Mission Information*

Microgravity effects on pollination and fertilization

Experiment Acronym: B-STIC
Principal Investigators: Dr. Mary Musgrave (United States) and Dr. Antonina Popova (Ukraine)
Hardware: PGF

A particularly sensitive time in the life cycle of a plant growing in microgravity seems to be the transition from the vegetative to the reproductive phase. In previous spaceflight experiments, most plants grown full term in space failed to produce any seed at all, and in one experiment in which seeds were produced, the seed quality was very poor. Dosimetry reading taken in flight have failed to explain this ubiquitous sterility in terms of radiation load, thus some developmental failure during plant reproduction seems to be triggered by the microgravity environment itself. Reproductive events in angiosperms have a number of stages which could potentially be influenced directly by gravity. Microsporogenesis (the production of pollen), megasporogenesis (the production of egg cells), pollination and fertilization are all complex developmental events. *Brassica rapa*, a compact plant with a short life cycle, is ideal for such studies.

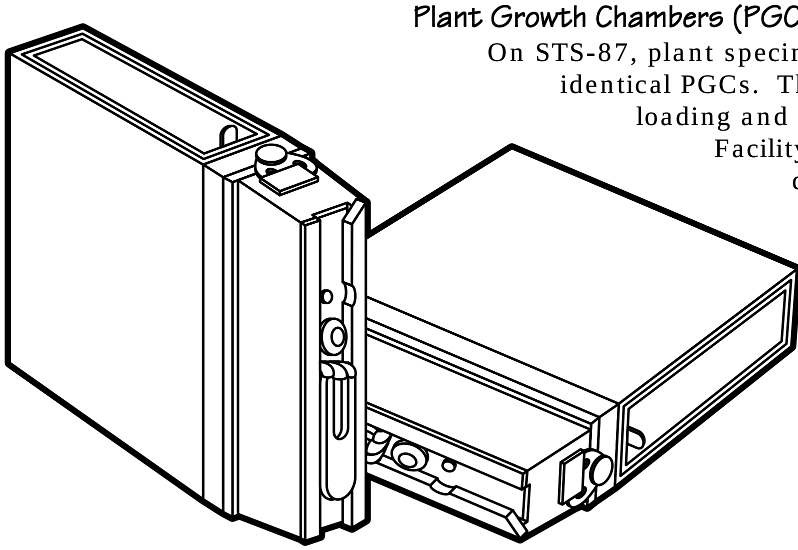
Close comparison of pollination and fertilization processes in microgravity with ground controls has not been possible before this experiment because we have not been able to control when pollination occurs. It is only through the availability of a trained participant for in-flight activities that controlled pollination and in-flight fixation of pollinated flowers will be possible. This will yield important information on pollen germination and maturation in microgravity, pollen-stigma interactions, pollen tube growth, fertilization and early embryo development.

Two plant populations are involved in this study. One population will be launched at the pre-flowering stage of growth. A second population will be seeds at time of launch. Using a pollination kit, the Payload Specialist will perform daily pollinations on the first population, and will mark the flowers pollinated with color-coded wire loops. The pollination wands will be stored with desiccant for subsequent viability assays on pollen. Several pollinated flowers will be fixed in-flight for microtubule studies, but the bulk of the flowers will be returned fresh for extensive processing on the ground. From the first population of plants which will be launched at the pre-flowering stage, siliques will be obtained. For high quality microscopy it will be necessary to dissect out the developing ovules prior to in-flight fixation. A small portion of the siliques will be placed in tissue culture for embryo rescue techniques.

From the second population of plants which were seeds at time of launch, flower buds will be obtained. In vivo tests on these buds will include pollen viability (fluorescein diacetate staining), pollen germination, pollen tube growth through the stigma (aniline blue staining), and staining for stigma esterases. Flower buds will be scored for size prior to dissection and processing for microscopy. In many ways, study of this single event in a plant life cycle integrates the many outstanding questions in gravitational biology.

* adapted from CUE Experiment Requirements Document (ERD), draft version (6/13/96)

PGF and PGC Schematics

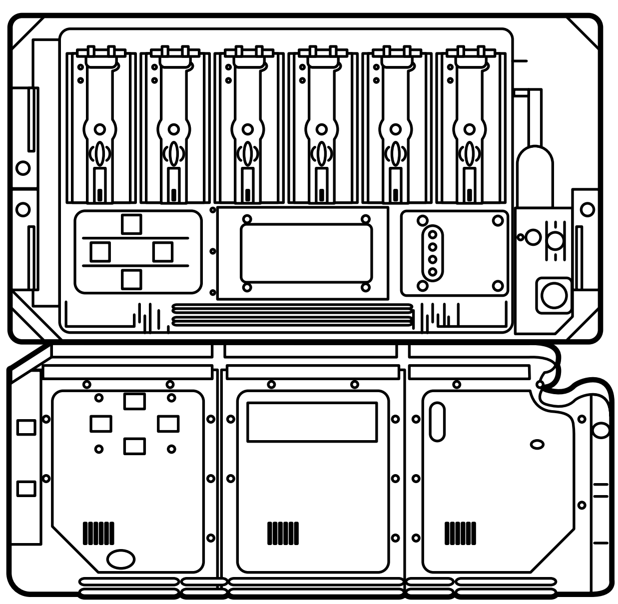


Plant Growth Chambers (PGCs):

On STS-87, plant specimens will be contained in six identical PGCs. The PGCs are designed for easy loading and unloading into the Plant Growth Facility (PGF), and are designed to be doubled or possibly tripled in width.

Front Elevation of PGF:

View of six Plant Growth Chambers (PGCs) and the Control Panel.



Sources of Supplies

Acrylic Sheets
The 2 mm thick, clear acrylic sheets of various size dimensions (e.g., 70 cm x 80 cm) are available from hardware and building supply stores.

AstroPlants Seed
Seed from the stock of Wisconsin Fast Plants known as "AstroPlants" can be purchased from Carolina Biological Supply Company (2700 York Road, Burlington, NC 27215, tel: 800-334-5551).

Bees
Honeybees can be obtained from local beekeepers, or commercially from Carolina Biological Supply Company (2700 York Road, Burlington, NC 27215, tel: 800-334-5551).

Capillary Wicking Material
Capillary wicking material is used in the CUE-TSIPS peatlite growing system. Pellon is available from fabric stores. WaterMat® is available local garden supply centers or from Florist Products (2242 N. Palmer Drive, Schaumburg, IL 60173, tel: 1-800-828-2242).

Chemical Fixatives
The components for mixing chemical fixative solutions are available from chemical supply companies; these components can be ordered for preparing solutions to fix brassica pods, embryos and ovules. A recipe for acetic alcohol fixative solution is included on page 98.

Drawer Organizers
Drawer organizers can be purchased at discount houseware stores.

Nutrient Solution Chemicals
The components for the Wisconsin Fast Plants nutrient solution can be ordered from commercial chemical supply companies. This solution is a modified Hoagland's basal salt mixture with the macro- and micronutrients as described by Hoagland and Arnon (1950). Hoagland's mixture is available premixed in volumes of 1 liter and 10 liters from Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178, tel: 800-835-3010). A recipe for Wisconsin Fast Plants Nutrient Solution is included on page 96. Liquid fertilizers such as Peters® are available from garden supply stores.

Film Cans
Both 35 mm black and clear film cans can be obtained in large quantities from film processing outlets or camera stores. The cans are usually discarded, so ask that they be saved.

Floral Foam
Floral foam is available from florist supply stores.

Indicating Silica Gel
Type III indicating silica gel changes color from blue to pink above 20% relative humidity. Silica gel is available in various quantities from Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178, tel: 800-835-3010) and Aldrich Chemical Company (1001 West Saint Paul Avenue, Milwaukee, WI 53233, tel: 800-558-9160).

Iodine Potassium Iodide Staining Solution
The components for the nutrient solution can be ordered from commercial chemical supply companies such as Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178, tel: 800-835-3010). Directions for mixing the solution and staining specimens is included on page 98.

Lenses
Double convex lenses for film can magnifiers are available from various suppliers, including Hamilton Bell (30 Craig Road, Montvale, NJ 07645, tel: 800-526-0864).

Light Banks
Materials for constructing light banks are available from hardware and building supply stores, or a light system can be ordered from Carolina Biological Supply Company (2700 York Road, Burlington, NC 27215, tel: 800-334-5551).

Microscopes
Illuminated hand-held microscopes are available in magnifications of 30X and 100X from local Radio Shack stores.

Mylar
Colored mylar filters (for the phototropism activity) are available from entertainment or theatre supply stores or directly from Roscolux.

Peatlite
Commercially available as JiffyMix® or Terra-lite Redi-earth®, peatlite mixtures can be obtained from garden supply stores.

Styrofoam Sheets
Builder's insulating styrofoam can be purchased as large sheets from building supply stores.

***** ***Note:*** The listing of proprietary names in this section is not an endorsement of the products. The brand names listed are suggestions only.

Wisconsin Fast Plants Nutrient Solution

Four stock solutions are made in 2-liter dispensing plastic soda bottles. Wisconsin Fast Plants Nutrient Solution is used in the reservoirs for continuous irrigation, mixed in the proportions listed. This nutrient solution is a modified half-strength (0.5X) Hoagland's solution. As an alternative to mixing the stock solutions and preparing the solution, premixed Hoagland's can be purchased from Sigma Chemical Company and diluted to half-strength for use in the CUE-TSIPS activities.

Stock Solutions

- Stock Solution 1: Mixture
  - Add gram amounts to distilled water to make 1.8 liters of stock solution:

    1.0 M KNO_3 (potassium nitrate) 182 grams
    0.2 M KH_2PO_4 (potassium phosphate monobasic) 49 grams
    0.4 M $MgSO_4 \cdot 7H_2O$ (magnesium sulfate) 177.5 grams (or 86.6 grams anhydrous)

- Stock Solution 2: Calcium Nitrate
  - Add gram amounts to distilled water to make 1.8 liters of stock solution:

    1.25 M $CaNO_3$ (calcium nitrate) 531.4 grams

- Stock Solution 3: A-Z (micronutrients)
  - Add gram amounts to distilled water to make 100 ml, then put 45 ml solution in 2-liter bottle, fill to 1.8 liters using distilled water (discard excess):

    H_3BO_3 (boric acid) 2.9 grams
    $MnCl_2 \cdot 4H_2O$ (manganese chloride) 1.8 grams
    $ZnSO_4 \cdot 7H_2O$ (zinc sulfate) 0.2 grams
    $CuSO_4 \cdot 5H_2O$ (copper sulfate) 0.08 grams
    $H_2MoO_4 \cdot H_2O$ (molybdic acid) 0.09 grams

- Stock Solution 4: Iron
  - Dissolve gram amounts in 400 ml of distilled water by heating to 80°C for one hour; let cool slightly and add distilled water to reach total volume of 1.8 liters:

    $FeSO_4 \cdot 7H_2O$ (iron sulfate) 2.5 grams
    Na EDTA (ethylene diamine tetraacetic acid) 3.3 grams

Wisconsin Fast Plants Nutrient Solution

To make half-strength Wisconsin Fast Plants Nutrient Solution, mix the following quantities of the four stock solutions and add distilled water to make a total volume of one liter:

2.0 ml Mixture (Stock Solution 1)
2.5 ml Calcium Nitrate (Stock Solution 2)
2.0 ml A-Z (Stock Solution 3)
2.0 ml Iron (Stock Solution 4)

Peters® Fertilizer Solution

Peters® Professional brand fertilizer solution (N-P-K 20-20-20, with minor elements) can be made in a dispensing plastic soda bottle. To mix the solution, dissolve one soda bottle cap of the Peters® crystals per liter of distilled water.

Film Can Magnifier

A simple hand lens can open the world of micro-exploration for a student. Through the use of an inexpensive lens, a film can and soda bottle cap, students can make their own magnifier.

Materials

- one clear film can (such as those made by Fuji®)
- one plastic soda bottle cap
- one double convex glass lens, 28 mm in diameter (***Tip:*** Use a lens with a focal length of 59 mm; you can use smaller lenses but drill correspondingly smaller holes in the film can.)
- 17 to 20 mm diameter Pyrex test tube or wood bit with spurs
- propane torch or Bunsen burner

Procedure

1. Drill or melt a 20 mm hole in a plastic soda bottle cap and in the bottom of a clear film can. To melt the hole, heat the lip of a Pyrex test tube in a propane torch or Bunsen burner flame. Carefully push the heated end of the tube through the cap and can.

   Caution: *Melting plastic can give off noxious fumes, perform this step in a well-ventilated area or under a hood*.

2. Use scissors to trim off the small protruding rim of the cap so that it fits into the film can.

3. Drop the lens into the drilled film can. Push the drilled soda bottle cap into the can until it holds the lens snugly in place against the film can. The focal length of this lens will bring an object into focus at the open edge of the film can.

Tips and Suggestions

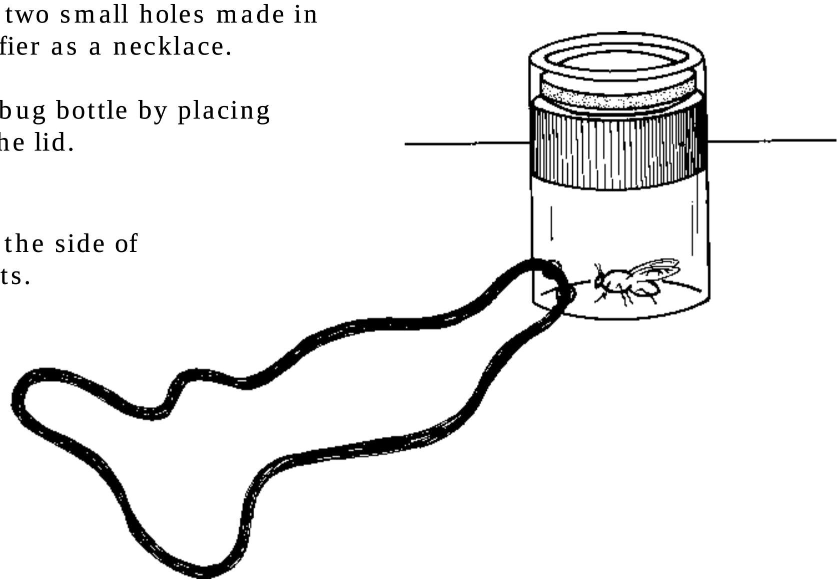
- A local film processing outlet or camera store is a good source for film cans. Ask that the cans be saved for you, since they are usually discarded.

- You can thread a string through two small holes made in the film can and wear the magnifier as a necklace.

- The hand lens can be used as a bug bottle by placing a bug in the can and replacing the lid.

For Dissections

- You can melt two larger holes in the side of the can to be used as access ports.



Chemical Fixative

To mix a fixative solution, combine 75 ml 95% ethanol and 25 ml glacial acetic acid for a total volume of 100 milliliters

Store the fixative solution in an airtight container. Use caution when mixing chemicals.

At the desired developmental stages, remove pods from AstroPlants. Place the pods in an airtight container. Add a depth of the chemical fixative to cover the pods. Seal and label the container with the date and the age of the pods. Pods can be stored for many years at room temperature in acetic alcohol fixative.

Use forceps to remove pods from the fixative and transfer them into a container of at least 100 ml of water for at least three minutes. Pods can then be safely handled with fingers or forceps. Wash hands thoroughly after working with the fixed pods.

Acetic alcohol fixative solution can be poured down a sink and followed with water for disposal. Check with lab supervisors for any special instructions.

Iodine Potassium Iodide (IKI) Staining Solution

To mix an iodine potassium iodide (IKI) staining solution, dissolve 2 g of KI in 100 ml distilled water. Then dissolve 0.2 g of iodine in the KI solution. Store the solution in an airtight container.

Starch will appear blue to black within a few minutes of staining. Newly formed starch may appear red to purple.

Black Line Masters

Dissection Card

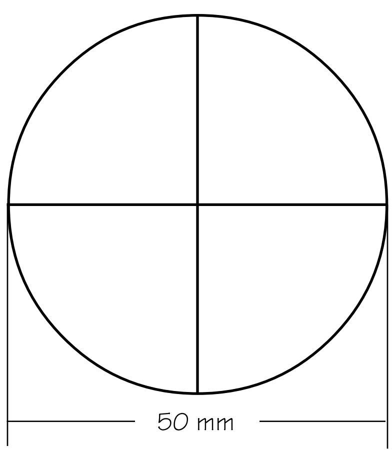
Use this card for activities in which students make observations, drawings and measurements.

Dissection Card

Name ______________________ Date __________

1. Draw a magnified scale on the horizontal axis of the field.
2. Accurately draw scale bar indicating length.
3. Draw the object in view to scale.

Object is: ______________



complete items below:

magnification of lens: _____ X

length represented by scale bar: ________ mm

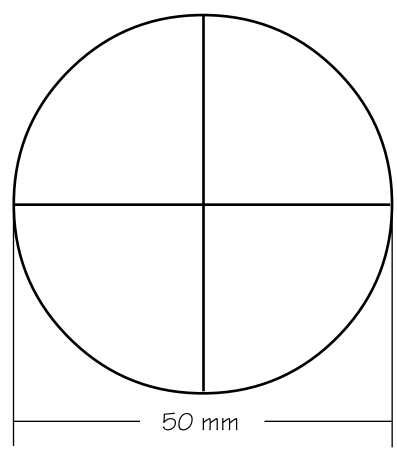
actual length of scale bar: ________ mm

estimated length of object: ________ mm

calculated magnification of drawing:
= ______
= ______ X

1. Draw a magnified scale on the horizontal axis of the field.
2. Accurately draw scale bar indicating length.
3. Draw the object in view to scale.

Object is: ______________



complete items below:

magnification of lens: _____ X

length represented by scale bar: ________ mm

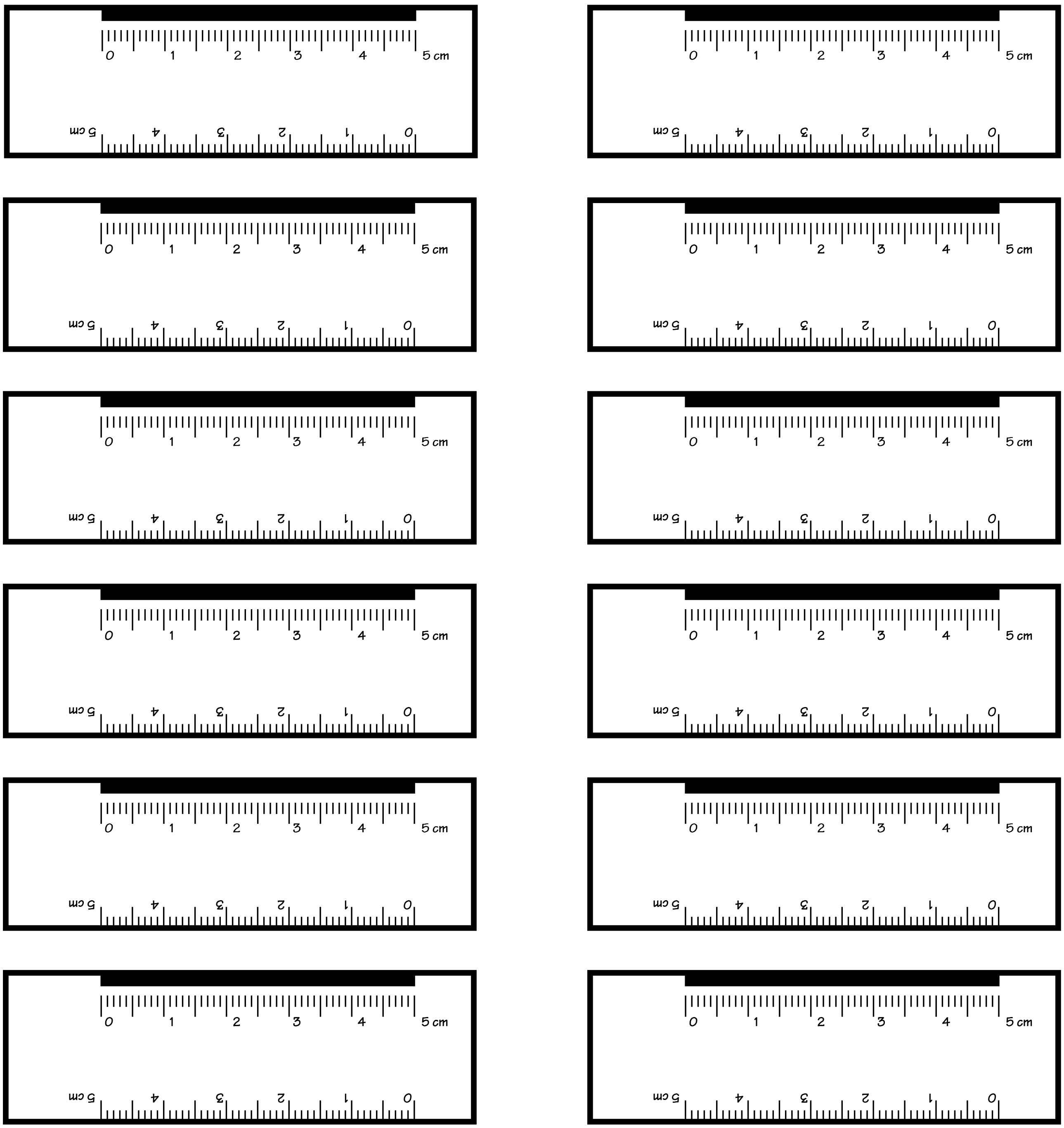
actual length of scale bar: ________ mm

estimated length of object: ________ mm

calculated magnification of drawing:
= ______
= ______ X

Dissection Strips

Photocopy this page onto an overhead transparency sheet. Stick the transparency sheet to a "do it yourself" laminating sheet or piece of clear contact paper, printed side down (this will protect the printing from being pulled of during use of the strip). Cut out the dissection strips. Use the strips for the activities in the pollination and fertilization sections.



NASA Space Life Sciences
Suggested Resources for Study of Plants

Websites

The following Internet addresses will provide users with links to the NASA Specialized Centers of Research and Training (NSCORTs) that focus on plant research and to NASA Life Sciences websites that include lessons and information on plants.

NSCORT in Gravitational Biology:
http://www2.nscu.edu/unity/lockers/project/ncsu-nscort/public/homepage.htm

NSCORT in Plant Biology
Co-sponsored by NASA and the National Science Foundation
http://trna.chem.yale.edu/pss/

Plant Growth Imaging Homepage
http://hideo.biosci.ohio-state.edu/

NSCORT in Bioregenerative Life Support
http://www.rci.rutgers.edu/~biorengg/njnscort

Web of Life
This site is designed as an open door to activities of public interest sponsored by NASA's Life Sciences Division.
http://webolife.arc.nasa.gov
OR email to: *UL_outreach@mail.arc.nasa.gov*

NASA Headquarters Homepage
http://www.hq.nasa.gov

Office of Life and Microgravity Sciences and Applications Homepage
http://www.hq.nasa.gov/office/olmsa

Kennedy Space Center Homepage
http://www.ksc.nasa.gov/ksc.html - Select Biomedical Homepage, Select Space Biology

Johnson Space Center Homepage
http://www/jsc.nasa.gov - Select search, type word "plants"

Ames Research Center Advanced Life Support (CELSS)
http:brad.arc.nasa.gov/Project/CELSSExplanation.html

Additional Websites

The AstroPlants
http://fastplants.cals.wisc.edu/ap.html

Wisconsin Fast Plants Information Documents
http://fastplants.cals.wisc.edu

Books

Bowman, J., Ed. 1994. *Arabidopsis, An Atlas of Morphology and Development*. Springer-Verlag, Inc. (New York).

Buchmann, S.L. and G.P. Nabhan. 1996. *The Forgotten Pollinators*. Island Press (Covelo, CA).

Darwin, C. 1880. *The Power of Movement in Plants*. J. Murray Publishing (London).

Hart, J.W. 1990. *Plant Tropisms and Other Growth Movements*. Unwin Hyman Publishing (London).

Kearns, C.A. and D.W. Inouye. 1993. *Techniques for Pollination Biologists*. Colorado University Press (Niewot, CO).

National Council for Agricultural Education. 1994. *Using Fast Plants and Bottle Biology in the Classroom*. National Association of Biology Teachers (Reston, VA).

Proctor, M., P. Yeo and A. Lack. 1996. *The Natural History of Pollination*. Harper Collins (London).

Suge, Hiroshi. (editor) 1996. *Plants in Space Biology*. Institute of Genetic Ecology, Tohoku University.

Ragnavan, V. 1986. *Embryogenesis in Angiosperms: A Developmental and Experimental Study*. Cambridge University Press (New York).

Raven, P.H., R.F. Evert and S.E. Eichorn. 1992. *Biology of Plants*. Worth Publishers (New York).

Vogt, G.L. and J.J. Wargo, Eds. 1992. *Microgravity*. National Aeronautics and Space Administration (Washington, D.C.).

Journal Articles

Evans, M.L., Moore, R., Hasenstein, K.H., 1986 How roots respond to gravity. Scientific American 254: 112–119.

Reiser, L. and R. Fischer. 1993. The ovule and embryo sac. The Plant Cell 5:1291–1301.

Russel, S. 1993. The egg cell: development and role in fertilization and early embryogenesis. The Plant Cell 5:1349–1359.

Salisbury, F.B. 1993. Gravitropism: Changing Ideas. In Offprints from Horticultural Reviews, Volume 15, pp. 233–278.

Salisbury, F.B. and B.G. Bugbee. 1988. Space farming in the 21st Century. 21st Century Science and Technology 1:32–41.

West, M. and J. Harada. 1993. Embryogenesis in higher plants: an overview. The Plant Cell 5:1361–1369.

Williams, P.H. 1986. Rapid-cycling populations of Brassica. Science 232:1385–1389.

Yeung, E. and D. Meinke. 1993. Embryogenesis in angiosperms: development of the suspensor. The Plant Cell 5:1371–1381.

Wisconsin Fast Plants Information Documents

(Documents available from the Wisconsin Fast Plants office or at http://fastplants.cals.wisc.edu.)

Wisconsin Fast Plants. 1987. "Around the World with Brassicas" Wisconsin Fast Plants (Madison, WI).

Wisconsin Fast Plants. 1994. "Hormone-Induced Parthenocarpy in Rapid-Cycling Brassica Rapa." Wisconsin Fast Plants (Madison, WI).

Wisconsin Fast Plants. 1996a. "The Hunt for Glucose—A Flower's Treasure." Wisconsin Fast Plants (Madison, WI).

Wisconsin Fast Plants. 1990. "Pollen Germination." Wisconsin Fast Plants (Madison, WI).

Wisconsin Fast Plants. 1996b. "Pollen-Stigma Interactions and Pollen Tube Growth." Wisconsin Fast Plants (Madison, WI).