NAGW-3672

PLANT METABOLISM AND CELL WALL FORMATION IN SPACE (MICROGRAVITY) AND ON EARTH

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Description of Research

Variations in cell wall chemistry provide vascular plants with the ability to withstand gravitational forces, as well as providing facile mechanisms for correctional responses to various gravitational stimuli, e.g., in reaction wood formation. A principal focus of our current research is to precisely and systematically dissect the essentially unknown mechanism(s) of vascular plant cell wall assembly, particularly with respect to formation of its phenolic constituents, i.e., lignins and suberins, and how gravity impacts upon these processes. Formation of these phenolic polymers is of particular interest, since it appears that elaboration of their biochemical pathways was essential for successful land adaptation. By extrapolation, we are also greatly intrigued as to how the microgravity environment impacts upon "normal" cell wall assembly mechanisms/metabolism.

The following section describes recent progress related to unravelling the effect of gravity on plant growth and development: the first area describes approaches to delineate the fundamental basis of secondary cell wall assembly mechanisms (ground-based studies), and the second examines the effect of microgravity proper (shuttle flight studies) on plant cell wall formation/metabolism.

Accomplishments and Significance

A. Ground-Based Studies: Our most recent research endeavors have addressed development of a model system to investigate early stages of lignin/secondary cell wall formation, the delineation of the sequential steps involved in lignin assembly and regulation of the pathway itself. The following has been discovered: using cell cultures of *Pinus taeda* (Eberhardt *et al.*, J. Biol. Chem. 1993), it has been possible to attain a cell line capable of undergoing a developmental-like transition from an unlignified primary wall to a lignified secondary wall (~S_1 deposition). Judicious carbon-13 labeling established that the lignin so formed was a relatively high fidelity copy of a softwood gymnosperm lignin. We have also discovered that lignin synthesis in these cell cultures can be totally inhibited using H_2O_2 scavengers (e.g., KI) (Nose *et al.*, Phytochemistry, 1994). In such instances, only the monolignols, *p*-coumaryl, and coniferyl alcohols were formed, but without lignin synthesis itself occurring. During lignification/secondary wall synthesis, it was also observed that *p*-coumaryl and coniferyl alcohols had different metabolic fates prior to polymerization (Bernards *et al.*, Phytochemistry, 1994). Thus, coniferyl alcohol underwent facile dimer formation prior to lignin synthesis proper, whereas *p*-coumaryl alcohol did not react until polymerization was initiated.

Additionally, using *Forsythia* species, we have shown that three types of phenol-coupling enzymes are present. The most remarkable is a hitherto uncharacterized stereoselective oxidase which catalyzes the coupling of two *E*-coniferyl alcohols to give (+)-pinoresinol (Paré *et al.*, Tetrahedron Letters, 1994); this is being purified to apparent homogeneity. The other coupling enzymes are O_2-requiring laccase(s) and H_2O_2-dependent peroxidase(s), respectively, both of which afford racemic products.

Significance: It has long been known that lignins vary with cell type and species, and that gravitational corrections result in altered lignin compositions in the tissues so affected (e.g., a

(NASA-CR-197618) PLANT METABOLISM AND CELL WALL FORMATION IN SPACE (MICROGRAVITY) AND ON EARTH Progress Report (Washington State Univ.) 5 p

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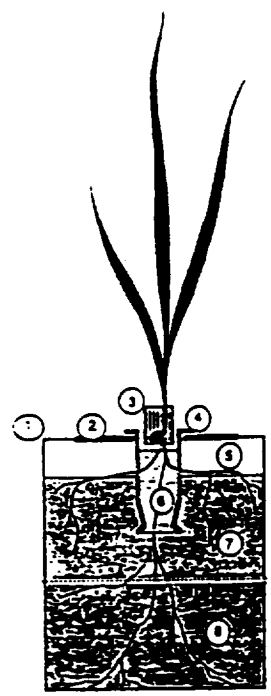
higher *p*-coumaryl alcohol content). Our recent findings now provide a means to systematically define how these compositional changes are regulated, i.e., what factors differentially control individual monomeric synthesis and how precise mechanisms of assembly are controlled in different cell types. A detailed understanding of such processes is essential if we are to define how lignin synthesis is induced during both "normal" and "gravitational correctional" developmental processes.

B. Space Shuttle Studies: Previous difficulties in growing plants in space have been partially overcome by development of a nutrient agar pack (Heyenga *et al.*, 1994) suitable for the uninterrupted growth/development of plants in space (Fig. 1). This obviated the need for sporadic replenishment of water/nutrients, the fluid parameters of which in microgravity have been extremely difficult to control.

Using this approach, it was found that *T. aestivum* wheat seedlings in the "nutrient pack" grew very well in space with little differences observed in shoot and root tissue growth/fresh weight over the 10-day flight when compared to the corresponding ground controls. Moreover, electron microscopic examination of cross-sections of serial sections of wheat shoots and roots revealed essentially no differences. That is, the organelles in the cytosol were very similar, and in the root cell walls there were no measurable changes in the cellulose microfibril orientation/cell wall thickness as revealed by freeze-fracture/TEM/SEM analyses (Figs. 2 and 3). This latter observation is particularly important since it strongly implies that either the process guiding microfibril orientation is fully sensitive to even a microgravity stimulus, or it occurs independent of the g-force experienced.

Figure 8. WSU "Nutrient Pack"

1. Polypropylene envelope
2. Gas diffusion membrane
3. Seed support matrix
4. Support column
5. Air phase
6. Germination medium
7. Basic nutrient medium
8. Enriched medium



Significance: This is the first data set that we are aware of that has been obtained whereby excellent growth, relative to the 1 g controls, was attained in space over a relatively "long duration". Importantly, cell wall assembly was not significantly affected; these results are in direct contradiction to previous studies whose plants, it can now be hypothesized, suffered from various stresses in microgravity. Consequently, these results underscore the growing need for development of reliable plant growth systems for the microgravity environment. [Note also that the greater significance of these findings must await more detailed chemical and biochemical analyses.]

Publications

Umezawa, T., Davin, L.B. and Lewis, N.G. 1991 Formation of Lignans, (-)-Secoisolariciresinol and (-)-Matairesinol with *Forsythia intermedia* Cell-Free Extracts. J. Biol. Chem. **266**:10210-10217.

Davin, L.B., Umezawa, T. and Lewis, N.G. 1991 Enantioselective Separations in Phytochemistry, Modern Phytochemical Methods, Rec. Adv. in Phytochem. **25**:75-112 (Stafford, H.A. and Fischer, K., eds.), Plenum Press, New York.

Lewis, N.G. and Davin, L.B. 1992 Phenylpropanoid Metabolism: Biosynthesis of Monolignols, Lignans, Lignins and Suberins. *In* "Recent Advances in Phytochemistry" (Stafford, H.A., ed.), **26**:325-375, Plenum Press, New York.

Lewis, N.G. and Davin, L.B. 1992 Stereoselectivity in Polyphenol Biosynthesis. *In* "Plant Polyphenols: Synthesis, Properties and Significance" (Hemingway, R.W. and Laks, P.E., eds.), Plenum Press, New York, pp. 73-95.

Lewis, N.G. and Ryan, C.A. 1992 Microgravity in Plant Biological Systems: Realizing the Potential of Microgravity Research in Molecular Biology. AIAA **92**:1347.

Lewis, N.G. 1993 Plant Phenolics. *In* "Antioxidants in Higher Plants" (Hess, J.L. and Alscher, R., eds.), CRC Press, pp. 135-169.

Eberhardt, T.L., Bernards, M., Davin, L.B., Yamamoto, E., Wooten, J.B. and Lewis, N.G. 1993 Lignification in Cell Suspension Cultures of *Pinus taeda* During Early Stages of Cell Wall Thickening. J. Biol. Chem. **268**:21088-21096.

Chu, A., Dinkova, A., Davin, L.B., Bedgar, D. and Lewis, N.G. 1993 Stereospecificity of Benzylic Aryl Ether Reductions in Lignan Formation: Reduction of (+)-Pinoresinol and (+)-Lariciresinol. J. Biol. Chem. **268**:27026-27033.

Bernards, M.A., Lopez, M.L., Zajicek, J. and Lewis, N.G. 1994 Hydroxycinnamate Polymers Constitute the Aromatic Domain of Suberin (in press).

Lewis, N.G. and Davin, L.B. 1994 Evolution of Lignan and Neolignan Biochemical Pathways. ACS Symp. Ser. (XXXX) (Nes, W.D., ed.).

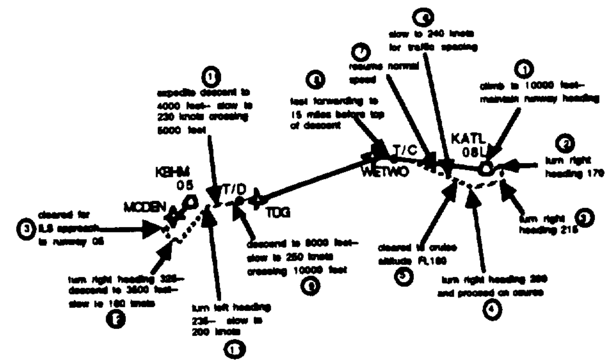
Paré, P.W., Wang, H.-B., Davin, L.B. and Lewis, N.G. 1994 (+)-Pinoresinol Synthase: A Stereoselective Oxidase Catalysing 8,8'-Lignan Formation in *Forsythia intermedia*. Tetrahedron Lett. (XXXX).

Nose, M. and Lewis, N.G. 1994 Monolignol Formation in Cell Culture. Phytochemistry (in press).

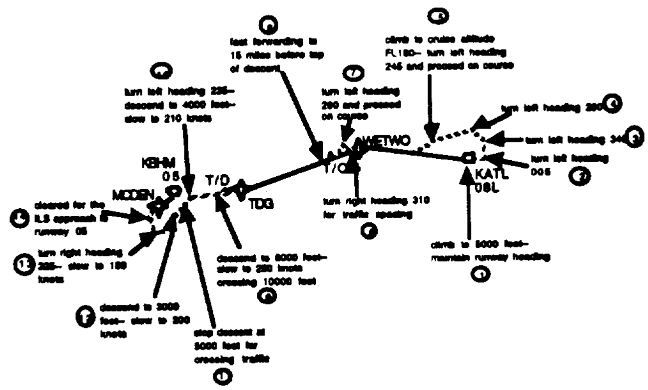
Bernards, M.A., Nose, M., Dettmering, A., Furlan, M., Zacijek, J. and Lewis, N.G. 1994 New Insights into Lignification. Phytochemistry (in press).

Heyenga, G., Davin, L.B., Brown, C. and Lewis, N.G. 1994 A Solid-State Nutrient Support System for Long-term Plant Cultures in Microgravity Conditions (submitted).

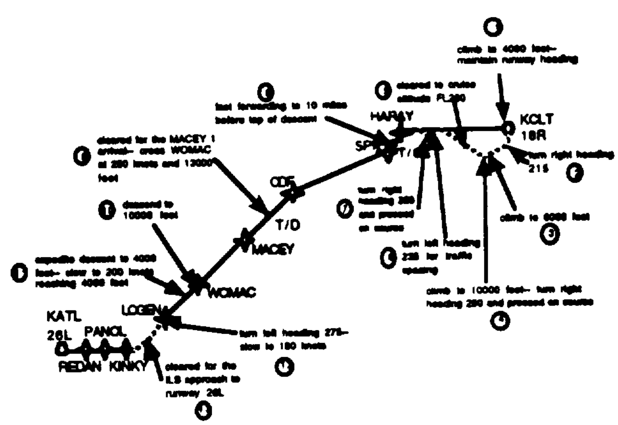
CATS was also given at the NASA Ames Training For Automation workshop (Appendix A3). Subsequent discussions explored possibilities for integrating GT-CATS into an intelligent tutoring system for advanced cockpit automation.



Scenario 1: KATL-KBHM

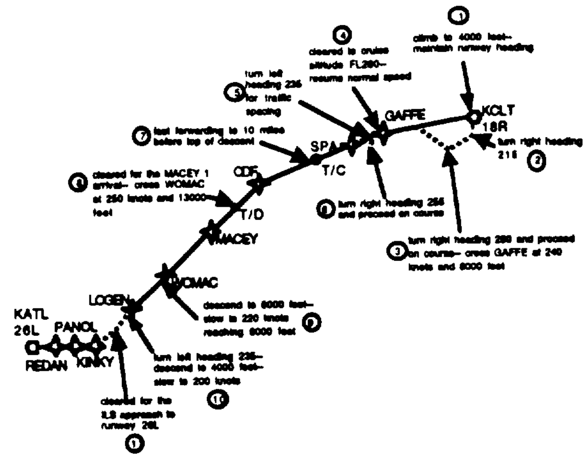


Scenario 2: KATL-KBHM1

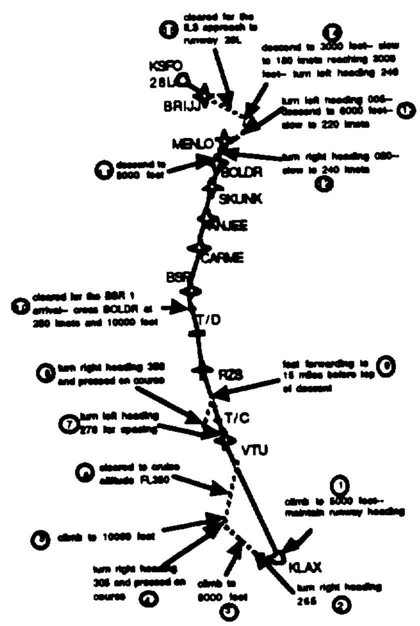


Scenario 3: KCLT-KATL

Figure 2a. Scenarios 1-3.



Scenario 4: KCLT-KATL1



Scenario 5: KLAX-KSFO

Figure 2b. Scenarios 4-5.

APPENDIX A1

GT-CATS SMC Paper

Callantine, T. J., and Mitchell, C. M. (1994). A methodology for understanding how operators use modes of automation to control complex dynamic systems. *Proceedings of the 1994 International Conference on Systems, Man, and Cybernetics*, San Antonio, TX.

APPENDIX A2

GT-CATS SMC Presentation

Presentation Entitled “A Methodology for Understanding How Operators Use Modes of Automation to Control Complex Dynamic Systems” presented at the 1994 International Conference on Systems, Man, and Cybernetics, San Antonio, TX, October, 1994.

APPENDIX A3

GT-CATS Training for Automation Workshop Presentation

Presentation Entitled "The Georgia Tech Crew Activity Tracking System" presented at the NASA Ames Training for Automation Workshop, Moffett Field, CA, August, 1994.

Training Systems for Automation

One of the primary research efforts for this grant is training systems for automation. In the past six months this effort included several complementary activities: evaluation and documentation of the VNAV Tutor, conceptual design of a case-based mode management tutor, and the establishment of a Silicon Graphics research environment, and participation in the Training for Automation Workshop. Progress on each of these activities is discussed below.

VNAV Tutor

A statistical analysis of the data from the initial evaluation of the VNAV Tutor was completed. This evaluation used five pilots transitioning from non-FMS aircraft to a glass cockpit aircraft. The major findings from this analysis show that students learn the actions associated with VNAV well (see Figure 1). In most cases they successfully learn the relationship between the MCP and the FMS as it relates to VNAV (see Figure 2). However, the students are less proficient at identifying data sources to confirm the accuracy of their actions or the accuracy of the VNAV control mode operation. The data supporting these findings and more detailed interpretation are presented in Appendix B1.

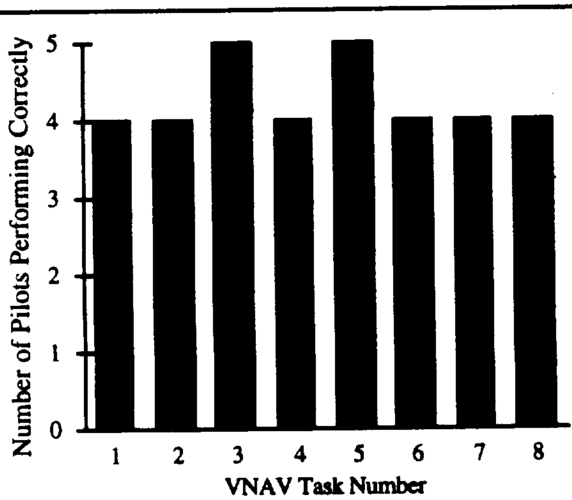


Figure 1. Number of pilots performing correctly on VNAV tasks.

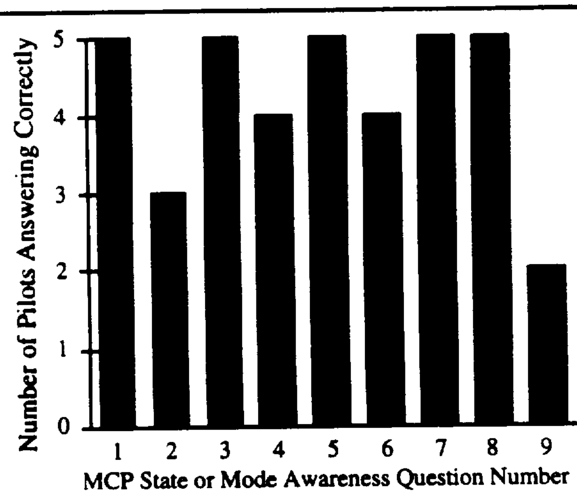


Figure 2. Number of pilots correctly answering questions about the MCP state and mode transitions.

A paper titled "VNAV Tutor: System Knowledge Training for Improving Pilots' Mode Awareness" which documents the VNAV Tutor and the results of the evaluation was written. This paper was presented at the 1994 IEEE International Conference on Systems, Man, and Cybernetics and published in the conference proceedings. A copy of this paper and the presentation appear in this report as Appendices B1 and B2, respectively.

In addition, we started the formulation of a plan for the follow-on evaluation of the VNAV Tutor. The VNAV related functions, data sources, and operations taught by the VNAV Tutor were identified and enumerated. This list extracts and summarizes the salient points of each tutor message in the four training scenarios. Such a set of training objectives defines the starting point for a thorough evaluation of the effectiveness of the VNAV tutor. Preliminary versions of the revised pre- and post- tutor questionnaires have been completed. The in-flight evaluation focusing on VNAV use and mode awareness has also been developed. These evaluation tools provide insights into the capabilities and limitations of the VNAV Tutor. The information gained through this evaluation will guide future Georgia Tech research in training systems.

Case-Based Mode Management Tutor

In order to build on the lessons learned from the VNAV Tutor and other developments in training systems research we initiated the conceptual design for a new tutoring system, a case-based mode management tutor. This proposed system incorporates a case-base of flight scenarios (i.e., incidents and accidents drawn from the relevant literature, such as the ASRS data base). These scenarios are used to configure focused tutoring scenarios that highlight mode management, transition, and interaction. The tutoring system monitors pilot actions in order to control training both within and between scenarios. Within a scenario, the tutor uses a combination of expert and student models to guide the pilot's focus of attention and flight control actions. Expert and student models also guide the selection of new scenarios to systematically broaden the pilot's exposure to mode operation and potential mode management problems. Such an instructional system, combining case-based reasoning (CBR) and intelligent tutoring system (ITS) technologies, may provide an environment and instructional content capable of teaching pilots the wide range of complex and varied modes of control in modern glass cockpit aircraft.

Silicon Graphics Research Environment

A Silicon Graphics Inc. (SGI) Indigo 2 was purchased and configured. This dual head graphics engine duplicates the Mini-ACFS environment at Ames Research Center. A comparable configuration promotes better software transfer from Georgia Tech to Ames and other Ames grantees. Future Georgia Tech aviation research will develop and evaluate proposed systems on the SGI . Experiments will be conducted with the research software running on the SGI communicating via UNIX interprocess protocols with the GT-EFIRT simulator running on a Sun SPARCstation 10.

APPENDIX B1

VNAV Tutor SMC Paper

Crowther, E. G., Chappell, A. R., and Mitchell, C. M. (1994). VNAV Tutor: System knowledge training for improving pilots' mode awareness. *Proceedings of the 1994 International Conference on Systems, Man, and Cybernetics*, San Antonio, TX.

APPENDIX B2

VNAV Tutor SMC Presentation

Presentation Entitled "VNAV Tutor: System Knowledge Training for Improving Pilots' Mode Awareness" presented at the 1994 International Conference on Systems, Man, and Cybernetics, San Antonio, TX, October, 1994.