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**The Cooperative U.S./Ukrainian
Experiment: An Overview**

HISTORY OF THE COOPERATIVE U.S./UKRAINIAN EXPERIMENT (*CUE*)

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INTRODUCTION

The Cooperative U.S./Ukrainian Experiment (*CUE*), a peer-reviewed middeck Shuttle flight experiment focused on plant cellular biology, has its history in the diplomatic relations between the United States and the member nations of the former Soviet Union. One goal of these diplomatic initiatives was to strengthen the ties between the U.S. and Ukraine, and early discussions at the State Department level identified cooperation in space science as an excellent beginning to an international dialogue. Thus, the initiation of discussions between the U.S. National Aeronautics and Space Administration (NASA) and the National Space Agency of Ukraine (NSAU) was a directive from each government. These discussions identified a suite of plant cellular biology experiments which had matured to the point where a spaceflight opportunity was appropriate and which focussed upon the mutual interests of U.S. and Ukrainian scientists.

HISTORY OF *CUE*

Signing of a Trilateral Statement between the U.S., Russia, and Ukraine in January, 1994, initiated the discussions between U.S. and Ukraine to collaborate in space research. NASA sent a delegation to Kiev in the spring of 1994 to explore collaborative options with NSAU scientists. In September, 1994, a discussion group of U.S. and Ukrainian space biologists was assembled in Washington, D.C. During this meeting, single-page collaborative preproposals, covering the range of space life sciences from human physiology to computational biology, were developed and submitted to NASA for consideration.

A "Joint State of Future Aerospace Cooperation Between the United States and Ukraine", dated November 22, 1994, directed NASA and NSAU to identify potential experiments and payloads which could qualify for flight and which could create an opportunity for a Ukrainian Payload Specialist to fly on Shuttle.

These early initiatives resulted in the construction of a multi-component proposal which was approved by the NSAU Coordinator for Space Biology in March of 1995 and selected by a NASA peer review panel in May, 1996. Authorization for payload development has resulted in a spaceflight experiment package which includes 5 middeck lockers, a vigorous bilateral collaboration between life scientists, and an educational overlay which will impact students in both nations.

THE COLLABORATIVE UKRAINIAN EXPERIMENT: SCIENCE OVERVIEW

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INTRODUCTION

Life on Earth originated in the relatively constant gravitational force we define as 1 g. Because of its constant nature and the difficulty in varying it in a reproducible manner (particularly downward), gravity has been relatively ignored in biological research. However, with the advent of manned spaceflight, opportunities for conducting significant biological experimentation in the microgravity environment of space are now available. This paper is an overview of the scientific rationale and objectives of one such opportunity involving multiple experiments proposed by investigators from Ukraine and the United States and is known as the Collaborative Ukrainian Experiment (CUE). CUE, manifested for flight on the Space Shuttle mission STS-87 in October 1997, and is scheduled for 16 days. The suite of experiments will utilize five mid-deck lockers and hardware including the Plant Growth Facility (PGF), Biological Research In Canisters (BRIC) chambers and BRIC chambers modified to include light emitting diodes. Four species will be flown, including *Brassica rapa* (rapeseed), *Glycine max* (soybean), *Ceratodon purpurea* (moss) and *Pottia intermedia* (moss). In the spaceflight environment, plants will be grown, observed, manipulated, fixed and frozen. Specimens will be returned to Earth for post-flight analysis.

RATIONALE AND OBJECTIVES

Plants respond to both the magnitude and direction of gravity. Plants which have been reoriented with respect to the gravity vector perceive the change, transduce the signal either inter- or intracellularly, and then respond via redirected growth and shifts in metabolism. Plants exposed to microgravity in the spaceflight environment exhibit changes in a number of growth, developmental and metabolic parameters. The mechanism(s) for these responses to gravity are not known with certainty at the present time and are the focus of the experiments of CUE.

Protonemata of the mosses *C. purpureus* and *P. intermedia* are tip growing cells which are negatively gravitropic in darkness. This is a unique model system since the perception of, and response to gravity purportedly occur within the same cell. Additionally, the protonemata are photo- as well as gravitropic. Vegetative fragments of the wild type and gravity mutant protonemata will be cultured in space and exposed to a number of light treatments. At various times during the mission, specimens will be fixed. Upon return to Earth, specimens will be measured and sectioned for light and electron microscopy to characterize plastid zonation, plastid morphology, subapical cell branching and degree of vacuolation. These experiments allow the study of the influence of gravity on cellular organization and offer an opportunity to resolve whether phototropism blocks gravitropism.

Plants grown in space exhibit a number of metabolic alterations in response to the stresses of spaceflight. In order to examine a number of these effects, both light-grown *B. rapa* and etiolated soybean seedlings will be germinated and grown in space. Tissue will be fixed and/or frozen on orbit and some will return to Earth. Post-flight analyses will focus on the influence of spaceflight on the photosynthetic apparatus of the leaves of *B. rapa* and measurement of compounds known to be altered under stress conditions. Etiolated soybean seeds will be germinated in space and used to determine the mechanism for spaceflight-induced altered carbohydrate and ethylene metabolism. A separate set of soybean seedlings will be challenged with a fungal pathogen, *Phytophthora sojae*, to determine if spaceflight results in altered plant/pathogen interactions. Tissue from both *B. rapa* and soybean will be used for the determination of the differential expression of stress-related genes and seedlings will be measured for growth to determine the impact of the spaceflight environment on biomass partitioning.

A particularly sensitive time in the life cycle of a plant seems to be the transition from the vegetative to the reproductive phase. Gravity may directly or indirectly influence reproductive events in plants. *B. rapa*, due to its compact size and short life cycle, will be used to examine the processes of pollination and fertilization. Plants will be launched at the pre-flowering stage of growth and as seeds which will germinate in space. Pollination will be performed on orbit on the older plants. Tissues from both populations of plants will be fixed in space and/or returned to Earth for extensive examination of parameters of reproductive success.

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DOUBLE FERTILIZATION OF INQUIRING MINDS: TEACHERS AND STUDENTS INVESTIGATING PLANTS IN SPACE FOR THE COLLABORATIVE UKRAINIAN EXPERIMENT

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Three ideas and situations came together in 1995 to create the Collaborative Ukrainian Experiment Education Project which is titled Teachers and Students Investigating Plants in Space. In May of 1995 the presidents of the United States and Ukraine issued a joint statement on cooperation in space via a joint Space Shuttle mission. Dr. Mary Musgrave of Louisiana State University became one of the plant scientists to be awarded space to run a controlled pollination and in-flight fixation of pollinated flowers of the special dwarf stock of rapid-cycling *Brassica rapa* known as "AstroPlants." The stock was developed by Paul Williams at the University of Wisconsin-Madison, where in the February of 1995 the Wisconsin Fast Plants Program had piloted an AstroPlants educational activity associated with the Astroculture experiments aboard Space Shuttle mission STS-63. Substantial educational benefit was experienced by 13 classrooms of students and teachers who shared this real time experiment with flight-based scientists. Dr. Musgrave had mentored one of those classrooms in Baton Rouge and she envisioned creating a similar educational project for STS-87. And thus CUE-TSIPS, with Paul Williams as Educational PI, was initiated. The STS-87 flight offers another real-time investigative opportunity to both United States and Ukrainian teachers and students.

An instructional manual for high school and advanced middle school levels has been written to include investigations on germination, orientation, growth and development, pollination, fertilization and embryogeny. The materials emphasize skills of observing, questioning, hypothesizing, experimenting, analyzing and communicating, and are alligned with the National Science Standards.

Plans for experimental classroom equipment have been designed using low cost, readily available materials that simulate the Shuttle's Plant Growth Chamber (PGC) and its environment. Students will grow their AstroPlants under 24 hour/day fluorescnet light, in 1g gravity.

Sixteen lead teachers in the U.S., four NASA Aerospace Specialists from various NASA centers and the Science Education Coordinator at KSC (sponsored by NASA and the Wisconsin Fast Plants Program) and 25 teachers and specialists in Ukraine (sponsored by the Ukrainian Junior Academy of Sciences), have been trained in the Fast Plants investigative exercises and techniques and are now training other colleagues. It is expected that CUE-TSIPS classrooms will generate a large set of experimental data that can serve as ground control information to be compared with information gathered from the flight experiments. Communication over the Internet will permit data sharing among classrooms and between the U.S. and Ukraine.

Some questions that teachers and students will be asking:

How responsive is the germinating seedling to Earth's gravity?

What would the orientation behavior of the seedlings be like in microgravity?

How is effective pollination carried out?

What are the indicators of normal fertilization and post-fertilization development that follow successful pollination?

Substantial interest from teachers and students to participate in this project has already been generated.

MISSION OPERATIONS FOR THE COLLABORATIVE UKRAINIAN EXPERIMENT

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The Collaborative Ukrainian Experiment (CUE) is a collection of plant space-biology experiments scheduled to fly on a 16-day space shuttle mission (STS-87) in October 1997. A Ukrainian Payload Specialist selected for the mission will conduct the manipulations and measurements required for the various experiments. The experiments will utilize the Plant Growth Facility (PGF) to grow *Brassica rapa* seedlings (a small mustard related to canola), while smaller sealed containers called Biological Research in Canisters (BRIC) will be used to grow soybean seedlings and moss plantlets. The CUE mission will be the first test of the newly developed Plant Growth Facility (PGF), a single suitcase-sized unit which contains six smaller plant chambers. Some of the environmental parameters controlled by the Plant Growth Facility include temperature (with the use of thermoelectric coolers), lighting (with fluorescent lamps), humidity (using Nafion membranes and introductions of dry cabin air), and carbon dioxide (using either LiOH scrubbing or additions of CO₂ -rich cabin air). The BRIC canisters will provide an enclosed, humid environment with passive ethylene scrubbing (potassium permanganate) for the soybean seedlings, and an enclosed environment with supplemental LED lighting for culturing the moss plants. In addition, specially developed hardware will be used for in-flight fixation of the plant tissue grown in the LED/BRICs and PGF.

Mission activities will include periodic pollination of flowers and watering of plants, harvesting, fixing, and freezing of plant tissue, gas sampling from the BRIC canisters, regular checks on system hardware performance, and several video downlinks to conduct educational sessions in support students and teachers following the mission. Science investigators and payload development staff will monitor progress of the experiments in the Experimental Monitoring Area of Hangar L, Kennedy Space Center, where mission activities and communications are monitored on a real time basis. In addition, science investigators and mission operations staff will conduct 48-h-delay ground controls in a second PGF unit and similar BRIC canisters maintained in the Orbital Environmental Simulator (OES) located in Hangar L. The OES can be programmed to provide environmental conditions similar to the Orbiter, except for gravity and atmospheric pressure. Landing for the mission is expected at Kennedy Space Center, after which the hardware and plant tissue will be returned to Hangar L for inspection and analysis.