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The Department of Biology, University of Waterloo

Biology

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Schedule/ *Agenda*

Sunday, December 10, 2000

Le dimanche 10 décembre 2000

Davis Centre

		Room/*Salle*
Registration	**8:00-12:00**	Davis Centre (DC) Foyer
Welcome	**8:50-9:00**	DC 1350
Oral Presentation Sessions 1A & 1B	**9:00-10:15**	**A. Metabolism** DC 1302 **B. Pathology** DC 1304
Coffee & Posters	**10:15-11:00**	DC 1301
Keynote Speaker **Dr. Jianming Li** **University of Michigan** "How can Plants Sense Steroids?"	**11:00-11:45**	DC 1350
Lunch with Invited Speaker **Larry Peterson** **University of Guelph** "Update of the Canadian Journal of Botany & How to Prepare a Manuscript"	**11:45-1:15**	Lunch Room

Oral Presentation Sessions 2A & 2B	**1:15-2:30**	**A. Stress 1** DC 1302 **B. Growth & Development** DC 1304
Coffee and posters	**2:30-3:15**	DC 1301
Oral Presentation Sessions 3A & 3B	**3:15-4:15**	**A. Stress 2** DC 1302 **B. Molecular** DC 1304
Invited Speaker **David Evans** **University of Guelph** "Exploring the Uses of DNA Chip Technology: Guelph Experience"	**4:15-5:00**	DC 1350
Social & Awards	**5:00-6:00**	DC 1301
Women's Supper	**6:00-7:00**	DC 1331

PLEASE NOTE:

Poster setup is from **8:00-10:00am** in DC 1301.

- **Odd numbered posters** (P1, P3, P5 etc) – presenters should be available in front of their posters for the first poster session at 10:15-11:00am.
- **Even numbered posters** (P2, P4, P6 etc) – presenters should be available in front of their posters for the second poster session at 2:30-3:15pm.

Oral presenters –A projector is available for previewing slides DC 1331 after 8:00am.

Oral Presentations

Session 1A Chair: Dr. T. Sudhakar Babu Room: DC 1302 Metabolism	Time	Session 1B Chair: Room: DC 1304 Pathology
Madore, Monica A., Xuan Liu and Mary Lu Arpaia. ROLES FOR SEVEN-CARBON SUGARS IN AVOCADO (*PERSEA AMERICANA* MILL.)	**9:00-9:15**	**Taylor, JH**, Pauls, KP MOLECULAR MAPPING OF *FUSARIUM* RESISTANCE IN CORN
Gray, G.R. OVEREXPRESSION OF MITOCHONDRIAL NADP+- DEPENTENT ISOCITRATE DEHYDROGENASE TARGETING AND IMPLICATIONS FOR REDOX MODULATION	**9:15-9:30**	**Wang, Chunxia**, Bernard R. Glick, and K. Peter Pauls THE USE OF TRANSGENIC TOMATO PLANTS TO UNDERSTAND THE ROLE OF ETHYLENE IN RESPONSE TO PATHOGEN ATTACK
Bhatti, S, Colman, B. INORGANIC CARBON UPTAKE IN THE MARINE ALGA *ISOCHRYSIS GALBANA*	**9:30-9:45**	**Cameron, Robin K**., Julianne V. Kus, Denny Mellersh AGE-RELATED RESISTANCE IN *ARABIDOPSIS THALIANA* IS A NOVEL DEVELOPMENTALLY REGULATED DEFENSE RESPONSE TO *PSEUDOMONAS SYRINGAE*.
Huertas E, Espie GS, Colman B ENERGIZATION OF CO_2 TRANSPORT IN A MARINE MICROALGA	**9:45-10:00**	**Shelp, BJ**, McLean, MD, Yevtusheko, D, Van Cauwenberghe, OR, Potter, JW, Bown, AW TRANSGENIC TOBACCO PLANTS WITH ELEVATED GAMMA-AMINOBUTYRIC ACID LEVELS ARE RESISTANT TO THE ROOT-KNOT NEMATODE.
McConnell, Michael D., Randy Koop and Doug H. Bruce MECHANISM OF THE LIGHT STATE TRANSITION IN CYANOBACTERIA. CHANGES IN THE DISTRIBUTION OF EXCITATION ENERGY ABSORBED BY CHLOROPHYLL *A* ARE INDEPENDENT OF CHANGES IN THE DISTRIBUTION OF EXCITATION ENERGY ABSORBED BY THE PHYCOBILISOME.	**10:00-10:15**	**Ann Oaks** GENETICALLY MODIFIED ORGANISMS: IS THE COMMERCIALIZATION OF GMOS PREMATURE?
Coffee & Posters	**10:15-11:00**	
Keynote Speaker Dr. Jianming Li	**11:00-11:45**	
Lunch & Invited Speaker Dr. Larry Peterson	**11:45-1:15**	

Session 2A **Chair:** **Room: DC 1302** **Stress I**	**Time**	**Session 2B** **Chair:** **Room: DC 1304** **Growth & Development**
Yu, XM, Griffith, M ANTIFREEZE ACTIVITY IS REGULATED BY ETHYLENE IN WINTER RYE LEAVES	**1:15-1:30**	Jeong, HSL, Hicklenton, PR, and **Kristie,DN** EVIDENCE FOR THE INVOLVEMENT OF AUXIN IN THE TEMPERATURE-MEDIATED CONTROL OF DIURNAL STEM ELONGATION RHYTHMS IN YOUNG ZINNIA.
Wiseman, SB, Griffith, M THE SEARCH FOR THE ICE-BINDING DOMAIN OF A RYE ANTIFREEZE PROTEIN	**1:30-1:45**	Chaffey, Nigel, **Ewa Cholewa**, Sharon Regan and Björn Sundberg WOOD FORMATION IN TREES: *ARABIDOPSIS* AS A MODEL SYSTEM
Pukacka, S., P.M. Pukacki and **A.M. Zobel**, CONCENTRATION OF GLUTATHIONE, PHENOLIC COMPOUNDS AND FLAVONOIDS IN SEVERAL GINKGO TREES.	**1:45-2:00**	**Glick, B.R.** THE EFFECTS OF LOWERING PLANT ETHYLENE LEVELS USING ACC DEAMINASE-CONTAINING PLANT GROWTH- PROMOTING BACTERIA
Van Cauwenberghe, OR, McLean MD, Shelp BJ IDENTIFICATION AND FUNCTIONAL EXPRESSION OF PLANT GABA TRANSAMINASE.	**2:00-2:15**	**Geil, R.D.,** Peterson, R.L., and Guinel, F.C EFFECTS OF EXOGENOUS ETHYLENE ON THE FORMATION OF LEEK (*Allium porrum* L.) ARBUSCULAR MYCORRHIZA
Breitkreuz, KE, Van Cauwenberghe, OR, Jakobs, C, McLean, MD, **Shelp, BJ** IDENTIFICATION AND FUNCTIONAL EXPRESSION OF PLANT GAMMA-HYDROXYBUTYRATE DEHYDROGENASE.	**2:15-2:30**	**Ferguson, B.,** Guinel, F.C. REDUCTION OF PEA NODULATION BY CYTOKININ TREATMENT
Coffee & Posters	**2:30-3:15**	

Session 3A **Chair:** **Room: DC 1302** **Stress II**	**Time**	**Session 3B** **Chair:** **Room: DC 1304** **Molecular**
Greenberg, B.M., **Huang, X.-D**., El-Alawi, Y. S. & Glick, B. R. A MULTI-COMPONENT PHYTOREMEDIATION SYSTEM TO INCREASE DEGRADATION KINETICS FOR REMOVAL OF PERSISTENT ORGANIC CONTAMINANTS FROM SOILS.	**3:15-3:30**	**Todorova, MI,** Pereira, LA, & Moffatt, BA GENE EXPRESSION CHANGES ASSOCIATED WITH THE METHYLATION REQUIREMENTS OF DEVELOPING SILIQUES AND STEMS
Babu, TS, Akhtar, T, Cosway, TC, Tripuranthakam, S, and Greenberg, BM EFFECTS OF THE HEAVY METAL COPPER ON THE AQUATIC PLANT *LEMMA GIBBA* GROWN UNDER PHOTOSYNTHETICALLY ACTIVE RADIATION AND SUJNATED SOLAR RADIATION	**3:30-3:45**	**Tian L**, Brown DCW, Foster E, Miki B, Ouellet T, Wu K, Malik K ISOLATION AND CHARACTERIZATION OF A CRYPTIC CONSTITUTIVE PROMOTER, tCUP, FROM TOBACCO
Stevens, Kevin J., Janne Lempe, and R. Larry Peterson SHOOT RESPONSES OF SIX LYTHRACEAE SPECIES TO FLOODING	**3:45-4:00**	**Rogers, Amanda C.** Wen-Jin Yu and John S. Greenwood A CYSTEINE PROTEINASE INVOLVED IN PROGRAMMED CELL DEATH IN *VICIA FABA* L.
West, LJA, Peterson, CA MEASUREMENTS OF RESISTANCE TO WATER FLOW IN CUT ROSE STEMS	**4:00-4:15**	**Chan, J.K.,** Pauls, K.P. THE ELUCIDATION OF G PROTEIN ACTIVITY IN *B. NAPUS* MICROSPORE CULTURE.
Invited Speaker David Evans	**4:15-5:00**	

Poster Presentations

P1 Boudreau, E, Lemaire, S, Vaistij, F, Nickelsen, J, Goldschmidt-Clermont, M, Ossenbuhl[3], F, Rochaix, J-D
CHARACTERIZATION OF NUCLEUS-ENCODED TPR PROTEINS REQUIRED FOR THE EXPRESSION OF SPECIFIC CHLOROPLAST GENES IN *CHLAMYDOMONAS REINHARDTII*

P2 Zobel, A.M., Wierzchowska-Renke, K. , Nighswander, J.E. and Furmanowa, M.
REPLANTING DINOSAURS?

P3 Marentes, Eduardo, and Wilfried E. Rauser
PARTITIONING OF CADMIUM IN COMPLEXES IN DURUM WHEAT SEEDLINGS DOES NOT ACCOUNT FOR HIGH OR LOW CADMIUM ACCUMULATION IN THE GRAIN

P4 Marentes, Eduardo, and Wilfried E. Rauser
METALLOTHIONEIN PROTEIN OCCURS IN AN EARLY CADMIUM-BINDING FRACTION IN LEAVES OF DURUM WHEAT

P5 Gerhardt, KE, Greenberg, BM
PHOTOMODIFICATION OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RUBISCO) BY ULTRAVIOLET-B (UVB) RADIATION AND UVB ACCLIMATION VIA FLAVONOID BIOSYNTHESIS

P6 Williams, Heather, A., J. Derek Bewley, John S. Greenwood, Richard Bourgault and Beixin Mo
THE MANNAN-CONTAINING CELL WALLS IN THE ENDOSPERM OF *ASPARAGUS OFFICINALIS* SEEDS DURING DEVELOPMENT AND FOLLOWING GERMINATION

P7 Hodson, JN, Williwns, JP, Khan, NW, Imperial, V
THE ROLE OF PHOSPHATIDYL-CHOLINE IN FATTY ACID EXCHANGE AND DESATURATION IN *B. NAPUS* LEAVES

P8 Holguin, G,, and Glick, B.R.
EXPRESSION OF THE ACC DEAMINASE GENE FROM *ENTEROBACTER CLOACAE* UW4 IN *AZOSPIRILLUM BRASILENSE*

P9 Bourgault, R., Banik M., Mo B., Bewley J. D.
ENDO-β-MANNANASE IS PRESENT IN AN INACTIVE FORM IN RIPENING TOMATO FRUITS OF THE CULTIVAR WALTER

P10 Wang, Chunxia, Edouard Knill, Bernard R. Glick, and Geneviève Défago
THE BIOCONTROL ABILITIES OF THE STRAIN *PSEUDOMONAS FLUORESCENS* CHAO ARE INFLUENCED BY EXPRESSION OF AN 1-AMINOCYCLOPROPANE- 1-CARBOXYLIC ACID (ACC) GENE

P11 Nicol, R.W., Bernards, M.A. and Traquair, J.
THE ANTIFUNGAL ACTIVITY OF SAPONINS FROM AMERICAN GINSENG

P12 Thomas, K, Pauls, KP
PATHOGENESIS-RELATED GENE EXPRESSION IN TOMATO, DURING INFECTION WITH *VERTICILLIUM DAHLIAE*

P13 Silva, NF, Stone SL, Christie LN, Sulaman W, Nazarian KAP, Burnett LA, Arnoldo M, Rothstein SJ, Goring DR
REJECTION OF SELF-INCOMPATIBLE *BRASSICA NAPUS* POLLEN BY *BRASSICA NAPUS CV*. WESTAR PLANTS EXPRESSING THE *S* RECEPTOR KINASE

P14 Vassiliev, Sergej, Doug Bruce and Richard Peterson
CHARACTERIZATION OF NON-PHOTOCHEMICAL QUENCHING IN ISOLATED THYLAKOIDS OF THE LSR5 MUTANT OF *ARABIDOPSIS THALIANA* BY PICOSECOND TIME-RESOLVED CHLOROPHYLL FLUORESCENCE ANALYSIS

P15 Pinhero, RG, Paliyath,G, Tanaka, T, Yada, RY
PARTIAL PURIFICATION OF NAD^+ KINASE FROM POTATO

P16 Patten, C.L., Glick, B.R.
CHARACTERIZATION OF THE IAA BIOSYNTHESIS PATHWAY IN PLANT GROWTH-PROMOTING BACTERIA

P17 Stewart, LI, Hamel, C, Hogue R, Driscoll, BT, and Moutogils, P
MYCORRHIZAL SPECIFICITY ON STRAWBERRY CULTIVARS

P18 Yau, KYF, Tout, NL, Trevors, JT, Lee, H, Hall, JC
PICLORAM SPECIFIC SINGLE CHAIN FV FROM SPLENOCYTES OF HYPERIMMUNIZED MOUSE USING PHAGE DISPLAY TECHNOLOGY

P19 Curtis, Jason, Stephen Brown & David Layzell
O_2 CONCENTRATION & LIMITATION IN THE VASCULAR TISSUE OF SOYBEAN STEMS

P20 Mykoo, Jeevan, Angela Guse and David Layzell
RELATIONSHIP BETWEEN REDUCTIVE METABOLISM AND THE EXCHANGES OF $C0_2$, O_2, H_2 AND N_2 IN LEGUME NODULES.

ORAL PRESENTATIONS

(Arranged by sessions)

Oral Presentations Session 1

Rm: DC 1302 — 9:00-9:15 — Rm: DC 1304

Metabolism A1

ROLES FOR SEVEN-CARBON SUGARS IN AVOCADO (*PERSEA AMERICANA* MILL.)

Madore, Monica A., Xuan Liu and Mary Lu Arpaia.
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521 U.S.A., madore@mail.ucr.edu

Avocado (*Persea americana* Mill.) tissues contain high levels of the seven-carbon (C7) ketosugar mannoheptulose and its polyol form, perseitol. $^{14}CO_2$ labeling of intact leaves indicated that both perseitol and mannoheptulose are not only primary products of photosynthetic CO_2 fixation but are also exported in the phloem. In cell-free extracts from mature source leaves, formation of the C7 backbone occurred by condensation of a 3-carbon metabolite (dihydroxyacetone-P) with a 4-carbon metabolite (erythrose-4-P) to form sedoheptulose-1,7-bis-P, followed by isomerization to a phosphorylated D-mannoheptulose derivative. A transketolase reaction was also observed which converted 5-carbon metabolites (ribose-5-P and xylulose-5-P) to form the C7 metabolite, sedoheptulose-7-P, but this compound was not further metabolized to mannoheptulose. This suggests that C7 sugars are formed from Calvin (PCR) cycle, not oxidative pentose phosphate (OPP) pathway, reactions in avocado leaves. In avocado fruit, C7 sugars were present in substantial quantities and the normal ripening processes (fruit softening, ethylene production, climacteric respiration burst), which occurs several days after the fruit is picked, did not occur until levels of C7 sugars dropped below an apparent threshold concentration of approximately 20 mg g-1 f.wt. The effect of picking could be mimicked by girdling the fruit peduncles, which resulted in ripening on the tree. Again, ripening followed a decline in C7 sugars to below a threshold level. Taken together, these data indicate that the C7 sugars play important roles in carbon allocation processes in the avocado tree, including a possible novel role as phloem-mobile ripening inhibitors .

Pathology B1

MOLECULAR MAPPING OF *FUSARIUM* RESISTANCE IN CORN

Taylor, JH, Pauls, KP
Department of Plant Agriculture, University of Guelph, Guelph, Ontario, N1G 2W1 jhtaylor@uoguelph.ca

Giberella ear rot, caused by *Fusarium graminearum*, reduces the crop yield and quality of corn. The disease is most severe in Canada (particularly Ontario), and thus has received little study by American research groups. As a result, little is known about the molecular basis of the infection or resistance mechanisms to this pathogen. In the research presented here, the primary goal was to identify molecular markers that correlate strongly with *Fusarium* resistance, and subsequently the map location of those markers on a linkage map. To do this, a cross was made between parents that were resistant (CO387) and susceptible (CG62) to *Fusarium* infection. From the individuals resulting from this cross, recombinant inbred breeding lines were established by selfing. These lines were planted in the field, and their resistance to giberella ear rot (from either silk or wounded kernel entry) was assessed. A variety of molecular markers (SSRs, RAPDs, RFLPs, etc.) were scored against this population for the purposes of A) establishing anchor loci throughout the genome upon which unanchored loci could be positioned and B) ascertaining those markers that correlated strongly with *Fusarium* resistance (based on the field trials). Through this work, we were successful in producing maps of the F_2 and F_5 generations that indicated those loci related to resistance to giberella ear rot. By determining those markers that are robust and consistently indicate loci that are highly related to *Fusarium* resistance, we hope to develop a simple system that allows marker-assisted selection to be used to breed for resistance to *Fusarium* in corn.

Rm: DC 1302	**9:15-9:30**	**Rm: DC 1304**
Metabolism A2		**Pathology B2**

OVEREXPRESSION OF MITOCHONDRIAL NADP+- DEPENDENT ISOCITRATE DEHYDROGENASE: DUAL ORGANELLE TARGETING AND IMPLICATIONS FOR REDOX MODULATION

Gray, G.R.
Department of Biochemistry, University of Saskatchewan, Saskatoon, SK 87N 5E5, gr.gray@usask.ca

The enzymatic action of NADP+-dependent isocitrate dehydrogenase (mtICDH; EC 1.1.1.42) is thought to provide the reducing power (NADPH) utilized for the reductive activation of a mitochondrial terminal oxidase, known as the alternative oxidase (AOX). The gene encoding mtICDH has been cloned from tobacco and used in the construction of transgenic plants. A sense line demonstrates increased mtICDH protein abundance and a 7-fold increase in specific activity in comparison to wild-type plants, supported by an increase in cellular NADPH level. However, non-reducing immunoblot analysis suggests a further level of control for the reductive activation of AOX. In addition, sequence examination of the mtICDH gene suggests the possibility of dual targeting of the gene product to both the mitochondria and chloroplast. Preliminary chloroplastic import experiments support this hypothesis and indicate an important role for this enzyme in organelle redox modulation.

THE USE OF TRANSGENIC TOMATO PLANTS TO UNDERSTAND THE ROLE OF ETHYLENE IN RESPONSE TO PATHOGEN ATTACK

Wang[1], Chunxia, Bernard R. Glick[2], and K. Peter Pauls[1]
[1]Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1; [2]Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, ON, N2l 3G1

Over the past few years, conflicting evidence has accumulated about the role of ethylene in plant-pathogen interactions. Since ACC is the direct precursor of ethylene in plants, ethylene synthesis can be greatly reduced by expression of antisense ACC synthase or ACC oxidase transgenes, or by the expression of a foreign ACC deaminase gene. The current project used transgenic tomatoes with a bacterial ACC deaminase gene under the control of three different promoters (CaMV 35S; rolD; or prb-1b) to gain further insights into the role of ethylene in disease development. We examined the effects of reduced ethylene synthesis on major tomato diseases caused by three fungal and two bacterial pathogens. The results demonstrate that different transgenic tomato plants respond differently to pathogen infection. Some of the lines exhibited a significant reduction in disease symptoms in comparison with nontransgenic lines (e.g. rolD promoter line R317 with *Fusarium* and *Verticillium*). With detached leaves similar results were obtained, for instance, leaves from R317 were more resistant to *F. oxysporum* and *Xanthomonas campestris* than nontransgenic plants. However, there was no significant difference between the transgenic and nontransgenic lines infected with *Pseudomonas syringae* and *Pythium aphanidermatum*. Moreover, in some instances, added ethylene can induce disease resistance, for example: 3.8ppm ethylene increased the resistance of R27 (CaMV 35S promoter) to *Fusarium* and *Verticillium* infection significantly. Our results suggest that stress-ethylene might play an important role in plant-pathogen interactions, but the effects depend on the host-pathogen system and the parts of the plant that are affected.

Rm: DC 1302

Metabolism A3

9:30-9:45

Rm: DC 1304

Pathology B3

INORGANIC CARBON UPTAKE IN THE MARINE ALGA *ISOCHRYSIS GALBANA*

Bhatti, S, Colman, B.
Department of Biology. York University. 4700 Keele Street. Toronto, ON. M3J 1P3. shabana@yorku.ca, colman@yorku.ca

The marine haptophyte microalga *Isochrysis galbana*, grown in low CO_2, has high external carbonic anhydrase (CA) activity which is not inhibited by high concentrations of acetazolamide (AZA; 500μM). Attempts to remove the CA by enzyme digestion were unsuccessful. Cells grown on high CO_2 have external CA activity but this can be inhibited by 600 μM AZA. AZA-treated cells photosynthesize at rates 20 times the rate of spontaneous CO_2 supply in seawater at pH 8.0, indicating that they take up bicarbonate and addition of bovine CA (1000μg/ml) stimulates photosynthesis by 45-50% indicating that they take up CO_2 by active CO_2 transport. The results show that growth on high CO_2 does not suppress active HCO_3^- and CO_2 transport as it does in microscopic freshwater algae.

AGE-RELATED RESISTANCE IN *ARABIDOPSIS THALIANA* IS A NOVEL DEVELOPMENTALLY REGULATED DEFENSE RESPONSE TO *PSEUDOMONAS SYRINGAE*.

Cameron, Robin K., Julianne V. Kus, Denny Mellersh
Department of Botany, University of Toronto, 25 Willcocks St, Toronto, Ontario, Canada, M5S 3B2, rcameron@botany.utoronto.ca

Age-Related Resistance (ARR) has been observed in a number of plant species including *Arabidopsis*, which becomes more resistant to virulent *Pseudomonas syringae* (pv. *tomato* or *maculicola*) as it develops over the 8 week experimental period (*in planta* bacterial growth reduction of 10 to 100-fold). We chose to study this ARR response because it often interfered with our studies on Systemic Acquired Resistance (SAR). The *Arabidopsis* mutants, *pad3-1*, *eds7-1*, *npr1-1* and *etr1-4*, exhibit ARR suggesting that ARR is different from both the SAR and Induced Systemic Resistance responses. ARR was not observed in NahG transgenic plants (cannot accumulate salicylic acid [SA]) and was not associated with PR-1 gene expression. Production of an intercellular heat stable, non-proteinaceous anti-bacterial compound in response to *Pst* infection was correlated with the ability to exhibit ARR (not observed in NahG). These data suggest that the ARR defect in NahG plants may not be due to the inability to accumulate SA, but rather to some other phenylpropanoid defect present in NahG plants.

Rm: DC 1302 — 9:45-10:00 — Rm: DC 1304

Metabolism A4 — Pathology B4

ENERGIZATION OF CO_2 TRANSPORT IN A MARINE MICROALGA

[1,2]**Huertas E,** [2]**Espie GS,** [1]**Colman B.**

1. Department of Biology. York University. Toronto. ON. M3J 1P3 2. Department of Botany. Erindale College. University of Toronto at Mississauga, Mississauga. ON. L5L 1C6. emma@yorku.ca, espie@credit.erin.utoronto.ca, colman@yorku.ca

The marine microalga *Nannochloris atomus* lacks external carbonic anhydrase (CA) and takes up CO_2 from the external medium by active transport. CO_2 transport was studied by mass spectrometry using inhibitors and artificial electron acceptors of photosynthesis in order to investigate the reactions providing energy for uptake. The capacity of cells to take up CO_2 was affected by light intensity, demonstrating that CO_2 transport was activated by light. Respiration rates were also dependent upon the light intensity to which cells were exposed during a pre-illumination period, indicating the presence of light-enhanced dark respiration. Addition of bovine CA to cells in which CO_2 fixation was inhibited with iodoacetamide, decreased the CO_2 concentration in the medium by restoring the HCO_3^-–CO_2 equilibrium, indicating that the CO_2 concentration was maintained above its equilibrium value during photosynthesis and thus the cells had lost the ability to transport CO_2 when CO_2 fixation was blocked. When DCMU was added to illuminated cells that had been allowed to concentrate Ci internally, a rapid burst of CO_2 occurred, demonstrating that CO_2 transport was blocked. A similar response was obtained when cell suspensions were treated with DMBQ, suggesting that linear electron flow was involved in supporting CO_2 transport. When methyl viologen was used to drain electrons from ferredoxin, cells were still able to take up CO_2 although fixation decreased with time. These results indicate that CO_2 transport in *Nannochloris atomus* is a photosynthesis-dependent process that is supported by linear electron transport.

TRANSGENIC TOBACCO PLANTS WITH ELEVATED GAMMA-AMINOBUTYRIC ACID LEVELS ARE RESISTANT TO THE ROOT-KNOT NEMATODE.

Shelp, BJ,[1] McLean, MD,[1] Yevtusheko, D,[1] Van Cauwenberghe, OR,[1] Potter, JW,[2] Bown, AW[3]

[1]Department of Plant Agriculture, Division of Biotechnology, University of Guelph, Guelph, ON N1G 2W1, bshelp@uoguelph.ca; [2] Agriculture & Agri-Food Canada, Vineland Station, ON L0R 2E0; [3]Department of Biological Sciences, Brock University, St. Catharines, ON L2S 3A1

Gamma-aminobutyric acid (GABA) is a four carbon, non-protein amino acid that is found in virtually all prokaryotic and eukaryotic organisms as a significant component of the free amino acid pool. In animals, GABA functions as an inhibitory neurotransmitter. The role of GABA in plants is uncertain; however, it does accumulate rapidly in response to a variety of biotic and abiotic stresses. Recent experimentation in our laboratories with the soybean/oblique-banded leafroller and transgenic tobacco/budworm model systems suggests that GABA plays a role in plant defense. In the present study, we produced transgenic tobacco plants which constitutively overexpress the primary synthetic enzyme, glutamate decarboxylase, possess elevated GABA levels and are more resistant than wild-type plants to the parasitic root-knot nematode. The use of GABA as part of a management strategy for invertebrate pests is a promising initiative.

Rm: DC 1302 — 10:00-10:15 — Rm: DC 1304

Metabolism A5 — Pathology B5

MECHANISM OF THE LIGHT STATE TRANSITION IN CYANOBACTERIA. CHANGES IN THE DISTRIBUTION OF EXCITATION ENERGY ABSORBED BY CHLOROPHYLL *A* ARE INDEPENDENT OF CHANGES IN THE DISTRIBUTION OF EXCITATION ENERGY ABSORBED BY THE PHYCOBILISOME.

McConnell, Michael D., Randy Koop and Doug H. Bruce Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1. Dbruce@spartan.ac.brocku.ca

The light state transition adjusts the balance of the distribution of absorbed excitation energy between the two photosystems of photosynthesis under varying environmental conditions and/or metabolic demands. Although no consensus for a mechanism in cyanobacteria exists, there is much evidence for the redistribution of energy absorbed by both Chl *a* and by phycobilin pigments. Proposed mechanisms differ greatly in the relative involvement of chlorophyll and phycobilin pigments. We have investigated the mechanisms of the state transition in both wt and mutant strains of the cyanobacterium *Synechococcus* sp 7002 and *Synechocystis* sp 6803. Changes in the distribution of excitation energy were assayed from 77K fluorescence emission spectroscopy and measures of PSII absorbance cross-sections for excitation of chlorophyll and phycobilin pigments. Action spectra for the redistribution of both chlorophyll and phycobilin pigments were obtained for both wt and mutant cyanobacteria previously characterized as state transition impaired. We found the action spectra for changes in the distribution of both phycobilin and chlorophyll pigments to be very similar to each other in both species of cyanobacteria. The two mutants previously characterized as state transition impaired both showed no redistribution of phycobilin absorbed excitation energy but did show a redistribution of chlorophyll absorbed excitation. Action spectra for the chlorophyll absorbed changes in excitation were very similar in the mutant and wt's. Our data show that the redistribution of absorbed excitation energy by Chl *a* is independent of the redistribution of absorbed excitation energy by phycobilin pigments but are triggered by very similar environmental light conditions.

GENETICALLY MODIFIED ORGANISMS: IS THE COMMERCIALIZATION OF GMOS PREMATURE?

Ann Oaks
Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1.

I am concerned about the premature introduction of genetically modified organisms (GM0s) into our food supply. I will consider four examples used in the pro-/anti-GMO debate which suggest that corporate greed and not good science is the motor driving the rush to commercialization. 1) Antibiotic resistance markers. Other markers are available and should be used. Antibiotic resistance is already a problem. It should not be enhanced by adding antibiotic resistant genes to the human gut. 2) Genes required for the expression of the Bt toxin should and can be modified so that the Bt toxin is expressed only in those tissues where the insect pest feeds. If that were done, then, the effect of the Bt toxin on monarch butterflies would be a non-issue. 3) Round-up ready canola should not be commercialized. Problems, which should have been anticipated, have arisen because canola seeds can survive the harsh Canadian winters. As a result the seeds germinate in the spring, and where crop rotation is practiced, they present a real problem. How does one eradicate round-up ready canola from wheat fields? There is also a problem resulting from our inability to keep GMO- and non-GMO seed separated. This has led to a major loss of income for the prairie farmers who used to export canola to countries which now ban the import of GMO-seed. 4) Golden rice has been engineered and is now ready to be used commercially. Questions arise: Will the poor people who will be the primary beneficiaries of this new crop be willing to eat yellow rice? or even be able to afford yellow rice? or will it do them any good? There were/are alternative ways to enhance the availability of beta-carotene, the precursor of vitamin A. Should the development of these potential alternatives not have been explored in depth before embarking on the transfer of genes conferring the ability for the synthesis of beta-carotene from daffodil flowers to rice endosperm?.

Keynote Speaker

11:00-11:45

HOW CAN PLANTS SENSE STEROIDS?

Dr. Jianming Li
University of Michigan

Brassinosteroids (BRs) are a special class of plant steroids that have wide distribution throughout the plant kingdom and cause pronounced growth-promoting effects on plants. Early physiological studies by exogenous application indicated that BRs could induce a broad spectrum of physiological and cellular responses and showed that BR applications in the field could increase yield and improve stress resistance of several major crop species. However, their roles as plant hormone were not widely accepted until 1996 when mutants that are defective in BR biosynthesis were isolated and their corresponding genes were cloned.

One of the mutants is the *Arabidopsis det*2 mutant, which was originally identified as a "de-etiolation" mutant that displays many characteristics of light-grown plants when grown in the dark. In the light, *det*2 mutants are dark green dwarfs with epinastic round leaves, have reduced male fertility and apical dominance, display delayed flowering and senescence, indicating that DET2 must play an important role throughout *Arabidopsis* development. The DET2 gene encodes a protein that shares significant sequence identity with mammalian steroid 5a-reductases that are important for animal steroid metabolism, suggesting the involvement of DET2 in the biosynthesis of plant steroids such as BRs. Indeed, BR treatment rescued the growth defects of det2 mutants. When expressed in human embryonic kidney 293 cells, DET2 protein was able to 5a-reduce several animal steroids including testosterone and progesterone. Moreover, the expression of human steroid 5a-reductase genes in *det*2 mutants complemented the *det*2 mutation, demonstrating that DET2 is indeed a functional homologue of mammalian steroid 5a-reductases. Loss-of-function mutations in DET2 inhibit a reaction expected to be catalysed by a steroid 5a-reducase in the BR biosynthetic pathway, leading to a ~90% reduction in BR biosynthesis. Thus, DET2 is a steroid biosynthetic enzyme catalysing an early step in the BR biosynthetic pathway. Consistent with the early claims that BR application could increase crop productivity, overexpression of DET2 led to a dramatic biomass increase in *Arabidopsis* and a ~10% yield increase (dry weight/l 000 seeds) in transgenic rice. These results, combined with studies of other BR-deficient dwarf mutants of Arabidopsis, pea, and tomato, have provided unequivocal evidence that BRs constitute a unique class of plant hormones that are essential for normal plant growth and development. To understand the molecular mechanisms) by which BRs regulate plant growth and development, we took a genetic approach to screen for mutants that display characteristic BR-deficient phenotypes but can not be rescued by BR treatment, and identified 18 new alleles of a previously characterized genetic locus, BRASSINOSTEROID-INSENSITIVE 1 (BRI1). The BRI1 gene was cloned by a chromosome-walking strategy and found to encode a leucine-rich repeat (LRR) receptor-like kinase that is composed of an extracellular domain containing 25 tandem LRRs that is disrupted by a 70-amino-acid island between 21st and 22nd LRRS, a transmembrane

α-helix, and a cytoplasmic kinase domain. Sequence analysis of various mutant *bri*1 alleles has identified the 70 amino-acid island and the kinase domain as the two most important functional domains required for BR signaling. BRI1 is a plasma membrane localized protein and can function as a serine/threonine kinase. Moreover, a BRI1-Xa21 chimeric receptor, which contains the extracellular domain, the transmembrane region, plus a short cytoplasmic juxtamembrane segment of the BRI1 protein fused to the cytoplasmic kinase portion of the rice disease resistance receptor kinase Xa21, responded to BR treatment and activated a plant defense response pathway. Based on these results, we hypothesized that BR binding to BRI1 would induce homo- or heterodimerization, which could in turn stimulate the receptor's intrinsic kinase activity, leading to recruitment of other BR signaling components to the phosphorylated receptor and activation of an intracellular phosphorylation signaling cascade.

To identify additional components of such a receptor-kinase-mediated BR signal transduction pathway, we have analyzed various dwarf and semi-dwarf mutants that were collected during a previous genetic screen for *bri*1 mutants and isolated an interesting semi-dwarf mutant that showed almost no response to the steroid treatment. Genetic and physiological analyses indicate that this mutant defines a novel genetic locus, BRASSINOSTEROID-INSENSITIVE 2 (BIN2), whose gain-of-function mutations interfere with the normal BR signal transduction. Recently, we cloned the BIN2 gene and found that it encodes a cytoplasmic serine/threonine kinase. A hypermorphic mutation within its coding sequence and its overexpression inhibit plant steroid signaling, resulting in plants that display phenotypes reminiscent of BR-deficient and BR response mutants, whereas its reduced expression by co-suppression suppresses a weak *bri*1 phenotype. Thus, BIN2 acts as a negative regulator to control brassinosteroid signaling. Identifying upstream regulator(s) and downstream target(s) is becoming the newest challenge in BR signaling research

Invited Speaker

11:45-1:15

UPDATE ON CANADIAN JOURNAL OF BOTANY AND HOW TO PREPARE A MANUSCRIPT.

Peterson, R. Larry, Editor.
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The editorial office has been located at Guelph since July, 1999 with myself as editor and Jane Gurney as Assistant to the Editor. Several changes to the Editorial Board make it more international in scope. The review process has been expedited by screening manuscripts before sending to associate editors, by centralizing mailing, by the use of a new guideline to reviewers form, and by the use of a new manuscript tracking program (Paper Path). Technical editing letters now go only to authors of papers accepted. The average time from acceptance to publication is four months. Manuscripts now appear on the web before the hard copy is issued. A new format for journal covers is in place so that colour can be used for each issue. Plans for next year involve a series of mini-reviews, an effort to identify papers for rapid communications, and soliciting papers in under-represented research areas.

The most common problems identified in submitted manuscripts are: no statement of purpose of the research; abstracts that do not reflect the contents of the paper; improper statistical analyses of data; manuscripts NOT conforming to the format of CJB; lifting of papers from theses without rigorous editing.

Oral Presentations Session 2

Rm: DC 1302 — 1:15-1:30 — Rm: DC 1304

Stress 1 A1

ANTIFREEZE ACTIVITY IS REGULATED BY ETHYLENE IN WINTER RYE LEAVES

Yu, XM, Griffith, M
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, griffith@uwaterloo.ca

Antifreeze activity is induced by cold and drought in winter rye leaves, but the regulatory pathway for this activity is unknown. Because the activity arises from dual-function pathogenesis-related (PR) proteins, we examined the roles of plant hormones known to act as secondary messengers in responses to cold and disease. Plants treated with abscisic acid (ABA) and salicylic acid (SA) at 20°C did not exhibit antifreeze activity. In contrast, plants treated with ethylene for one week at 20°C developed antifreeze activity to the same high level that is normally observed in plants that have been cold acclimated for 2 months. Moreover, plants exposed to cold and drought evolved ethylene at a rate far higher than control plants, thus confirming that ethylene may be important as a messenger in cold-induced processes *in planta*. The unusual part of the response is that ABA, SA and pathogens induce equivalent accumulations of six apoplastic proteins that are immunologically the same as the six apoplastic antifreeze proteins induced by ethylene, cold and drought. At this time, our explanation for these results relies on the fact that PR proteins are encoded by large gene families. We hypothesize that different members of the gene families encoding PR proteins are induced by cold, drought and ethylene, and that only these specific PR proteins have dual functions in freezing and disease resistance.

Growth & Development B1

EVIDENCE FOR THE INVOLVEMENT OF AUXIN IN THE TEMPERATURE-MEDIATED CONTROL OF DIURNAL STEM ELONGATION RHYTHMS IN YOUNG ZINNIA.

Jeong, HSL, Hicklenton, PR, and Kristie, DN
Atlantic Food and Horticulture Research Centre, Kentville, N.S., B4N I JS; Department of Biology, Acadia University, Wolfville, N.S., BOP IXO, David.Kristie@AcadiaU.ca

The role of auxin in promoting stem growth and influencing daily growth rhythms in Zinnia (*Zinnia elegans* cv. Pompon) was studied in relation to day/night temperature differential (DIF) and developmental stage. Plants were treated with the auxin transport inhibitor TIBA, and the synthetic auxin 2,4D, under 3 DIF regimes (positive DIF - 21.5C DT/16.5C NT; zero DIF - constant 18.7C; negative DIF -16.5C DT/21.5C NT) at two developmental stages (vegetative and pre-flowering). In vegetative plants most internode growth occurred during the day period and growth rates were highest under pos DIF. TIBA application just below the apical meristem inhibited internode elongation for up to 48 hours, after which growth rates slowly recovered. During this recovery, TIBA induced growth rate patterns under 0 DIF and positive DIF, which closely resembled those of negative DIF plants. Normal pos DIF growth rates and patterns were restored by application of 2,4D. Internode growth at the pre-flowering stage differed from that in vegetative plants in that most growth occurred during the night period, and patterns were unaffected by either neg DIF or TIBA. It appears that DIF and auxin have similar effects on internode growth in vegetative zinnia and we propose that auxin is at least partly involved in expression of the DIF effect in this species. The lack of response to both DIF and TIBA at a later developmental stage provides support for this hypothesis.

Rm: DC 1302 **1:30-1:45** **Rm: DC 1304**

Stress 1 A2 **Growth & Development B2**

THE SEARCH FOR THE ICE-BINDING DOMAIN OF A RYE ANTIFREEZE PROTEIN

Wiseman, SB, Griffith, M
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AFPs similar to pathogenesis-related (PR) proteins accumulate in winter rye (*Secale cereale* L.) leaves during cold acclimation (CA). These dual-function proteins are glucanases, chitinases and thaumatin-like proteins. PR proteins lacking antifreeze activity are also present in the apoplast of ABA-treated nonacclimated rye leaves. We cloned two full-length chitinases: *CHT46* is a 998 bp cDNA cloned from a CA rye leaf cDNA library. *ABA-CHT46* is a 996 bp cDNA cloned by RT-PCR, 5'- and 3'RACE from ABA-treated rye leaves that is 99% identical to the coding region of *CHT46* at the nucleotide level. We first hypothesized that PR proteins acquire antifreeze activity through post-translational modifications. However, mass spectrometry showed that the CHT46 purified from CA rye leaves is not post-translationally modified, although ABA-CHT46 may be modified to prevent formation of an ice-binding domain. Alternatively, AFPs may be encoded by distinct genes. *CHT46* has antifreeze activity when expressed in *E. coli*, which supports the possibility of a novel gene. We are now comparing the 3-D structure of CHT46 from winter rye with similar proteins lacking antifreeze activity to identify structural differences that may confer ice-binding ability. Because the rye AFPs form oligomers, we are also examining the structure of native ABA-induced PR proteins. In conclusion, antifreeze activity may result from subtractive modifications to PR proteins or from conformational changes that arise in the native proteins.

WOOD FORMATION IN TREES: *ARABIDOPSIS* AS A MODEL SYSTEM

Chaffey[1], Nigel, Ewa Cholewa[2] , Sharon Regan[3] and Björn Sundberg[2]
[1]IACR Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF, United Kingdom; [2] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden; [3]Present address: Department of Biology, College of Natural Sciences, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

Our understanding of the molecular controls regulating the identity of the vascular cambium and the development of secondary xylem and phloem have not yet benefited from the use of *Arabidopsis* as a genetic system. Under short day growth conditions, *Arabidopsis* undergoes extensive secondary growth in the hypocotyl, with the development of both a vascular and a cork cambium. The secondary xylem of the hypocotyl develops in two phases, an early phase in which only vessel elements mature and a later stage in which both vessel elements and fibres are found. During this second phase the secondary xylem of *Arabidopsis* closely resembles the anatomy of the wood of an angiosperm tree, and can be used to address basic questions about wood formation. We are reporting here a detailed description of secondary xylem in *Arabidopsis* and the use of new fast staining technique that can be easily employed in screening for effect of mutations on *Arabidopsis* anatomy.

Rm: DC 1302 — 1:45-2:00 — Rm: DC 1304

Stress 1 A3

CONCENTRATION OF GLUTATHIONE, PHENOLIC COMPOUNDS AND FLAVONOIDS IN SEVERAL GINKGO TREES.

Pukacka, S.[1], P.M. Pukacki[1] and A.M. Zobel[2]
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In six Ginkgo trees the total glutathione level varied from 1.61 to 25.89 mg/g dry weight, a variation of over 18-fold, suggesting that, even within a species, separate organisms may have a wide range of concentrations of this master antioxidant. There was a correlation between total and reduced glutathione levels. The total glutathione level depended on the sex of the plant, with male individuals containing more than females: 21.2 vs. 1.57mg/g, about a 13-fold difference. In comparison, in *Pinus sylvestris* the total and reduced glutathione levels were at least twice as high as in *Ginkgo*, and in *Picea abies* at least tenfold lower than in *P. sylvestris*. The variation of concentration of the master antioxidant was exhibited both within and between different tree species.

Growth & Development B3

THE EFFECTS OF LOWERING PLANT ETHYLENE LEVELS USING ACC DEAMINASE-CONTAINING PLANT GROWTH- PROMOTING BACTERIA

Glick, B.R.
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Direct promotion of plant growth by plant growth-promoting bacteria may involve: fixation of atmospheric nitrogen; production of siderophores which can solubilize and sequester iron and provide it to plants; synthesis of phytohormones, including auxins, cytokinins, and gibberellins; solubilization of minerals such as phosphorus; and synthesis of enzymes that can modulate plant growth and development. A particular bacterium may affect plant growth and development using any one, or more, of these mechanisms. Moreover, a bacterium may utilize different traits at various times during the life cycle of the plant. Among the direct mechanisms used by soil bacteria to promote plant growth is the lowering of plant ethylene levels by the bacterial enzyme ACC deaminase. This enzyme takes up seed or root exuded ACC, the immediate precursor of ethylene in plants, and cleaves it to ammonia and α-ketobutyrate. Bacteria that possess this enzyme stimulate plant root elongation and protect ethylene sensitive plants from a wide range of abiotic and biotic stresses including flooding, heavy metals and bacterial and fungal pathogens.

Rm: DC 1302 **2:00-2:15** **Rm: DC 1304**

Stress 1 A4 **Development B4**

IDENTIFICATION AND FUNCTIONAL EXPRESSION OF PLANT GABA TRANSAMINASE.

Van Cauwenberghe, OR, McLean MD, Shelp BJ,
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GABA accumulates in response to various biotic and abiotic stresses. In plants, the fate of GABA is determined by two possible transaminase reactions that use either pyruvate or 2-oxoglutarate as amino acceptors producing alanine or glutamate, respectively. Earlier, we showed that these two activities were separable in plants. In this study, GABA transaminase (GABA-T) from tobacco (*Nicotiana tabacum* L. cv. Samsun) leaf tissue was purified to homogeneity by a combination of mitochondrial isolation, FPLC anion-exchange-, GABA affinity-, and gel filtration-chromatography, and a number of PAGE steps. Sequence from the purified protein was used to identify an *Arabidopsis thaliana* expressed sequence tag (EST) with greater than 30% identity to many known non-plant *GABA-T*s. PCR techniques were employed to acquire a full-length clone of this gene from an *Arabidopsis thaliana* [L.] Heynh (*Landsberg erecta* ecotype) cDNA library. The 1515 bp open reading frame codes for a 504-amino acid polypeptide with several interesting features, including a putative mitochondrial signal sequence, three membrane spanning domains, and a putative pyridoxal-5-phosphate binding domain. In addition, functional expression of this cDNA in *Escherichia coli* confirmed that the protein coded by this gene confers pyruvate-dependent GABA-T activity in plants. The importance of these materials for study of in planta GABA metabolism will be discussed.

EFFECTS OF EXOGENOUS ETHYLENE ON THE FORMATION OF LEEK (*Allium porrum* L.) ARBUSCULAR MYCORRHIZA

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In general, ethylene is inhibitory to arbuscular mycorrhiza (AM) formation; however Ishii *et al.* (1996) have reported stimulation of AM formation at 0.05 ppm ethylene. In this study, leeks inoculated with the AM fungus *Glomus aggregatum* were subjected to a continuous flow of 0.3 or 0.6 ppm substrate-ethylene to determine if ethylene concentration-dependent alterations in AM formation could be induced. Treatment with 0.6 ppm ethylene inhibited colonization by intraradical hyphae and arbuscules. This level of ethylene did not affect fungal morphology nor did it cause a block to colonization. It is suspected that restricted extension of colonization units along the longitudinal axes of roots caused the reduction in colonization. Although treatment with 0.3 ppm ethylene did not significantly stimulate colonization compared to controls, it did increase the level of colonization enough to cause a more significant difference between the two ethylene treatments than between the 0.6 ppm ethylene and control treatments.

Rm: DC 1302 **2:15-2:30** **Rm: DC 1304**

Stress 1 A5 **Growth & Development B5**

IDENTIFICATION AND FUNCTIONAL EXPRESSION OF PLANT GAMMA-HYDROXYBUTYRATE DEHYDROGENASE.

Breitkreuz, KE,[1] Van Cauwenberghe, OR,[1] Jakobs, C,[2] McLean, MD,[1] Shelp, BJ[1]

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In plants, gamma-aminobutyric acid (GABA), a non-protein amino acid, accumulates rapidly in response to a variety of abiotic stresses such as hypoxia. Complementation of a succinic semialdehyde dehydrogenase-deficient yeast mutant with an *Arabidopsis* cDNA library allowed the identification of a novel gene for gamma-hydroxybutyrate dehydrogenase (*GHBDH*). The recombinant protein, isolated from an insoluble *Esherichia coli* fraction, catalyzed the reduction of succinic semialdehyde to gamma-hydroxybutyrate, with a preference for NADPH over NADH, in an essentially irreversible reaction. We propose that GHBDH functions as a fermentation enzyme, thereby facilitating the catabolism of GABA in plants.

REDUCTION OF PEA NODULATION BY CYTOKININ TREATMENT

Ferguson, B., Guinel, F.C.

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The synthetic cytokinin 6-benzylaminopurine (BAP) affects nodule organogenesis of *Pisum sativum*. BAP concentrations higher than 5 μM inhibit modulation. Infection occurs in plants treated with 15 μM BAP, but infection threads are blocked in the outer cortex and cell division centers in the inner cortex are rare. In separate experiments, it was found that the higher the concentration of BAP applied, the more ethylene evolved by the root. Preliminary evidence for another synthetic cytokinin, kinetin, and the naturally-occurring cytokinin, zeatin, suggest that they too affect modulation and ethylene production. *P. sativum* seedlings inoculated with *Rhizobium leguminosarum* were treated with concentrations (0.5 to 25 μM) of either kinetin or zeatin to determine their effects on modulation and C_2H_4 production. The results are similar to those obtained with BAP. In our discussion, we will take into account that C_2H_4 is a known inhibitor of modulation and that cytokinin has been recently proposed to stimulate the expression of nodulin genes.

Oral Presentations Session 3

Rm: DC 1302 — 3:15-3:30 — Rm: DC 1304

Stress 2 A1 — Molecular B1

A MULTI-COMPONENT PHYTOREMEDIATION SYSTEM TO INCREASE DEGRADATION KINETICS FOR REMOVAL OF PERSISTENT ORGANIC CONTAMINANTS FROM SOILS.

Greenberg, B.M., Huang, X.-D., El-Alawi, Y. S. and Glick, B. R.
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The level of persistent organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs) are increasing due to anthropogenic release related to industrial activities. Their toxicity, mutagenicity and carcinogenicity are of significantly environmental concern. Clearly, effective remediation processes for aromatic organic contaminants would be valuable. Phytoremediation has the potential to be an effective route for remediation of persistent organic contaminants. Plants can provide a large amount of biomass as a sink for the contaminants. Further, plants can work synergistically with contaminant-digesting rhizobacteria and mechanical remediation. A phytoremediation system employed multiple processes for removal of persistent organic contaminants (PAHs and TPHs) in contaminated soils was developed in our laboratory. The system was composed of land farming (aeration and light exposure), fertilizing, inoculation of contaminant degrading bacteria and plant growth with rhizobacteria, resulted in an enhanced physical, photochemical, microbiological and phyto-biological remediation process. The land farming (aeration and light exposure) was effective for removal of smaller compounds; bacteria were effective for removal of relatively soluble compounds; plants with rhizosphere bacteria together effectively remediated insoluble and soil bound compounds. We were able to use this technique to remediate creosote up to 3 g/kg in contaminated soils. The results showed that more than 90% of creosote was removed from soil within a four-month period in a laboratory setting. Therefore, we believe that the multiple process system could be the optimal solution for remediating persistent aromatic organic contaminants from soils. There are three advantages to this process: it is faster than any individual process; it is economically sound; and cleanup and restoration occur simultaneously.

GENE EXPRESSION CHANGES ASSOCIATED WITH THE METHYLATION REQUIREMENTS OF DEVELOPING SILIQUES AND STEMS

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Many compounds in plants, including phosphocholine, lignin and pectin, are methylated. The vast majority of the methylation reactions involved in the synthesis of these compounds rely on the methyl donor, *S*-adenosylmethionine (SAM). Each methylation event produces one molecule of *S*-adenosylhomocysteine (SAH) that must be rapidly metabolized to prevent feedback inhibition of the transmethylase and maintenance of the transmethylation cycle. Our current goal is to understand the metabolic signals that adjust the availability of SAM and SAH metabolism in response to methyl requirements. We are studying the contributions of two enzymes to SAH metabolism, adenosine kinase (ADK) and *S*-adenosylhomocysteine hydrolase (SAHH). Recent results (Weretilnyk *et al*., Plant Physiol 2000, in press) show that the expression of each gene increases in plants that accumulate glycine betaine, a methylated osmolyte. We are now examining ADK and SAHH enzyme activity, transcript abundance and protein levels in *Arabidopsis* in several developmental conditions that incur an increased methyl demand. *In situ* hybridization and immunoblotting analysis of developing siliques and stems reveal increased accumulation of ADK and SAHH transcripts prior to lignin accumulation. Similar analysis of mutant plants that ectopically lignify as well as transgenic lines that express glycine betaine are also being investigated.

Rm: DC 1302 **3:30-3:45** **Rm: DC 1304**

Stress 2 A2 **Molecular B2**

EFFECTS OF THE HEAVY METAL COPPER ON THE AQUATIC PLANT LEMMA GIBBA GROWN UNDER PHOTOSYNTHETICALLY ACTIVE RADIATION AND SUJNATED SOLAR RADIATION

Babu, TS, Akhtar, T, Cosway, TC, Tripuranthakam, S, and Greenberg, BM
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Heavy metals, beyond a threshold concentration pose a danger to plants, animals and microorganisms. Because, heavy metals exist in the polluted natural environment, it becomes imperative to study the combined effects of heavy metals and solar radiation on plants. Therefore, in the present study, we examined for the responses of the aquatic plant *Lemna gibba* exposed to the heavy metal, Cu^{2+} in the presence of photosynthetically active radiation (PAR) and simulated solar radiation (SSR). It was observed that the growth and chlorophyll content of plants exposed to Cu^{2+} plus SSR were more retarded than plants exposed to Cu^{2+} plus PAR. Because, Cu^{2+} is a redox active metal, we tested for the activities of antioxidant enzymes in plants treated with Cu^{2+} under PAR or SSR. Indeed, we found that Cu^{2+} treatment of plants caused enhanced activities of superoxide dismutase and glutathione reductase. However, beyond a threshold concentration of Cu^{2+} (6 μM), the GR activity of plants grown under SSR started decreasing. Assay of reactive oxygen species (ROS) using DCF fluorescence showed that Cu^{2+} caused formation of ROS both under PAR and SSR. We report here for the first time that plants exposed to Cu^{2+} in the presence or absence of SSR exhibited induction of flavonoid compounds. The induction of flavonoid compounds was much greater in plants grown under PAR than under SSR when compared with their respective controls. Similar to the GR activity profile, plants exposed to Cu^{2+} beyond 6 μM, exhibited a decrease in the induction of flavonoid compounds only under SSR. In conclusion, the results indicated that the toxicity of Cu^{2+} under either PAR or SSR was due to the formation of ROS. However, the enhanced toxicity of Cu^{2+} to plants under SSR could have been due to accelerated reduction of copper by UV chromophores that have got over-reduced under SSR and/or repressed gene expression involved in the formation of antioxidants. We propose that the induced flavonoid compounds in *L. gibba* in response to Cu^{2+} treatment may be acting as antioxidants and/or chelators of the heavy metal Cu^{2+}.

ISOLATION AND CHARACTERIZATION OF A CRYPTIC CONSTITUTIVE PROMOTER, tCUP, FROM TOBACCO

Tian L, Brown DCW, Foster E, Miki B, Ouellet T, Wu K, Malik K
Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, Ontario N5V 4T3 and Eastern Cereal & Oilseed Research Centre, Ottawa, Ontario, Canada. K1A 0C6 Tianl@em.agr.ca

Cryptic promoters are sequences that do not act as promoters at their native locations in the genome but have the capability of being functional when positioned adjacent to genes. A cryptic promoter, tCUP, was isolated from tobacco by T-DNA tagging. Characterization of the tCUP promoter sequence revealed the differences in tCUP from those of other plant constitutive promoters; for example, the tCUP promoter lacks a TATA box. Transcription initiates at a single site within the tCUP sequence, which is similar to transcription start site consensus sequence (*Inr*) determined for plant genes. The tCUP promoter is cryptic, as the transcriptional start site is not used in untransformed tobacco plants. tCUP is the first example of a cryptic, constitutive promoter isolated from plants. tCUP directed GUS gene expression in a wide range of plant species, including dicots, monocots, and conifers. The tCUP promoter activated reporter gene constitutively at the levels comparable to that generated by the widely used CaMV 35S promoter. The composition and the elements of tCUP were also analyzed and characterized. Results indicate cryptic promoter elements can influence the expression pattern of plant genes through DNA rearrangement.

Rm: DC 1302 **3:45-4:00** **Rm: DC 1304**

Stress 2 A3 **Molecular B3**

SHOOT RESPONSES OF SIX LYTHRACEAE SPECIES TO FLOODING

Stevens[1], Kevin J., Janne Lempe[2], and R. Larry Peterson[1]

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Flood responses of three *Lythrum* species (*L. salicaria*, *L. hyssopifolia*, *L. alatum*), *Decodon verticillatum*, *Pleurophora anomola* and *Heimia myrticifolia* were assessed. All species, except *L. hyssopifolia*, responded to flooding by increasing total plant height. All species, except *H. myrticifolia*, formed a phellem of significantly wider diameter at the stem base of flooded plants compared to controls. This phellem consisted of alternating bands of small, isodiametric cells and radially elongated cells separated by large air lacunae forming a very specialized aerenchyma, which may increase the air space continuum from shoot to root in plants that have undergone secondary growth. The small cells had Casparian band-like wall modifications and occasionally displayed modifications that included all cell wall surfaces. Although the significance of these flood responses was not determined, the purported "highly invasive nature" of *Lythrum salicaria* cannot be attributed to the absence of these characteristics in Lythraceae endemic to North America.

A CYSTEINE PROTEINASE INVOLVED IN PROGRAMMED CELL DEATH IN *VICIA FABA* L.

Rogers, Amanda C. Wen-Jin Yu and John S. Greenwood

Dept. of Botany, University of Guelph, Guelph ON

A 31 kDa cysteine proteinase, vfcyspro, from germinated *Vicia faba* was thought to be critical to storage protein mobilization following germination. However, both enzyme amount and activity peaked after the majority of seed storage proteins were degraded. Using a cDNA encoding vfcyspro, we found that the accumulation of transcripts in the germinated cotyledons mimicked the pattern of cysteine proteinase accumulation and activity, being first noticeable at 2 days after imbibition (DAI) and peaking between 14 and 18 DAI. The late peaks in expression, enzyme accumulation and activity suggested that this particular enzyme may have roles in addition to storage protein mobilization. *Vfcyspro* transcripts and translation products are not restricted to the cotyledons of the germinated seeds but are also found in leaves, shoots and roots of young plants, and in the pod wall of more mature plants. *Vfcyspro* is not expressed in developing seeds. Immunochemical assays and *in situ* hybridization reveal that expression is not specific to cell or tissue type. Although present in the storage parenchyma of the germinated cotyledon, the enzyme is also present in developing vasculature of younger roots and shoots. Transcripts and translation products are also present in the parenchyma cells of the pith of the youngest and second youngest shoot internodes, the pith becoming hollow as the stem matures. Based on these findings, we speculate that vfcyspro is not only involved in storage protein mobilization but also plays a critical role in multiple developmental processes involving programmed cell death in *V. faba*.

Rm: DC 1302 **4:00-4:15** **Rm: DC 1304**

Stress 2 A4 **Molecular B4**

MEASUREMENTS OF RESISTANCE TO WATER FLOW IN CUT ROSE STEMS

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Increasing the vase life of cut roses is an important goal for the rose industry in Canada. Vase life is sometimes prematurely terminated by diminished water supply through the stem resulting in wilt. It is not clear whether this reduction in water flow through the stem is due to embolisms in combination with bacterial plugs, or to bacterial plugs alone. Hydraulic conductivities of 'Kardinal' and 'Escimo' *Rosa hybrida* stems were measured along their length and over time up to 18 days after cutting. Stem segments were examined for bacterial plugs, anatomical differences, and embolism formation. While no time effects were observed for the middle and upper portions of the stems, the resistance to water transport rates in the basal segments increased significantly for both varieties after the first two days. Bacterial plugs were observed in the lower segments only, and embolisms were not observed in the stems for the duration of the studies. These results indicate that bacterial plugs and not copious embolisms are responsible for the observed increase in water flow resistance. Attempts to avoid the increase in hydraulic resistance by removing the bacterial plugs by regular re-cutting of the basal 20-30 mm of the stem were unsuccessful. Further experiments are planned to test the involvement of embolisms under stress conditions.

THE ELUCIDATION OF G PROTEIN ACTIVITY IN *B. NAPUS* MICROSPORE CULTURE.

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The activity of G proteins is being studied in the embryogenic *Brassica napus* cv Topas microspore culture model. A PCR strategy using degenerate primers has been employed to amplify and clone putative genes belonging the ROP small G protein family from a pollen-derived cDNA library. A total of six unique sequences were found. RT PCR was performed utilizing sequence specific primers to monitor the expression of one of these genes in culture. Preliminary results with ORF 5 ROP gene suggests its expression may be differentially regulated in microspore culture. Futhermore, a fluorescent-labeled G protein stain has been used to examine GTP-binding activity of cultured microspores. Preliminary results from the staining study suggest that G protein activity is different in different types of cells in the microspore cultures. It is possible that this work will lead to the elucidation of other classes of genes that may be differentially regulated during androgenesis.

Invited Speaker

4:15-5:00

EXPLORING THE USES OF DNA CHIP TECHNOLOGY: GUELPH EXPERIENCE

Dr. David Evans
University of Guelph, Guelph, Ontario, N1G 2W1

DNA array technology is currently revolutionizing our understanding of cellular gene regulatory networks. The method uses glass slides upon which have been fixed DNA fragments in well-defined arrays. Up to 20,000 different DNAs can be spotted in these arrays using precision robotics. Typically, mRNA is extracted from the cell or organism of choice, converted into a fluorescent cDNA and these molecules hybridized to DNA on the chip surface. The bound fluorescent products are then detected and quantitated using digital fluorescence microscopy. By analysing changes in fluorescence patterns in response to various stimuli, one gains an understanding of global gene regulatory networks. In this presentation I will describe some of the new technology being assembled at the University of Guelph which permits the construction of DNA arrays and facilitates acquisition and analysis of DNA array data. Some examples from on-going research will also be presented, to illustrate applications in such areas as virus diagnosis and genetic analysis of gene function.

POSTER PRESENTATIONS

Poster Abstracts

P1: CHARACTERIZATION OF NUCLEUS-ENCODED TPR PROTEINS REQUIRED FOR THE EXPRESSION OF SPECIFIC CHLOROPLAST GENES IN *CHLAMYDOMONAS REINHARDTII*

Boudreau[1], E, Lemaire[2], S, Vaistij[2], F, Nickelsen[3], J, Goldschmidt-Clermont[2], M, Ossenbuhl[3], F, Rochaix[2], J-D

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The *psbD* and *psbB* chloroplast genes encodes the D2 and P5 subunits of the photosystem II (PSII) reaction center. In the green alga *Chlamydomonas reinhartdii* nuclear mutant strains *Nac2* and *222E* (*mbb1*) the *psbD* and *psbB* mRNA, respectively, are unstable and the PSII is not assemble. Using genomic complementation strategies the *nac2*and *mbb1* genes were identified. Both genes encode for hydrophilic polypeptides which contains tetratricopeptide repeats (TPR). TPR proteins consist of degenerate 34 amino acids repeats which are believed to be involved in protein-protein interactions. Nac2 (140 kDa) and Mbb1 (70 kDa) proteins were both found to be located in the stromal compartment of the chloroplast. Size fractionation experiments revealed that they are part of different high molecular complex which are associated with RNA. Change of a conserved alanine in the fourth TPR domain of the NAC2 protein by site–directed mutagenesis leads to aggregation of the protein and completely abrogates its function. The Nac2 and Mbb1 protein complexes are most likely involved in processing, stability and/or translation of *psbD* and *psbB* mRNA, respectively.

P2: REPLANTING DINOSAURS?

Zobel, A.M.[1], Wierzchowska-Renke, K.[2], Nighswander, J.E.[3] and Furmanowa, M.[4]

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Plants grow in ever-changing environments, where both abiotic conditions vary every second and coexisting organisms change and influence each other. Annuals need to survive only a few months, and can thus afford production of a wide variety of toxic compounds, such as phenolics, in huge quantities. But trees, which have to stay in such conditions over tens and even hundreds of years, probably cannot. Plants extrude different active compounds to their surfaces, as their first defense, as well as to the surface of every cell within the plant body. Over 100 000 phenolics have been identified, of which a given species may produce several hundred; thus great variation is possible. Humans have selected species according to their wishes, with the required genome and phenotype lowering diversification. Forests are changing from multispecies to large stands of a single species. Are we on the path to planting highly selected organisms, perfect for today's environmental conditions, which, like the dinosaurs, could vanish with any environmental change or the arrival of a new pathogen? We wish to discuss planting such tree species and their fate in North America.

P3: PARTITIONING OF CADMIUM IN COMPLEXES IN DURUM WHEAT SEEDLINGS DOES NOT ACCOUNT FOR HIGH OR LOW CADMIUM ACCUMULATION IN THE GRAIN

Marentes, Eduardo, and Wilfried E. Rauser,
Departments of Plant Agriculture and Botany, University of Guelph, Guelph, ON, N1G 2W1

Five pairs of near isogenic lines of durum wheat (*Triticum turgidum* L. var. *durum*) differing in high (H) or low (L) Cd accumulation in the grain were grown hydroponically with 0.5 µM $CdSO_4$ for 21 days. The distribution of Cd between roots and leaves was determined. Only two isoline pairs had higher concentrations of Cd in the leaves of H isolines than in the L isolines. Although extractability of Cd with buffer was the same for H and L isolines, more of the tissue Cd was extracted from roots (~84%) than from old leaves (~53%) and young leaves (~69%). The buffer soluble Cd was separated by gel filtration into three Cd-containing complexes. In young and old leaves more Cd eluted as complex I near the void volume with lesser amounts as the later eluting complexes II and III. However, in roots most Cd occurred as complex II and little as complexes I and III. The partitioning of Cd into the three complexes was similar for H and L isoline pairs. Conclusion: the partitioning of Cd as complexes in the seedling stage does not account for high or low Cd accumulation in the grain at maturity.

P4: METALLOTHIONEIN PROTEIN OCCURS IN AN EARLY CADMIUM-BINDING FRACTION IN LEAVES OF DURUM WHEAT

Marentes, Eduardo, and Wilfried E. Rauser,
Departments of Plant Agriculture and Botany, University of Guelph, Guelph, ON N1G 2W1

Seedlings of durum wheat (*Triticum turgidum* L. var. *durum* cv. Arcola) were grown hydroponically with 1 µM $CdSO_4$ for 21 days. Buffer soluble Cd extracted from roots and leaves was separated by gel filtration into complex I eluting near the void volume and complexes II and III eluting later. For roots little Cd occurred in complex I (7%), most was in complex II (75%) and less in complex III (18%). In leaves most Cd occurred as complex I (56% in old leaves, 70% in young leaves) with lesser amounts as complexes II and III. The presence of Wali-1 protein, a class II type 2 metallo- thionein (MT), was tested immunologically in the three Cd-binding complexes. Polyclonal antibodies were generated using Wali-1 protein purified from the over expression of a *GST/Wali-1* fusion construct. Wali-1 protein was found in several Cd-containing fractions of complex I in old and young leaves, but not in complex I from roots. No Wali-1 protein was detected in Cd-binding complexes II and III in leaves or roots. Conclusion: a class II type 2 MT protein is associated with Cd in wheat leaves and not in roots.

P5: PHOTOMODIFICATION OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE (RUBISCO) BY ULTRAVIOLET-B (UVB) RADIATION AND UVB ACCLIMATION VIA FLAVONOID BIOSYNTHESIS

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There is evidence that damage to the ozone layer is allowing increased levels of UVB to reach the earth's surface, thereby increasing the potential for damage to susceptible biological targets such as proteins, DNA and membranes. Proteins are susceptible to photodamage due to UVB absorption by aromatic amino acid residues, particularly tryptophan (Trp). As a model system for investigating UVB-damage to proteins, UVB exposure of Rubisco was examined. UVB causes formation of a specific photoproduct that is a covalent crosslink between a large and small subunit within the holoenzyme. The mechanism of this photoproduct formation involves photolysis of a Trp residue. *In vitro* experiments, using purified Rubisco and active oxygen quenchers and scavengers, suggest that the mechanism of photoproduct formation involves a reactive oxygen species. Since UVB has always been present in the environment, plants have evolved ways to detect incident UVB and to mitigate damage to their molecular targets by employing acclimation mechanisms. One mechanism by which plants can prevent UVB from reaching sensitive targets in the mesophyll is to synthesize epidermal flavonoids, which act as UVB screens. UVB irradiation of *Brassica napus* (canola) results in the accumulation of numerous flavonoid species, mainly quercetin and kaempferol derivatives. Consequently, another objective of this study was to determine if flavonoid biosynthesis is correlated with the prevention of Rubisco photoproduct formation *in vivo*; this would suggest that flavonoids prevent UVB-induced damage to Rubisco in acclimated plants. We are currently investigating the photobiology of UVB-induced flavonoid accumulation, with particular attention to the kinetics of induction, and the light quality required for accumulation.

P6: THE MANNAN-CONTAINING CELL WALLS IN THE ENDOSPERM OF *ASPARAGUS OFFICINALIS* SEEDS DURING DEVELOPMENT AND FOLLOWING GERMINATION.

Williams, Heather, A., J. Derek Bewley, John S. Greenwood, Richard Bourgault and Beixin Mo
Dept. Botany, University of Guelph, Guelph, ON NIG 2W1 Canada

The thickened cell walls of endosperm cells of asparagus (*Asparagus officinalis*) seed are composed of glucomannans. During development, glucomannan deposition is specific to the lateral region of the endosperm and not to the micropylar region. The micropylar endosperm may thus be predisposed to facilitate germination. Following germination there is a progressive mobilization of the reserves in the cells of the lateral endosperm in a wave-like manner away from the cotyledons, accompanied by the loss of cytoplasm and the crushing of the lateral endosperm cells. The micropylar endosperm cells, however, remain unchanged during the process. These may form a living barrier to prevent the loss of the soluble products of the hydrolysis of the lateral endosperm to the surrounding environment. The location and timing of endo-β-mannanase production and the increase in activity of β-mannoside mannohydrolase in seeds of germinated asparagus was followed. Endo-β-mannanase activity increases greatly in the endosperm until the mid point of mobilization process and is about 45 times higher than in the embryo on a per seed part basis. Unlike endo-β-mannanase, which is extractable in low-salt buffer, β-mannoside mannohydrolase requires high salt (0.5M NACI) for extraction. This enzyme continually increases in activity in both the endosperm and embryo following germination, with the majority of the activity being concentrated in the embryo when considered on a fresh weight basis.

P7: THE ROLE OF PHOSPHATIDYL-CHOLINE IN FATTY ACID EXCHANGE AND DESATURATION IN *B. NAPUS* LEAVES

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Phosphatidylcholine (PC) plays a key role in lipid biosynthesis in leaves of higher plants, but not necessarily the role that is generally assumed. Fatty acid exchange and desaturation were examined and compared between the chloroplast and extra- chloroplastic compartments in *Brassica napus* leaves using ^{14}C- labeling *in vivo*. Data presented here indicate that in the chloroplast, newly synthesized palmitic and oleic acids are incorporated into monogalactosyldiacylglycerol (MGDG), and desaturated *in situ*, but that in extra-chloroplastic membranes, a different pattern was found. Newly formed fatty acids are exchanged with polyunsaturated fatty acids in PC, and subsequently desaturated *in situ*. The polyunsaturated fatty acids released from PC rejoin the acyl-CoA pool in the extra-chloroplastic membranes, and are used in the formation of diacylglycerol (DAG). This highly unsaturated DAG is, in turn, utilized for the biosynthesis of new PC and may be the source of other cellular glycerolipids. We question the generally accepted theory in which PC synthesized from DAG containing palmitic and oleic acid undergoes sequential desaturation of oleic acid to linoleic and linolenic acid, with no exchange. Our results clearly indicate the existence of a deacylation/reacylation cycle of fatty acids between PC and the acyl-CoA pool.

P8: EXPRESSION OF THE ACC DEAMINASE GENE FROM *ENTEROBACTER CLOACAE* UW4 IN *AZOSPIRILLUM BRASILENSE*

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Many plant growth promoting bacteria (PGPB) may promote plant growth through the activity of the enzyme 1-aminocyclopropane-l-carboxylate (ACC) deaminase, an enzyme that hydrolyses ACC, the immediate precursor of ethylene. *Azospirillum*, a PGPB that promotes the growth of numerous plant species, does not produce ACC deaminase. The ACC deaminase gene from *Enterobacter cloacae* UW4 was transferred into *Azospirillum brasilense* Cd, however, the transformants did not show any ACC deaminase activity. The wild type promoter of the ACC deaminase structural gene (*acd*S) was replaced either with the *lac* promoter or with the Tet^R gene promoter, and transferred into *Azospirillum brasilense* Cd. The Cd/pRKLACC transformants (*acd*S under the control of the *lac* promoter) showed higher ACC deaminase activity as compared to Cd/pRKTACC (*acd*S under the control of the Tet^R promoter). However, the former produced less IAA and showed a significantly lower growth rate. Flooded tomato plants treated with *A. brasilense* Cd/pRKTACC showed lower levels of epinasty than plants treated with the wild type strain or with Cd/pRKLACC probably due to the ability of the cells to degrade ACC. The better performance of Cd/pRKTACC over Cd/pRKLACC may be due to the metabolic load imposed on the cells by the *lac* promoter, which is stronger than the Tet^R gene promoter.

P9: ENDO-β-MANNANASE IS PRESENT IN AN INACTIVE FORM IN RIPENING TOMATO FRUITS OF THE CULTIVAR WALTER

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Mannans are a hemicellulosic component of tomato fruit cell walls and endo-β-mannanase, the enzyme which hydrolyses these polymers, increases during ripening in various cultivars. The cv. Walter, however, exhibits no endo-β-mannanase activity at any time throughout ripening yet Southern, northern and western analyses show that the gene, mRNA, and protein are all present. Furthermore, using immunocytochemical laser scanning confocal microscopy, it was determined that the endo-β-mannanase protein is present in the cell walls of the outer fruit tissues, as is the case in other cultivars exhibiting enzyme activity. The enzyme from the cv. Walter is therefore synthesized and present in the expected location in the fruit during ripening, but is inactive. The lack of endo-β-mannanase activity is not compensated for by an increase in β-mannosidase activity. Unlike the situation in the fruit, the seed contains active endo-β-mannanase activity.

P10: THE BIOCONTROL ABILITIES OF THE STRAIN *PSEUDOMONAS FLUORESCENS* CHAO ARE INFLUENCED BY EXPRESSION OF AN 1-AMINOCYCLOPROPANE- 1-CARBOXYLIC ACID (ACC) GENE

Wang[1], Chunxia, Edouard Knill[1], Bernard R. Glick[2], and Geneviève Défago [1]
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Pseudomonas fluorescens strain CHAO, a root colonizing bacterium, has a broad spectrum of biocontrol activity against plant diseases. Strain CHAO is unable to utilize 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of plant ethylene, as a sole source of nitrogen. This suggests that CHAO does not produce ACC deaminase. Therefore ACC deaminase genes were transferred into CHAO and CHA96, a global regulatory *gac*A mutant of CHAO. ACC deaminase activity was expressed in both CHAO and CHA96. Strains with ACC deaminase genes increased root length of canola plants under gnotobiotic conditions, whereas strains without the genes had no effect. Introduction of ACC deaminase genes into strain CHAO improved its ability to protect cucumber against Pythium damping-off and potato tubers against Erwinia soft rot in small hermetically-sealed container. In contrast, ACC deaminase genes slightly reduced the ability of CHAO to protect tomato against Fusarium crown and root rot, and potato tubers against soft rot in large hermetically-sealed containers. Our results suggest that (i) ACC deaminase activity may have lowered the level of plant ethylene thereby increasing root length, and (ii) the role of stress-generated plant ethylene in susceptibility or resistance depends on the host-pathogen system, and on the experimental conditions used and (iii) the constructed strains could be developed as biosensors for the role of ethylene in plant diseases.

P11: THE ANTIFUNGAL ACTIVITY OF SAPONINS FROM AMERICAN GINSENG.

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Saponins are secondary plant metabolites composed of a triterpenoid or steroid aglycone plus various sugar moieties. The ecological function of these compounds is not known, although research indicates that saponins can act as antifungal defenses. For example, the idea that oat saponins confer resistance to "take-all" disease is well-characterized. Ginseng, (*Panax quinquefolius* L.) has a high content of triterpenoid saponins (6 to 8 % [w/w] in the roots), but can be attacked by several fungal species when grown commercially. The fungitoxicity of the ginseng saponins (ginsenosides) was evaluated *in vitro* for 10 species of fungi, 7 of these were pathogens, and 3 belonged to the genus *Trichoderma*, which may act as antagonists to the pathogens. Also, *Trichoderma* spp. are known to be sensitive to this class of compounds. We found that two species of *Fusarium* were the most resistant to the ginsenosides, as growth was only reduced by ≈ 3 % at ginsenoside levels of 1 mg/mL. Contrary to numerous reports, the *Trichoderma* spp. were more resistant to the saponins than several of the pathogenic fungi.

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P12: PATHOGENESIS-RELATED GENE EXPRESSION IN TOMATO, DURING INFECTION WITH *VERTICILLIUM DAHLIAE*

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Ethylene biosynthesis increases in tomato (*Lycopersicon esculentum*) plants following infection with *Verticillium dahliae*, contributing to wilting, chlorosis and leaf abscission. To identify stress-related genes expressed during this process, three-week-old plants were inoculated with a Verticillium spore suspension using a root-dip method. 72 hours post inoculation, total RNA was extracted from leaves of infected and control plants, and reverse transcription performed. Magnetic bead mediated subtraction hybridization was used to isolate cDNAs upregulated during infection. Subtracted cDNA was cloned for sequencing, and twenty clones with inserts larger than 400bp were selected for sequencing. Four cDNAs have been sequenced, and two have shown identity to a histone protein of *Arabidopsis* and an alpha-glucosidase protein of potato. The remaining cDNAs of interest will be sequenced and differential expression confirmed by reverse Northern. To reduce ethylene stress during fungal infection, promoters for differentially-expressed genes corresponding to these cDNAs will be isolated from genomic DNA, and used to drive expression of genes for disease resistance in transgenic tomato plants.

P13: REJECTION OF SELF-INCOMPATIBLE *BRASSICA NAPUS* POLLEN BY *BRASSICA NAPUS CV*. WESTAR PLANTS EXPRESSING THE *S* RECEPTOR KINASE

Silva, NF[1§], Stone SL[1§], Christie LN[1§], Sulaman W[1], Nazarian KAP[2], Burnett LA [2], Arnoldo M[2], Rothstein SJ[3], Goring DR[1].

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Expression of an *S* receptor kinase (SRK_{910}) transgene in the self-compatible *Brassica napus cv*. Westar conferred the ability of the transgenic pistil to reject pollen from the self-incompatible *Brassica napus* W1 line which carries the S_{910} allele. In one of the *SRK* transgenic lines, 1C, virtually no seeds were produced when the transgenic pistils were pollinated with W1 pollen (Mean seeds/pod = 1.22). This response was specific to the W1 pollen since pollen from a different self-incompatible *Brassica napus* line, T2, and self-pollinations were fully compatible. Westar plants expressing an *S* locus glycoprotein transgene (SLG_{910}) did not show any self-incompatibility response towards W1 pollen. Transgenic Westar plants resulting from crosses between the 1C *SRK* transgenic line and three SLG_{910} transgenic lines were also tested for rejection of W1 pollen. The additional expression of the SLG_{910} transgene in the SRK_{910} transgenic plants did not significantly cause a further reduction in seed production (Mean seeds/pod = 1.04) or have any detectable effects on the number of pollen grains adhered to the pistil. Thus, while the *SLG* gene was previously reported to have an enhancing effect on the self-incompatibility response, no evidence for such a role was found in this study.

P14: CHARACTERIZATION OF NON-PHOTOCHEMICAL QUENCHING IN ISOLATED THYLAKOIDS OF THE LSR5 MUTANT OF *ARABIDOPSIS THALIANA* BY PICOSECOND TIME-RESOLVED CHLOROPHYLL FLUORESCENCE ANALYSIS.

Vassiliev[1], Sergej, Doug Bruce[1] and Richard Peterson[2]

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Recent studies of the non-photochemical quenching (NPQ) deficient mutants of *Arabidopsis thaliana* [Li et al., Nature (2000) 403:391-395, Peterson and Havir, Planta (2000) 210:205-214] have revealed that the PsbS gene encoding chlorophyll a/b binding PS II core polypeptide CP22 is required for NPQ. Several PsbS mutants (lsr1, lsr5 and npq4) appeared to have normal quantum yield of PS II electron transport and normal levels and pigment composition of the other PS II light-harvesting proteins but reduced NPQ level compared to the wild type. Based on these findings it was suggested that CP22 is either the site of ΔpH - dependent excitation quenching or is necessary for conformational changes and quenching that occur in adjacent light-harvesting proteins. In an attempt to clarify the mechanism of NPQ we studied quenching of chlorophyll fluorescence in thylakoids isolated from the wild type and lsr5 mutant of *Arabidopsis thaliana.* Picosecond time-resolved chlorophyll fluorescence decay kinetics were collected at F_m and F_m - quenched states in the spectral region 675-720 nm and analyzed using the global lifetime analysis. We found that two fluorescence decay components which are usually ascribed to PS IIβ (τ_4 = 3 ns) and PS I (τ_1 = 80 ps) exhibit the same changes between quenched and unquenched states in both the wild type and the mutant while changes in the two other (PS IIα) components (τ_2 = 300 ps and τ_3 = 1.5 ns) in lsr5 mutant are 50% smaller compared to the wild type. Further we analyzed the PS IIα fluorescence decay components in terms of the exciton/radical pair equilibrium model. Experimental data was described well by kinetic model with variable rate of excitation decay in the antenna. Thus mutation in CP22 was found to affect only a part of NPQ related to enhanced non-radiative dissipation of excitation in PS IIα antenna while another part of quenching that may originate in PS II reaction centers or alternatively in PS IIβ antenna complexes remained unaffected.

P15: PARTIAL PURIFICATION OF NAD^+ KINASE FROM POTATO

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The low temperature sweetening (LTS) of cold-stored potato tubers cause accumulation of reducing sugars resulting in the formation of dark brown colour of potato chips and French fries. Potato clones resistant to LTS has been shown to possess higher antioxidant enzyme activities. Antioxidant system, the Halliwell-Asada pathway needs NADPH to recycle ascorbate and glutathione and its activity is related to NAD^+ kinase (ATP:NAD 2'phosphotransferase, EC 2.7.1.23). This kinase catalyzes the only known reaction leading to the production of $NADP^+$ in cells. To characterize the relation between antioxidant potential and LTS, calmodulin-sensitive NAD^+ kinase was partially purified and characterized. Four thousand and eight hundred fold purification was obtained after DEAE cellulose and calmodulin affinity chromatography. Further purification was tried with Mono-Q FPLCcolumn chromatography, loosing most of the activities with 145-fold purification factor. The partially purified NAD^+ kinase after the affinity column showed the highest activity at pH 7. At this pH, Km values for NAD^+ and ATP were 40 and 6 μM respectively.

P16: CHARACTERIZATION OF THE IAA BIOSYNTHESIS PATHWAY IN PLANT GROWTH-PROMOTING BACTERIA.

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Inoculation of crop plants with the rhizobacteria *Pseudomonas putida* GR12-2 and *Enterobacter cloacae* CAL3 results in substantial promotion of root growth. Both strains produce the phytohormone indole-3-acetic acid (IAA), which may contribute to the ability of these bacteria to enhance plant growth. While phytopathogenic bacteria primarily utilize the indoleacetamide pathway for IAA synthesis, plant growth-promoting bacteria seem to synthesize IAA through the intermediate indolepyruvic acid. Southern and colony hybridization, and PCR confirmed that *P. putida* GR12-2 carries the *ipdc* gene encoding indolepyruvate decarboxylase, a key enzyme in the indolepyruvic acid pathway; this gene is similar to that from other plant growth-promoting bacteria. In *E. cloacae* CAL3, the *ipdc* gene is different as it only weakly hybridized to the probe and could not be amplified with the PCR primers used to amplify the *ipdc* gene from *P. putida* GR12-2

P17: MYCORRHIZAL SPECIFICITY ON STRAWBERRY CULTIVARS

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We investigated the fate and effects of mycorrhizal inoculation on strawberries in the field under high P conditions. Three strawberry cultivars (Glooscap, Joliette, and Kent) commonly grown for fruit production in Quebec, were inoculated in the field with three mycorrhizal treatments (control, *Glomus intraradices*, and *Glomus mosseae*). Fruit yield and other agronomic parameters were measured. In 1999, the yield was affected by the interaction of cultivar and mycorrhizal treatment with Glooscap-G. *mosseae*, Glooscap-G. *intraradices*, Kent-G. *mosseae*, and Kent-control producing the highest yields. None of the inoculum treatments affected the development dates of buds, flower or fruit. Root colonization levels were the same among cultivars and treatments when sampled at 4, 8 or 12 weeks following the beginning of the growing season. The fruit yield in 2000, although greater than 1999 results overall, was not affected by the cultivar, mycorrhizal treatment or interation effects. Currently foliar nutrient analysis is being measured to determine if mycorrhizal colonization enhanced nutrient uptake. The persistence of the introduced species is also being measured.

P18: PICLORAM SPECIFIC SINGLE CHAIN FV FROM SPLENOCYTES OF HYPERIMMUNIZED MOUSE USING PHAGE DISPLAY TECHNOLOGY.

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Immunoglobin genes were directly isolated from the splenocytes of a mouse hyperimmunized with picloram conjugated to bovine serum albumin, thereby, circumventing the need to synthesize hybridoma cell lines. Variable light and heavy domain DNA were spliced together to produce the scFv DNA, which were cloned into phage vector fd-tet-GIIID to display multiple copies of scfv on the filamentous phage minor coat protein glllp. The phage display scFv library (10^4 clones) was selected against picloram conjugated to ovalbumin. After 5 rounds of panning individual clones were analyzed. ScFv with different affinities to picloram (IC_{50} ranged from 20 ppm to 10 ppm) were present in the final enriched pool. Compared to phagemid vector, the increased avidity of phage vector selected for picloram-specific recombinant antibodies of polyclonal nature. Nucleotide sequence analysis from the isolated clones revealed that all the V_L belonged to the *Vκ9A* family joined to *Jκ2* segments. All the V_H belonged to the V_H7l83 family but joined to two different J segments (J_H2 and J_H4). Different from the immune response to large molecular weight molecules, which requires both VDJ segment rearrangement and somatic hypermutations, we show in this study that production of high affinity antibodies to small molecules (MW < 500 Da) predominantly requires somatic hypermutations.

P19: O_2 CONCENTRATION & LIMITATION IN THE VASCULAR TISSUE OF SOYBEAN STEMS

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In legume nodules, the N_2 fixing enzyme, nitrogenase, is irreversibly inhibited by 0_2, yet it requires large amounts of ATP produced via oxidative phosphorylation. To provide the bacteroids with a high 0_2 supply at low [0_2], nodules have evolved the ability to regulate their permeability to 0_2 diffusion. Similar control over 0_2 may have evolved in other metabolically-active plant tissues (e.g. vascular tissue) to protect against 0_2 free radical damage. To test this hypothesis, an 0_2 sensitive fluorescent dye (Bathophenanthroline Ruthenium Chloride) was pumped through the xylem of a soybean stem and the fluorescence monitored at 610 nm using an excitation of pulse-modulated light (470 nm). In air, the xylem [0_2] was estimated to be <1% (12 μM). Step changes in external p0_2 were used to assess whether [0_2] limits respiratory 0_2 consumption.

P20: RELATIONSHIP BETWEEN REDUCTIVE METABOLISM AND THE EXCHANGES OF $C0_2$, O_2, H_2 AND N_2 IN LEGUME NODULES.

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Real-time measurements of $C0_2$, 0_2, and H_2 exchanges of legume nodules, and calculation of N_2 exchange from H_2, should provide a non-invasive measure of the net metabolic pathways involved in the synthesis of compounds that are less reduced (e.g. ureides) or more reduced (e.g. Polyhydoxybutryric acid or PHB) than glucose. If so, this approach could provide a powerful tool for studying how environmental, physiological and molecular factors regulate these biosynthetic pathways within intact, attached nodules. To test this hypothesis, gas exchange measurements were compared with a mathematical model that was developed to calculate the theoretical $C0_2$ and 0_2 exchanges associated with N_2 fixation, H_2 evolution, growth respiration, maintenance respiration and the synthesis of ureides, amides and PHB. The model predicted that nodules should have a respiratory quotient ($RQ=-CO_2/0_2$) which was much lower (RQ=1.08) than that measured in excised soybean nodules (RQ=1.25). A sensitivity analysis of the model revealed that this discrepancy was most likely related to the biochemical pathways that were assumed to be involved in ureide biosynthesis. An experimental approach will be described to test these assumptions.

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