Canadian Society of Plant Physiologists

2000 Western Regional Meeting

University of Alberta
May 5-6, 2000

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University of Alberta
Edmonton, AB
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Call for Abstracts

The local organizing committee would like to extend an invitation to you to attend the Western Regional Meeting of the Canadian Society of Plant Physiologists. The meeting is hosted by the University of Alberta and will take place at the **University of Alberta - Lister Hall**, Friday, May 5 to Saturday, May 6. The scientific program will include plenary lectures, 15 minute oral presentations and a poster session. **Student presentations are highly encouraged**. The regional meeting has traditionally been a venue for the discussion of "work in progress" and thus your presentation need not be limited to completed projects. Supervisors, please encourage you students to attend and to make a presentation, even if you can not attend yourself.

Location

The University of Alberta is located on the southside of Edmonton. The location of Lister Hall is 116 Street and 87 Avenue. Parking availability is indicated on the University of Alberta Map.

University of Alberta Map

City of Edmonton Map

Accommodations:

A block of rooms at **St. Joseph's College** (a dormitory - a 3 minute walk from Lister Hall) at the University of Alberta has been reserved for May 5 and 6. Single rooms are available for a flat rate of $28.90. Washrooms are shared at this facility. This price includes a daily continental breakfast. To guarantee a room in the block, please make your reservation by April 5 and indicate that you are with CSPP. Reservations can be made by calling 780-492-7681 ext. 221 or email rae.beaumont@ualberta.ca.

A block of rooms at **Lister Hall** (a dormitory) at the University of Alberta has been reserved for May 5 and 6. Single rooms are available for $31.36. Washrooms are centrally located on each floor; linen and towel services are provided. To guarantee a room in this block, please make your reservation by April 5 and indicate that you are with CSPP. You can contact them at guest.services@ualberta.ca or call 780-492-4281 and they will fax a reservation form to you.

There are a few additional rooms at **Lister Hall** that have a private washroom that are $45.00 per night. These rooms are quite popular and availability at any given time in not guaranteed. They're reserved on a first come, first serve basis.

Registration Procedure

Please complete the registration form on this web site. The fees are: Full Member, $120; Non-member, $ 170; and Student Member, $ 75. **A late fee of $20 will be applied to registrations received after April 24.** The registration fee includes snacks at the Friday mixer, refreshments between sessions, and a dinner buffet Saturday evening.

If you are paying by cheque or money order make it payable to "**University of Alberta/CSPP**", and mail to: Francine Hodder, Department of Agricultural, Food and Nutritional Science, University of Alberta, 4-10 Agriculture Forestry Centre, Edmonton, AB T6G 2P5.

If you are paying by Visa or Mastercard you can indicate the card number and expiry date on the registration form or phone Francine Hodder directly at 780-492-7101.

Tentative Program

Friday, May 5	**Venue: The Ship - Lister Hall**
7:00 - 9:00 pm	Conference Mixer (Cash Bar)
Saturday, May 6	**Venue: The Alberta Room - Lister Hall**
7:30 - 8:30 am	Registration
8:00 am	Poster Set-up in the **Ryan Room - Lister Hall**
9:00 am - 12:00 pm	Main Symposium: Physiological, Biochemical and Molecular Analysis of Fruit Development
Speakers:	
9:00 - 9:45 am	**Dr. Jerry Cohen**, USDA, Beltsville MD and University of Minnesota *"Alteration of auxin regulation and its potential for the control of fruit ripening"*
9:45 - 10:30 am	**Dr. Dennis Reinecke**, University of Alberta *"Hormonal interaction in early fruit development"*
10:30 - 11:00 am	Break
11:00 - 11:45 am	**Dr. Karen Koch**, University of Florida *"Carbohydrate-regulated genes and resource allocation during early fruit development"*
12:00 - 1:30 pm	Lunch (on your own)
1:30 - 2:15 pm	**Dr. Douglas L. Godbold**, University of Wales *"How does soil acidification affect roots of norway spruce"*
2:30 - 3:45 pm	15 min oral research presentations
3:45 - 4:00 pm	Break
4:00 - 6:00 pm	Poster viewing
6:00 pm	Buffet Dinner
7:00 pm	Awards Presentations

Abstract Preparation and Submission

To submit an abstract, email Francine Hodder at francine.hodder@ualberta.ca. Submission of an abstract will only be accepted through email. Please review the following instructions below.

DEADLINE FOR RECEIPT: April 24, 2000

Time allowed: 15 min for oral presentations, please leave 1 or 2 minutes after your presentation for questions.

1. For papers submitted from Canadian institutions, one author must be a member of CSPP (not applicable to non-Canadian institutions).

2. Corrections cannot be made after submission.

Instructions for Preparation of your Abstract

Authors and Institutions are to be cited as follows:

B. Concise and T.O. La Pointe*, Department of Botany, University of Toronto at Mississauga, Mississauga, ON L5L 1C6

Your entire abstract including title, author(s), location, and text **must be no longer than 2000 characters including spaces**. Capitalize the title, underline the authors' names, and indicate the speaker's name with an asterisk when the speaker is not listed first. **Use font "Times Roman" and size no larger than 12 point.** Submit the abstract as a **Word Document** to Francine Hodder at francine.hodder@ualberta.ca. Once again, submission of an abstract will only be accepted through email.

Click to Register

Registrants for the 2000 CSPP Western Regional Meeting

Last Name	FirstName	Address	Address	Phone
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Fashu-Kanu	Samuel	Biological Sciences	University of Alberta	492-0181
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Canadian Society of Plant Physiologists
2000 Western Regional Meeting
University of Alberta
Saturday May 6, 2000

7:30 – 8:30 am	Registration (Alberta Room – Lister Hall)
8:00 am	Poster Set-up (Ryan Room – Lister Hall)
	Main Symposium: Physiological, Biochemical and Molecular Analysis of Fruit Development
9:00 – 9:45 am	**Jerry Cohen**, University of Minnesota,"Alteration of Auxin Regulation and its Potential for the Control of Fruit Ripening"
9:45 – 10:30 am	**Dennis Reinecke**, University of Alberta "Hormonal Interaction in Early Fruit Development"
10:30 – 11:00 am	Coffee Break
11:00 – 11:45 am	**Karen Koch**, University of Florida "Carbohydrate-Regulated Genes and Resource Allocation During Early Fruit Development"
12:00 – 1:30 pm	Lunch (on your own)
1:30 – 2:15 pm	**Douglas L. Godbold**, University of Wales "How Does Soil Acidification Affect Roots of Norway Spruce"
2:30 – 2:45 pm	**Urmila Basu**, Biological Sciences "Investigating the Relationship Between Oxidative Stress and Aluminum Toxicity by Transgenic Approach – Overexpression of MnSOD in *Brassica napus*"
2:45 – 3:00 pm	**Neil Harris**, Biological Sciences "Transport of Cadmium and Zinc from the Flag Leaf Lamina to the Maturing Grain of Near Isogenic Lines of Durum Wheat that Differ in Grain Cadmium Accumulation"
3:00 – 3:15 pm	**Julie Stephens**, Biological Sciences "Direct Measurement of Aluminum Uptake and Distribution in Single Cells of *Chara Corallina*"
3:15 – 3:30 pm	**Samuel Fashu-Kanu**, Biological Sciences "Mobilization of the Storage Protein Reserves in Germinated Radiata Pine Seeds"
3:30 – 3:45 pm	**Chris Todd**, Biological Sciences "Regulation of Loblolly Pine Arginase During Germination and Early Seedling Growth"
3:45 - 4:00 pm	Coffee Break
4:00 - 6:00 pm	Poster viewing
6:00 pm	Buffet Dinner

ALTERATION OF AUXIN REGULATION AND ITS POTENTIAL FOR THE CONTROL OF FRUIT RIPENING

Jerry D. Cohen, Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108 USA

Recent advances in the genetics, biochemistry, and molecular understanding of auxin metabolism have provided important perspectives on the hormonal control of plant development. Two aspects of our work on auxin metabolism will be discussed from the prospective of the use of this information for control of fruit ripening:

1) Mutant and normal plants of several species (maize, *Arabidopsis*, carrot, tomato and *Lemna gibba*) are able to make indole-3-acetic acid (IAA) by a previously unknown tryptophan-independent route. An *in vitro* system from maize seedlings will make ^{14}C-IAA from ^{14}C-indole in a tryptophan-independent reaction, thus providing the potential for isolation of the enzymes and genes involved in this pathway. Interestingly, the use of the tryptophan degradation pathway to IAA versus the tryptophan-independent pathway is under developmental control. We now have several examples where a change from one pathway to the other has been demonstrated: during embryogenesis in carrot, following wounding in bean, and during fruit ripening in tomato. The significance of these changes in understanding auxin's role in developmental regulation will be discussed.

2) Three genes are being studied for their potential to alter IAA regulation in plants. First, we are using antisense technology to block the formation of IAA-glucose, a reaction catalyzed by the product of the *iaglu* gene. Antisense and overexpressing *iaglu* tomato lines show altered rates of fruit ripening. Second, we have isolated and cloned a gene from *Enterobacter agglomerans* that encodes an indole-3-acetyl-L-aspartic acid hydrolase. This enzyme shows unusual substrate specificity in that it only hydrolyzes IAA-asp, but not other conjugates. Third, the major IAA conjugates of bean consist of a series of proteins of molecular masses from 17-60 kDa with IAA attached, but the function of these proteins remains unknown. A partial cDNA clone for a 42 kDa IAA-protein was isolated and used to screen a genomic DNA library in order to obtain the 5' end of the gene and its promotor sequence. A full length 1182 bp sequence was obtained with an open reading frame of 393 amino acids. Southern hybridization indicated that this IAA modified protein is encoded by a single copy gene. Northern blot analysis is being conducted to study the developmental and tissue-specific expression pattern of this gene in order to begin to understand the role of IAA modification of proteins in plant processes. The long term objective of this work is to have an array of molecular tools for modification of IAA metabolism in specific tissues and at specific times during development. *Supported by DOE grant DE-AI02-94-ER20153 and by National Science Foundation grant IBN97-23999.*

HORMONAL REGULATION OF EARLY FRUIT DEVELOPMENT

Dennis Reinecke and Jocelyn Ozga, University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, AB T6G 2P5

Pea (*Pisum sativum* L.) fruit is a model system for studying the effect of seeds on fruit growth and coordination of organ development. In pea, as in many economically important plants, normal fruit growth requires the presence of developing seeds. The effect of seeds on pea pod (pericarp) growth is assumed to involve hormones [gibberellins (GAs) and auxins]. But the exact role for these hormones in fruit growth is being clarified.

We have developed a method to open the pericarp of plant-attached pea fruit, and expose the pericarp/seeds to specific treatments to understand their effect on growth and development. Data obtained using the split-pericarp method strongly suggests that seed removal inhibits a key step in the GA biosynthesis pathway in the pericarp, conversion of GA_{19} to GA_{20}. Furthermore, an application of a naturally occurring auxin (4-chloroindole-3-acetic acid, 4-Cl-IAA, an auxin of pea fruit) induces the conversion of GA_{19} to GA_{20}, increases GA 20-oxidase message levels (codes for the enzyme: $GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20}$), and stimulates growth of deseeded pericarp. However, another naturally occurring pea auxin, IAA does not stimulate any of these processes suggesting a unique receptor/signal transduction pathway for 4-Cl-IAA action. GA treatment effects the metabolism of GAs, reduces GA 20-oxidase message level, and stimulates the growth of deseeded pericarp. GA plus 4-Cl-IAA treatment to deseeded pericarp can mimic many processes induced by the presence of seeds in pericarp development, and the physiological interactions induced by GA and 4-Cl-IAA are thought to play a significant role in normal pea fruit development.

CARBOHYDRATE-REGULATED GENES AND RESOURCE ALLOCATION DURING EARLY FRUIT DEVELOPMENT

K. E. Koch, Y Wu, and Y Zeng. Plant Mol. and Cell Biol. Program. - Hort Sci. Dept., University of Florida, Gainesville, FL, 32611 (kek@gnv.ifas.ufL.edu)

Many genes affecting fruit development can be either up- or down- regulated by sugars. However, these ancient sugar responses at the cell-level must be integrated into those of development at the organ and whole plant levels. We therefore examined the interface between sugar and developmental signals in *Zea mays* L., using genes for invertases and sucrose synthases. These are not only central to sucrose import into developing fruit, but are also sugar-responsive. In addition, the balance between them can affect the extent of hexose-based sugar signals to other genes (invertase produces 2x more "sensible" hexoses). Changes in expression were examined via enzyme activity, mRNA levels, and in situ localization of mRNA. During pollination, invertase genes were rapidly repressed in silks (styles) but rose in elongating pollen tubes (favoring sucrose transfer). In ovules, a burst in invertase expression followed fertilization. This narrow window of expression coincided with periods of kernel set and rapid expansion by maternal tissues. The potential involvement of hormonal signals in these and other responses was tested using excised root tips. A pronounced interaction was observed between kinetin and sugar availability for invertase expression, with kinetin at least partially ameliorating effects of C-deprivation. Finally, analysis of invertase promoters in these root tips and transgenic cell cultures indicated that kinetin/sugar interactions involved at least some transcriptional control by both effectors, as well as influence of introns and mRNA turnover. Multiple mechanisms thus integrate single-celled sugar signals into whole plant responses via hormonal control.

INVESTIGATING THE RELATIONSHIP BETWEEN OXIDATIVE STRESS AND ALUMINUM TOXICITY BY TRANSGENIC APPROACH – OVEREXPRESSION OF MnSOD IN *BRASSICA NAPUS*

U Basu, A Good, G J Taylor, Department of Biological Sciences,
University of Alberta, Edmonton, AB, Canada

Activated oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals have been associated with a number of physiological disorders in plants. Superoxide dismutases (SODs) are considered as key enzymes within the antioxidative stress defense mechanism, directly determining the cellular concentration of O_2 and H_2O_2. Metal ion toxicity has been strongly associated with the induction of enzymes of antioxidant pathway. In this study, we have used transgenic approach to investigate the potential role of SOD in Al toxicity. The full length MnSOD cDNA clone was isolated from a cDNA library prepared from Al-induced *Triticum aestivum* cv. Maringa. Expression of MnSOD was found to be up regulated in response to Al in root tips of Maringa. The MnSOD cDNA from wheat was placed under the control of CaMV35S promoter and introduced into *Brassica napus* cv. Westar using *Agrobacterium* mediated transformation. Putative transgenic plants were selected for resistance to kanamycin and confirmed by genomic DNA PCR. Northern analysis of primary (T0) and T1 transformants indicated enhanced SOD-Gus transcript. Total SOD activity in the transformants (T0 and T1) was about 1.5-2 fold that of the wild type. The presence of MnSOD resulted in increased retention of chlorophyll and a significant reduction in electrolyte leakage in transgenic leaf tissue following methyl viologen (MV) and H_2O_2 induced treatment. Further work is in progress to test homozygous T2 plants with Al and other metal stresses, using lipid peroxidation and root growth as key parameters.

DIRECT MEASUREMENT OF ALUMINUM UPTAKE AND DISTRIBUTION IN SINGLE CELLS *OF CHARA CORALLINA*

J. L. Stephens and G. J. Taylor, Department of Biological Sciences,
University of Alberta, Edmonton, AB T6L 5E7

Quantitative information on the uptake and distribution of aluminum (Al) at the cellular level is required to understand mechanisms of Al toxicity, but direct measurement of uptake across the plasma membrane has remained elusive. We measured rates of Al transport across membranes in single cells of *Chara corallina* using the rare ^{26}Al isotope, an emerging technology (accelerator mass spectrometry), and a surgical technique for isolating subcellular compartments. Accumulation of Al in the cell wall dominated total uptake (71-318 $\mu g\ m^{-2}\ min^{-1}$), although transport across the plasma membrane was detectable (71-540 $ng\ m^{-2}\ min^{-1}$) within 30 min of exposure. Transport across the tonoplast was initially negligible, but accelerated to rates approximating uptake across the plasma membrane. The avacuolate protoplasm showed signs of saturation after 60 min, but continued movement across the plasma membrane was supported by sequestration in the vacuole. Saturation of all compartments was observed after 12-24 h. Accumulation of Al in the cell wall reflected variation in $\{Al^{3+}\}$ induced by changes in Al supply or complexing ligands, but was unaffected by pH. In contrast, transport across the plasma membrane peaked at pH 4.3 and increased when $\{Al^{3+}\}$ was reduced by complexing ligands. Cold temperature (4°C) reduced accumulation in the cell wall and protoplasm, while DNP and CCCP increased membrane transport by 12-13 fold. Our data suggest that the cell wall is the major site of Al accumulation. Nonetheless, membrane transport occurs within minutes of exposure and is supported by subsequent sequestration in the vacuole. The rapid delivery of Al to the protoplasm suggests that intracellular lesions may be possible.

TRANSPORT OF CADMIUM AND ZINC FROM THE FLAG LEAF LAMINA TO THE MATURING GRAIN OF NEAR ISOGENIC LINES OF DURUM WHEAT THAT DIFFER IN GRAIN CADMIUM ACCUMULATION

N. S. Harris and G. J. Taylor, Department of Biological Sciences,
University of Alberta, Edmonton, AB, T6G 2E9

High cadmium content of Canadian durum wheat grain represents a potential economic risk to growers. In an effort to understand the physiological processes that mediate low Cd-accumulation, we studied the transport of non-toxic concentrations of radiolabelled cadmium (5×10^{-11} M ^{109}Cd) from the flag leaf lamina to the maturing grain in two near isogenic lines of durum wheat that differ in grain Cd accumulation. We also determined how this transfer was affected by zinc (^{65}Zn). Most of the ^{109}Cd was retained in the labelling leaf-flap (70% retention 14 d after labelling). Upward transport (peduncle and above) of ^{109}Cd was strongly directed to the grain, with 16 to 17% of total absorbed ^{109}Cd occurring in the grain after 14 d. Little ^{109}Cd accumulated in other parts of the shoot. The high Cd-accumulating isoline accumulated 1.5 to 2-fold more ^{109}Cd in the grain than the low accumulating isoline. Differential accumulation was unrelated to variations in the number of grains per spike, or to grain weight. Transport of ^{65}Zn (5×10^{-7} M) applied to the flag leaf was strongly directed to the grain, with between 65 and 70% of ^{65}Zn accumulating in the grain 7 d following application. Grain ^{65}Zn accumulation was significantly higher in the high Cd-accumulating isoline than in the low accumulating isoline. Transport of ^{109}Cd was not inhibited when jointly applied with ^{65}Zn. Our results show that differential accumulation of Cd in the grain of high- and low-accumulating isolines is correlated with flag leaf to grain transport of Cd. Dual labelling with ^{109}Cd and ^{65}Zn suggests that differences between isolines in Cd transport may be related to Zn transport efficiency, although Zn did not competitively inhibit Cd transport.

MOBILIZATION OF THE STORAGE PROTEIN RESERVES IN GERMINATED RADIATA PINE SEEDS

Fashu-Kanu Samuel, Sandra L. Stone and David J. Gifford, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9

The haploid megagametophyte is the main storage site in radiata pine seeds. Storage protein reserves were classified according to their solubility in phosphate buffer as soluble and insoluble. Quantitative and qualitative study of soluble and insoluble reserve protein showed insoluble protein as the main constituent. Insoluble proteins formed about 85% of the total protein content in the megagametophyte of radiata pine seed. Rapid breakdown of megagametophyte insoluble protein reserve was observed following completion of germination, defined as emergence of the radicle from the seed coat. The depletion continued in the megagametophyte during early seedling growth. This correlates with a substantial increase in the free amino acid pool in the seedling. Increases in aminopeptidase activity in the megagametophyte also appear to correlate with storage protein breakdown. The results of this research provide basic information about the storage protein reserves and enzymes involved in their breakdown. This study serves as an information base for future studies of the molecular and biochemical events during radiata pine seed germination and early seedling growth.

REGULATION OF LOBLOLLY PINE ARGINASE DURING GERMINATION AND EARLY SEEDLING GROWTH

C.D. Todd and D.J. Gifford Department of Biological Sciences,
University of Alberta, Edmonton, Alberta, T6G 2E9

As seeds germinate storage protein hydrolysis provides a nitrogen source to the developing seedlings. In the conifer seed these proteins are found primarily in the living megagametophyte tissue. Following germination of loblolly pine seeds there is a major influx of free amino acids from the megagametophyte to the seedling. A major component of this pool is arginine, which is transported rapidly and efficiently without prior conversion. In the seedling the enzyme arginase is responsible for liberating nitrogen, in the form of urea, from free arginine. Using antibodies raised against an arginase subunit a full length cDNA was isolated from a loblolly pine expression library. This represents the third plant arginase cloned from plants and the first from a conifer. Using this clone and the antibodies as tools, developmental regulation of arginase enzyme activity, protein levels and RNA accumulation in the seedling was investigated. In loblolly pine seedlings arginase activity increases due to de novo synthesis of the protein following germination and is temporally coordinated with increases of free amino acids, including arginine. The regulation of arginase and its importance to early seedling nitrogen metabolism will be discussed.

POSTERS

EXPRESSION OF FLC IN 6-202, A *BRASSICA NAPUS* L. WINTER LINE

Nancy E. Go, Glen P. Hawkins and Anne M. Flanagan
Molecular Biology and Biotechnology Centre
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The FLC (Flowering Locus C) gene encodes a MADS-box class of transcription factor that represses flowering. In *Arabidopsis*, FLC is thought to be the central gene in the regulation of flowering response to vernalization. It is down-regulated by exposure to cold temperatures and there is a direct correlation between level of FLC transcript and flowering time in late-flowering mutants and ecotypes. We checked FLC expression in 6-202, a *Brassica napus* line that has an absolute vernalization requirement. As compared to spring lines, the level of FLC transcript is much higher in 6-202. We then checked if FLC expression is correlated with the quantitative flowering response of 6-202 to vernalization. No such correlation was observed. This indicates that gene(s) other than FLC may play a significant role in the vernalization response of *B. napus* winter lines.

OSMOTICUM ADJUSTMENT DURING COLD ACCLIMATION IN *BRASSICA NAPUS* LINES

Z. Hao, A.M. Johnson-Flanagan, Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB T6G 2P5

Freezing tolerance of *Brassica napus* will increase dramatically after exposure to cold acclimation. Osmotic adjustment is one of the most important mechanisms of the cold acclimation process. In our experiment, double haploids were generated from the crossing of parental Cascade and Rebel lines. Six of these offspring lines were selected based on their cold acclimation performance (TL50). These lines were subject to 21 days of cold acclimation exposure and sampled for osmolality, total soluble sugar, and five specific sugars, including stachyose, raffinose, sucrose, glucose and fructose, measured at 0, 2, 4, 7, 12 and 21 days. In addition, total amino acids including proline were measured at 0, 2, 4, 7, 12 and 21 days. Results for the full time span showed that gradual increases in osmolality and total soluble sugar levels are highly correlated to increased freezing tolerance (TL50) during this cold acclimation period (cc is 0.80 and 0.75, $p<0.01$). Specifically, total soluble sugar contributed significantly to osmolality (from 30% to 90%), while total amino acids contributed to a lesser extent (<6%). Furthermore, proline levels reached a maximum on day 4 or 7 then declined to basal levels observed on day 0. The relationship between the levels of total soluble sugar, proline and cold acclimation will be discussed.

VARIATION IN THE NITROGEN STABLE ISOTOPE COMPOSITION OF WHITE SPRUCE UNDER CONTROLLED CONDITIONS

E.S. Pritchard and R.D. Guy*, Department of Forest Sciences, University of British Columbia, Vancouver, BC V6T 1Z4

Physiological and genetic control of whole-plant $\delta^{15}N$ was examined using 10 full-sib families of white spruce (*Picea glauca*) in two hydroponic experiments. Experiment 1 tested effects of N source (100 μM N as NH_4^+, NO_3^- or NH_4NO_3), while Experiment 2 tested the effects of N supply regime (200 μM NH_4^+, draw-down vs steady-state). The NH_4^+ treatment enhanced growth, which correlated negatively with C/N ratio. $\delta^{15}N$ values were higher in the NH_4^+ and NH_4NO_3 treatments, possibly reflecting the efflux/influx ratio associated with each N source, which, in gymnosperms, is higher for NO_3^- than for NH_4^+. Families differed significantly in biomass, C/N, and $\delta^{15}N$, and their ranking was maintained across treatments. Genetic differences in $\delta^{15}N$ and $\delta^{13}C$ were positively correlated. Biomass and $\delta^{15}N$ did not differ between treatments in Experiment 2, but a higher root/shoot ratio in the draw-down treatment indicated some N-stress. Substrate depletion analysis, however, indicated that average net discrimination changed as a function of the NH_4^+ concentration in the medium, decreasing from >9 ‰ at ~100-200 μM to <1 ‰ at ~20-50 μM. Under steady-state conditions, discrimination was largely countered by an inevitable increase in the $^{15}N/^{14}N$ ratio of the media. Across these treatments there was a strong positive environmental correlation between $\delta^{15}N$ and $\delta^{13}C$. As in Experiment 1, family differences in $\delta^{15}N$ were evident and may be related to the effect of uptake and assimilation capacity on the balance of efflux/influx. An almost significant interaction term ($P<0.1$) indicated that genetic control of these processes may vary with supply regime.

INVOLVEMENT OF VACUOLAR ATPASE AND ATP SYNTHASE IN ALUMINUM RESISTANCE IN AN ALUMINUM RESISTANT CULTIVAR OT WHEAT (*TRITICUM AESTIVUM* L.)

Hamilton, Christie A., Gregory J. Taylor and Allen Good. Department of Biological Sciences, University of Alberta, Edmonton AB, T6G 2E9

Two aluminum-induced proteins (RMP51) have recently been discovered in an Al-resistant cultivar (PT 741) of wheat, *Triticum aestivum* L., and shown to segregate with the Al resistance phenotype. The purified peptides share sequence homology with the B subunit of the vacuolar H+ATPase (V-ATPase) and the α and β subunits of ATP synthase. Aluminum induces activities of both V-ATPase and ATP synthase in a dose-dependent manner. In contrast, activity of the plasma membrane ATPase (P-ATPase) is reduced, even under relatively low-stress conditions. To determine whether or not this is a general phenomenon, responses of cellular ATPases to Al stress were examined in a variety of Al-resistant and Al-sensitive cultivars. Induction of V-ATPase and ATP synthase was only observed in PT 741, the Al-resistant cultivar in which induction of these proteins was originally observed.

To test the hypothesis that V-ATPase and/or ATP synthase contribute to Al resistance, the effects of altered ATPase activity levels on the Al resistance phenotype in yeast are being measured. We hypothesize that V-ATPase mutants, and not ATP synthase mutants, will be hypersensitive to Al. Complementation experiments are underway to confirm the role of the missing V-ATPase subunits. We hope this will restore the wild-type phenotype of V-ATPase mutants and confirm that V-ATPase is required for Al resistance. Experiments using antisense transgenic constructs to disable the V- ATPase in *Arabidopsis* are also underway to study the role of these enzymes in mediating Al resistance in plants. Together, we expect these experiments to provide evidence suggesting that maintenance of vacuolar membrane energization is essential for Al resistance in plants. This may reflect a requirement for transport across the tonoplast or for maintenance of cytoplasmic pH for Al resistance.

DIRECT MEASUREMENT OF CADMIUM UPTAKE AND DISTRIBUTION IN CELLS OF *CHARA CORALLINA*

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Cadmium (Cd) is receiving worldwide attention due to its presence as a contaminant on agricultural lands, bioaccumulation in crops, and subsequent health risks posed by human consumption. In an effort to reduce human exposure, an extensive effort has been undertaken to understand uptake of Cd by plants. Uptake of Cd has been investigated in roots of various plant species using metabolic inhibitors, fractionation techniques, and kinetic analysis. These studies suggest that Cd is capable of crossing the plasma membrane and that accumulation is dependent on metabolism. Unfortunately, these results relied on indirect interpretation of kinetic and temporal data to assign pools of Cd to subcellular locations. We are using a more *direct* method to examine Cd accumulation and localization at the cellular level. Our model system consists of single cells (50 - 120 mm in length, diameter ~1 mm) of *Chara corallina* which can be surgically separated into distinct components (cell wall, protoplasm, and vacuole). Accumulation of radioactive ^{109}Cd can be measured independently in each of these fractions. Uptake over short time periods (0-3 h, 0-100 nM) was dominated by accumulation into the cell wall (~90%), and was concentration dependent. As cell wall levels measured in this study were higher than previously reported the nature of accumulation in the cell wall has been investigated. Results from experiments which compared accumulation by intact and isolated cell walls suggest that Cd accumulation into this subcellular compartment is dependent on metabolism. In addition, cell wall bound Cd is readily removed by exchange with excess $CdCl_2$.

ISOLATION AND CHARACTERIZATION OF A WOUND-SIGNAL MOLECULE FROM HYBRID POPLAR INVOLVED IN INSECT DEFENSE MECHANISMS

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Poplars and aspen (*Populus sp.)* are economically and ecologically important forest trees in North America, but are subject to severe defoliation by insect pests. Induced defenses, which occur via activation of defense genes after insect attack, are thought to have a significant function to reduce subsequent herbivory. In a previous study, we characterized two defense genes, trypsin inhibitor (TI) and polyphenol oxidase (PPO), that were induced by forest tent caterpillar damage, as well as methyl jasmonate (a known local plant wound signal). Induction of TI and PPO mRNA by mechanical wounding was observed in both the wounded and unwounded leaves on the same tree, suggesting the presence of a systemic wound-signaling system in hybrid poplar. Therefore, we are working to isolate and characterize this mobile wound-signal from hybrid poplar. Recently, we have established two bioassay systems to monitor signaling activity during purification: 1) an excised poplar leaf feeding test and 2) poplar cell culture test. Using these assay systems, poplar leaf extracts were fractionated by several chromatographic steps. The elution patterns of the active fractions from these chromatographic steps suggests similarity to those of the tomato systemin, which is a known wound-signaling peptide. We will further purify the wound-signaling molecule and characterize the chemical structure and biological features of the compound. This project will contribute to establishing the importance of systemic wound-signals in trees and will provide a potential strategy for improving endogenous herbivore-defenses in plantation trees.

CHEMICAL DEFENSE AGAINST INSECTS: DIHYDROFLAVONOL REDUCTASE AND CONDENSED TANNINS IN TREMBLING ASPEN (*POPULUS TREMULOIDES*)

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Trembling aspen (*Populus tremuloides*) has many constitutive and induced defenses that prevent infection and pest damage. One major group of defense compounds is the condensed tannins. Tannins are toxic polymers that strongly bind and precipitate proteins. The enzyme dihydroflavonol reductase (DFR) is the last known enzyme involved in the synthesis of condensed tannins. Performance of the forest tent caterpillar (*Malacosoma disstria*) correlates inversely with the concentrations of condensed tannins in trembling aspen, indicating that condensed tannins may deter herbivory by foliage-eating insects (unpublished data). The objectives of this research are to clone and characterize a DFR cDNA from aspen, study the expression of DFR and correlate DFR expression with the concentration of condensed tannins in aspen. The DFR gene was isolated and sequenced from an aspen cDNA library. The number of DFR genes in the aspen genome will be determined using the isolated DFR cDNA as a probe. The expression of DFR will be studied with respect to biotic stress, environmental conditions, and in different tissues. Preliminary results indicate that wounding induces DFR, which may lead to increased concentrations of condensed tannins. This is the first genetic study of DFR with respect to condensed tannins and plant defense.

AUXIN AND ETHYLENE IN EARLY PEA FRUIT DEVELOPMENT

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Ethylene appears to be a negative regulator of pea fruit development and is involved in senescence of unpollinated pea ovaries. Pea fruit contain two auxins, indole-3-acetic acid (IAA) and 4-chloro-indole acetic acid (4-Cl-IAA). Only 4-Cl-IAA stimulates pea pericarp (pod) elongation while IAA has no effect or is inhibitory. Silver thiosulfate (STS), an ethylene action inhibitor reverses the negative effect of higher concentrations of IAA on pericarp growth. We are interested in understanding whether pea fruit hormones stimulate ethylene release and how fruits respond to this release when fruit are actively growing. The current study describes an *in situ* closed vessel method to measure ethylene levels in control and auxin-treated pericarps that remain attached to the plant. Initial studies measuring ethylene levels from control pericarp treatments (no hormone application) from 1 to 24 h after apparatus setup show that maximum ethylene release occurs between 1 to 6 hours after placing the fruit in the vessels. Application of 4-Cl-IAA and IAA to pericarps immediately after deseeding resulted in significantly higher ethylene release than in deseeded control pericarps (treated with 0.1% Tween 80). Currently we are testing ways to reduce fruit to fruit variability in the observed ethylene release from treated fruit by delaying hormone treatment to 12 h after deseeding when wound ethylene lessens.

REGULATION OF GIBBERELLIN BIOSYNTHESIS BY AUXIN

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Normal pea pericarp growth requires the presence of seeds. Removal of seeds results in reduced pericarp growth and subsequent abscission. It is proposed that pea seeds may promote pericarp growth by maintaining gibberellin (GA) biosynthesis in the pericarp through seed transmittable factors such as auxin. Application of 4-Cl-IAA to deseeded pericarp stimulates pericarp growth (Reinecke et al., 1995, Phytochem. 40:1361), GA 20-oxidase mRNA levels (van Huizen et al. 1997, Plant Physiol. 115:1), and GA 20-oxidase in vivo enzyme activity (conversion of GA19 to GA20)(van Huizen et al., 1995, Plant Physiol. 109:1213). This study focused on the use of 4-substituted auxins as molecular tools to determine the specificity of auxin regulation of [14C]GA19 metabolism in pea pericarp. Pericarp growth and conversion of [14C]GA19 to [14C]GA20 was greatest in deseeded pericarp treated with 4-Cl-IAA followed by 4-Me-IAA. IAA treatment did not stimulate conversion of [14C]GA19 to [14C]GA20 or pericarp growth. Therefore, regulation of the conversion of GA19 to GA20 in deseeded pea pericarp was specific to the biologically active (growth) auxins in this tissue. These results support the hypothesis that auxins and gibberellins are involved in early pea fruit development.

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METABOLISM OF ARGININE IN LOBLOLLY PINE SEEDLINGS

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Storage protein reserves of loblolly pine (*Pinus taeda* L.) megagametophytes are composed of about 23% arginine, representing about 45% of the seed's stored nitrogen. Following germination, breakdown of this storage protein accelerates and the liberated amino acids are transported to the growing seedling. In 9-day-old seeds, which have a high rate of reserve mobilization, levels of free arginine in both the megagametophyte and the shoot pole (cotyledons and epicotyl) of the seedling are comparable to arginine levels observed in storage proteins. However, the free amino acid profile is significantly different in the root pole (hypocotyl and radicle) of seedlings at the same stage, since little arginine is present. This localization of arginine to the shoot pole of the seedling corresponds to the location of arginase, the enzyme which metabolizes arginine into urea and ornithine. Similarly, the rate of storage protein reserve breakdown corresponds to the peak arginase specific activity. Significance of arginase activity with respect to free arginine levels in the seedling will be discussed.

PLASTOQUINONE REDOX-STATUS REGULATES RNA HELICASE EXPRESSION

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In photosynthetic organisms it is becoming evident that light driven shifts in redox potential act as a sensor that initiates alterations in gene expression at both the level of transcription and translation. This report provides evidence that the expression of a cyanobacterial RNA helicase gene, crhR, is controlled at the level of transcription and mRNA stability by a complex series of interacting mechanisms that are redox regulated. Transcript accumulation correlates with a net reduction of the plastoquinone pool, when Synechocystis is cultured photoautotrophically or photomixotrophically and subjected to darkness and/or electron transport inhibitors or illumination that preferentially excites PSII.

Redox-modulation of crhR mRNA stability also regulates transcript accumulation through a mechanism which responds to the cellular redox state and not specifically plastoquinone or signaling by photoreceptors. Our data are consistent with CrhR RNA helicase activity functioning as a linker between redox regulated transcription and translation. The potential for translational regulation of redox-induced gene expression through RNA helicase catalyzed modulation of RNA secondary structure will be discussed.

PLANT GENETIC ENGINEERING AT ARC-VEGREVILLE

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The Alberta Research Council established a new plant genetic engineering laboratory at their Vegreville site in early 1998. The current research projects include molecular farming, development of novel plant promoters, improvement of quality characters and disease resistance in crop species. Our facilities enable us to manage most of the stages necessary to develop this technology and include a state-of-the-art recombinant DNA lab, tissue culture lab, growth chambers, greenhouses, and field plot sites. The lab is focusing on areas that support ARC's mandate of bringing technology developments into commercial, practical use for the benefit of Albertans. We are also working to create partnership with other public and private sector organizations.