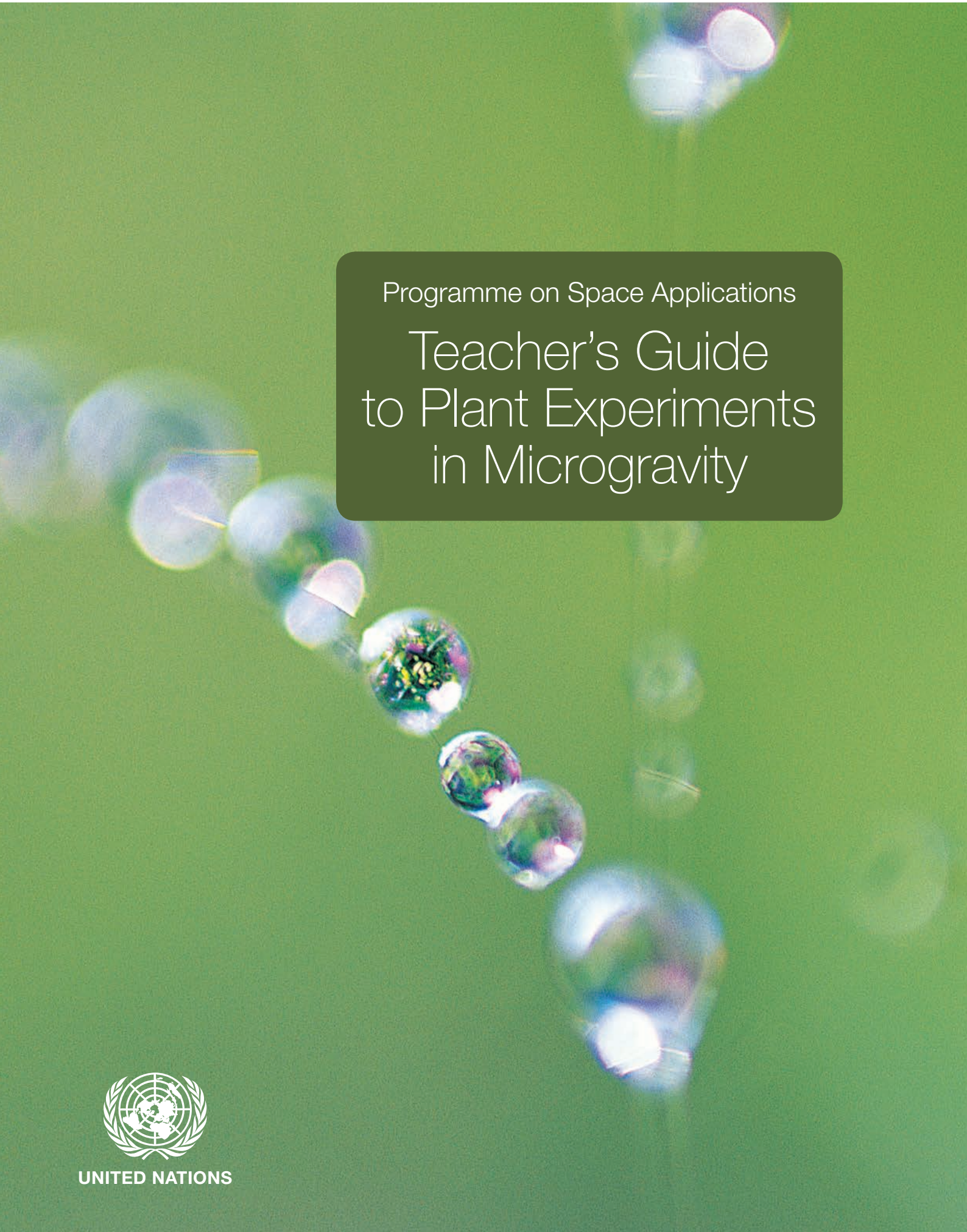
UNITED NATIONS
OFFICE FOR OUTER SPACE AFFAIRS



Programme on Space Applications

Teacher's Guide to Plant Experiments in Microgravity

UNITED NATIONS

OFFICE FOR OUTER SPACE AFFAIRS
UNITED NATIONS OFFICE AT VIENNA

United Nations Programme on Space Applications

Teacher's Guide to Plant Experiments in Microgravity

Human Space Technology Initiative

UNITED NATIONS
New York, 2013

ST/SPACE/63

Acknowledgements

The *Teacher's Guide to Plant Experiments in Microgravity* was made possible by the intensive work of many people. We would like to express our sincere appreciation to Ingrid Dietlein, Takao Doi, Hans Haubold, Jens Hauslage, Ruth Hemmersbach, Takayuki Hoson, Sarah Lammens, Yinghui Li, Mian Long, Jack J. W. A. van Loon, Aimin Niu, Hideyuki Takahashi, Mika Ochiai, Ahmed Osman and Heike Steffens.

Publishing production: English, Publishing and Library Section, United Nations Office at Vienna

DISCOVER THE EFFECTS OF GRAVITY ON PLANTS

Teachers and students can now perform experiments on plants in simulated microgravity conditions in the classroom: the clinostat is an experimental platform that can enable you to simulate microgravity easily and effectively in your own Earth laboratory. Use the instructions provided to conduct experiments on plant growth in order to understand topics in gravitational biology such as the influence of gravity on the spatial orientation and growth direction of plants. Tell us about your experiences and send us your ideas for new experiments. We hope that the clinostat will open up an exciting new world of discovery for both teachers and students.

CONTENTS

INTRODUCTION

The Zero-Gravity Instrument Project is aimed at providing teachers and students with the opportunity to perform experiments under simulated microgravity conditions. This will inspire students to become interested in what life is like in space by demonstrating the influence of a unique environmental stimulus—gravity—which is used by organisms for their spatial orientation and growth direction.[1] Although experiments in real microgravity in space are rare and expensive, similar experiments can be conducted on the ground.[2] This teacher's guide provides straightforward instructions for teachers and students to perform experiments on plant growth using the clinostat, an experimental device which can create simulated microgravity conditions in a school laboratory. Teachers should also try to let students develop their own ideas on how to use this device to test other systems and to measure parameters of interest.

GRAVITY AND MICROGRAVITY

Gravity

Gravitation is a natural phenomenon by which physical bodies attract each other with a force proportional to their respective mass and inversely proportional to the square of the distance between them. Every planetary body is surrounded by its own gravitational field, which in turn exerts an attractive force on all objects. The strength of the gravitational field on the Earth's surface is given as a form of acceleration corresponding to 9.81 metres per second squared (m/s^2) and is often expressed as "1 g".

---

[1] G. Perbal, "The role of gravity in plant development", in *A World Without Gravity*, G. Seibert and others (Noordwijk, Netherlands, European Space Agency Publications Division, 2001).

[2] R. Herranz and others, "Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology", *Astrobiology*, vol. 13, No. 1 (2013), pp. 1-17.

Microgravity

Microgravity is very small compared to gravity on the surface of the Earth. By definition, it is equal to one millionth of the Earth's gravity and is expressed as "µg" (the Greek letter "µ" means one millionth). Mathematically, µg corresponds to 10^{-6} g; however, the term microgravity is used more generally to describe acceleration of less than 1 g.

Microgravity is a condition which takes place in a free-falling body. For example, it is realized inside a satellite orbiting the Earth. The satellite is actually free-falling towards the Earth because of the Earth's gravitational pull. The reason it does not fall to the ground is that the satellite also has enough horizontal velocity to simultaneously travel around the Earth.

In order to distinguish the effects of simulated or real microgravity from other effects that might be induced by space flight conditions, such as cosmic radiation, control experiments are absolutely necessary to avoid the misinterpretation of results. With respect to ground-based simulation approaches, an experiment under real microgravity conditions is necessary for the validation of the quality of the simulation.

Hypergravity

An acceleration force larger than 1 g is called hypergravity. It can be generated in a laboratory by means of a centrifuge, which can simulate the acceleration and deceleration forces that occur during the launch and landing of space vehicles. In addition, hypergravity experiments help to detect and understand gravity-related phenomena, thereby contributing to studies in microgravity.

STUDIES IN MICROGRAVITY

Gravity is always present on Earth. However, the influence of gravity can be modified or, in some cases, compensated for. Today, different experimental and technical approaches can be used to study the effects of gravity, as explained below.

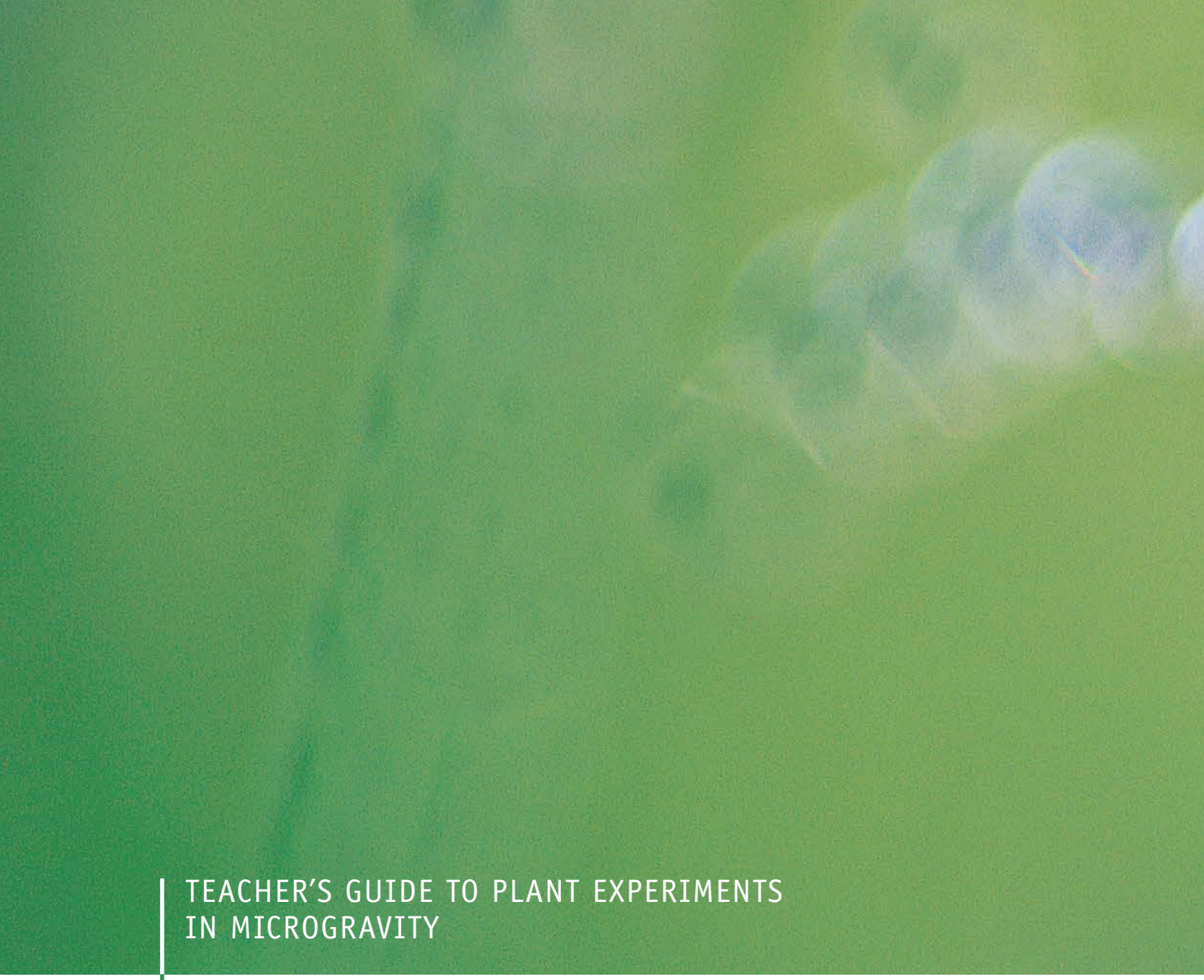
Real microgravity conditions

Short-term microgravity can be provided in drop towers or drop shafts (for 2-10 seconds), balloons (30-60 seconds), parabolic flights of aircraft (20-25 seconds) or sounding rockets (up to 15 minutes). These methods are suitable fast-responding systems. In order to study the long-term effects of microgravity, however, satellites or human-tended space laboratories have to be used. The development of space stations fulfilled the dream of a long-term stay by humans in space. The Russian MIR space station orbited at a height of 300-400 km above the Earth, and more than 100 astronauts and cosmonauts had the opportunity to visit the space station. Since 1998, the International Space Station (ISS) has been in space, providing living and working accommodation for up to six astronauts. The ISS offers laboratory conditions for systematic studies in microgravity.[3]

Simulated microgravity conditions

Scientists have developed various kinds of ground-based facilities and equipment to achieve the condition of functional weightlessness. For example, astronauts are trained underwater, since buoyancy can compensate for gravity and create simulated microgravity conditions in a pool. A clinostat is an experimental device which can equalize the gravity vector around one or two rotational axes if it is for a slow-reacting phenomenon. Research under simulated microgravity conditions must, however, be verified by research under real microgravity conditions.

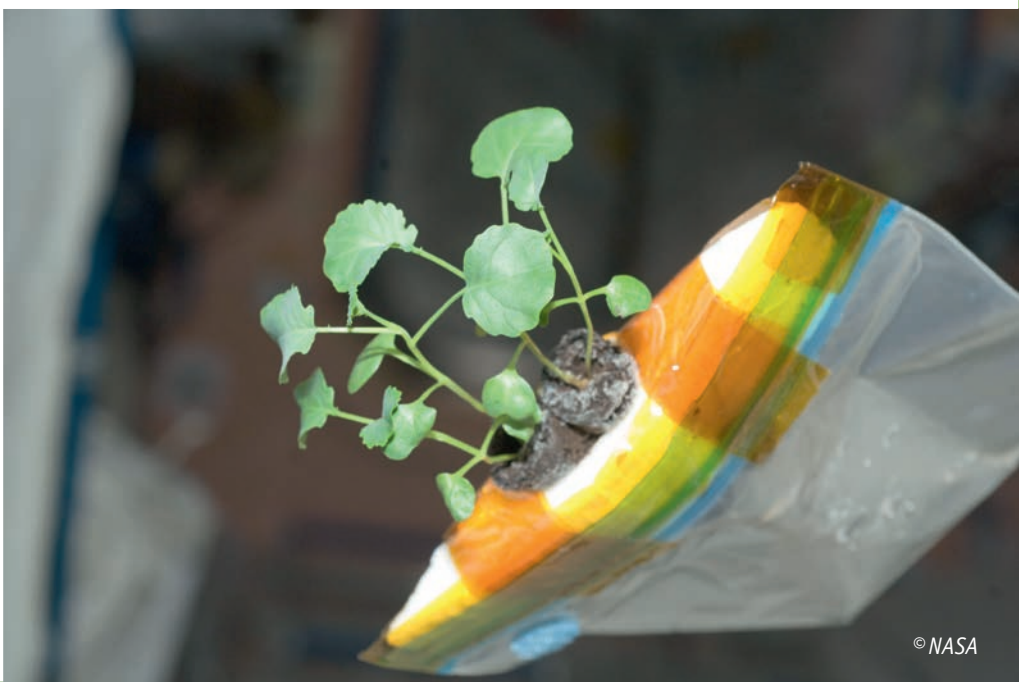
[3] United States of America, National Aeronautics and Space Administration, Reference Guide to the International Space Station (Washington, D.C., 2010).



TEACHER'S GUIDE TO PLANT EXPERIMENTS IN MICROGRAVITY

1.

PLANTS IN SPACE



In this teacher's guide, we will focus on plant research with respect to gravity. Plants were chosen as test systems since they are easily available and their experiment demands are quite easy to meet. Plants are very important for space-flight. They provide us with information on fundamental biological processes.[4] Understanding the molecular and cellular basis of mechanisms in plants for responding to gravity is important with respect not only to plant breeding and agriculture on Earth but also to growing plants in space (space farming) and ensuring a supply of oxygen and food during long-term space missions.[5]

[4] A. L. Paul and others, "Fundamental plant biology enabled by the space shuttle", *American Journal of Botany*, vol. 100, No. 1 (2013), pp. 226-234.

[5] R. Ferl and others, "Plants in space", *Current Opinion in Plant Biology*, vol. 5, No. 3 (2002), pp. 258-263.

1.1 GRAVITATIONAL BIOLOGY

The main objective of gravitational biology is the identification and understanding of the effects of gravity on organisms. This includes the identification of the underlying mechanisms and of the role of gravity not only in individual development but also during evolution in general.

Gravitational biology as a discipline started during the nineteenth century, when Sir Thomas Knight, Charles Darwin, Julius Sachs and Wilhelm Pfeffer investigated the influence of gravity on plants. They had already demonstrated the role of the root cap in downward-growing plants. Knight, Sachs and Pfeffer constructed machines (centrifuges and simple clinostats) in order to change the influence of gravity and to study its impact on the growth of plants. Today, various experimental platforms—on the ground and in space—have been developed to study the influence of altered gravity. Consequently, our knowledge of the impact of gravity and microgravity has increased greatly. Key research findings in gravitational biology cover all levels of biology, ranging from isolated proteins, single cells and tissues to complex organisms.

Gravity-sensing mechanisms developed early in the evolutionary process. Free-moving organisms, even unicellular ones, use gravity for their orientation, such as for their swimming direction, a behaviour called gravitaxis. In addition, the growth and orientation response of sessile organisms is called gravitropism. [6] The direction with respect to the gravity vector is defined as either positive (parallel to gravity) or negative (against the direction of gravity).

1.2 EFFECTS OF GRAVITY ON PLANTS

Gravity is the stimulus that a plant uses to grow its root in the direction of the gravity vector (down), anchoring the plant in the ground, and to grow the shoot in the opposite direction of the gravity vector (up), out of the soil in the direction of the sun. To understand "up" and "down" is mandatory for the survival of plants on Earth.[7] It is also indispensable for all life on Earth because photosynthesis is needed for food and oxygen production.

By growing plants, one can easily observe the impact of gravity on orientation and growth. Considerable progress has been achieved with regard to basic knowledge of gravity-sensing in plants and their final responses in the form of gravitropism.[8] Experiments performed in microgravity have greatly contributed to the understanding of how plants sense the direction of gravity and respond to it. However, the complete signal transduction process is not yet understood in detail.

---

[6] R. Chen, E. Rosen and P. H. Masson, "Gravitropism in higher plants", *Plant Physiology*, vol. 120, No. 2 (1999), pp. 343-350.

[7] E. B. Blancaflor and P. H. Masson, "Plant gravitropism: unraveling the ups and downs of a complex process", *Plant Physiology*, vol. 133, No. 4 (2003), pp. 1677-1690.

[8] D. Sack, "Plastids and gravitropic sensing", *Planta*, vol. 203, No. 1 (1997), pp. S63-S68.

1.3 GRAVITY SENSING AND RESPONSES

Gravity-dependent growth is based on a highly complicated stimulus-response chain. The physical signal of gravity has to be transformed into a biochemical signal, which leads to a physiological response. Gravity is a force that acts on mass. A mass has to be transported in the gravitational field in order to create sufficient energy for the activation of a biological sensor. In order to transfer this signal at the level of a single cell, a mass which is denser than the surrounding medium must exist. This heavier mass sediments under the influence of gravity, thereby activating gravity-specific receptors. Candidates for sedimenting mass are either intracellular statoliths or the entire cell mass (protoplast).[9] The process that perceives gravity is called graviperception. Figure I shows a typical gravitropic response of a plant.

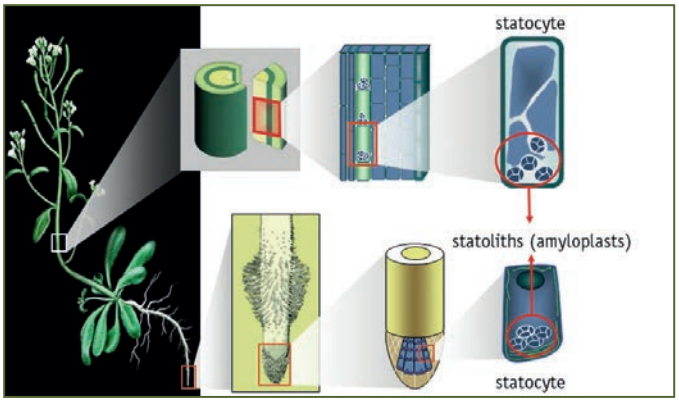


Figure I. The primary root of a plant grows in the direction of the gravity vector, while the shoot grows in the opposite direction. The growth and orientation response of sessile organisms to gravity is called gravitropism.

Courtesy: Markus Braun

In more highly evolved plants, graviperception takes place in specialized gravity-sensing cells (statocytes) in the root cap. Vacuoles filled with starch, called amyloplasts, move in the direction of gravity and activate the gravity-specific receptors. This causes an increase in the local concentration of calcium and also results in a differential concentration of the hormone auxin. As a consequence, a distinct growth of plant organs with respect to gravity is initiated.[10]

[9] M. Braun, B. Buchen and A. Sievers, "Actomyosin-mediated statolith positioning in gravisensing plant cells studied in microgravity", *Journal of Plant Growth Regulation*, vol. 21, No. 2 (2002), pp. 137-145.

[10] M. T. Morita, "Directional gravity sensing in gravitropism", *Annual Review of Plant Biology*, vol. 61, 2010, pp. 705-720.

The green algae *Chara* is an ideal model system to study gravitropism, since its root consists of just one cell (a rhizoid). As a consequence, all of the phases, from the perception of gravity to the response, occur in a single cell. In *Chara*, the sedimenting mass is represented by barium sulphate crystals (statoliths) within vacuoles. After reorienting rhizoids by 90°, the statoliths sediment along the gravity vector and settle in the lower cell flank, where a differential growth of the cell flank is induced, resulting in gravitropic curvature growth in the direction of the gravity vector (positive gravitropic response), as shown in figure II.

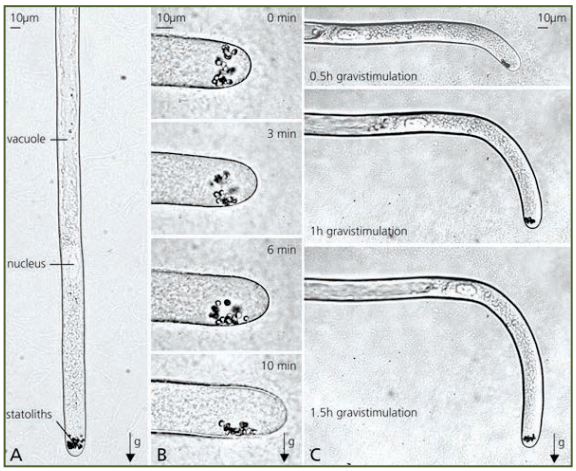
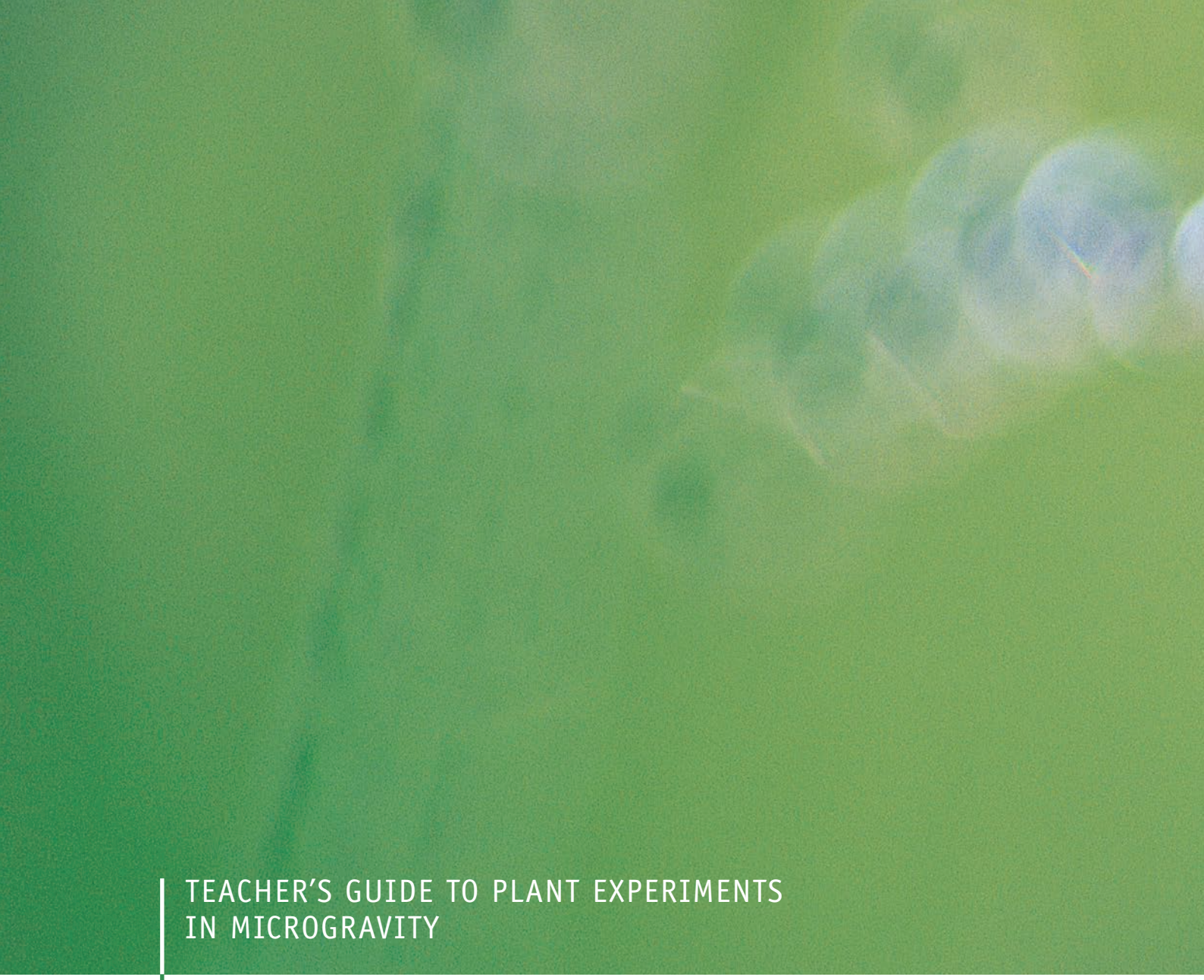


Figure II. Displacement of statoliths induces the bending of the rhizoid (root-like cell) of *Chara*.

Courtesy: Jens Hauslage.



TEACHER'S GUIDE TO PLANT EXPERIMENTS IN MICROGRAVITY

2.

CLINOSTATS

© NASA

The clinostat was invented to enable the constant rotation of an object, such as a plant, around an axis perpendicular to the force of gravity. Experimenters use this device to cancel the effect of gravity by equalizing the gravity vector around the horizontal axis.

2.1 PRINCIPLES OF A CLINOSTAT

Various kinds of clinostats have been developed, differing in the number of rotational axes and the modes of operation with respect to the speed and direction of the rotation. A two-dimensional (2-D), or one-axis, clinostat has a single rotational axis, which runs perpendicular to the direction of the gravity vector.[11] A three-dimensional (3-D) clinostat has two rotational axes, which are perpendicular to each other. If this kind of clinostat is running at constant speed and in a constant direction, it is specifically called a 3-D clinostat.[12] However, if both axes rotate at different speeds and in different directions, the term "random positioning machine" is used. Current studies concentrate on a comparison between these different devices in order to define appropriate simulation conditions for the exposed objects. Clinostats can be equipped with capacities for microscopy, online kinetic measurements or chemical fixation of the sample during rotation. A rotation on a clinostat is often called "clinorotation".

For the Zero-Gravity Instrument Project, a one-axis clinostat will be used. It can provide a rotational speed in a range of 0 to 20 revolutions per minute (rpm) for educational purposes and 0 to 90 rpm for research purposes. The specifications of the one-axis clinostat are shown in table 1, while figure III shows what a clinostat looks like.

Table 1. Specification of the one-axis clinostat to be provided in the Project

1.	Equipment size (cm):	Main body: 25 x 25 x 25 Control box: 23 x 20 x 11
2.	No. of rotational axes:	One
3.	Rotational speed:	0-90 rpm 0-20 rpm: 0.5 rpm increments 20-90 rpm: 5 rpm increments Accuracy: 1 per cent
4.	Rotational axis angle:	0° (parallel to the ground) to 90° (perpendicular to the ground)
5.	Rotation direction:	Clockwise or counterclockwise
6.	Input voltage:	100V-240V
7.	Building material:	Aluminium
8.	Experiment conditions:	Maximum weight o f samples: 500 g Maximum diameter of a sample container: 10 cm

[11] W. Briegleb, "Some qualitative and quantitative aspects of the fast-rotating clinostat as a research tool", ASGSB Bulletin, vol. 5, No. 2 (1992), pp. 23-30; R. R. Dedolph and M. H. Dipert, "The physical basis of gravity stimulus nullification by clinostat rotation", *Plant Physiology*, vol. 47, No. 6 (1971), pp. 756-764; and D. Klaus, "Clinostats and bioreactors", *Gravitational and Space Biology Bulletin*, vol. 14, No. 2 (2001), pp. 55-64.

[12] T. Hoson and others, "Evaluation of the three-dimensional clinostat as a simulator of weightlessness", *Planta*, vol. 203, No. 1 (1997), pp. S187-S197; and J. J. van Loon, "Some history and use of the Random Positioning Machine, RPM, in gravity related research", *Advances in Space Research*, vol. 39, No. 7 (2007), pp. 1161-1165.

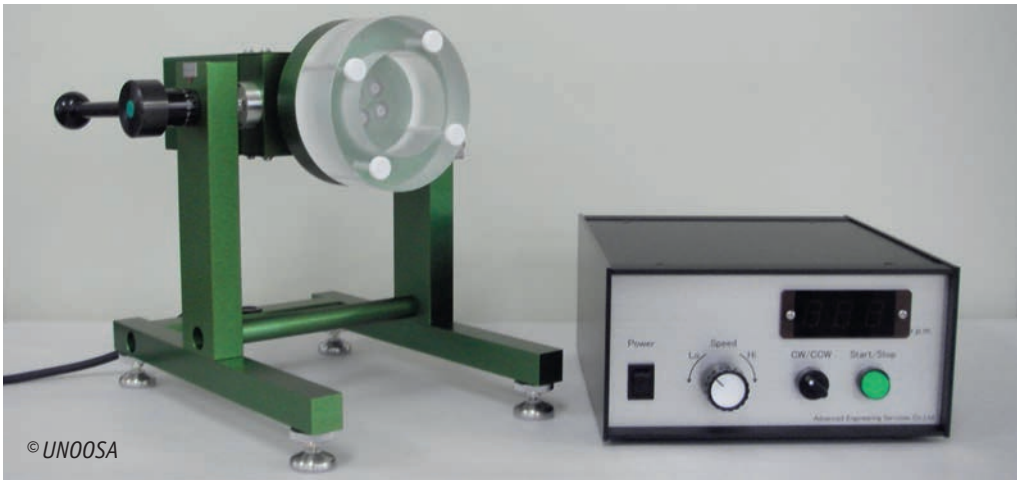


Figure III. Picture of the one-axis clinostat provided in the Project.

2.2 MODES OF OPERATION

When using a clinostat, the speed of rotation, the diameter and time sensitivity of the sample and the horizontal placement of the clinostat are essential parameters which determine the effectiveness of the microgravity simulation.

The first factor to be considered is the speed of rotation of the clinostat. Figure IV explains the effects of gravity and clinorotation compared with true microgravity conditions. Under a 1 g condition, particles fall and become sediment. Under a free-fall condition, there is no sedimentation and particles homogeneously distribute. On Earth, this situation can be achieved by rotating a vertically positioned object. Under this condition, particles will fall along the gravity vector but will also be forced into circular paths because of the clinorotation. The faster the system rotates, the more the radii of the circles decrease. If the rotational speed is too high, however, particles will disperse due to the centrifugal force. At an ideal rotational speed, particle movement due to sedimentation and centrifugal force is kept within the limits of Brownian motion. Transferring this image to the level of gravity-perceiving cells (statocytes), we can predict that the sedimentation of statoliths can be suppressed under the ideal simulation conditions in a clinostat.

A slow-rotating clinostat is operated with rotational speeds of 1-2 rpm and is mainly used by plant physiologists to investigate the gravitropism of plants. Due to the relatively long response time of plants (in the range of minutes), the gravitropic response is suppressed under these conditions. However, recent research has revealed that the slow-rotating clinostat may produce a stressful situation in a cell due to the fact that the radius of the circular path of a statolith may be equal to or greater than the size of the cell itself and that the statolith may continually hit the cell wall and thus overload gravitational stimulation.

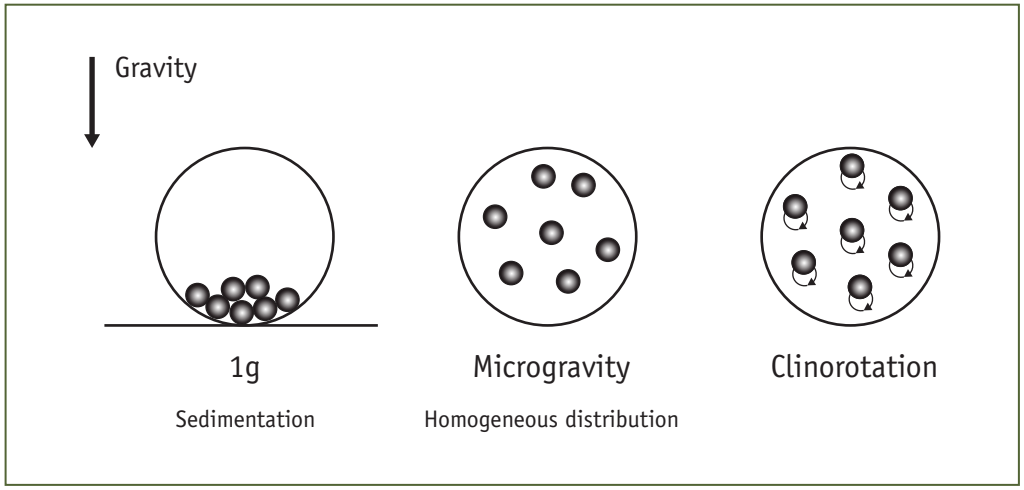


Figure IV. This scheme explains the clinostat principle with liquid media in a clinostat with one rotational axis. Sedimentation takes place under 1 g. In microgravity, there is no sedimentation and particles homogeneously distribute. During clinorotation, particles still fall along the gravity vector but will also be forced into circular paths. A condition can be achieved in which particles have no relative movements.

The second factor to be considered when using a clinostat is centrifugal force, which is in proportion to the distance between the sample and the axis of rotation and the rotational speed (revolutions per minute) squared. If the rotational speed is too high, then the centrifugal force acting on statoliths will move them towards the cell wall. The relationship between centrifugal force and speed and radius are shown in table 2, which can be used to calculate the centrifugal acceleration acting on objects rotated in a one-axis clinostat.

The third factor to be considered is the horizontal placement of the rotational axis of the clinostat. The rotational axis must be placed horizontally as accurately as possible. An error of 0.5 degrees can create an axial acceleration on the order of 10^{-2} g. The kind of accuracy needed can be achieved by using a commercially available bubble-level gauge when setting up the clinostat in your school laboratory.

Try to come up with a good rule of thumb for deciding the rotational speed for your experiment. If the sample involved is small enough to be placed on the clinostat within five centimetres of the rotational axis, and the time sensitivity of the sample is on the order of a minute or longer, a rotational speed of 10 rpm is a good value with which to start the experiment. This speed will guarantee that all the residual acceleration exerted on the sample is on the order of 10^{-2} g. If you would like the residual acceleration to be on the order of 10^{-3} g, you have to place your sample within one centimetre of the rotational axis, as well as place the rotational axis horizontally within a margin of error of 0.1 degree.

Table 2. Calculation of acceleration during clinorotation

Centrifugal force calculation:

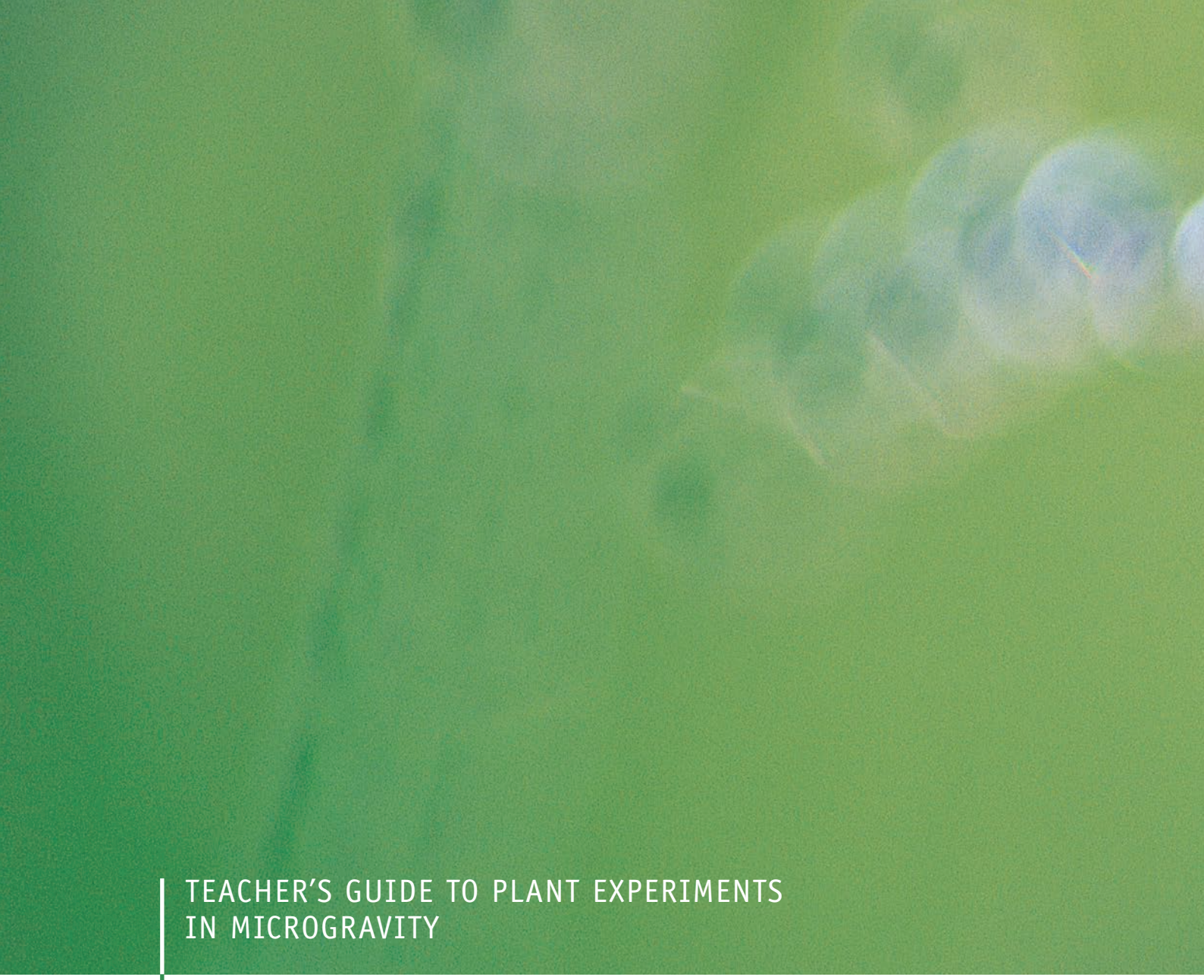
$$\frac{Fc}{g} = \frac{v^2}{r} * \frac{1}{g}$$

Fc: acceleration due to centrifugation [m/s²]
g: gravitational acceleration g = 9.81[m/s²]
v: rotational velocity [m/s]
r: radius [m]

Set ω as the rotational speed (revolutions per minute)

$$v = 2\pi r(\frac{\omega}{60}) = (\frac{\pi}{30})r\omega \qquad \frac{Fc}{g} = \frac{(\frac{\pi}{30})^2 r^2}{r} * \frac{\omega^2}{9.81} = 1.12 * 10^{-3} r\omega^2$$

Radius [cm]	1	2	3	4	5
ω [rpm]	Fc/g	Fc/g	Fc/g	Fc/g	Fc/g
1.0	1.12×10^{-5}	2.24×10^{-5}	3.35×10^{-5}	4.47×10^{-5}	5.59×10^{-5}
10.0	1.12×10^{-3}	2.24×10^{-3}	3.35×10^{-3}	4.47×10^{-3}	5.59×10^{-3}
20.0	4.47×10^{-3}	8.94×10^{-3}	1.34×10^{-2}	1.79×10^{-2}	2.24×10^{-2}
30.0	1.01×10^{-2}	2.01×10^{-2}	3.02×10^{-2}	4.02×10^{-2}	5.03×10^{-2}
40.0	1.79×10^{-2}	3.58×10^{-2}	5.37×10^{-2}	7.15×10^{-2}	8.94×10^{-2}
50.0	2.80×10^{-2}	5.59×10^{-2}	8.39×10^{-2}	1.12×10^{-1}	1.40×10^{-1}
60.0	4.02×10^{-2}	8.05×10^{-2}	1.21×10^{-1}	1.61×10^{-1}	2.01×10^{-1}
70.0	5.48×10^{-2}	1.10×10^{-1}	1.64×10^{-1}	2.19×10^{-1}	2.74×10^{-1}
80.0	7.15×10^{-2}	1.43×10^{-1}	2.15×10^{-1}	2.86×10^{-1}	3.58×10^{-1}
90.0	9.06×10^{-2}	1.81×10^{-1}	2.72×10^{-1}	3.62×10^{-1}	4.53×10^{-1}
100.0	1.12×10^{-1}	2.24×10^{-1}	3.35×10^{-1}	4.47×10^{-1}	5.59×10^{-1}



TEACHER'S GUIDE TO PLANT EXPERIMENTS IN MICROGRAVITY

3.

EXPERIMENT WITH THE ONE-AXIS CLINOSTAT


© UNOOSA

The one-axis clinostat provided by the United Nations Office for Outer Space Affairs is a tool used to study the impact of altered gravity conditions on organisms such as plants, fungi and other small organisms. The quality of the simulation is determined by the size of the test system chosen.

In the following sections, a model experiment using cress seedlings and cress roots will be introduced. This experiment will demonstrate the function of and methods for using the clinostat. In addition, a great deal of information is available on the Internet and in scientific literature about the impact of gravity and microgravity on other biological systems, ranging from bacteria and cells to small organisms. This can serve as a foundation for further experiments with the clinostat.

3.1 EXPERIMENT OBJECTIVES

The main objective of the experiment is to understand the impact of gravity on plant growth. The idea behind studying the "gravitropism" of plants is to determine what their orientation will be in space, where there is no gravity, as well as to identify the underlying mechanisms. With clinostat experiments, the importance and impact of gravity can be demonstrated.

One approach to demonstrating the impact of gravity on plants is to stimulate them by changing the direction of gravity (gravistimulation). Plants are able to sense changes with respect to the influence of the gravity vector. If the plant's position is shifted away from the vertical, it is "gravistimulated", which means a signal transduction cascade is initiated, resulting in a correction of the growth direction. In nature, gravistimulation is done by wind and rain forces. In a laboratory, roots and shoots can be gravistimulated by displacing them from the horizontal direction by 90°, i.e. to the vertical direction (see figure V), at which point the impact of gravity on plant gravitropism can then be studied.

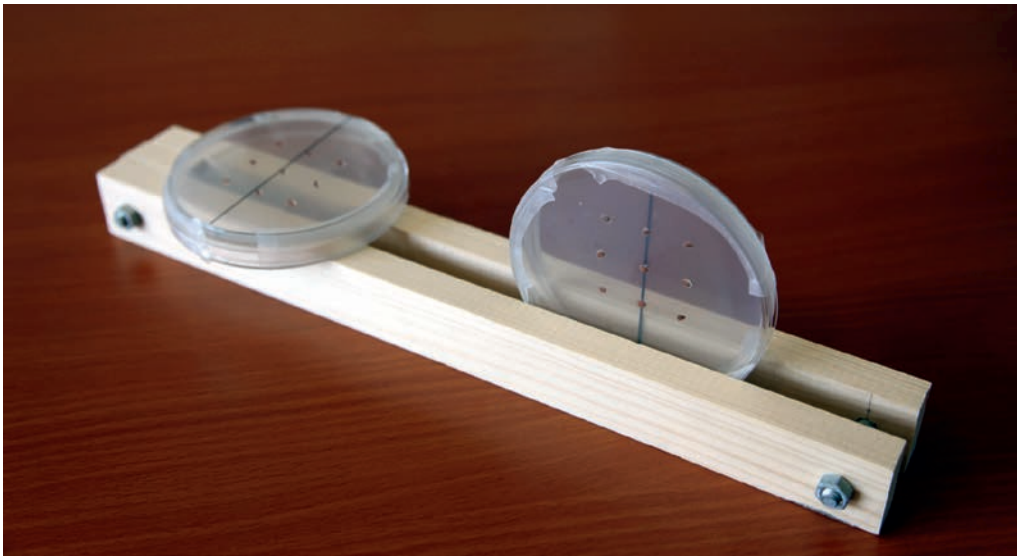


Figure V. A 1 g control (cress) is placed horizontally or vertically depending on the objectives of the experiment.

Learning all of the necessary steps

The steps necessary for preparing an experiment using the clinostat with plants include the preparation of a substrate for seeds in Petri dishes, the planting of seeds into the substrate, cultivation inside a wet chamber, placement of the seeds on the clinostat and possible methods for getting results with a further analysis of observed graviresponses.

Selection of plant seeds

One of the scientific goals of the Project is to create a dataset of world plant growth and the responses of plants to gravity. The Project will provide opportunities for students and researchers from different regions to use their indigenous plant seeds to conduct experiments under simulated microgravity conditions.

If you are planning experiments with roots or shoots, you should know the size and approximate growth velocity of your chosen seeds. It is quite difficult, because of their size, to use coconuts (too big) or orchid seeds (too small) for clinostat experiments. Also, seeds with a long germination period, such as those of many conifers, are not suitable for such experiments. For that reason, keep in mind that your seeds have to be small, easy to handle and fast-growing. Seeds with germination times of longer than three to seven days may not be used. The following seeds are suitable for plant experiments with the clinostat:

Garden cress *(Lepidium sativum)*

One of the plants that has been most studied in relation to microgravity is cress. If this is your first plant experiment, cress may be a good first choice. With garden cress (*Lepidium sativum*), it is very easy to get roots and shoots growing. They only need water for the first days of growth and no special substrate. You can just use wet paper or cellulose tissues for germination.

Rice *(Oryza sativa)*

Rice feeds over two billion people on Earth. The study of rice in space has been conducted by many researchers in Asia. With respect to the exploration of other planets or long-term travel in closed habitats, food plants are very important, and thus knowledge of them is essential. When preparing experiments with rice seeds, handle them like cress seeds, with the difference being that first you have to soak them in tap water for one night. After that, you can select uniformly germinated seeds and put them on a suitable substrate in a Petri dish.

Peas *(Pisum sativum)*

Peas are a good protein source. They are also very easy to prepare and grow for experiments conducted under simulated microgravity conditions. When using peas, you have to put them in a thick layer of a substrate. For preparation, you also have to soak them in tap water for one night. After one day of germination, short roots will appear; they can then be placed on the clinostat.

3.2 MATERIALS AND REQUIREMENTS

Please confirm that you have received in your starter kit all of the items listed below and that the items are in good working condition. Prepare a suitable location to perform your experiments with the additional user-provided items listed below (see also figure VI).

STARTER KIT (PROVIDED BY THE UNITED NATIONS)

1 clinostat

35 Petri dishes (1 package)

Double-sided tape for fixation of the Petri dishes

Agar-agar

Parafilm M (laboratory film for closing the Petri dishes)

Biological/medical tweezers (optimally type 7)

ROOM/LABORATORY INFRASTRUCTURE AND MATERIALS (USER-PROVIDED)

Room with a minimum temperature of 20°C and a maximum temperature of 30°C

Timing device

Fine permanent markers

Heating plate

Small cooking pot or beaker glass (at least 500 ml) for cooking the agar-agar

Thermometer

Hygrometer (0-100% relative humidity)

Scale (for weighing the agar-agar), with an accuracy of 0.1 g

Wet chamber (incubator) for the preparation of the Petri dishes, to keep humidity at around 60-100 per cent

Clinostat chamber (minimum dimensions of 30 cm x 30 cm x 30 cm) to accommodate the clinostat

Optional: paper towels for preparation of the wet chamber

Photo camera with a minimum of 3.5 megapixels

Computer with a Windows, Apple OS X or Linux operating system

ImageJ in the latest version (see http://rsb.info.nih.gov/ij)

Figure VI. This is an experiment set up for plant growth using the clinostat. The white plastic box is a wet chamber, used to keep the humidity at 60-100 per cent, in which Petri dishes with seeds are placed for germination. A small heating plate is used to cook an agar-agar solution in a small cooking pot. A digital camera with macro capability is useful to record the growth of the cress roots.

3.3 EXPERIMENT: PREPARATION

The following procedures describe an experiment with cress roots using the clinostat. The aim of this experiment is to observe and measure the influence of clinorotation on roots compared with a 1 g control (vertical position) sample and a sample that has been turned 90°.

The present section explains how to prepare a Petri dish with a seed-supporting substrate and how to place seeds on it. A common substrate for germination experiments is agar-agar, which is made of seaweed and is transparent for easier observation. You can use agar-agar in tap water (depending on the type of agar-agar). For all experiments, tap water should be used, as it contains residual minerals that will support the germination in the first stage. Distilled water will disturb the osmotic processes, leading to a mineral deficiency.

PREPARATION OF FOUR PETRI DISHES

1. Place a Petri dish on the template for seed placement shown in annex II, with the back side facing towards you. Using a permanent marker, draw a vertical reference line on each Petri dish and put a mark at the top of the line. This line will indicate the direction of the gravity vector. The mark at the top will indicate the up side of the Petri dish.
2. Prepare 100 ml of 1-1.5% Duchefa Biochemie Plant Agar-Agar in tap water (1.5 g agar-agar in 100 ml of tap water), as shown in figure VII.
3. Boil the agar-agar until no visible particles are left (up to two minutes). The solution must be clear. Stir well. Caution: it will be hot! (see figure VIII)
4. Wait for five minutes for the solution to cool down to about 60°C. Agar-agar becomes solid at a temperature lower than 37°C.
5. Fill four Petri dishes with 10 ml to 25 ml (depending on the chosen seeds) of the agar-agar solution. The right depth of the agar-agar solution is such that your seed can be embedded only halfway in the agar-agar, thus guaranteeing a supply of oxygen for the seeds. Cover the Petri dishes with the lid. It is important that the complete bottom surface of the Petri dishes be covered homogenously with agar-agar, so make sure to turn them slightly on the table when pouring the agar-agar, as shown in figure IX.
6. Wait until the agar-agar has become solid. This can take from 2 to 30 minutes.
7. After the agar-agar cools down, if there is condensation of water on the underside of the lid, remove the condensation by tapping the lid.
8. Put each Petri dish on the template for seed placement in the same way that you did in step 1.
9. In each Petri dish, place nine cress seeds on the agar-agar by using the tweezers. All seeds should be planted in the same direction, as shown in figure X. This makes it easier to identify the black line (micropyle) on each seed in order to orient it.
10. Use a lid to close the Petri dishes. After seeding the cress seeds on the agar-agar surface, the cress seeds take up water in the outer cell layer. After this stage, you can rotate the Petri dishes vertically.
11. After you are sure that the seeds are stuck on the agar-agar surface, use parafilm or transparent sticky tape to affix each lid onto the Petri dishes, as shown in figure XI. There should be some gaps between the lid and the dish for proper oxygen support.
12. Next, position the Petri dishes in a vertical position. Use a wooden holder or other device to support the Petri dishes, as shown in figure XII. Make sure the vertical reference line on each Petri dish is parallel to the gravity vector.
13. Prepare a wet chamber in a plastic box with a size of about 40 cm x 40 cm x 40 cm, as shown in figure XIII. The humidity of the wet chamber should be maintained at 60-100 per cent.
14. After about 20 to 30 hours, depending on the temperature and the humidity, short roots will appear on the agar-agar surface.
15. For optimal results, you need roots between 5 mm and 10 mm in length, as shown in figure XIV.

With four Petri dishes, you can prepare your first clinostat experiment: one Petri dish for the 1 g control, one dish for a sample that has been turned 90° and one for the clinostat. The one Petri dish left over will be a backup.

Figure VII. When you cook the agar-agar, stir it well to prevent the formation of lumps.

Figure VIII. Boil the agar-agar until no particles are left. You will then have a clear solution.

Figure IX. This shows agar-agar being poured into a Petri dish. Caution: the liquid agar-agar is hot! When cooked in the right manner, no air bubbles will be left in the solid agar-agar.

Figure X. Place nine cress seeds in a Petri dish so that they face in the same direction, using the template for seed placement in annex II. Use the micropyle (black line) to orient the seeds in the same direction.

Figure XI. Use parafilm or transparent sticky tape to cover the Petri dishes around the lid. Do not seal them completely. That way, oxygen can diffuse and support the germination.

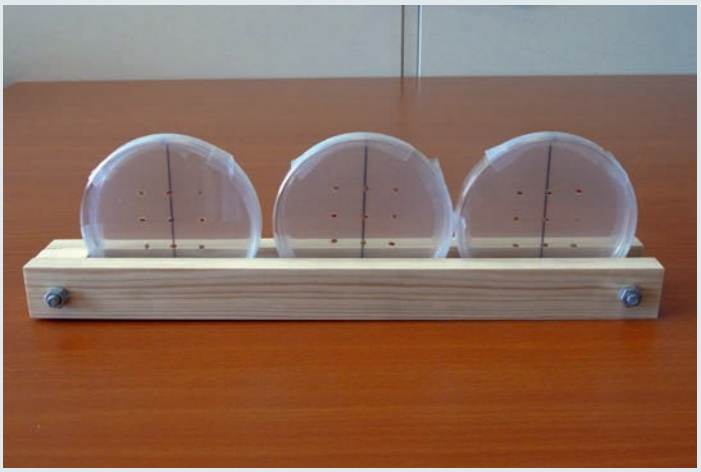


Figure XII. This shows Petri dishes in a wooden holder. You can see that the reference line (black) is parallel to the gravity vector (vertical).

Figure XIII. This is a wet chamber with Petri dishes in an upright position. The wet chamber has two sections. The lower section is filled with water. The humidity in the wet chamber should be maintained at 60-100 per cent.

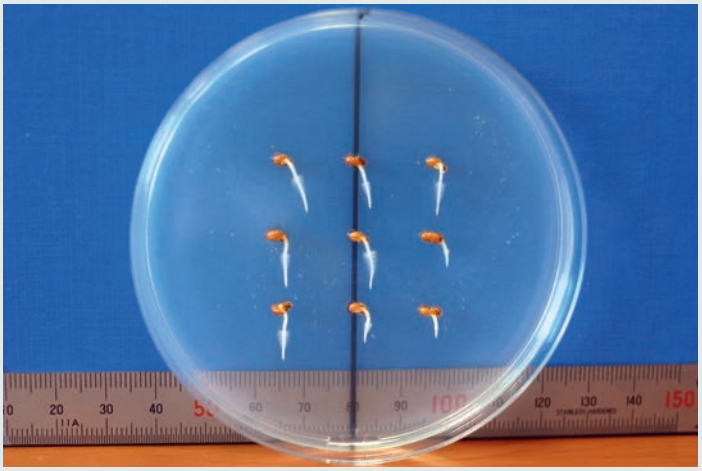


Figure XIV. This is a Petri dish (1 g control) after approximately 28 hours in a wet chamber at room temperature (23°C). You can clearly see the short roots growing in the direction of gravity. This is a good result and starting condition for your first experiment using the clinostat.

3.4 EXPERIMENT: OBSERVATION

The aim of this section is to observe the influence of clinorotation on gravitropism in cress roots.

1. Take three comparable Petri dishes with short roots and label them “1 g control”, “90°-turned” and “clinostat”.

2. Rotate the 90°-turned Petri dish by 90° in one direction. It does not matter in which direction. Draw the new vertical line that is parallel to the gravity vector. (It is useful to use a different colour for this step.) Take a photo of the 90°-turned Petri dish for further analysis.

3. Using double-sided tape, mount the “clinostat” Petri dish in the centre of the clinostat. Make sure that the vertical orientation of the Petri dish is maintained until the clinorotation starts, as shown in figure XV.

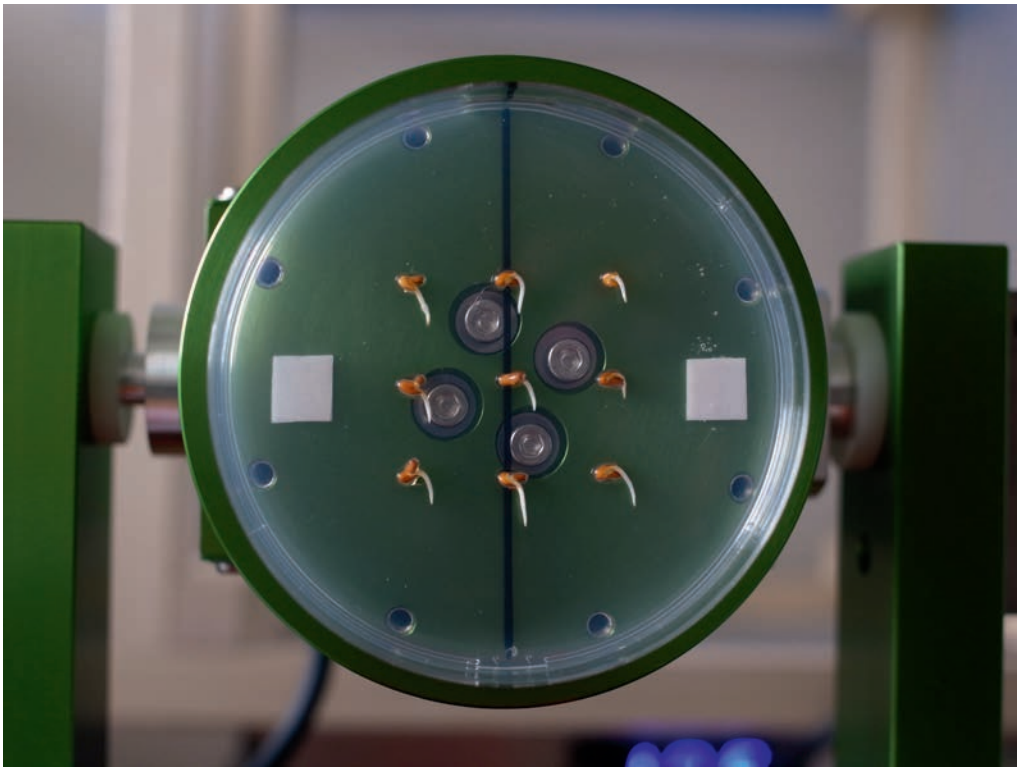


Figure XV. This is a Petri dish attached to a clinostat with double-sided tape. Make sure that you centre the Petri dish as precisely as possible.

4. Take photos of your three Petri dishes (see, for example, figure XVI). The results of the experiment can be recorded with a digital camera. The following points are important for taking photos:

- Use a dark and homogeneous background so that you can see the roots with a high contrast.
- Use artificial illumination from beside or above the samples (but not from behind you) when taking photos of your samples.
- Use the macro setting on the camera for better resolution.

5. Start the clinostat with your pre-selected speed of between 1 and 20 rpm and record the time. The planned experiment time is between two and four hours.

6. Take photos of your three Petri dishes every 30 minutes. When taking pictures of the clinostat sample, stop the clinostat for a very short time with the vertical orientation as it was in step 3. Take the photo and then restart the clinostat. Keep the time for stopping the clinostat as short as possible, as gravity is now acting on the clinostat sample and might have an influence. Observe and describe the status of your samples.

7. After between two and four hours, stop the clinostat and mark the end of the observation time.

8. For an analysis of the root curvature, go to section 4.1. For an analysis of the growth rate of the root, go to section 4.2.

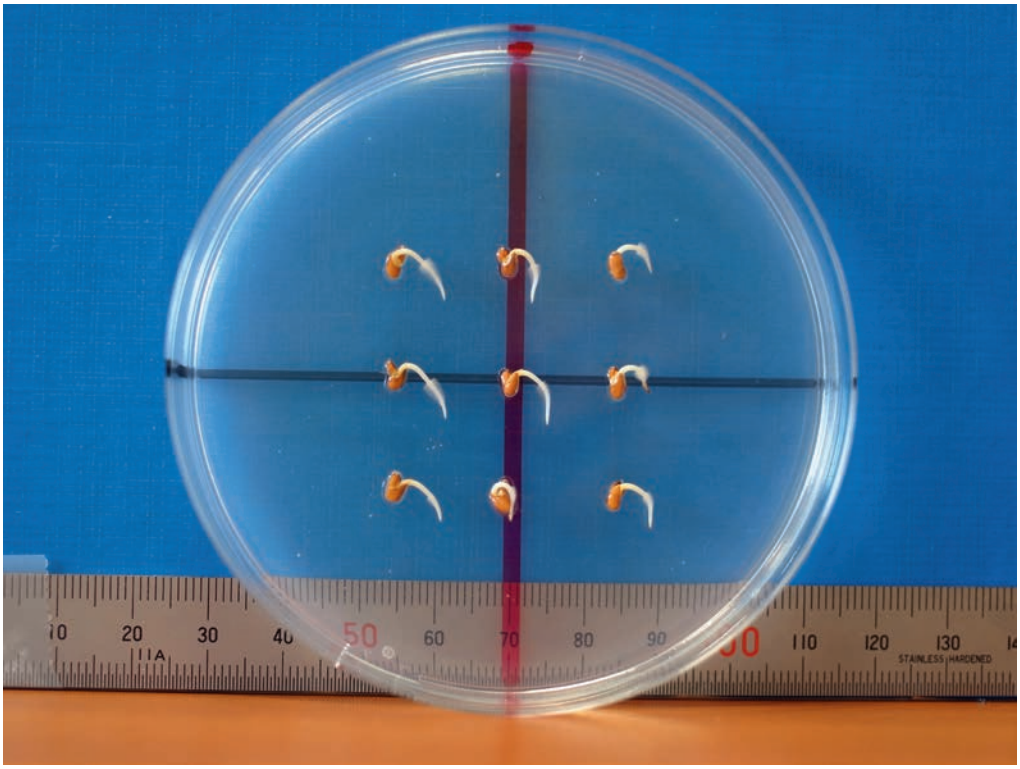
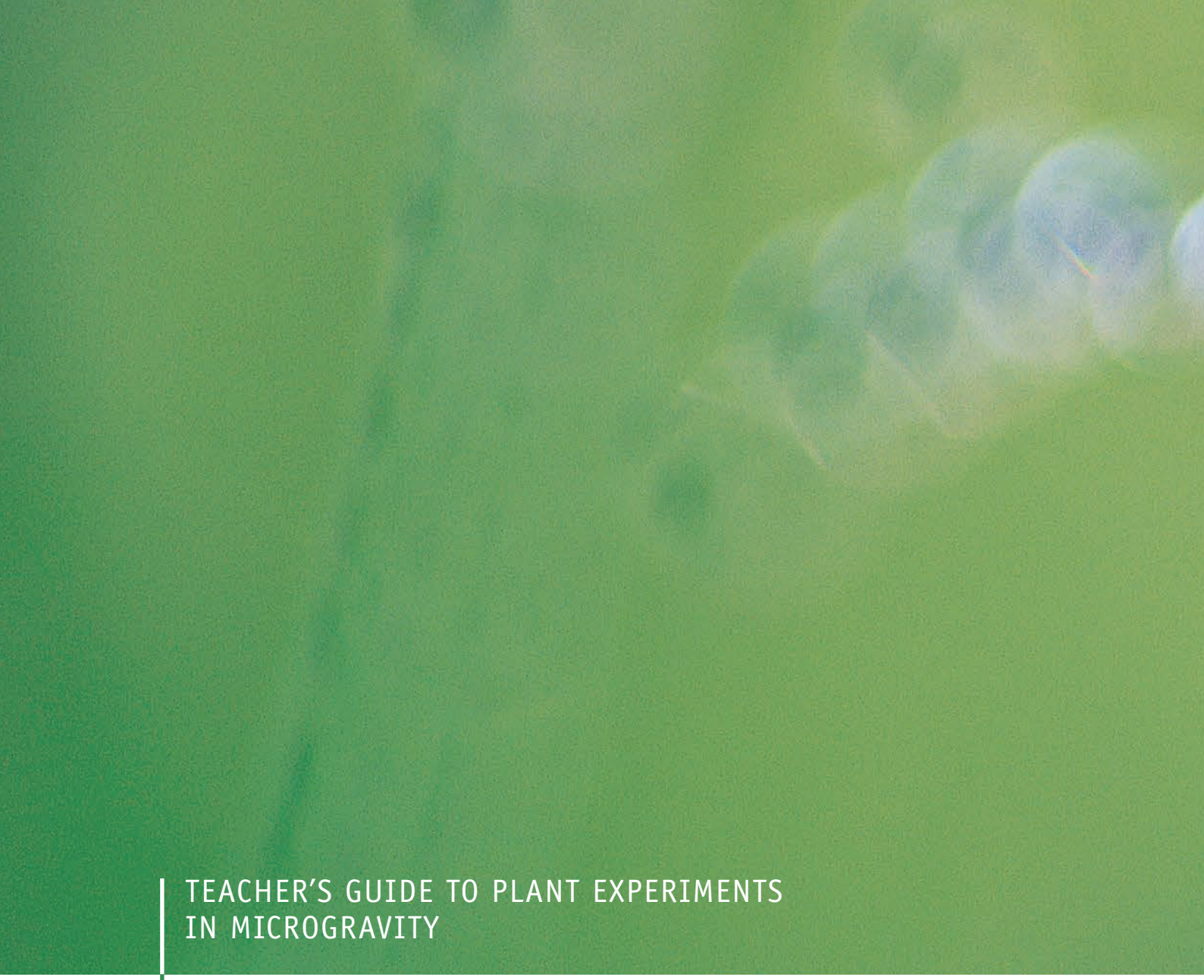


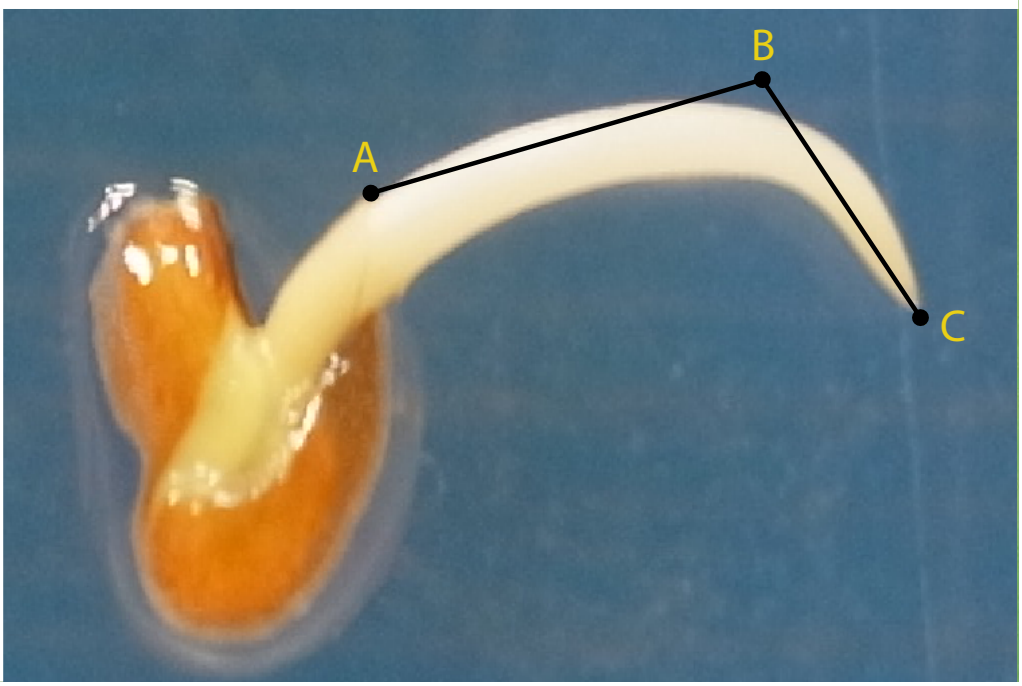
Figure XVI. This is the "90°-turned" sample. You can see a bending of the cress roots after turning the Petri dish by 90°.



TEACHER'S GUIDE TO PLANT EXPERIMENTS IN MICROGRAVITY

4.

ANALYSIS



This section will show how to analyse the data you obtained in section 3. The data are the three sets of photos of the cress roots which show the 1 g control, 90°-turned and clinorotated roots. We are going to introduce an image-processing application to analyse these photos. Section 4.1 focuses on the curvature of the roots. Section 4.2 deals with the growth rate of the roots.

4.1 ANALYSIS 1: ROOT CURVATURE

We are going to use the photos of the 90°-turned and the clinorotated roots for the first analysis. The photo of the 90°-turned sample shows that the roots started bending in the direction of gravity after the Petri dish was turned by 90°. This is evidence of gravitropism of the cress roots. On the other hand, the clinorotated roots do not clearly show bending in any one direction. You may see a little oscillation of the root tips in any direction. This is evidence that the clinorotation confused the cress roots and created simulated microgravity conditions for them.

There are many excellent image-processing applications available. One of them is called ImageJ, which is an open-source image-processing application. It offers a range of tools and applications for analysing digital images and can be downloaded free of charge from http://rsb.info.nih.gov/ij/ (It is also important to download the ImageJ User Guide to become familiar with the application.)

Let's start the first analysis.

1. Download ImageJ. Make sure that you download the right version for your operating system. (You can choose between Apple OS X, Linux and Windows operating systems.) Also, make sure that your Java system is correctly installed.

2. Open the photo with the root curvatures and zoom into the photo.

3. Measure all curvature angles of the roots using the angle measurement tool. The angle measurement tool is below the Process button in the upper control row. You can use Ctrl+M to list each angle in your picture, as shown in figure XVII. For more information, please refer to the ImageJ documentation.

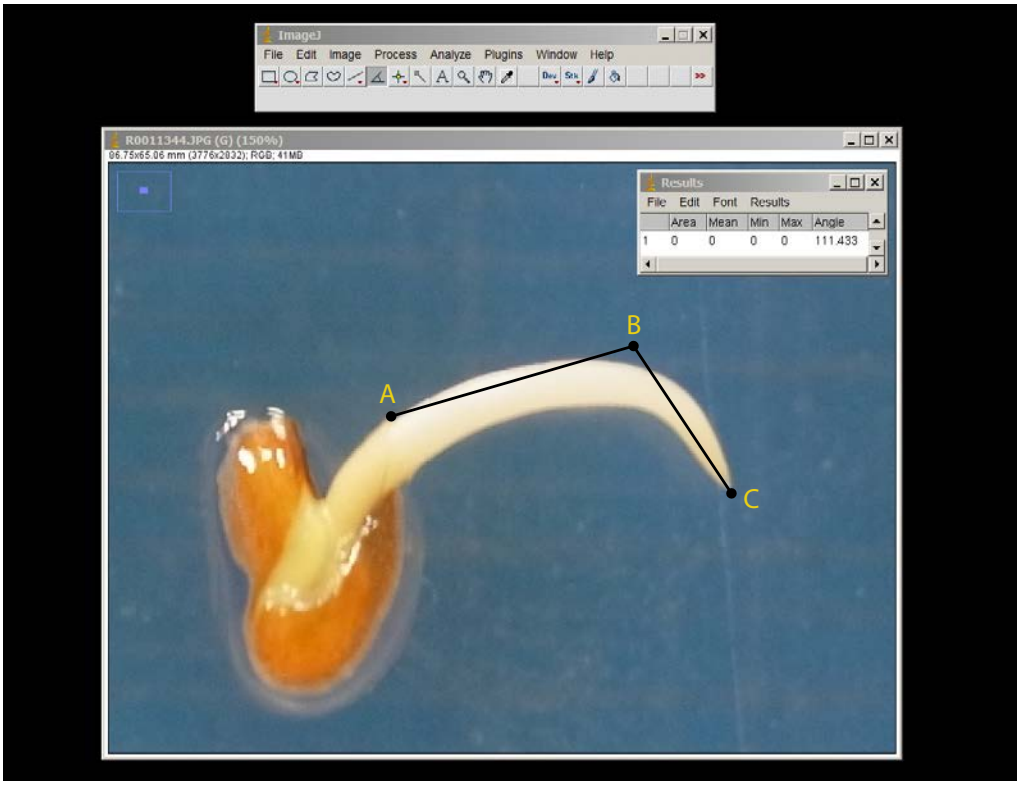


Figure XVII. This is ImageJ with an angle measurement tool. The image shows the root bending after the Petri dish has been turned by 90°. In order to measure the bending angle, first place the cursor at point A, then drag a line that is parallel to the root and click again at point B. From there, again drag a line that is parallel to the second part of the root and click at point C. Finally, hit Ctrl+M to show the angle in the picture.

4. When you have obtained the curvature angle of the roots, you then have to calculate the real curvature angle by subtracting the measured angle from 180°. For example, if you get a curvature angle measured with ImageJ of 117°, the real curvature angle is 63° (180°-117°).

5. Measure all of the curvature angles of the 90°-turned and clinorotated roots for each time point and calculate the average value.

6. Compare your curvature angles from the 90°-turned and clinorotated roots and discuss your results.

7. Calculate the average angular rate of the root bending in degrees per hour.

One common problem is where to put the measurement tool in the picture initially. The best way to measure a root angle is to start in the middle of the root tip and follow along the root until the curvature begins. Then click and follow with a second line that is parallel to the upper root part.

The results of this experiment should show the influence of clinorotation on gravitropism in roots. When we compare the average curvature angle of the clinorotated roots to that of the 90°-turned roots, we can see that the clinorotated roots show no response to gravity (see figure XVIII).

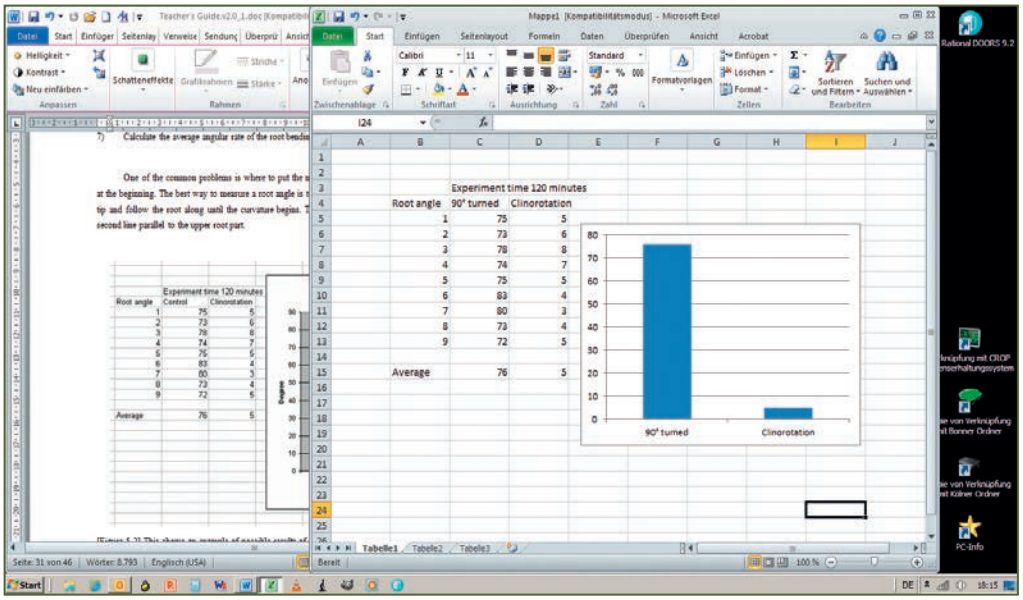


Figure XVIII. This shows an example of possible root curvatures after two hours of clinorotation compared with the curvatures of roots that were turned by 90°.

4.2 ANALYSIS 2: GROWTH RATE

We are going to use the pictures of the 1 g control and the clinorotated roots for a second analysis. The photos of the 1 g control show that the roots continuously grow in the direction of gravity. The roots of the 1 g control and clinorotated roots may appear to be similar, but the mechanisms for stimulating their growth are totally different in each case. For the 1 g control, the Earth's gravity continuously stimulates the growth of the roots in the direction of gravity. For the clinorotated roots, however, nothing stimulates their growth in any direction. We are going to analyse the difference between the two cases by measuring the length of the roots, which thereby allows their growth rate to be determined. In order to perform an analysis of the length of the roots, it is important that you record the length of the roots with a ruler placed at the same depth as the roots in the picture. Other ways, such as drawing a line which is exactly 1 cm long on the Petri dish, would also be acceptable.

Let's start the second analysis:

1. Open the photo of the roots and zoom into the photo.

2. Set the scale in the photo. This is done by using the length measurement tool and measuring a fixed length in the photo. Then set the scale by going to Analyze -> Set Scale and filling in the distance you just measured.

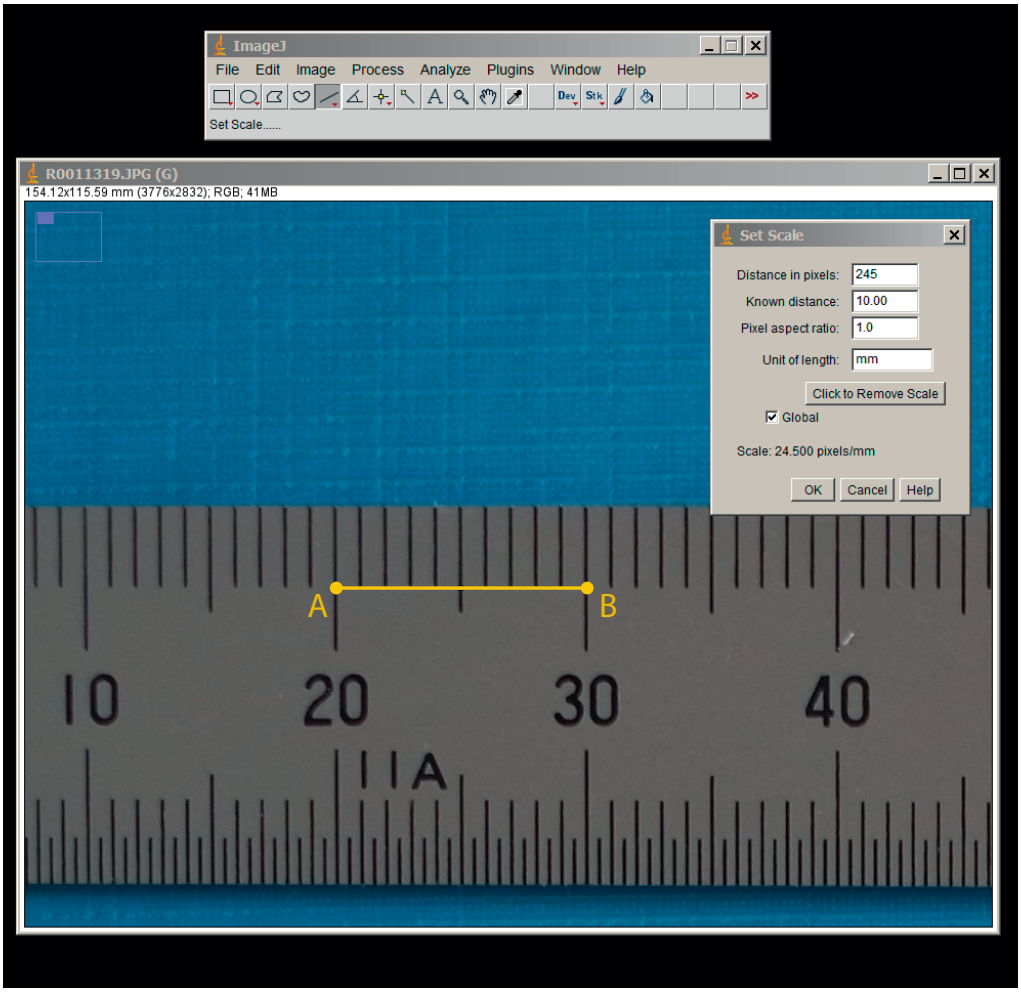


Figure XIX. This is ImageJ with Analyze -> Set Scale command. First, select the straight line selection tool, put the cursor at the initial point of the ruler: Point A and then drag the line to the end point: Point B. Second, select "Analyze" in the top menu bar and then select "Set Scale". In the pop-up window of "Set Scale", put the "Known distance", which you just measured on the ruler, "Pixel aspect ratio", and "Unit of length". Check the box next to "Global" and then hit "OK".

3. Place the cursor at the initial measurement point and then drag it to the end measurement point. Hit Ctrl+M to show the length of the root in your picture, as shown in figure XX. For more information, please refer to the ImageJ User Guide.

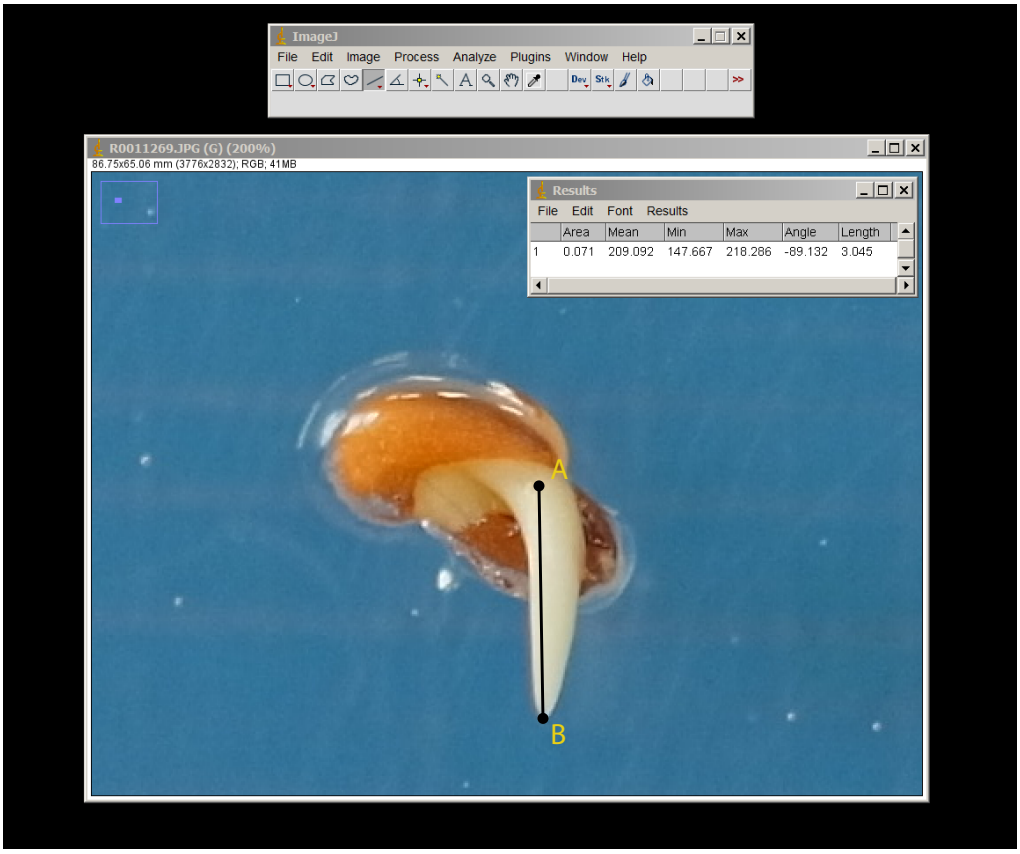


Figure XX. This is ImageJ with the length measurement tool. Put the cursor at the initial measurement point (point A) and then drag the line to the end measurement point (point B). Hit Ctrl+M to show the length of the root in your picture.

4. Measure all the lengths of the 1 g control and the clinorotated roots for each time point and calculate the average value. Figure XXI shows the lengths of the 1 g control cress roots versus the time since germination.

5. Compare and discuss the measured lengths for the 1 g control and clinorotated roots.

6. Calculate the average growth rate (millimetres/hour) for the 1 g control and the clinorotated roots.

We are going to compare the growth rates of the clinorotated cress roots with those of the 1 g control. Let's see what we can say about the effects of gravity and a lack of gravity on the growth rate of the roots.

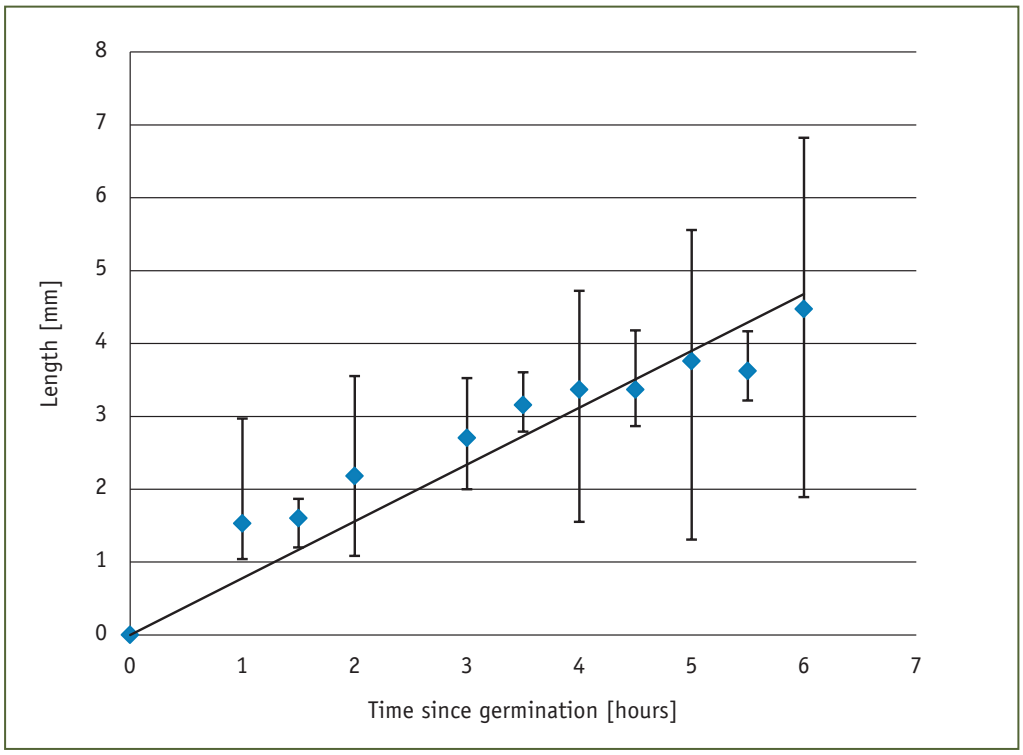


Figure XXI. This shows an experimental result with the length of the 1 g control cress roots versus the time since germination. The short vertical bar at each given time is an error bar which shows a range of actual measured lengths. The blue dot is the average length of the cress roots. The solid line represents the result of a least regression analysis of all the data points, which were taken at an average temperature of 23°C.

4.3 IDEAS AND TIPS

Further experiment ideas involving cress roots

What we did in section 3 represents just the beginning of the study of the gravitropism of plants. There are still so many questions to ask and so many answers to be found. In section 4.1, we analysed how gravity affected the bending of cress roots and how they behaved under simulated microgravity conditions.

In the gravity-sensing cells in the roots, statoliths indicate the direction of gravity as they move to the lower cell wall. Due to the contact between the statoliths and the cell wall, a still unknown gravireceptor is activated. This activation is transferred into a signal that is transmitted to the neighbouring cells to start the differential flank growth. The result is a bended

root, a so-called gravitropic root curvature. In the clinorotated root, the gravireceptor is, depending on the speed of the clinostat rotation, either intermittently activated due to many contacts with statoliths or, in the best case, not activated at all. In both cases, the result is that the cell no longer knows what is up or down.

This raises a question: what is the best clinostat rotational speed to create simulated microgravity conditions? You may be able to answer this question by changing the speed of the clinostat and observing how the roots react at different speeds.

In section 4.2, we analysed the growth of roots under 1 g and simulated microgravity conditions. The direction of the growth of the roots in both cases looked similar, however. This may be caused by the fact that we had the same preconditioning: a 1 g environment until germination and early growth started. What would have happened if we had put the seeds on the clinostat before germination started? This would have eliminated the 1 g preconditioning. The question is: in which direction does a root initially start growing under simulated microgravity conditions? Such an experiment would need longer hours of observation of the clinorotated roots, and you would have to prepare a good environment for germination and early growth of the roots on the clinostat by providing a clinostat chamber, as shown in figure XXII.

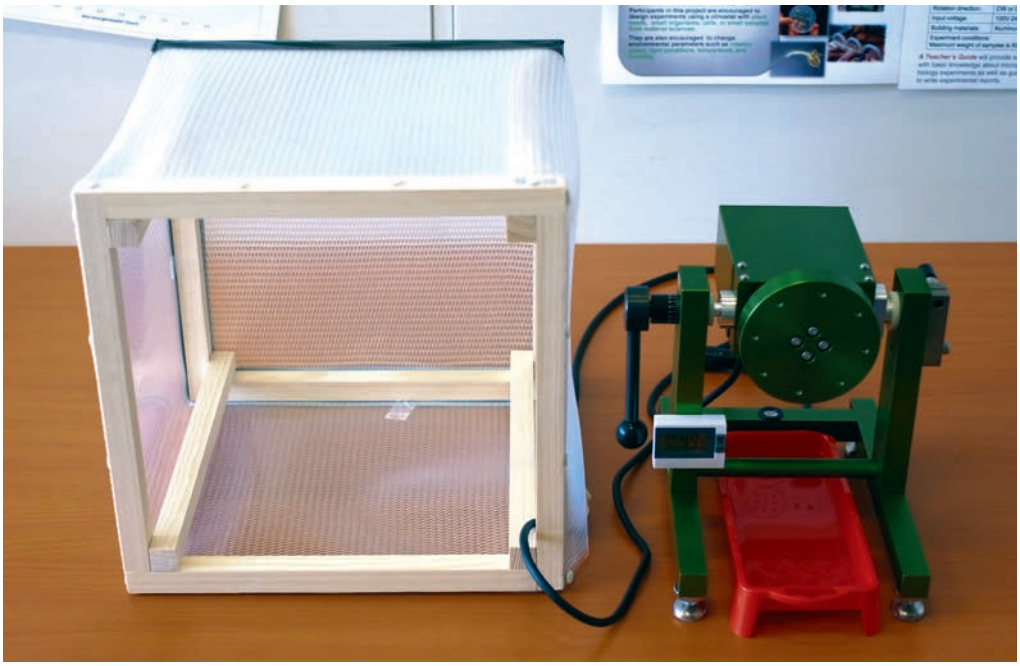


Figure XXII. A clinostat chamber is needed to keep the humidity high enough during the germination and early growth of seedlings that are placed on the clinostat. A small tray filled with water can be placed inside the chamber to add moisture.

Tips for experiments

Roots: Shorter roots are better than longer ones, so plants with roots with a length of about 5 to 10 mm should be used. Longer roots will also react to gravity but, in most cases, much more slowly than shorter roots.

Shoots: Short cress shoots are good subjects for studying gravitropism. They can be grown very easily on an agar-agar surface in a wet chamber without a Petri dish lid. When you are conducting experiments with shoots on the clinostat, use a clinostat chamber to prevent drying of the substrate.

Oxygen: Seeds need oxygen for germination. When closing your Petri dishes, leave at least one third of the space between the Petri dish and its lid open (see figure XI).

Wet chamber: Inside a Petri dish that has been covered with a lid, the roots have favourable conditions for growth. However, if you are using open Petri dishes (for big shoots or bigger seeds like peas), you need a chamber to prevent the roots or shoots from drying out. You can use commercially available plastic boxes, as shown in figure XIII. Some wet tissues or a small bowl with water will help to keep the humidity high in the chamber.

Clinostat chamber: You can make a clinostat chamber, as shown in figure XXII. To keep humidity high, you can place a small bowl of water under the clinostat. To achieve the best results, cover all the inner walls with wet tissues.

Light: Most seeds do not need light during the first days of germination. Some, however, like bell pepper seeds, do. Always remember that light is a strong stimulus for shoots and also for roots. To prevent influence from light, be sure that light is coming from above and is not too bright. The shoots will grow in the direction of the light, and the roots will always grow away from it (phototropism).

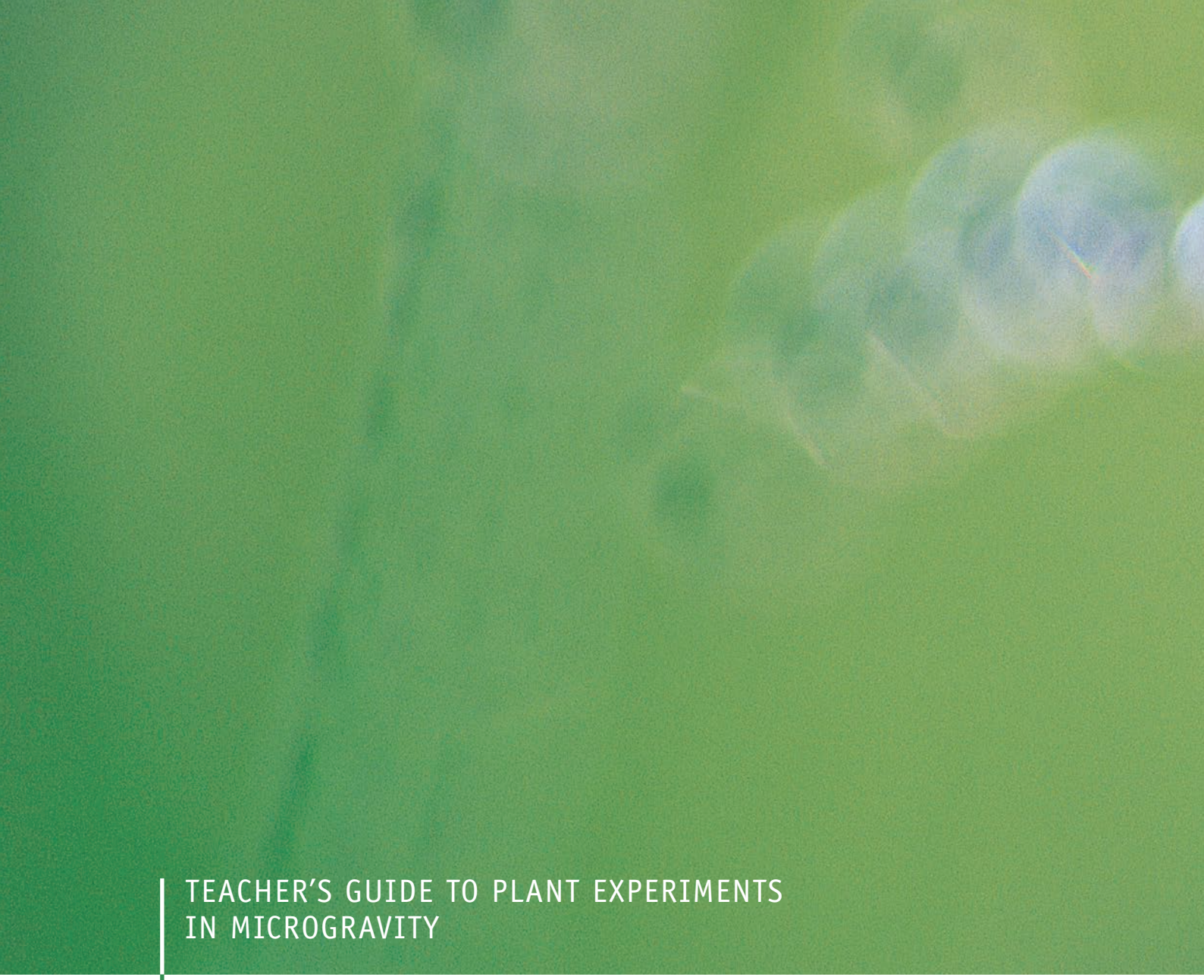
Water: For all experiments, tap water is the best choice. The residual minerals in tap water support the first stages of germination.

Substrate: You can substitute the agar-agar with other kinds of gel such as gelatine or phytogel. Gelatine has some disadvantages: it can be occupied by microorganisms in a very short time, and its physical properties are not as ideal as those of agar-agar. Phytogel is difficult to buy, but it is the best substitute. You can also try alginate as a substrate. For some experiments with cress seeds, only wet tissues are needed, and can be placed in a Petri dish.

Time: A young cress root needs about two hours to get a 90° curvature, so think about the time needed for your experiment.

4.4 REPORTING EXPERIMENTS

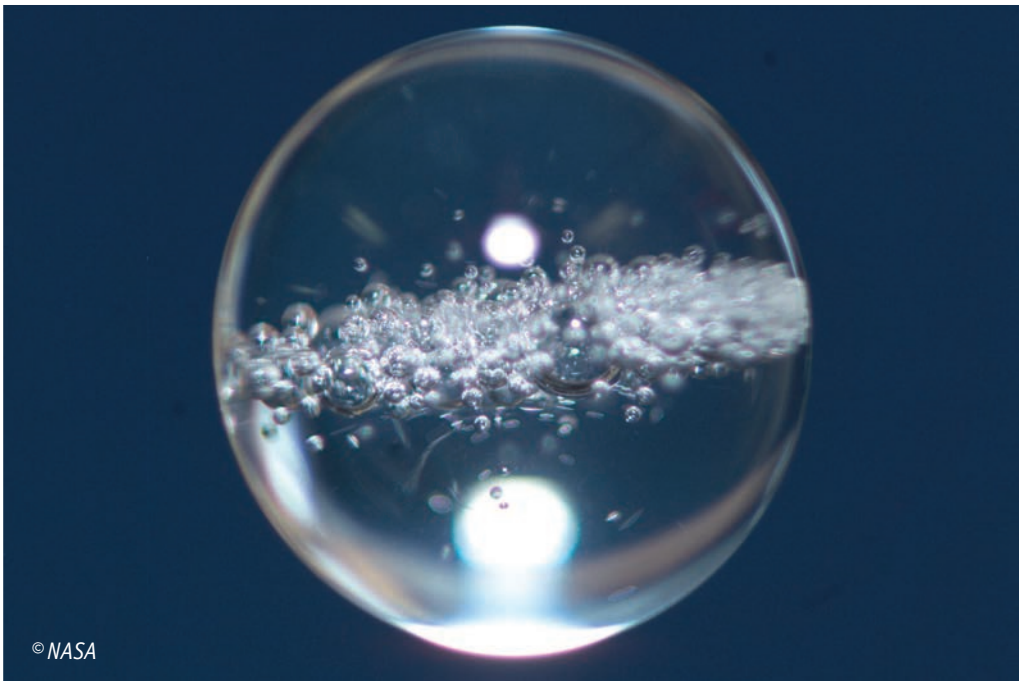
After conducting experiments and analysing all the data, please report your experimental results and analyses to the United Nations Office for Outer Space Affairs. Please refer to annex III of this guide for information on how to write a report on your experiments.



TEACHER'S GUIDE TO PLANT EXPERIMENTS IN MICROGRAVITY

5.

FURTHER STUDIES USING CLINOSTATS



© NASA

Always keep in mind that rotating samples on a clinostat is only the beginning of your investigation. After clinorotation, you can use a wide range of observational and measurement tools and methods, depending on the level of your study. In this teacher's guide, the growth of cress roots is observed with the naked eye, and the data is recorded by a digital camera. If you want to go to the next level of study, you may want to start using microscopes to observe and measure the activities on a cellular level, or to make movies with object-tracking software. In a morphological study, the most advanced step is to apply electron microscopy with magnifications of up to 1:1,000,000 to see structures and cell organelles related to gravitropism. Further experimental approaches include studies of genes and ion channels by means of molecular biology.

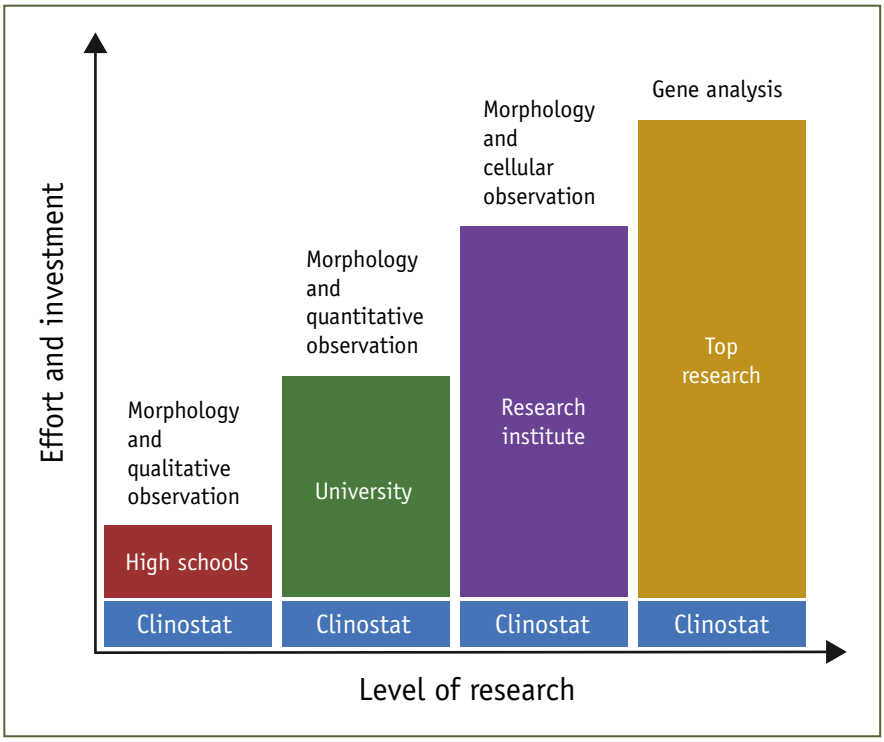


Figure XXIII. This shows the amount of effort and investment versus the level of research. Experiments with clinostats are the first step in the field of gravitropism research. Clinorotation is the first treatment in a long series of research activities. If you want to go further and into more detail, specialized observational and measurement methods are needed.

High schools: If you are teaching in a high school, simple observational experiments with respect to gravitropic reactions can be conducted. You can measure the curvature angles and growth rates of roots and shoots and compare them with those of control experiments. This is sufficient to introduce students to gravitational biology.

University: At the university level, you might use microscopes to study cellular behaviour after clinorotation. You have to fixate the roots directly after stopping the clinostat and cut them for microscopic observation with a microtome, as shown in figure XXIV. Some dyes, such as iodine solution, are also very useful to mark the starch in the amyloplasts located at the root tip. At this level, you can also study living root cells after preparation.

Research institution: At this level, you can employ more complex methods, like laser scanning microscopy or living cell video observation, or even use genetically modified organisms to compare their gravitropic reactions to those of wild organisms. Using inhibitors to block channels or disrupt the cytoskeleton is also a common method of obtaining more details about the

mechanisms involved in gravitropism. You can teach introductory lessons about the pathways and molecules involved in the gravity signal transduction chain that results in gravitropism.[13]

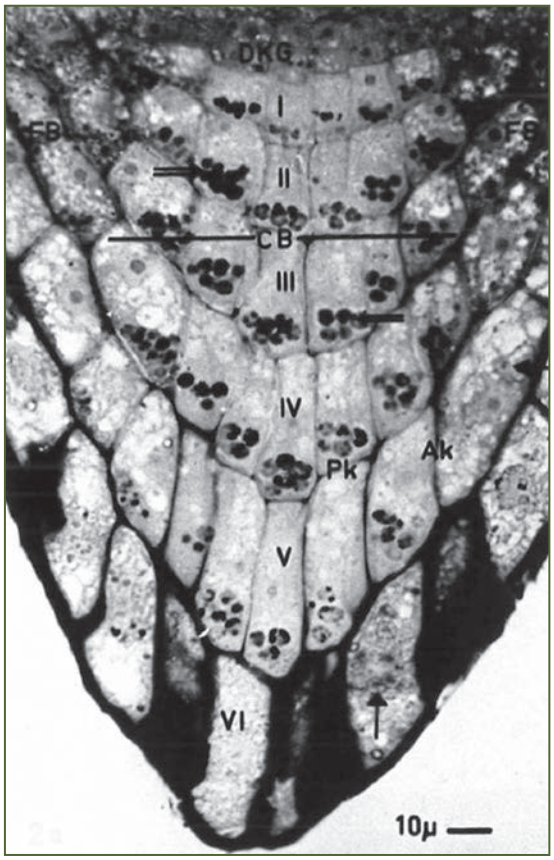


Figure XXIV. Gravisensing cells (statocytes) in a cress root with clearly visible statoliths (amyloplasts). *Courtesy:* Dieter Volkmann.

Top research: At the highest level of gravitropism research, molecular biology methods combined with laser scanning microscopy and/or transmission electron microscopy are mandatory. Using genetically modified organisms with specialized constructs to show interactions between molecules and receptors is also standard at this level. Three-dimensional reconstruction of a cell cluster in the root tip is one state-of-the-art method. This can be performed only in a research laboratory that has all of the equipment mentioned above.

[13] K. Boonsirichai and others, "Root gravitropism: an experimental tool to investigate basic cellular and molecular processes underlying mechanosensing and signal transmission in plants", *Annual Review of Plant Biology*, vol. 53, June 2002, pp. 421-447.

ANNEX I. GLOSSARY

Actin microfilament: structural element of the cytoskeleton inside of cells

Brownian motion: temperature-dependent random motion of particles suspended in a fluid caused by a collision of fast-moving atoms or molecules

Centrifugal force: a force acting on a rotating system that moves the object in the radial direction

Clinostat: a device which enables the rotation of an object around one axis or two axes which are perpendicular to each other to cancel the effect of the force of gravity on the object

Graviperception: sensing of gravity by organisms

Gravireceptor: biological sensor for transducing a physical signal (gravity) into a physiological response

Gravitational biology: a field of biology in which the effects of gravity on organisms are studied

Gravitaxis: special orientation of free-moving organisms with respect to the gravity vector

Gravitropism: growth or turning movement of a sessile organism with respect to the gravity vector

Hypergravity: acceleration larger than 1 g

ImageJ: an image-processing application which provides software tools for measuring a bending angle and the length of a root

Phototropism, phototaxis: orientation with respect to light

Microgravity: acceleration smaller than 1 g

Micropyle: small stigmata in seeds from which the root grows out

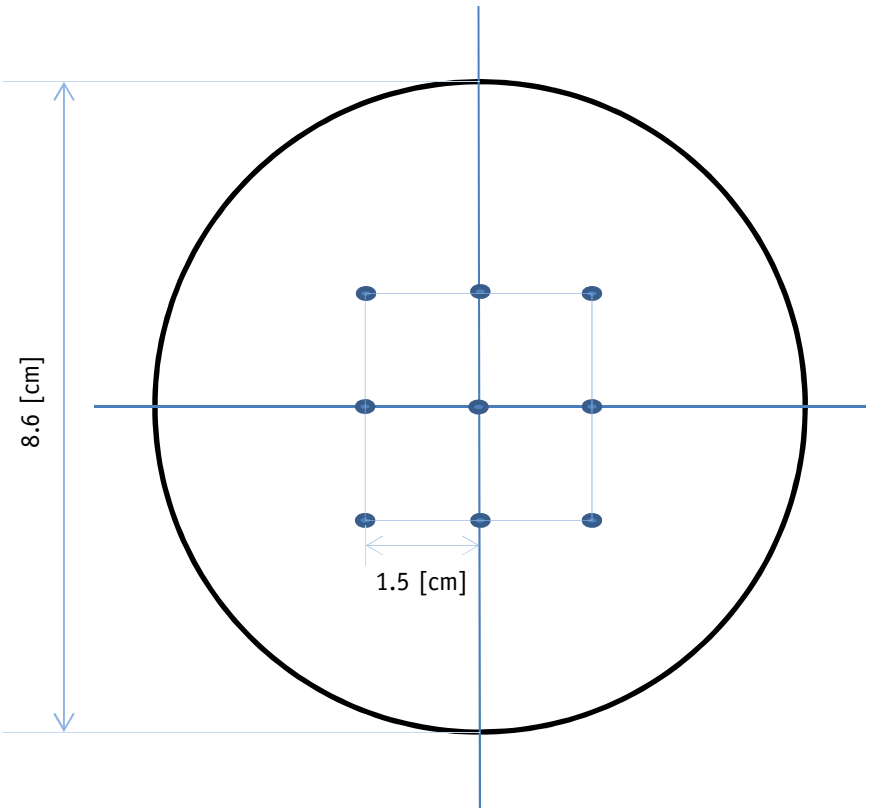
Mustard glycoside: chemical substance found in mustard, horseradish and wasabi

Rhizoid: root-like cell of a highly developed green algae (e.g. *Chara*)

Statocytes: cells that contain statoliths

Statoliths: dense particles in specialized cells. The typical material to form a statolith is starch in taller plants and barium sulphate (BaSO4) in *Chara* rhizoids.

ANNEX II. TEMPLATE FOR SEED PLACEMENT



ANNEX III. HOW TO REPORT ON EXPERIMENTS

Provide a written report on your experiment, which should include the following:

1. Cover sheet

The cover sheet should list the topic, the date, the participants and their affiliations.

2. Aim of the project (short introduction)

What is the question for the project? What biological materials are used and why? What is the rationale for performing the experiment as planned?

3. Materials and methods

Explain how the experiment was performed, including by discussing experimental conditions such as duration, temperature and humidity, and how the analysis was done.

4. Results

This part includes tables, figures and photos. The most important ones could be inserted in the main text, with additional ones put in the appendix.

5. Discussion and conclusion

Discuss your data and conclude your experiment by summarizing it and analysing its success and effectiveness. Include your experiences using the clinostat and any comments or suggestions for the Zero-Gravity Instrument Project.

6. References

Include the literature and sources you used for information and to gain background knowledge.

7. Appendix

Insert supplementary data and pictures.

www.unoosa.org

The United Nations Office for Outer Space Affairs (OOSA) is responsible for promoting international cooperation in the peaceful uses of outer space and assisting developing countries in using space science and technology.

The Zero-Gravity Instrument Project is aimed at providing teachers and students with the opportunity to perform experiments under simulated microgravity conditions.

FOR FURTHER INFORMATION,
PLEASE CONTACT:
United Nations Office for Outer Space Affairs
Vienna International Centre, P.O. Box 500,
1400 Vienna, Austria

Website: www.unoosa.org

For enquiries about the Human Space Technology Initiative and its Zero-Gravity Instrument Project, please send an e-mail to: hsti@unoosa.org

Website: www.unoosa.org/en/SAP/hsti/index.html

United Nations publication

V.13-84163—August 2013