

Title: **NSCORT. Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology**

PI Name (include prefix (e.g., Dr. Prof), middle initial, and suffix (e.g., Ph.D., M.D.)

**Prof. Eric Davies Ph.D.**

PI Mailing Address (include department, mail code, institution, city, state, zip):

Department of Botany, Box 7612  
College of Agriculture and Life Sciences  
North Carolina State University  
Raleigh, NC 27695-7612

Phone: (919) 515-2727  
Fax: (919) 515-3436  
Email: eric\_davies@ncsu.edu

Project Type (Ground/Flight): Ground  
If flight: Hardware:

Co-Investigators/Affiliation

1. Allen, Nina S. North Carolina State University
2. Boss, Wendy F. North Carolina State University
3. Brown, Christopher S. Dynamac Corporation
4. Huber, Joan L. North Carolina State University
5. Huber, Steven C. North Carolina State University
6. Muday, Gloria K. Wake Forest University
7. Robertson, Dominique North Carolina State University
8. Sederoff, Ronald R. North Carolina State University
9. Thompson, William F. North Carolina State University
10. Tucker, Edward B. Baruch College, City University of New York
11. Whetten, Ross W. North Carolina State University
12. Davies, Eric North Carolina State University

Number of Funded Students:

Pre-College:  
Undergraduate: 10  
Graduate: 5  
Post Doctoral: 7

## **Task Progress and Bibliography for FY98**

(this report prepared by Christopher Brown, cbrown@unity.ncsu.edu)

**PI: Eric Davies**

### **Title: NSCORT. Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology**

#### **ABSTRACT** (PLEASE UPDATE AS NECESSARY)

This program in gravitational biology involves 12 faculty members from North Carolina State University, Wake Forest University, Baruch College, and the Dynamac Corporation through the Kennedy Space Center. The overall goal is to study calcium as a central focal point in the gravity response. The group uses an integrated molecular, cellular, and physiological approach to plant gravitational biology.

The precise modulation of calcium homeostasis will be achieved using transgenic technologies and monitored using sophisticated imaging techniques to verify the specificity and extent of transgenic expression. These efforts, in combination with our expertise in local and long-distance signalling, will make a major contribution to understanding the fundamental role of calcium in orchestrating the transduction of the gravity stimulus into an autopoietic (self-regulated) response.

The project brings together experts in a range of specially-selected fields to address a single major research problem, i.e., the fundamental role of calcium in regulating gravity-stimulated signal transduction in plants. The expertise to be called on includes molecular biologists to produce transgenic plants with altered calcium homeostasis (Thompson, Robertson, Sederoff); cell biologists to image calcium and other components of the signal transduction pathway (Allen, Tucker); physiologists to study signal transduction (Boss, Davies, Muday); and biochemists to study calcium-modulated carbon/nitrogen metabolism (Brown, Huber, Huber). By fostering interdisciplinary collaborations among these diverse laboratories, the proposed program will create a multi-faceted approach to the study of plant gravitational biology.

#### **TASK PROGRESS**

For the **Education/Outreach** component, programs which were established during the first months of the NSCORT were refined and continued and new initiatives were begun. "Space Biology" was offered as an undergraduate course at NC State University and attracted extramural and intramural funding to utilize distance learning technology to enhance the students' experience. Workshops for school teachers dealing with techniques for teaching topics related to plants, gravity and space again were offered and attended by teachers from several states. Other opportunities for outreach, in partnership with existing programs, were explored as well. In all, an estimated 500 students, educators and members of the general public were directly influenced by programs involving the NSCORT. Finally, our homepage was updated and enhanced which will increase advertisement of

this program to the public (<http://www2.ncsu.edu/ncsu/cals/nscort>).

For the **Research** component we focused on two model systems, the maize pulvinus and *Arabidopsis thaliana*. These systems were characterized and utilized for studies of gravity-induced biochemical alterations involving carbon and inositol lipid metabolism, auxin transport, polysome distribution and MAP kinases. Additionally, we progressed in our efforts to image changes in cytoskeleton, calcium, pH and ion currents during gravistimulation through continued research and participation in extramural training. We identified target proteins (calmodulin, calreticulin, PIP-5-kinase and the ER Ca<sup>2+</sup>-ATPase) for a reverse genetics approach to alter calcium homeostasis and the clones are now in hand. This year we have XX publications (published or in press), XX published abstracts, XX seminars/lectures. All of this should benefit NASA in their efforts to understand the role that gravity plays in the development and physiology of plants.

The NSCORT played a major role in the establishment of NC State University as an Affiliate Campus of the International Space University (ISU). ISU is an interdisciplinary, international, and intercultural university dedicated to excellence in space education. The affiliate campus status will allow us to become integrated in the world-wide international intellectual community involving space and its applications. This will provide opportunities such as exchange of faculty, postdoctoral fellows and graduate students, sabbatical leave, off-campus scholarly assignments, joint research development, joint teaching, and outreach program development. This relationship should be of great benefit to ISU, NCSU and NASA as more highly trained scientists, engineers, policy makers and business leaders will be needed to continue our quest to explore the solar system and the universe.

## **EARTH BENEFITS** (PLEASE UPDATE AS NECESSARY)

This research will determine the mechanisms by which plants perceive and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions will improve our understanding of how they grow in various space conditions (e.g. Earth orbit, Mars) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products of enhanced paper quality (e.g. pine seedling system and its formation of compression wood) and yield of seed grains (e.g. reorientation of corn plants blown over in strong winds).

## BIBLIOGRAPHY

### Abstracts

- Brady, S.R., Reed, R.C., and Muday, G.K. 1997. The role of basipetal auxin transport in root elongation, gravitropism, and waving in *Arabidopsis thaliana*. *Grav and Space Biol. Bull.*, 11(1):170.
- Brown, C.S. Sanwo, M.M., Goins, G.D. Wheeler, R.M., Croxdale, J.G., and Tibbitts, T.W. 1997. Potato tuber growth and carbohydrate metabolism in space. *Grav. and Space Biol. Bull.* 11(1):74.
- Collings, D., Winter, H., and Allen, N.S. 1997. Analysis of microtubules and cell wall structure in gravistimulated maize stems. *Grav. and Space Biol. Bull.* 11(1):78.
- Davies, E., Vian, A., and Vian, C. 1997. Electrical signals and gene expression in plants. *Grav. and Space Biol. Bull.* 11(1):74.
- Holzwarth, G. Webb, S.J., Kubinski, D.J., Allen, N.S. 1996. Improving DIC Microscopy with Polarization Modulation. *Molecular Biology of the Cell* 7:158A.
- Huber, J.L., Winter, H., and Huber, S.C. 1997. Gravistimulation induces changes in sucrose phosphate synthase activity in maize pulvini. *Grav. and Space Biol. Bull.* 11(1):76.
- Kuang, A, Brown, C.S., Matthews, S.W., and Musgrave, M.E. 1997. Effect of spaceflight on ultrastructure, chlorophyll and carbohydrate content of *Arabidopsis* leaves. *Grav. and Space Biol. Bull.* 11(1):38.
- Kuznetsov, O.A., Brown, C.S., Sanwo, M.M., and Hasenstein, K.H. 1997. Magnetograviphoretic studies show differences in starch metabolism of space-flown soybean. *Grav. and Space Biol. Bull.* 11(1):24.
- Love, J., Allen, G.C. and Thompson, W.F. 1997. Stringent inducible transgene expression using the Top10-tetracycline promoter in plant cells. *Grav. and Space Biol. Bull.* 11(1):42.
- m-chlorophenylhydrazone (CCCP) application on the gravitropic response in roots and stamen hairs. *Grav. and Space Biol. Bull.* 10(1):17.
- Muday, G., Brown, D., Cowan, L.L., Roberston, D., and Wyatt, S.E. 1997. Gravity perception and response in the inflorescence stems of *Arabidopsis thaliana* *Grav. and Space Biol. Bull.* 11(1):44.
- Perera, I.Y. Heilmann, I., and Boss, W.F. 1997. Gravity-induced changes in phosphorylation of inositol-containing phospholipids in plasma membranes of maize pulvini. *Grav. and Space Biol. Bull.* 11:46.
- Perera, I.Y., Heilmann, I.H, and Boss, W.F. 1997. Gravity-induced changes in phosphorylation of inositol containing lipids from plasma membranes of maize pulvini. *Grav. and Space Biol. Bull.* 11(1):46.
- Scott, A., Wyatt, S., Sampson, K. and Allen, N. 1997. GFP-labeled endoplasmic reticulum in plant cells: the effects of mas-7 and gravity. *Mol. Biol. of the Cell.* 8 S: 226a.
- Scott, A.C., and Allen, N.S. 1997. The effect of carbonylcyanide
- Winter, H., Huber, J.L. and Huber, S.C 1997. Gravistimulation induces membrane-association of sucrose synthase. *Grav. and Space Biol. Bull.* 11(1):44.
- Wyatt, S.E., Tsou, P-L., and Robertson, D. 1997. Manipulation of calcium homeostasis with calreticulin. *Grav. and Space Biol. Bull.* 11:66.

## Articles in peer reviewed journals

- Allona, I., Quinn, M., Shoop, E., Swope, K., St. Cyr, S., Carliss, J., Reidl, J., Retzel, E., Campbell, M.M., Sederoff, R., and Whetten, R.W. 1998 Analysis of xylem formation in pine by cDNA sequencing. *Proc. Natl. Acad. Sci. USA* 95:9693-9698.
- Brown, C.S., Tibbitts, T.W., Croxdale, J.G., and Wheeler, R.M. 1997. Potato tuber formation in the spaceflight environment. *J. Life Support and Biosphere Science* 4(2):71-76.
- Collings, D.A., Winter, H., Wyatt, S.E., Allen, N.S. 1998. Growth dynamics and cytoskeleton organization during stem maturation and gravity-induced stem bending in *Zea mays* L. *Planta*, (in press).
- Cook, M.E., Croxdale, J.G., Tibbitts, T.W., and Brown, C.S. 1998. Development and growth of potato tubers in microgravity. *Adv. Space Res.* 21(8/9):1103-1110.
- Croxdale, J., Cook, M., Tibbitts, T.W., Brown C.S., and Wheeler, R.M. 1997. Structure of potato tubers formed during spaceflight. *J. Exp. Bot.* 48(317):2037-2043.
- Goins, G.D., Yorio, N.C., Sanwo-Lewandowski, M.M., and Brown, C.S. 1998. Life cycle experiments with *Arabidopsis* grown under red light-emitting diodes (LEDs). *J. Life Supp. Biosphere Sci.* 5:143-149.
- Kuzma, M.M., Winter, H., Storer, P., Oresnik, I., Atkins, C.A., Layzell, D.B. 1998. The site of O<sub>2</sub> limitation in soybean nodules. *Plant Phys.* (in press).
- Musgrave, M.E., Kuang, A., Brown, C.S., and Matthews, S.W. 1998. Leaf development in *Arabidopsis thaliana* during spaceflight. *Ann. Bot.* 81:503-512.
- Winter, H., Huber, J.L., Huber, S.C. 1998. Membrane association of sucrose synthase: Changes during the graviresponse and possible control by phosphorylation. *FEBS letters*, 420:151-155.
- Winter, H., Huber, J.L., Huber, S.C. 1998. Identification of sucrose synthase as an actin-binding protein. *FEBS letters* 430: 205-208.

## Articles in other journals (non peer reviewed)

### Meeting papers / Proceedings

- Heilmann, I., Perera, I.Y., Stevenson, J.M., Ransom, W.D., Gross, W., and Boss, W.F. 1998. Inositol lipid signaling: What can we learn from plants? *Proceedings of the 13th International Symposium on Plant Lipids* (in press).

### Academic dissertations or theses

### Books or book chapters

- Lohaus, G., Heineke D., Kruse, A., Leidreiter K., Riens, B., Robinson, D.G., Winter, H., Winzer, T., Heldt, H.-W. 1998. Compartmentation of metabolites between the subcellular

compartments of leaves, the apoplast, the phloem and the storage tissue of different crop plants. In: *Regulation of Primary Metabolic Pathways in Plants*. Kluwer Academic Publisher, (in press).

## Government Publications

Tibbitts, TW, CS Brown, JG Croxdale and RM Wheeler 1998. Experiment IX. Astroculture: Growth and starch accumulation in potato tuber. NASA TM-1998-208697. pp. 9.221-9.228.

## Presentations / Lectures

- Allen, Nina S., Sarah E. Wyatt, Pei-Lan Tsou, and Dominique Robertson. Calreticulin as a tool to manipulate cellular calcium. *Cell Biology of Calreticulin Workshop*, Banff AB, Canada April 1-4, 1998.
- Boss, W.F. Inositol Lipid Signaling: What can we learn from plants?" *The 13th International Symposium on Plant Lipids*, Sevilla, Spain, July, 1998. Invited lecture.
- Brown, C.S. Plants, gravity and space. US Air Force Academy, Ft. Collins, CO, 30 January 1998.
- Brown, C.S. Space Biology: An undergraduate course at NC State University. Fall 1997 and Fall 1998.
- Brown, C.S., and Wyatt, S. "Plants in Space (and on Earth) & the NSCU/NSCORT"  $\Delta\text{K}\Gamma$ \_Service Sorority, Raleigh, NC Oct. 30, 1997.
- Brown, C.S., Keller, M.A., Peterson, B.V., Piastuch, W.C., Sanwo-Lewandowski, M.M. Ethylene increases in plants grown in space and on the clinostat. *American Society of Plant Physiologists Annual Meeting*, June 27 - July 1, 1998, Madison, WI.
- Brown, C.S., Keller, M.A., Peterson, B.V., Piastuch, W.C., Sanwo-Lewandowski, M.M. Ethylene increases in plants grown in space and on the clinostat. *Gordon Research Conference "Effects of gravity on living systems"*, July 12-17, 1998, New London, NH.
- Cowan, Linda L., Sarah E. Wyatt, and Dominique Robertson. Localization of calreticulin in plant cells. *Sigma Xi Undergraduate Research Symposium*, NC State University, April 23, 1998. **(Winner of the undergraduate award for research in the life sciences)**
- Cowan, Linda L., Sarah E. Wyatt, and Dominique Robertson. Localization of calreticulin in plant cells. *American Society of Plant Physiologists-Southern Section Meeting*, Roanoke, VA, March 21-23, 1998. **(Winner of the undergraduate research award)**
- Davies, E., T.J. Whitlock, H. Winter, B. Stankovic. Gravity-induced increases in polysomes in the maize pulvinus. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.
- Heilmann, Ingo, Imara Y. Perera, Wolfgang Gross and Wendy F. Boss. Osmostimulation of *Galdieria sulphuraria*: Effects of cell growth on PIP2-mediated signaling. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.
- Heilmann, Ingo, Imara Y. Perera, Wolfgang Gross and Wendy F. Boss. Life in an acid hot tub: *Galdieria sulphuraria* animal or plant? *32nd Southeastern Regional Lipid Conference*, Cashiers, NC, November 1997.
- Huber, J.L., H. Winter, S.C. Huber. Sucrose-phosphate synthase in graviresponding maize pulvini. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.

- Perera, Imara Y., Ingo Heilmann and Wendy F. Boss. Phosphoinositide signaling during gravity perception and response of maize pulvini. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.
- Perera, Imara Y. and Wendy F. Boss. Molecular characterization of PIP 5-kinase in Arabidopsis. *32nd Southeastern Regional Lipid Conference*, Cashiers, NC, November 1997.
- Perera, Imara Y., Ingo Heilmann and Wendy F. Boss. Phosphoinositide signaling during gravity perception and response of Maize pulvini. *Gordon Research Conference "Effects of gravity on living systems"*, July 12-17, 1998, New London, NH.
- Sanwo-Lewandowski, M., Richards, J.T. Schuerger, A.C. and Brown, C.S. Starch and sucrose metabolism in pepper plants grown under red and blue light. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.
- Tsou, Pei-Lan, Sarah E. Wyatt, and Dominique Robertson. Calreticulin as a tool to manipulate cellular calcium. *American Society of Plant Physiologists-Southern Section Meeting*, Roanoke, VA, March 21-23, 1998.
- Winter, H. Regulation of sucrose synthase by protein phosphorylation: new aspects of its role in carbon partitioning. Texas A&M University, College Station, TX, September 24, 1998. Invited seminar.
- Winter, H. Where is SuSy? The Association of Sucrose Synthase with Membranes and the Actin Cytoskeleton. Plant Physiology Seminar, Dept. of Botany, Duke University, Durham, NC, February 20, 1998. Invited seminar.
- Winter, H. Wo ist SuSy - Regulation der Saccharose Synthase durch Protein Phosphorylierung. Pflanzenphysiologisches Institut der Universitaet Osnabrueck, Germany, August 27, 1998. Invited seminar.
- Winter, H., J.L. Huber, S.C. Huber. Membrane Association of Sucrose Synthase. *Plant Cell Biology, Keystone Conference*, Taos, NM, February 1998.
- Winter, H., S.A. Wyatt, J.L. Huber, D. Robertson, S.C. Huber. Phosphorylation of Sucrose Synthase: membrane binding and actin association. *American Society of Plant Physiologists Annual Meeting*, 27 June – 1 July 1998, Madison, WI.
- Wyatt, S. "Clinorotation: simulating microgravity and more" In "Plants and Gravity Workshop" for Daniels Middle School, at NSCU, Raleigh, NC ca. 100 children, December 18, 1997
- Wyatt, S. "NSCORT & Outreach/Education Programs", EMS 203 North Carolina State University, Raleigh, NC April 14, 1998.
- Wyatt, S. "Plants in Space (& on Earth), 6th grade, Mayfield Middle 2x(100) "Plants in Space (& on Earth), Mayfield High School 2x(60), Mayfield, KY April 9, 1998.
- Wyatt, S. "Plants in Space", 15th Annual Daniels Science Symposium, Meredith College, Raleigh, NC, March 12, 1998.
- Wyatt, S. "Plants on the Move" Longfellow Elem., 1st grade (25) "Plants in Space (& on Earth), Sparks Elem., 4th grade, (28) Mayfield, KY April 8, 1998.
- Wyatt, S. "Plants in Space (& on Earth)", Mayfield High School, 4x(135) "DNA Science: Living by the code," Mayfield High School, 2x (80), Mayfield, KY Sept. 28, 1998.
- Wyatt, S. "The Gravity Persistence Signal (GyPSi) mutant screen in the inflorescence stems of Arabidopsis. *Gordon Research Conference "Effects of gravity on living systems"*, July 12-17, 1998, New London, NH.
- Wyatt, S. and Robertson D. PCR Workshop. School of Veterinary Medicine, Heredia, Costa Rica Feb 9-13, 1998

- Wyatt, S. Molecular analysis on the gravitropic response – new approaches to an old problem. *North Carolina Biotechnology Center (NCBC) Plant Molecular Biology Retreat*, Asheville, NC, September 17, 1997.
- Wyatt, S. Plants on the Move. Longfellow Elem., 2nd grade (23) “Plant the Green Side Up,” East College Early Childhood, K(18), "Plants in Space (& on Earth), Sparks Elem., 5th grade, 2x (112), Mayfield, KY Sept. 25, 1998.
- Wyatt, Sarah E., Pei-Lan Tsou, and Dominique Robertson. Calreticulin: A tool for altering cellular calcium. *Calcium and Gravitational Biology Symposium*, NC State University, Raleigh, NC, March 21-23, 1998.
- Wyatt, Sarah E., Linda Cowan, Dana Brown, Dominique Robertson, and Gloria Muday. The gravitropic response of the inflorescence stems of Arabidopsis. *Calcium and Gravitational Biology Symposium*, NC State University, Raleigh, NC, March 21-23, 1998.
- Wyatt, S. “Plants in Space (& on Earth)”. 6th grade, Mayfield Middle 2x(100) "DNA Science: Living by the code,” 7<sup>th</sup>, Mayfield Middle 5x(97) Mayfield, KY Sept. 24, 1998.

## Patents

## Awards

- Brown, C.S. “The use of videoconferencing to enhance the content of the course Space Biology at NC State University”. Awarded from the NC Space Grant Consortium for Teaching Enhancement, May 1998.
- Cowan, Linda L., Sarah E. Wyatt and Dominique Robertson. Localization of calreticulin in plant cells. Awarded by the NC Space Grant Consortium for Undergraduate Research, May 1998.
- Geary, Reathel, Sarah Wyatt and Dominique Robertson. Ligation of DNA molecules in microgravity...chosen for Undergraduate Space Flight Opportunity on STS-95 (awarded July 1998).
- Shimps, E., Davies, E. Calcium changes in plants during spaceflight conditions” A preliminary study. Awarded by the NC Space Grant Consortium for Undergraduate Research, May 1998.

## Impact on America

### Research Results

Representatives of several companies (including Union Camp Corporation and Westvaco Corporation) have asked to obtain cDNA clones involved in process of xylem formation. These clones are available through the American Type Culture Collection.

### Industrial Affiliates

Dynamac Corporation, Rockville MD.

### New Technologies

**Graduate Student Employment**

**Acronyms**

**Magazine Covers**

Journal of Experimental Botany 48(317), December 1997.

**Popular Press**

*For student, science is out of this world.* Raleigh News and Observer, September 15, 1998

*A new way of seeing: Images from a microscopic world.* Perspectives, the Magazine of the NC State University College of Agriculture and Life Sciences, Fall 1998.