wall degradation is fundamentally important. The characterization of the suite of cell wall modifying enzymes that are expressed in ripening fruit would therefore provide valuable mechanistic insights into fruit softening. European pear, 'La France', fruit softening is dependent on ethylene, as fruit undergo softening during ripening concomitant with ethylene biosynthesis, while 1-MCP, a potent inhibitor of ethylene perception interrupts softening. On the other hand, Chinese pear, 'Yali' fruit dose not soften during ripening despite high levels of ethylene production. We have used the yeast-based signal sequence trap (YSST), a relatively high throughput strategy for identification of eukaryotic secreted and cell surface proteins to clone putative cell wall-related genes from 'La France' fruit. A total of 175 unique genes was isolated, including those encoding polygalacturonase and expansin, which have previously been associated with cell wall modification,. Many of the clones had no homology to database sequences or any obvious features that might be used to predict function. Macroarray analysis was used to determine which of these genes are expressed in an ethylene-dependent manner in 'La France' or differentially between the 'La France' and non- softening 'Yali' cultivar.

Transcriptional regulation of Banana EIN3-like genes expression in fruit

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Ethylene signal transduction initiates with ethylene binding at receptor proteins and terminates in a transcription cascade involving the EIN3/EIL transcription factors. In order to get more insights into the ethylene responsiveness process of banana fruit, we have isolated from banana fruit four cDNA homologs of the Arabidopsis EIN3/EIN3-Like gene, *MaEILs* (*Musa acuminata* ethylene insensitive 3-like). Sequence comparison with other banana EIL genes already registered in the database led us to conclude that, at this day, at least 5 different genes namely *MaEIL1*, *MaEIL2/AB266318*, *MaEIL3/AB266319*, *MaEIL4/AB266320* and *AB266321* exist in banana. Expression of these genes were further analysed in peel and pulp tissues, in relationship with changes of fruit ethylene responsiveness and ripening processes. *MaEIL* mRNAs were detected in all examined tissues but at lower level in peel than in pulp. According to tissues, *MaEIL* genes were differentially regulated by ripening and ethylene in mature green fruit. *MaEIL2/AB266318* was the unique ripening- and ethylene-induced gene, *MaEIL1*, *MaEIL4/AB266320* and *AB266321* genes were down-regulated while *MA-EIL3/AB266319* presented an unusual pattern of expression. Interestingly, a marked change was observed mainly on *MaEIL1* and *MaEIL3/AB266319* mRNA accumulation, concomitantly with changes in ethylene responsiveness of fruit. Data presented in this study suggest the importance of a transcriptionally step control in the regulation of *EIL* genes during banana fruit ripening.

Characterization of ethylene regulation and softening during fruit ripening in kiwifruit, *Actinidia chinensis* 'Sanuki Gold'

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Ethylene biosynthesis and softening in kiwifruit, *Actinidia chinensis* 'Sanuki Gold' were characterized using propylene, ethylene analog, and 1-methylcyclopropene (1-MCP), inhibitor of ethylene perception. In fruits harvested early between 66 days after pollination (DAP) and 143 DAP, 2 days of exposure to 5000µl l^{-1} of propylene was enough to initiate ethylene biosynthesis while in fruits harvested at 154 DAP, 5 days of treatment were required to initiate ethylene production. This observation suggests that kiwifruit ethylene sensitivity decreases with fruit maturity. Propylene treatment up-regulated expression of *AC-ACO1, AC-ACO2, AC-SAM1*, *AC-SAM2, CK-PGC2, AC-XET5, AC-EXP2* and *AC-PL* prior to the induction of *AC-ACS1* and ethylene production confirming that *AC-ACS1* is the rate limiting step in kiwifruit ethylene biosynthesis. A single shot of 1-MCP treatment before continuous propylene treatment delayed the induction of ethylene production and *AC-ACS1* expression for 5 days. Treatment of fruits with more than 5µl l^{-1} of 1-MCP after the induction of ripening ethylene subsequently suppressed ethylene production and expression of ethylene biosynthesis and softening related genes successfully. These observations suggest that in 'Sanuki Gold', 1-MCP treatment would be useful in improving fruit shelf-life and that ethylene biosynthesis in ripening kiwifruit is regulated by positive feedback mechanism.

Ethylene negatively regulates lateral root formation in tomato.

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Lateral root branching is a genetically defined and environmentally regulated process. Auxin is required for lateral root formation and mutants altered in auxin synthesis, transport, or signaling often have lateral root defects. In Arabidopsis, cross talk between auxin and ethylene in lateral root formation has been demonstrated, but in tomato ethylene signaling studies are more focused on the role of ethylene in fruit ripening. We have explored the role of ethylene in lateral root formation in tomato ethylene signaling and synthesis mutants. We find that enhanced ethylene synthesis or signaling in the *epi* (Epinastic) mutant, or after ACC application, negatively impacts lateral root formation. In contrast, mutations that block ethylene responses *Nr* (Never ripe), *gr* (green ripe), *nor* (non ripening) and *rin* (ripening inhibitor) have enhanced root formation. The effect of ethylene on lateral root formation was lost in ethylene insensitive mutants. In cleared tomato roots, it is evident that ethylene inhibits initiation as well as elongation of lateral roots. Cleared *Nr* roots had greater numbers of primordia and emerged lateral roots than WT. We asked whether ACC alters auxin transport and find ACC treatments or the *epi* mutation positively regulate long distance acropetal IAA transport in tomato. *Nr* mutant has less auxin transport and transport is no longer regulated by ACC. In addition expression of DR5-GUS, an auxin reporter, was enhanced by ACC treatment, consistent with the ethylene positively regulating auxin transport. Currently we are investigating the possible involvement of ethylene signaling in a profuse branching mutant (*pbr*) which is defective in root formation. Our results show that ethylene has similar effects on root formation in two widely different species, Arabidopsis and tomato, revealing the vital role of ethylene signaling in root architecture. This work is supported by 2006-35304-17311 grant from USDA.

Differential expression of *TR-ACO1* and *TR-ACO2* during water deficit in the leaves of white clover (*Trifolium repens* L.)

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The committed steps in the ethylene biosynthetic pathway comprise the conversion of *S*-adenosyl-L-methionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS) and the oxidative cleavage of ACC by the enzyme ACC oxidase (ACO) into ethylene. Expression of the ACS and ACO multigene families is known to be developmentally regulated and responsive to environmental cues. However, their expression in response to water deficit is not that well characterised. In this study, two white clover varieties with differing sensitivity to water deficit, a drought-tolerant Tienshan and a drought-susceptible Kopu, were exposed to a water deficit in controlled atmosphere conditions by withholding water. Growth responses, the expression of white clover ACO genes, *TR-ACO1* and *TR-ACO2*, and the accumulation of the corresponding proteins were then examined. *TR-ACO1* is expressed predominantly in the apical structure of stolon, while *TR-ACO2* is expressed in newly-initiated leaves. For the treatments, plants were either exposed directly to the water deficit (designated non pre-stressed; NPS) or exposed to the water deficit, then re-watered and then re-exposed to the water deficit (designated pre-stressed; PS). As the water deficit progresses, a gradual increase in the *TR-ACO1* expression and protein accumulation was observed in the apical structures of both NPS- and PS-treated Tienshan and in the PS-treated Kopu. However, a discernable decline in expression and accumulation was observed in the NPS-treated Kopu. In the first fully-expanded leaves, the expression of *TR-ACO2* transcript and protein accumulation decreased as the water deficit progressed in both the NPS- and PS-treated Tienshan, and in the PS-treated Kopu. By contrast, in the NPS-treated Kopu, *TR-ACO2* expression and protein accumulation increased. These results suggest that a pre-stress treatment of the drought-susceptible Kopu may result in a degree of acclimation to the water deficit such that the responses become similar to those observed in the drought-tolerant Tienshan

Characterization of the *enhanced ethylene response 6* mutant in Arabidopsis.

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Ethylene is a gaseous plant hormone that regulates many important growth and developmental responses, including fruit ripening and tissue senescence. While significant progress has been made regarding how ethylene is perceived, little is known regarding how the ethylene-signaling pathway is dampened or reset following a signaling event. Several Arabidopsis mutants that have enhanced ethylene responses, including the previously undescribed *eer6*, have been isolated with these likely representing factors that are required to reset this pathway. Physiological analysis of *eer6* shows that ethylene treated *eer6* seedlings have the well-established characteristics of enhanced ethylene response including severe hypocotyl shortening with saturating ethylene. Molecular analysis of ethylene regulated gene expression in *eer6* seedlings and leaves reveals aberrant levels of *AtEBP*, *ACO2*, *chiB* and *PDF1.2* following ethylene treatment, suggesting that EER6 is required for proper transcriptional regulation including a suite of unknown genes involved in a proposed reset mechanism. As with other *eer* mutants such as *eer3* and *eer4*, loss of expression of genes involved in this reset mechanism would subsequently give rise to the exaggeration of response to ethylene seen in these *eer* mutants. Mapped based cloning revealed that the *eer6- 1* mutation results in an amino acid substitution in a factor required for mRNA processing. While this factor may not be directly related to ethylene signaling, it is likely that it is responsible for proper processing of transcripts that encode proteins essential for resetting the ethylene response pathway. Work to identify the targets of EER6 is in progress in order to begin to define the suite of genes that are required for proper dampening of the ethylene signaling pathway. In addition, genetic analysis including construction of double and triple mutants with other ethylene response mutants including *ctr1-3*, *ein2-5*, and *ein3-1* is underway.

Ethylene insensitivity alters ripening-associated metabolomic changes in apple peel

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Metabolomic changes were compared in untreated, diphenylamine treated, and 1-methylcyclopropene treated 'Granny Smith' apples stored for up to 6 months at 1 ºC in air. Metabolomic evaluation, including 600+ metabolites, was employed to characterize ripening-related metabolism. Partial Least Square Discriminant Analysis (PLS-DA) models revealed metabolomic differentiation based on treatment and storage duration. The 1-MCP treated fruit peel metabolome differentiated from the other treatments within the initial 2 weeks of storage and continued to do so until 6 months. The trajectory of metabolomic development in 1-MCP treated peel illustrated by the statistical model was different than both those of the untreated and DPA treated peel, which were both similar with respect to ripening-associated components of the model. Specific metabolites linked with increasing storage duration in 1-MCP treated peel included, but were not limited to, organic and amino acids. These finding demonstrate ethylene insensitivity does not halt but, instead, alters ripening during apple storage.

Monitoring the Expression of Ethylene Related Genes in Tomato Mutant by Microarray

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Fruit ripening is a complex, genetically programmed process. And it has been shown that many of ripening processes of climacteric fruit are regulated by ethylene. Despite the understanding of ethylene biosynthesis and perception, the mechanism regulating fruit ripening, including factors for the ethylene climacteric, remain obscure. Identifying the factors that control these processes is important for understanding the mechanisms of fruit ripening. Mutational approaches have been highly instrumental for the study of the genetic and molecular bases of traits. To obtain information on the genetic mechanism of fruit ripening, we induced mutations in the tomato cultivar 'Micro-Tom' by irradiation with gamma ray or accelerated heavy ions, and screened for associated phenotypes and tried to identify loss-of-function mutations in some genes that involve in the process. To obtain detailed information on the molecular mechanism, we designed an oligonucleotide-based microarray from whole set of tomato unigenes. The microarray consisted over 41,000 probe sets. Now we start to monitor the differences of gene expression level in the various stages of fruit ripening between mutagenized lines and wild type. We here report the expression profile of genes involving ethylene biosynthesis and signal transduction, and also will report the genes that co-express with these genes.
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Post-translational regulation of the turn-over of different types of ACC Synthase.

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ACC Synthase (ACS) catalyzes the committed, and in most cases, rate-limiting step in the pathway of ethylene biosynthesis. In *Arabidopsis thaliana* ACS is encoded by a small gene family composed of 9 genes. The different ACSs are divided to 3 types primarily based on the character of their C terminus. The C terminal domain of ACS was shown to play a central role in the post-translational regulation of ACS protein stability. In the current study we aim to follow the turn-over of the different ACS types in *Arabidopsis thaliana* expressed under a dexamethasone-induced promoter. The effect of numerous signals including: cytokinin, brassinosteroids and light on the turn-over of the different ACSs will be examined.

Ethylene production by *Fusarium oxysporum* f.sp. tulipae in 42 tulip cultivars

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Fusarium oxysporum f.sp. tulipae (F.o.t) is a soil fungus that causes the basal rot disease in tulip bulbs and also produces ethylene during the infection process. F.o.t. causes direct economic losses by killing bulbs due to infection, and indirectly as a result of ethylene effects on healthy bulbs and related physiological disorders and production problems. Ethylene evolution from *Fusarium* inoculated bulbs was measured for 28 days in 22 tulip cultivars and two bulb species. Maximal ethylene production was reached between 21 and 28 days post inoculation. Cultivars were grouped in three categories: 8 cultivars (33%) in the lowproducing rank (<0.5 ul·g-1FW·h-1); 13 cultivars (54%) in the medium rank (0.5-1.0 ul·g-1FW·h-1), and 3 cultivars (13%) were in the high ethylene rank (>1 ul·g-1FW·h-1). The ethylene variation between the lowest and the highest cultivars was 110 fold, and between the tulip species, the difference was 17 fold.

In a second experiment we inoculated eighteen cultivars from six tulip lineages, each with one to three generations of naturally occurring color mutants (sports) that are used in commercial forcing. Cultivars of the same lineage did not differ greatly from each other in the ethylene evolution caused by *Fusarium*. While the variation in ethylene production between the highest (Markant) and the lowest cultivars (Pink Star) was 11 fold, the difference between individuals of the same lineage only ranged from 1.2 to 2.2 fold.

These data will be useful in designing better storage and transportation protocols for tulip bulbs and could also prove useful for future tulip breeding efforts.

Ethylene-auxin cross talk regulates adventitious root formation in Arabidopsis and Tomato

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We examined the molecular mechanisms and hormonal controls of adventitious root formation in Arabidopsis and tomato. Tomato and Arabidopsis plants were grown under low light to elongate the hypocotyl and then transferred to high light. After seven days, adventitious roots form along intact hypocotyls, but removal of the root tissue from hypocotyls enhanced the frequency of root formation in both species. Ethylene insensitive mutants show altered adventitious root formation, with Arabidopsis *ein2-5* forming more adventitious roots than wild-type, while tomato *Neverripe* has fewer than wild-type. ACC treatment inhibits adventitious root formation in Arabidopsis, while in tomato enhances adventitious root formation. These results are consistent with ethylene oppositely regulating adventitious root formation in these two species. In contrast, both species show enhanced adventitious root formation in response to exogenous auxin. In addition, in both species there were local changes in auxin induced GUS reporter expression after root excision that precede adventitious root formation, suggesting that local auxin accumulation may drive adventitious root formation. In parallel, transport of auxin was found to be increased in stem explants of Arabidopsis and tomato. In the Arabidopsis *mdr1* mutant, the excision dependent enhancement of adventitious root formation is lost and MDR1-GFP expression was found to be increased in excised Arabidopsis hypocotyls. These results support the hypothesis that the ABCB19/MDR1 auxin efflux carrier may play an important role in adventitious root formation. These results reveal cross talk between auxin and ethylene during adventitious root formation. Although auxin shares a common positive role in both species, ethylene oppositely regulates this process in Arabidopsis and tomato.

Characterization of the *ETHYLENE FEEDBACK MEDIATOR* (*EFM*) Gene Family of Arabidopsis

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Negative feedback loops are a common feature of signal transduction pathways, and play important roles in limiting signal output and adaptive responses. In an attempt to identify such feedback regulators of ethylene signaling in plants, we have begun characterizing the *EFM* gene family of Arabidopsis. All four members of the *EFM* family are rapidly induced by ethylene, but this induction is blocked in ethylene-insensitive mutants or by treatment of plants with 1-methylcyclopropene. Direct support that *EFM* family members could function as a part of a negative feedback loop comes from the finding that over-expression of *EFM1* reduces the sensitivity of seedlings to ethylene in a triple response assay. We also find that the kinetics for ethylene-induction vary among the different family members, suggesting that they could modulate responses across a range of ethylene concentrations. Current studies are aimed at determining subcellular localization of the EFM proteins as well as where in the signaling pathway they act.

Spatial temporal gene expression of ethylene biosynthesis enzymes and receptors in *Hibiscus rosa-sinensis* L. flowers

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Flower senescence is regulated by internal and internal factors that may act synergistically leading the cells to death. Senescence signals are strictly correlated with flower pollination. The visible symptoms of flower senescence are shown by petals, but trigger events may take place in different flower organs Hibiscus flowers senesce in 24 hours and flower organs are very well distinct that can be easily separated. Therefore, these flowers can be used as model system for spatial-temporal gene expression and activation. The aim of this work was to study gene expression of enzymes and receptors involved in ethylene biosynthesis. Buds and fully open flowers were treated with 100 μM ABA or 500 ppb 1-methylciclopropene (1-MCP). Genes involved in the ethylene biosynthesis were isolated using degenerate primers and used for expression analyses. Ethylene production and endogenous ABA levels were also measured. Ethylene production was lower in both 1-MCP and ABA treatment. Endogenous ABA content was not affected by 1-MCP treatment. Gene expression studies showed that ABA reduced expression of ethylene receptors and increased the ACC synthase (ACS) and ACC oxidase (ACO). The 1-MCP reduced the ACS and ACO, but enhanced the transcript accumulation of ERS.

Ethylene up-regulates α-farnesene synthase gene *AFS1* in scald-susceptible pome fruits

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Several commercial cultivars of apple and pear fruits are prone to superficial scald, a physiological storage disorder that appears as brown or black patches on the skin and results from necrosis of hypodermal cortical tissue. Cell damage is thought to be induced or exacerbated by conjugated trienol (CTol) oxidation products of the sesquiterpene α-farnesene, which accumulate in the fruit skin during cold storage. Ethylene is known to play a key role in scald development. Pre-storage treatment with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, greatly reduces α-farnesene production and CTol accumulation, and largely prevents scald development after 4-6 months of air storage at 0–1 °C. A marked increase in expression of *AFS1*, the gene encoding α-farnesene synthase (AFS), precedes the rapid accumulation of α-farnesene in cold-stored apples and pears, and 1-MCP treatment blocks the up-regulation of *AFS1* expression. AFS catalyzes the last step in the synthesis of α-farnesene via the mevalonic acid pathway, and *AFS1* appears to be the primary control point in the pathway for ethylene-induced up-regulation of α-farnesene production during the first weeks of storage. We have cloned a 3.1-kb genomic DNA fragment including the *AFS1* gene promoter from the scald-susceptible Law Rome apple cultivar. A BLAST search of the *AFS1* promoter sequence against the Plant Cis-acting Regulatory DNA Elements (PLACE) database (http://www.dna.affrc.go.jp/PLACE/) identified several putative ethylene responsive elements (EREs), one or more of which may be responsible for up-regulation of *AFS1* expression in peel tissue of stored fruit. Deletion analysis of *AFS1* promoter fragments fused to the GUS reporter gene and transformed into Arabidopsis is planned to identify key EREs involved in ethylene-induced up-regulation. In addition to control of α-farnesene production in relation to superficial scald development, this work may more generally provide insight into regulation of aroma volatile production in pome fruits by ethylene.

Perspective of utilizing ethylene-responsive SR/CAMTA for postharvest improvement

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Both ethylene and calcium have been documented to play important roles in ripening and senescence. We have identified a gene family from *Arabidopsis* encoding a novel transcription factor, SR/CAMTA, which is regulated by calmodulin, a calcium-binding protein. Gene expression of SR and its orthologs was shown to be induced by ethylene and other stress signals in tobacco, tomato and *Arabidopsis*. These genes are also highly expressed during ripening and senescence. It has been reported that knockout of the SR gene in *Arabidopsis* led to an increased accumulation of salicylic acid and enhanced disease resistance, as well as increased sensitivity to low temperature. Salicylic acid can reduce the expression of ACC synthase and thereby affect ethylene biosynthesis. Thus SR/CAMTAs sit on the crossroads where calcium signaling intersects the ethylene, salicylic acid, and cold response signaling pathways. Further characterization of SR in horticultural crops will help in understanding the mechanisms underlying fruit ripening, senescence and disease resistance, and in improving the postharvest traits of horticultural crops.