Text for Slides on The Gravitational and Space Biology of Plants

1. shuttle lift-off
Man's curiosity about the space environment is centuries old, but only within the last three decades have people been able to go into space to satisfy that curiosity directly.

2. text slide
As people have gone into space, they have taken other organisms, including plants, with him. There are both practical and basic research reasons for people to take plants with them into space.

3. Why bring plants along?
The benefits of space plant research can be seen both in space and on earth. First, lets look at the benefits in space.

4. Cycle with plants and humans
Plants are able to convert the energy of sunlight into a food supply. They can also be used to purify air and water. In addition, plants are aesthetically pleasing so their presence could help mitigate the detrimental long-term psychological effects of working in a machine-filled stainless steel environment. Although certainly not needed for short-term flights, plants will play an important role in providing a food supply and in creating a hospitable environment for people in any long-term space colony they establish.

5. Minitron Plant Life Support system
Since plants can provide food and purify air and water, they could be an important part of the astronaut's life support system. In order to learn whether certain crop plants could be grown in space the CELSS program of NASA has tested these crops in different scaled-down growth chambers called
a Minitron Plant Life Support System.

6. Benefits on earth
Less evident but no less real are the benefits that will accrue for us on earth from plant studies in space. Examples of these benefits would be in the area of crop improvement and new kinds of products, such as possible medicinal products, that would result from microgravity-induced changes in secondary plant metabolism.

7. For example, many millions of dollars are lost each year in the US because wind and rain cause crops to fall over making them difficult or impossible to harvest. This weather-induced leveling of plants, called lodging is illustrated in this slide. Plants recover from this by a gravity response that allows them to reorient their shoot growth in an upright direction. Discovering the mechanisms by which this happens could allow scientists to develop crop plants that have stronger and faster gravity responses and thus are less susceptible to lodging.

8. Key questions to be answered

Driven by both these practical considerations and the desire to learn more about how plants respond to their environment, scientists study the gravitational biology of plants to answer two fundamental questions:
How does the gravity environment shape the way plants grow and reproduce?
and How will the microgravity of space alter the way plants grow and reproduce?

9. How does the gravity environment...(I)
From our everyday experience we are aware of some obvious ways gravity affects the way plants grow and develop; shoots grow up and roots grow down. When a plant is placed on its side, the plant responds with growth curvature until the shoots again grow up and roots grow down.

10. Corn shoots time-lapse
An example of this can be seen in corn shoots. Horizontally positioned shoots respond rapidly and dramatically to the gravity stimulus, reorienting their growth to the vertical in about an hours. What is not completely understood, as of yet, is how plants perceive gravity and then cause this curvature of growth. In other words, we know *that* plants respond to gravity, but we're not sure *how* they do it. Some of the possible theories will be discussed later.

11. How does the gravity environment...(II)
Scientists know a few other responses of plants to gravity besides gravitropism. But it is quite possible that there are still other ways gravity affects plants that we do not know about. These we may only discover when we try growing a plant in the virtual absence of gravity, in the microgravity environment of space. Theoretically, all aspects of plant physiology and development may be influenced by gravity in subtle and not-so-subtle ways. If we want to have plants with us as we explore the reaches of outer space, we need to find out just what are the ways gravity (and the absence of gravity) influences how plants grow and reproduce. This investigation has already begun and the next group of slides will describe some what we have learned so far.

12. How will the microgravity environment....
How will the microgravity of space alter the way plants grow and reproduce? An obvious effect is the disoriented growth of roots and shoots. However, scientists are also studying the less obvious effects on development, reproduction, and metabolism. In order to understand the effects of gravity and microgravity, studies are being done both in space and on Earth. First, we'll look at some space-based research.

13. Astronaut preparing experiment
The limited amount of space aboard the space shuttle means that experiments must be simple and compact. Here, astronaut Franklin Chang conducts a botany experiment aboard the space shuttle Columbia. This experiment involved chemically fixing seedlings grown in microgravity for later microscopic study by scientists on earth. More recent technical developments will soon allow scientists to view plants live and in color while they are responding to the microgravity environment.

14. Examples of studies in space

Most of the initial studies on plants in space were done by Russian scientists, beginning about 20 years ago, but more recently American scientists have completed some innovative and ground-breaking experiments, and the next group of slides will review the exciting results of these studies.

15. Examples: changes in root cells

The first study we'll look at was carried out with the participation of undergraduates from Baylor University working in the laboratory of Dr. Randy Moore. The question being asked was, "How does the microgravity environment affect the structure of root cells?".

16. EM of root cell normal on Earth

This is an electron micrograph of a cortical root cell from a corn plant grown on Earth. Plant cells store food mainly as starch in organelles called amyloplasts. Some is stored as oil. Note that in this slide the amyloplasts each contain many granules of starch, the white structures inside the amyloplasts, and there are relatively few droplets of oil.

17. EM of root cell from micro-g

This next slide is an electron micrograph of a cortical root cell from a corn plant grown in the microgravity of outer space. Compare this cell with the one shown in the previous slide. Note that the amyloplasts contain less starch and that there is an abundance of oil droplets. What would you conclude are the effects of the space environment on these cells? Biologists believe that these kind of cytological changes are indications that the space environment somehow disrupts normal carbohydrate metabolism.

18. Examples: chromosome damage

Another group of experiments deals with the possible genetic changes caused by space flight. Dr. Abe Krikorian at SUNY Stony Brook is studying whether mitosis and chromosome behavior in developing plant cells is modified by the space environment.

19. Cells on Grid

For these experiments, Dr. Krikorian used aseptically cultured, embryogenic cells of the garden daylily as a model for the study of development in higher plants both on earth and in the space environment, or microgravity. He chose to use these cells, which can develop and pass through all stages of embryogenesis, rather than seedlings or fully mature plants is mainly because the lily material is clonal, or genetically identical, and doesn't take up much room. Embryogenic daylily cells were flown on an eight day space shuttle flight.

This is a close-up of daylily somatic embryos on an agar surface. A honeycomb-like grid is visible in the background. This grid serves as a support for the agar nutrient medium and prevents dislodging of the medium during spaceflight. The cells are separated from the medium by a membrane which prevents growth of the cells into the medium, but allows nutrient to pass through it. This facilitates removal of somatic embryos at the end of the flight for analysis. After the flight, the cells were recovered quickly and assayed for chromosomal changes.

20. chromosomes

The assay for chromosomal changes showed evidence of progressive damage in developing somatic embryos of daylily. In the upper left-hand corner, you can see metaphase division figure showing morphology of control or undamaged cells. In the upper right, a division figure shows perturbations in structure of the chromosomes. Lower left and lower right pictures show chromosome structural deterioration and fracturing that signifies serious damage to the integrity of the cell's genetic material. Cells as badly damaged as these would not survive to divide again.

21. Daylily plants

The effects of space flight were also seen when daylily plants were grown from the somatic embryos exposed to the space environment. The plant on the left is a normal daylily. The plant on the right grown from space-exposed cells is clearly different. All the indications are that a mutational event or events occurred in the space-flown material. A key unanswered question in these daylily experiments is whether the chromosomal damage observed was due to microgravity or due to some other aspect of the space environment, for example higher ionizing radiation. This question can be answered only by including a 1-g control in microgravity. If changes observed in space are *not* observed when plants are kept on a 1-g centrifuge in space, then the changes are most likely attributable to the lack of a 1-g stimulus. Plans to include a 1-g centrifuge on the Space Station to be built in the next few years are being discussed.

22. Examples: Pollen and seed development

An additional area which is being studied is pollen and seed-development. Plant reproduction under spaceflight conditions is an important component of a proposed closed ecological life support system for long-duration manned missions. Reproduction is a complex developmental event likely to be disrupted by the unusual environmental conditions in orbital spacecraft. In these experiments, carried out by the laboratory of Dr. Mary Musgrave at LSU, plant reproduction was studied in *Arabidopsis thaliana* during three Space Shuttle missions: STS-54, STS-51 and STS-68. The question being asked was whether Arabidopsis would carry out normal reproductive processes in space.

23. SEM of pollen and flowers

This slide shows scanning electron microscope pictures of pollen grains and flowers from several STS missions. In (A) is shown typical pollen grains taken from plants flown in the STS-54 mission. The pollen grains are in a collapsed condition and are presumably sterile. Flowers on these plants, shown in C, were also abnormal, with their pistils, the large, central structure, and anthers, pointed out by arrows, collapsed at an early stage. Scientists conducting this study noted that their carbohydrate analysis of the foliage indicated very low levels of fructose in the spaceflight material, and that total carbohydrate, including starch, was only 61% of the ground control. They suspected that one possible cause of the reproductive failure on STS-54 was the low carbohydrate status of the plant, and in the subsequent experiments on STS-51 and STS-68, they supplemented the medium with additional sucrose and raised the carbon dioxide level in the growth chamber to 8000 ppm. These countermeasures resulted in morphologically

normal pollen production, (B), and normal flowers, (D), on STS-51. However pollination failed in these plants and no seeds developed.
As shown in (E), on STS-68 better air exchange was provided for the plants, and normal pollen grains were observed on the stigma of their flowers. The plants flown on this mission developed numerous normal appearing seed pods (siliques), indicating that successful pollination and fertilization occurred.

24. SEM of pollen grains
Examination with a scanning electron microscope showed that Arabidopsis seeds that developed during spaceflight on STS-68 (shown on bottom), were similar to those which developed on ground-control plants (shown on top).

25. Embryogenesis
Embryogenesis in seeds of plants grown on STS-68, shown in panels A, C and E, is similar to that of control plants on Earth, shown in panels B, D. The STS-68 results represent the first report of successful plant reproduction on the Space Shuttle. In comparison to the success on STS-68, the earlier failures point to the importance of enhancing carbohydrate nutrition and gas exchange for plants growing in the spaceflight environment. The absence of buoyance-driven convective air movement at microgravity probably results in a limitation on the rate of movement of metabolic gases, and this in turn, negatively affects reproductive development.

26. Examples of Space
Now that we have looked at some examples of plant gravity studies in microgravity, let's look at some examples of studies which take place under normal gravity conditions. Fundamentally useful information about how plants respond to gravity can be obtained from studies on Earth. The next group of slides will highlight the results of some of these studies. They include studies of gravity responses in roots, including mutant roots, and studies in single-cell systems.

27. The root as an experimental system
The root is a favored system for investigating gravitational mechanisms, because of the spatial separation of where it is believed gravity is perceived, the root cap, and where the growth response occurs, further back along the root.

28. curved and straight root
When oriented horizontally, roots adjust their growth pattern. The growth rate along the top of the root exceeds that along the bottom. This pattern of unequal growth causes the root to bend downward. This slide demonstrates the normal positive gravitropism of roots. The upper (straight) root was photographed immediately after placing it horizontally (before any gravitropic curvature occurred). The lower (curved) root was photographed 90 min after gravistimulation and is well into the curvature response.

29. root cap removal
Here, the root cap was removed from the upper (straight) root. The lower (curved) root is intact. Both roots were placed horizontally and photographed 90 min later. The intact root

shows a gravity response. The root from which the cap has been removed continues to grow but fails to respond to gravity. Hence, the root cap is thought to be the site of gravity perception. There is evidence that the cap is a source of a growth hormone (auxin) that can inhibit growth in roots at low concentrations. One theory is that, when a root is gravistimulated, auxin moving back into the root from the root cap, accumulates on the lower side of the growing region of the root inhibiting growth there. This would contribute to the differential growth pattern causing the root to bend downward. Consistent with this hypothesis, application of auxin can substitute for the presence of a root cap in inducing gravitropic curvature, as we will see in the next slide.

30. agar block
In these studies, carried out in the laboratory of Michael Evans at Ohio State, the root cap was removed from both of these roots before they were placed horizontally. The upper root was not treated further and failed to respond to gravity. On the lower root, a small amount of auxin was applied to its lower side by a small block of agar containing the hormone. This caused the root to bend downward, showing that artificially established gradients of auxin are able to induce curvature in roots. This result supports the idea that in normal intact roots, gravitropic curvature might result from accumulation of auxin along the lower side of the root.

31. Control plants on agar plates
Further evidence that auxin is an important messenger in the gravity response of roots comes from the lab of Dr. Gloria Muday. When tomato seedlings are grown on the surface of a vertical agar plate, the roots grow down along the surface of the agar. The seedling on the left were grown in the same orientation for 48 hours. The seedlings on the right were rotated 90^{o} after 24 hours. Under these conditions, the first gravitropic bending is obvious to the naked eye in less than 30 minutes.

32. Plants on Auxin inhibitor plates
These tomato roots were grown under the same conditions, except the agar contained an auxin transport inhibitor. This caused a slight reduction in growth rate and a total inhibition of gravity response, as shown on the right. These roots will not bend in response to gravity, even if left on these plates for several days. Again, this is evidence that auxin movement is critical for the gravitropic response.

33. Examples: plant mutants: mucilage
Mutants are often used by biologists to help elucidate functions of the mutated characteristic in wild-type, or non-mutant, organisms.

34. mucilage on root
Another known function of the root cap is secretion of mucilage which helps the root force its way thought the soil. A mutant in which the root cap does not secrete mucilage is being studied in the lab of Randy Moore.

35. mucilage free mutant

The roots of this mucilage free corn mutant do not bend downward when placed in a horizontal position. Assuming a signal must move from the cap to the growing zone a millimeter or so back of the cap for normal gravitropism, one theory to explain this effect is that the lack of mucilage may somehow interfere with the movement of the signal from the cap to the growing zone.

36. Examples: mutant: low starch
Another type of mutant being used to study gravity responses are starchless mutants of tobacco. Remember the studies discussed earlier in which space flown corn root cells had less starch? The starchless mutant studies give further evidence that starch filled amyloplasts may be an important part of full plant gravity sensing. However, they may not be *required*, but simply enhance the signal.

37. EM of two root tips
Let's look first at the electron micrograph of the wild-type root pictured on the left. Prominent in the structure of root caps is a central zone of cells that are characterized by having amyloplasts filled with starch , pointed out by the arrow. Notice that the amyloplasts tend to be in the lower part of the cell. Because these organelles are so dense and because the organelles fall in the direction of gravity when the root is oriented horizontally, they have been postulated to serve as sensors of the gravity stimulus. To test this hypothesis the gravity response of mutants that could not make starch, such as that on the right, was examined by a number of laboratories. Both roots respond to gravity by growing downward, but Dr. John Kiss and Dr. Fred Sack, working at Ohio State, found that roots missing amyloplasts respond more sluggishly. These results indicate that amyloplasts are not required for the gravity response but they contribute to it and facilitate it in some important way.

38. plants on agar plates; starchless mutants
The lack of full gravity response can be seen by looking at seedlings of the wild-type and mutant corn plants. The wild-type plants on the left are growing more straight down than the starchless mutants on the right.

39. Example: single: chara
Another class of systems being used to study gravity responses in plants are single cells systems. Single cells represent the simplest system in which to study gravity responses in plants. In these cells, all the sensing steps and all the responding steps occur in the same cell. The next group of slides will summarize some recent important findings using these simple systems.

40. Photo of Chara cells
Dr. Randy Wayne and Dr. Mark Staves are using the giant internodal cells of the *Chara* alga plant as a model system to study gravity responses. These cells are so large, (fill in size here), that they must have cytoplasmic streaming because diffusion cannot move molecules through the cell in a physiologically meaningful time.

41 & 42. Gravity response in Chara cells

Chara cells possess a very interesting gravity response. In a horizontal cell, streaming proceeds right and left at about 100 mmeters/sec. In a vertical cell, however, there is a gravity-induced polarity of cytoplasmic streaming such that the down stream moves about 10% faster than the up stream. To compensate for the different rates of streaming, a larger volume flows up than flows down. In the absence of any obvious sedimentation of organelles, such as amyloplasts, Wayne and Staves have suggested that instead of an organelles sedimenting to detect gravity, the entire protoplast of the cell settles and exerts compressive pressure on the bottom of the cell wall and pulls on the top of the cell wall. Indeed, when *Chara* cells are floated, by putting them in an extracellular medium with a higher density, the opposite response is seen and cytoplasm streams up faster than it streams down.

43. Examples: single: Ceratopteris
A new single cell system is being studied by Dr. Stan Roux and Dr. Erin Edwards at the University of Texas at Austin. In this new system, the rhizoids, a type of single celled root of germinating spores, grow down just as would be expected. This response can be seen in the next slide.

44. oriented growth of rhizoids
Oriented downward growth of primary rhizoids shows orientation of growth by gravity. However, after emergence, the rhizoids of *Ceratopteris* spores are surprisingly unresponsive to gravity. When a rhizoid is placed in a horizontal position, it does not change its direction of growth and curve downward, like the typical root gravitropic response. Instead, the rhizoid continues to grow in its original direction, as if there was no orientation change. Further investigation of the unusual occurrence led to the discovery of the unique gravity response of *Ceratopteris.*

45. flow of events
Ceratopteris appears to sense and respond to gravity only as a single-celled spore, before any division takes place. Additionally, gravity detection takes place at a very crucial time that allows it to influence subsequent cell polarity, cell differentiation, direction of nuclear migration, and direction of rhizoid growth. After initiation of germination by light, gravity is sensed during a limited period of time, the "polarity-determination window". In response, the nucleus migrates down to the bottom of the front face of the spore to positions itself for an asymmetric division. Because the two daughter cells are of unequal size, one of the cells is fated, or differentiated, to become the prothallus, or shoot, and the other becomes the rhizoid, or root. Nuclear migration positions the root cell underneath the shoot cell, and the emerging rhizoid grows down.

46. nuclear movement
One of the advantages of this system is that the nucleus can be seen through the spore wall of the intact cell. Shown on the left is a light microscopy picture of a *Ceratopteris* spore with the nucleus in its original central position. In the next picture, the image has been pseudo colored, with the nucleus colored yellow, to enhance the visibility of the nucleus. In the picture on the far right, the nucleus can be seen after its migration

following the polarity determination window. The nucleus is now positioned for the unequal primary division.

47. shuttle landing

Answering questions about the effects of gravity in plants is an ongoing and exciting process. If people want to have plants with them as they explore and colonize space, they will have to answer many more important questions about how the space environment effects plant growth and development. Answering these questions will also provide fundamental new insights on how plants sense and respond to the gravity signals that are always stimulating them on Earth.