Chapter 5

Centrifuges: Evolution of Their Uses in Plant Gravitational Biology and New Directions for Research on the Ground and in Spaceflight

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ABSTRACT

The use of centrifugation as a tool for exploring qualitative and quantitative features of plant responses to gravity and to other body forces can be traced to plant scientists' early 19th century experiments. To study how plants perceive and respond to gravitational stimuli requires experimental manipulation of the force or acceleration vector direction and magnitude. As technology advanced, especially during the past half-century, so did the sophistication of experimental designs and of the scientific questions that could be addressed. The most significant improvement in methodology probably will prove to be attainment of experimental access to the hypogravity range of inertial accelerations by the combination of centrifugation and spaceflight.

INTRODUCTION

To review uses of the centrifuge for plant research may seem straightforward, but to compare results of their uses on Earth and in space is more difficult; first, because so little has been done with centrifuges in space, and second, because all centrifuges on Earth can only maintain hyper-g conditions, while in space there are even more interesting applications in the hypo-g range of accelerations. We shall try to make clear some of what has been done with the centrifuge (on Earth and in orbit) and to suggest some things that can be done, especially by combining the centrifuge with other devices in the plant scientists' armamentarium.

As its title implies, the "Clinostats and Centrifuges" symposium was conceived as a review of scientific uses of two kinds of rotating machines, the clinostat and the centrifuge. But our research tools include a third (and even a fourth) kind of rotating machine that warrants our consideration. The spacecraft in Earth-orbit is a third kind of rotating machine, ideally one that can establish a nearly zero-g (free fall) platform for experiments, including those that may require centrifugation. A number of pioneering, and in some cases scientifically very fruitful, experiments have been performed either on Earth or in space using each of these three rotating machines alone, but also, in the case of plants, we shall argue that an especially powerful research technique is one that employs two of them together either on Earth—centrifuge plus clinostat—or in space—centrifuge plus spacecraft. Figure 1 charts the ranges of accelerations that can be provided as environmental information inputs to test subjects, achieved by combinations of these three rotating machines.

A discussion of scientifically significant results attributable to uses of centrifuges that also emphasizes technological limitations of manipulating experimentally applied g-forces must include some mention of clinostatting—hopefully without intruding too seriously on a subject area assigned to other chapters.

FIRST, SOME COMMENTS ABOUT TERMINOLGY: WHAT IS A CENTRIFUGE?

A centrifuge is one of a large population of rotating machines and, whether or not the machine is called a centrifuge, any device that imparts circular motion obeys the same physical laws that describe quantitatively the acceleration it generates. Any wheel, fan, centrifugal pump, top, clinostat, ballet dancer, acrobat, merry-go-round, NASA space shuttle in circular orbit, even the Earth itself—all are rotating machines and behave as centrifuges.

HOW SHOULD WE QUANTIFY THE ACCELERATION THAT CAN BE CREATED BY A CENTRIFUGE?

The universal (not geocentric) gravitational constant is denoted by G and relates the attractive force between any two bodies to the product of their masses and the distance between their centers, according to Newton's law of gravitational attraction. Dimensionally, this G is not an acceleration but is used to derive the acceleration caused by the gravitational attraction of a planet or other body. The acceleration so derived has the same dimensions as the acceleration produced by uniform circular motion on a centrifuge. During the better part of two centuries gravitational physiologists have used different
Figure 1. Chart showing the ranges of g-forces that can be achieved or simulated by various combinations of three rotating machines: centrifuge, clinostat, and space vehicle.

systems of units to express quantitatively the accelerations generated by centrifuges and by other rotating machines. Within about the last four decades it has become the nearly universal convention to express these accelerations in "Earth-g" units.

The important variables are rotation rate and radius of rotation. (If the centrifuge operates on Earth, the orientation of the centrifuge axis with respect to the plumb line and the constant value of Earth's gravity also may have to be considered.) Rotation rates often are expressed as rpm but a more convenient unit is rps, or hertz, abbreviated as Hz. Let R be the radius and ω²•R the acceleration generated by the rotation. With the acceleration expressed in g-units, the relationship can be written:

\[ g = k \cdot (Hz)^2 \cdot R \]  \hspace{1cm} (1)

The value of the dimensional constant, k, depends on our choice of units for radius, R. k also embodies the conversion factor \((2\pi)^2\). It is especially convenient to express R in metres, in which case k is approximately 4 (within 1%). Thus, a "unit g" centrifuge (product of R and Hz² equal to 1/4) produces an acceleration of about 1 g.

In microgravity science and in common biological jargon, many authors refer to a "gravity force," a "centripetal force," and a "centrifugal force" without seriously confusing the reader who also has been willing to accept the obvious dimensional inconsistency and to use "centripetal force" or "centrifugal force" to refer to what in truth are accelerations. Nevertheless, we know the term force cannot be physically (dimensionally) correct when it refers to an inertial acceleration. In the case of forces relating to uniform circular motion (centrifugation with constant angular velocity, ω), there is a radial force \(F_r\) (directed inward), properly referred to as centripetal, and an opposing force \(F_o\) (directed outward), termed centrifugal. \(F_o\) is a tensile force, exerted on the centrifuge by the object which it constrains to follow a circular path, while \(F_r\) is the force that the centrifuge imposes on the object. Under these conditions, \(ω\) and \(R\) are held constant, and

\[ F_o = mω^2 R. \]  \hspace{1cm} (2)

By convention we express accelerations quantitatively in units peculiar to our terrestrial environment in which an acceleration (due to the Earth-based gravitational attraction) is expressed in g units. \(g\) is roughly 10 m s⁻²; the handbook value of 9.81 m s⁻² is an approximation—correct for Winnipeg, Manitoba and for Land's End, England, but over the globe its value is latitude-dependent, and it varies from 978 to 983 m s⁻².

"Centrifugal force" is at least intuitively compatible with the noun, centrifuge. The etymology of "centrifugal" and of "centrifuge" is unfortunate, since the function of a centrifuge is to apply an increased acceleration to a rotated object, but who might have the temerity to argue in favor of calling the machine a centripetuge?

WE USED TO SAY GEOTROPIC; NOW WE SAY GRATITROPIC

Already most of us are getting used to referring to g-induced tropistic reactions as gravitropic instead of by the older term, geotropic. The latter term, coined by Frank (1868), does not seem quite adequate to describe plant growth changes in response to modified accelerations, especially when they occur onboard a space vehicle where the free fall condition results in an acceleration close to zero in the space vehicle reference frame. One might argue that neither term is ideal, but it is the fashion of our time to call the tropistic reaction gravitropism.

HOW DOES THE CENTRIFUGE WORK?

A centrifuge for plant research usually operates by rotating subjects about a vertical axis. The historical reasons for this are mostly trivial and, for tests at relatively high g-levels (say over 10 or 20 × g), the orientation probably does not matter. However, for low speed centrifugation on Earth, the centrifuge design of choice has been the "swinging bucket" type for which the resultant
acceleration, $A_R$, experienced by a test subject may be calculated from the "parallelogram of forces":

$$A_R = \sqrt{(\omega^2 r)^2 + (g)^2} \quad (3)$$

**HOW IS THE CENTRIFUGE USED?**

Biological uses of centrifuges fall easily into two distinct categories: for application of (a) very high accelerations ($g >> 1$), which patently induce stress and, in excess, can damage a plant’s structural integrity, and (b) low accelerations (approximately in the range, $g = 1 \pm 1$), which provide a special kind of physical *environmental information* to the plant—information that it can store for a short time (how we do not know) and, after transduction of the information into some biologically meaningful form, the plant can respond in some characteristic manner—tropistically, nastically, morphologically, biochemically, or physiologically. By its response we know that the plant has perceived the environmental information that we call the g-stimulus.

The research question that has been asked when very high accelerations were employed could be stated as “How much was too much?” High accelerations (orders of magnitude greater than 1 g) were induced by high speed centrifugation on small radii in order to establish large g-force *gradients*, thereby bringing about separation of cellular components (usually of small cells) because of only small differences in density. By this means cellular components could be stratified within the cell. The biologically interesting result was that some cells could endure that condition and later were able to restore much of their normal, somewhat higher-entropy, state of organization. Bouck (1963) centrifuged *Pisum sativum* roots at 20,000 g for up to 24 hr and observed that “within 12 hr after removal of the root from the centrifuge growth (in length) of the whole root could be measured.”

That plants can endure such large accelerations without irreversible injury is not really surprising. Some cells of higher plants under natural conditions often withstand very high internal pressures (by virtue of their strong, elastic, cells walls)—in extreme cases, as high as 3,000 kPa. Other cells of nearby tissues frequently experience (without cavitation) negative pressures as low as ~2,000 kPa. (For comparison, the pressure in a passenger car tire is only about 200 kPa above ambient.) In growing tissues, very large tensions (on a micro-scale) develop. The resistance of the “typical” plant cell to pressure extremes presumably also confers the ability to endure high accelerations. However, although cells may be undamaged, under high pressures tissues can be split, cracked, or squashed.

In one study (Brown, 1983), *Arabidopsis* plants were grown on nutrient agar in centrifuge tubes, and at age 21 days they were centrifuged for 10 min at well-controlled centrifuge speeds. Figure 2 shows that these small herbaceous plants withstood deformation by accelerations up to about 35 g. The acceleration needed to induce an “average” degree of mechanical distortion was about 60 g. When even higher accelerations were applied, maximal deformation was insufficient to cause permanent damage. The maximum acceleration tested was 390 g from which 100% of the test plants recovered. That performance was far better than what could be expected from most animal test subjects of comparable size—all attributable to the plant cell’s biologically unique structural component, its cell wall.

**Orientation of Axis of Rotation**

If the axis of a unit-g centrifuge is horizontal, the test subject will experience (from the sum of centripetal acceleration and Earth’s gravity) a net acceleration that varies sinusoidally over each revolution from zero to 2 g. To illustrate, if the applied centripetal acceleration is 1 g and the rotation rate is 1 Hz (60 rpm), and if the reference point on a small object is at a radius of 25 cm (0.25 m), then during each 1/2 revolution of the centrifuge (0.5 second) the reference point on the object is subjected to accelerations from zero to 2 g. In the example chosen, the average acceleration would be 1 g, but we do not know that the plant responds to a rapidly fluctuating environmental g-signal in the same way as it does to a steady stimulus.

**Speed of Rotation**

Continuing the above line of reasoning, with horizontal axis of rotation, with Earth’s gravity adding...
its vertical component, and with a slow rotation rate (5 rpm = 0.083 Hz), then a given reference location on the test subject 10 cm from the axis of rotation will experience at any one instant a net acceleration that may have a value in the range 0.997 to 1.003 g, presumably a negligible variation. However, within each complete revolution any given point on the subject will have experienced equal and opposite stimulations by Earth’s 1 g from every direction in the vertical plane. If we make the comforting assumption that opposing stimulations cancel, then all stimulations should sum to zero over each rotational period. By reducing the speed of our hypothetical centrifuge to only 5 rpm it has become a clinostat. The question arises: When does a centrifuge become a clinostat? In principle, when the centripetal acceleration generated by its rotation becomes experimentally negligible—as far as we now know, if it remains somewhat below about $10^{-4}$ or $10^{-3}$ g.

Consider a slowly rotating clinostat while we gradually increase its speed. At some point the centripetal acceleration will exceed our provisional limit of about $10^{-3}$ g. However, to determine that limit the radius of rotation also has to be considered; the smaller the radius the faster can be the acceptable rotation rate. If the machine’s radius is made very small, we can convert our now slowly rotating clinostat (SRC) into a “fast rotating clinostat” (FRC). The FRC usually has been operated at about 50 rpm (= 0.83 Hz). The SRC usually has been operated at from about 0.2 rpm (0.003 Hz) to about 4 rpm (0.07 Hz). It already has been explained in earlier chapters of this volume why, in theory, the FRC has an important advantage over the SRC design. Again in principle: When does a clinostat become a centrifuge? When centripetal acceleration becomes large enough to introduce an unacceptable ambiguity in the interpretation of a clinorotation experiment, i.e., when the product, $\omega^2 R$, becomes large enough to exceed the subject’s threshold for detection of a sustained inertial force. Therefore, as illustrated by Figure 3, if we wish to keep the centripetal acceleration at every point on the test subject below $10^{-3}$ g, its diameter must be no greater than about 0.7 mm and it must be centered exactly on the axis of rotation. If the specification is $< 10^{-4}$ g, the diameter must not exceed 71 $\mu$m. Therefore, only by restricting our choice of test subjects to quite small organisms can we confidently take advantage of the superior qualities of fast rotating clinostats.

**HOW DID THE USE OF A CENTRIFUGE IN SPACE COME ABOUT?**

When scientists speculated on what could be studied advantageously in Earth orbit (long before opportunities for those studies became possible), one of the most often repeated recommendations of advisory committees was the need for a “one-g control” onboard the space vehicle. The reason for this was that most committee members tended to distrust predicted effects of what were then unknowns—the space radiation environment, the consequences of prolonged sensory deprivation in higher animals including hominids, the potential disruption of processes controlled by circadian rhythms and, most of all, the possibility of some unpredicted artefact related to the spacecraft itself—which might exert an unwanted influence on a biological experiment.

The risk that an experimenter might be misled by such unidentified and perhaps unidentified spaceflight-related factors would be reduced, so the argument went, if the experiment could be performed on the spacecraft at “zero g” at the same time as an inflight “control” with centrifugation at 1 g. If all was well, the 1 g centrifuged specimens should behave in the same manner as a ground control. More often than not weightlessness was considered potentially very stressful, even dangerous, and therefore a hazard to be counteracted. Thus, only two g-levels were given high experimental priority, zero-g and unit-g.

Only a few scientists spoke out against that limited view of what a centrifuge in space could do. For example, 30 years ago when NASA was just beginning to prioritize areas of science for projected space experiments a National Academy of Sciences, National Research Council summer study report (1962) included the following “findings”:

"[g is viewed] as a continuous variable which the centrifuge can extend in one direction as the satellite can in the other . . . Probably the most interesting changes to be anticipated will relate to the lowest regions of the g variable . . ."
As more became known about spaceflight conditions and as interesting results from space experiments began to trickle in and to be assimilated by scientists in different disciplines, a fundamental change in outlook emerged. Conducting zero-g endurance contests with test organisms was put in reasonable perspective and more thought was given to devising ways to exploit, for a number of different scientific purposes, the so-called μg environment. Even then many biologists were thinking of g, not as one value but as a continuum from zero to well above unity becoming accessible in its entirety for research exploitation. From that perspective the appropriate “control condition” would be the absence of any stimulation by the factor under study, i.e., zero g. (The obvious analogy for a control condition in the case of studies on phototropism is darkness.) With that viewpoint the question: What is the “effect of weightlessness?” has only a limited meaning, since zero-g is only one point on a stimulus/response curve. Salient questions now can take the form: “What will be the g-function of whatever behavior of the test organism is under consideration?” By use of simulation (BIAXRO method, see Figure 4A) that kind of question can be asked also in our home laboratories, and it has become commonplace for biologists who propose spaceflight experiments to undertake such ground-based simulation studies prior to their tests in Earth orbit.

In our laboratory we have used a relatively large centrifuge (6 m in diameter) as the “turntable” with vertical axis of rotation (see Figure 4B). The swinging buckets were removed and the clinostat was mounted directly on top of the centrifuge arm with the clinostat axis of rotation aligned with the radius of centrifuge rotation.

In one such application (Finn and Brown, 1961), probably the first of its kind, the question was: What is the g-function of leaf epinasty (or, since variable g can begin at nominal zero, the more logical phraseology would have been: What is the g-function of g-induced hyponasty)?

In another case the question was: What are the parameters of a plant hypocotyl’s circumnutation as functions of g? To answer that question circumnutational parameters were measured on a series of plants held at a number of simulated g-levels over an unprecedented range, −1 g to +15 g (Chapman et al., 1980). Their results are reproduced in Figure 5.

In both of the above cases, all of the appropriate confirmatory tests in Earth orbit have not yet been accomplished. Other than simulation data we have measurements applicable to these examples from spaceflight experiments only at that one very important point, zero g (Johnson and Tibbitts, 1971; Brown et al., 1990). Biological exploration in space of the remainder of the hypogravity range is only just beginning.

Table I lists some major scientific questions about fundamental aspects of plant gravitational physiology. Centrifugation has contributed in important ways to the majority of the researches that have addressed six of the seven major questions listed.
In retrospect, there is some virtue in the argument that, until recently, the centrifuge has been the most widely used physical gadget contributing to advances in our knowledge of plant gravitational physiology. (Perhaps second place should go to the clinostat.)

We shall highlight some of these researches more or less in their historical sequence. Space does not permit an exhaustive review of the subject, but the following examples emphasize the diversity of research questions that were addressed by methods that included centrifugation.

Knight’s Wheel

Probably the first use of centrifugation to implement carefully designed scientific experiments with plants was that of Thomas A. Knight (1806) who wanted to demonstrate that what we now call tropistic responses of plant shoots and roots were truly responses to gravity. For that purpose Knight wanted to disorient his test plants with respect to the Earth’s gravitational vector. His apparatus was a rotating machine driven by water power. He referred to the apparatus as a “Wheel” of which he built several. His first experiments were with the wheel spinning on a horizontal axis. He wanted to alter so quickly the direction in which his test plants were exposed to Earth’s gravity that their growth movements could not keep up with the rapidly changing orientation. In Knight’s words, “Some naturalists have supposed these [tropistic] effects to be produced by gravitation; . . . [T]he hypothesis of these naturalists does not, however, appear to have been much strengthened by any facts they were able to adduce in support of it, nor much weakened by the arguments of their opponents; and therefore, as the phenomena observable during the conversion of a seed into a plant are amongst the most interesting that occur in vegetation, I commenced the experiments. . . . I conceived that if gravitation were the cause. . . . it could produce these effects only whilst the seed remained at rest, and in the same position relative to the attraction of the Earth, I imagined that its operation would become suspended by constant and rapid change of the position of the germinating seed, and that it might be counteracted by the agency of centrifugal force.” That is a fair statement of the concept that underlies clinorotation and, in the context of Knight’s own explanation of his experiments, it becomes quite clear that he could just as well have called his Wheel a fast rotating clinostat.

Knight wanted to achieve what is in today’s terminology “gravity compensation” although the “clinostat” was (officially) invented by Julius von Sachs 76 years later (Sachs 1882a; 1882b). One might argue that Knight was on the right track and should be given credit for conceiving the principle of clinorotation, even though what he built and used was functionally a cen-

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Table 1. Salient Questions about How Plants Grow That Have Been Addressed† by Plant Gravitational Physiologists.

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
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<tbody>
<tr>
<td>1. WHERE IS (ARE) THE PLANT’S BIOACCELEROMETER(S)?</td>
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<td>What biological structures are involved?</td>
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<td>2. HOW RESPONSIVE IS (ARE) THE PLANT’S g-SENSOR(S)?</td>
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<td>What are the thresholds for g-detection?</td>
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<td>Why are seedling shoots about an order of magnitude less responsive than roots?</td>
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<td>3. HOW ARE PLANT CIRCUMNUTATIONS DRIVEN AND CONTROLLED?</td>
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<td>Why are circumnutations ubiquitous?</td>
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<td>What is gravity’s role?</td>
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<td>4. WHAT PRESERVES PLANT ORGAN POLARITY?</td>
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<td>What maintains polarity?</td>
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<td>By what means can polarity be subverted or reversed?</td>
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<td>5. HOW DOES PLANT MEMORY WORK?</td>
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<td>How do plants add and subtract?</td>
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<td>Over what g-dose range does reciprocity prevail?</td>
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<td>6. HOW SENSITIVE ARE THE PLANT’S g-SENSORS?</td>
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<td>How can one explain the changes in sensitivity observed at different levels of the unidirectional acceleration?</td>
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<td>7. HOW IS THE PLANT’S PERCEIVED g-STIMULUS TRANSDUCED INTO OPERABLE BIOCHEMICAL INFORMATION?</td>
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<td>How is g-related information processed?</td>
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<td>What growth regulators are involved?</td>
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<td>How do “promoters” and “inhibitors” interact?</td>
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<tr>
<td>How are growth regulators transported?</td>
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†Centrifuges have played a prominent role in addressing six of the seven major questions listed.

Figure 5. Variation of nutational frequency over a range of axially imposed centripetal accelerations between -1 and +15 g. In all tests, the transverse component of Earth’s gravity either was actually zero (achieved in spaceflight) or was compensated by clinorotation. (Reproduced with permission from Plant Physiology, Vol. 65, page 535, 1980.)
trifuge. This point seems not to be generally recognized although it has been noted elsewhere (Mitchell, 1989).

To continue what Knight said, “The wheels performed something more than 150 revolutions in a minute; and the position of the seeds relative to the earth was of course as often perfectly inverted, within the same period of time; by which I conceived that the influence of gravitation must have been wholly suspended... I added... another wheel, which moved horizontally over the vertical wheels; and to this, by multiplying wheels of different powers, I was enabled to give many different degrees of velocity.” We note that the rotation rates used by Knight were much higher than what now would be acceptable even for a fast rotating clinostat, so his Wheel was indeed a centrifuge.

Implicit in Knight’s discussion of his experiments was that gravitational and centripetal forces should exert the same effect, if their magnitudes and vectors were rightly considered. He reported that, at 80 rpm (horizontal wheel with vertical axis) and a radius of 5.5 inches, the shoots and roots were inclined (in opposite radial directions) at an angle of 45° relative to the plane of the wheel. That works out to a centripetal acceleration of 1.0 g. One might say (with tongue in cheek) that Knight’s experiments included an empirical test of Einstein’s Principle of Equivalence, although his results were published 73 years before Einstein was born.

August Piccard (1904) used centrifugation in the first attempt of this century to localize the plant’s g-sensor. He was, of course, aware of the work of Charles Darwin (1867, 1880), who had emphasized that shoot and root tips appeared to be those plant regions that were most (or even exclusively) responsive to gravitational stimulations. Piccard arranged for plant organs to grow across the axis of rotation of his centrifuge (Figure 6) whereupon they would reverse growth direction and behave as if their guidance information had been derived from detection of a centripetal force. His conclusions included a rejection of the concept that the tips of growing organs are the sole sites of the plant’s g-sensors, an opinion now shared by other iconoclastic plant physiologists.

Freier and Anderson (1971) also used a variation of Piccard’s method to describe in physical terms the tropistic behavior of oat seedling shoots and roots responding to centripetal accelerations on a centrifuge rotating on an axis that was horizontal. Since they did not quote either Piccard or Knight we may assume that they reinvented the “Wheel.”

PLANTS’ G THRESHOLDS

Schwarz (1881) used centrifugation to demonstrate that a gravitropic response was g-related. He reported that the tropistic response to 17 g was stronger than that to 1 g. Czapek (1895) used a centrifuge to measure seedlings’ g-response threshold. Both acceleration and duration of application were varied. Eight hours at $5 \times 10^{-4}$ g (0.24 g-min) produced no detectable curvature. Six hours at $1 \times 10^{-3}$ g (0.36 g-min) produced a small curvature. These numbers are roughly one order of magnitude lower than those found by what we believe were even better controlled twentieth century experiments. Perhaps that should be taken as reasonably good agreement.

Charles Darwin did not make use of centrifugation but Francis Darwin and D.F.M. Pertz (1904) reported results of experiments on the statolith theory of geotropism during which they studied effects of centripetal forces. Their report of those mainly exploratory experiments included the observation that the g-threshold of their test plants was in the range of 0.02 to 0.05 g.

Ruten-Pekelharing (1910) reported in a 96-page paper (her thesis) the results of a large number of tests at different g-levels that allowed a quantitative assessment of the g-response threshold and also of the range over which $g \times t$ “reciprocity” prevailed. The apparatus (centrifuge) was described. Its cost was estimated as “120 Gulden” [Guilders?] which at today’s exchange rate would be equivalent to $63.13 (but, of course, the apparatus did not have to be subjected to NASA’s necessary but infamous Reliability & Quality Assurance
requirements). More than one species were used, but the most relevant data were obtained with *Avena sativa*.

The experimental program was well planned, although it is unfortunate that the author did not summarize her results in a lucid sentence or two. The reader is presented with a wealth of data, much of which is not relevant, and from which it is not easy to appreciate how best to “reduce” the data in order to calculate the threshold for a bending response. Both *g* and duration of application were varied (from 0.04 to 46.0 g and from 5 sec to 130 hr). Results were reported as percent of plant organs bending toward and away from the direction of the applied centripetal forces. Because of inherent variability, after responding to stimulation perfectly straight plants were rare. If a *g*-dose (acceleration × time product) was too weak to be detected, the average result to be expected would be 50% in either direction. If the force were detected, more than 50% of the test plants would exhibit a tropistic response in the same direction. For maximal precision of estimating the 50% response most of the results (very low or very high *g*-doses) were not very important. But one can calculate (or plot) responses against corresponding doses and establish that the slope of a fitted curve intersects the 50% response level at 5.7 *g*-min.

Many—perhaps a majority—of the attempts to measure a plant’s *g*-threshold have not employed centrifugation; stimulations were of the same intensity, viz., transverse application of Earth’s 1 g for which the plant organ had to be set at 90° from the plumb line for varying exposure times. This method yielded *g*-dose thresholds that often were quite different from those calculated by applying transverse stimulations that varied by 5 or 6 orders of magnitude and (after many hours of exposure to each treatment) by determining the threshold as the least acceleration dose that had produced a detectable response. There is no obvious reason why results from the two methods should not agree, if the response is strictly dose dependent. The disagreement may be related to a plant’s “forgetting function,” which perhaps is important for tests that take many hours, but insignificant for tests that are completed within a very few or tens of minutes.

A confident determination of the threshold is of considerable theoretical interest, and we may expect to hear of more experiments on this topic. For small cells the “ultimate” threshold has been predicted by a number of authors of theoretical papers based on sound physics and necessarily shaky assumptions of critical properties of biological models. We note that the threshold might be determined experimentally with less ambiguity in a spaceflight experiment as was attempted in 1985 during the D-1 Shuttle mission, STS-22 (Volkmann et al., 1986; Perbal et al., 1987). Unfortunately, the tests had not been accorded a high priority on the D-1 mission and were not implemented by a sophisticated method. They probably will be repeated on a later mission. Another attempt to establish a *g*-threshold by empirical tests in space was one of several objectives of the GTHRES (Gravity Threshold) experiment on the 1st International Microgravity Laboratory (IML-1) Space Shuttle mission (STS-42), which was launched in January 1992.

### EFFECTS OF INVERSION

Without using a centrifuge, several experimenters were able to contrast effects of exposure of plants to +1 g compared with effects of exposure to −1 g, i.e., to test the effect of inversion. Numerous contributions testify that inversion of the plant in the Earth’s 1-g field can have a dramatic effect on growth and morphogenesis. For example, among the earliest works actually called experimental plant morphology was that of Vöchting (1878) who reported that inversion had no effect on the polarity of root formation in willow cuttings.

Centrifugation can be used to increase the acceleration of normally positioned and of inverted subjects, so it is not surprising that it has been used for that purpose. Goebel (1908) reported that centrifugation produced root initiation at the apical end of inverted stem cuttings. Beams and King (1944) reported the alteration of polarity of *Vinca rosea* pollen by centrifugation at 20,000 g.

Various authors have tried to explain observations in which inversion was effective in terms of an influence on transport of growth regulator, but the mechanism for it is unknown and, while it may involve auxin transport, that could be a result far removed from the primary effect of the altered *g*-vector. Ouittrakul and Hertel (1969) used gravitational (1 g) and centripetal accelerations (greater than 1 g) to alter the rate of auxin (NAA) transport in maize coleoptiles. Ten g in the normal direction increased transport by 22%. Ten g in the inverse direction decreased transport by 37%.

Jones (1925) also questioned whether hyper- *g* could alter (suppress or reverse) growth regulator transport. He centrifuged seakale and reported reversal of polarity induced by what he considered a moderate centripetal force. Unfortunately, interpretation of his result is complicated since he seems to have made an error in calculating his experimentally applied centripetal accelerations. According to what he reported in his section on methods, Jones’ centrifuge applied a force well above what might be considered moderate.
CENTRIFUGES FOR PLANT GRAVITATIONAL BIOLOGY

Most botanical passengers on centrifuges have been higher plants but some studies have used fungi, e.g., Dennison (1961) studied tropistic responses of *Phycomyces* sporangiophores to centripetal accelerations, and Kawasaki et al. (1990) studied effects of hypergravity (and clinorotation) on different stages of the life cycle of *Dictyostelium discoideum*.

In one perhaps unique study (Chance and Smith, 1946), tropistic responses of plants to centripetal and gravitational accelerations were balanced against photore sponses, so one might calculate the equivalence of g-units per photon as measured by a population of buckwheat plants.

CAUSE OF DEVELOPMENT OF COMPRESSION WOOD

Beginning in the early part of this century, centrifuges were used to determine whether gravity is the principal or even the exclusive cause of the development of reaction wood (compression wood, red wood). Perhaps the most recent such study was the 591-day test by Scott and Preston (1955) in which they rotated potted 4-year-old white pine trees on a horizontal table at a rate that produced "a centrifugal force approximating gravity." The evidence is strong, but not conclusive, that gravity is the exclusive cause for the formation of reaction wood. A definitive test could be made on Space Station Freedom where seedlings of woody plants could be maintained at unit g, at zero g, and at other g levels for times long enough to achieve convincing results. (The maximal flight duration for the space shuttle is borderline for such experiments.)

APPLICATIONS OF THE BIAXRO TECHNIQUE

The BIAXRO procedure was used by J. Finn and O.D.R. Brown (1961), who probably were the originators of this combination of clinostat and centrifuge, which they developed at the Laboratories of North American Aviation, Inc., Downey, California. They wanted to simulate on Earth protracted accelerations in the hypogravity realm (zero to 1 g). They measured the g-function of what they termed "petiole epinasty" as ground-based information to support a Biosatellite experiment. Sachs (1882a, 1882b) had reported that the liminal angle of secondary roots was decreased by centrifugation when the application of centripetal acceleration was in the direction coincident with the plant axis. It also was known that clinorotation would increase the liminal angle. To measure this quantitatively was to be the object of an experiment on Biosatellites I and II. Unfortunately, the intramural report of Finn's work no longer is retrievable. Only a brief abstract was published. Finn's data survive only as an undergraduate textbook entry—Salisbury and Ross (1969).

Finn and Brown explored the simulated hypogravity range of centripetal accelerations and found that the greatest change of petiole angle (compared with the angle of unit g) was at simulated zero g. As they increased g incrementally from zero toward simulated unit g, the mean petiole angle changed progressively toward its normal value. From that result they predicted that in weightlessness the same extreme change of petiole angle would be observed (a prediction that would be confirmed qualitatively but not quantitatively).

Taken together, the work of Finn and the subsequent flight test by Johnson and Tibbitts (1968; 1971) constituted the first attempt to evaluate the validity of clinostat simulation of any hypogravity condition (in this case, weightlessness) by making the same kind of measurements first with centrifuge/clinostats on Earth and then during spaceflight.

The BIAXRO (centrifuge/clinostat) technique, in which gravity-compensated plants are centrifuged in the direction of their longitudinal axes, has been exploited in other laboratories—usually by investigators who probably invented the technique independently.

Gordon and Shen-Miller (1966; 1971) and Shen-Miller et al. (1968) centrifuged clinostated *Avena* coleoptile sections with centripetal accelerations acting in line with the long axis of the sections that were paired: one set with an "apex out," the other with an "apex in" orientation. The rate of elongation was measured. Their principal objective was not to study the mechanism of transport (although that may have been important to explain their results); it was to determine whether the coleoptile sections were capable of detecting the applied acceleration regardless of mechanism. When both sets exhibited the same elongation rate, that was taken as evidence that the growth process (almost all due to cell enlargement) was not responsive to whatever acceleration had been applied by the centrifuge over several days of growth. But when the "apex in" sections (exposed to a controlled g-stimulus in the natural direction) grew significantly longer than did the "apex out" sections, that demonstrated detection of the force. This was an early attempt to establish a g-threshold using the BIAXRO method for simulating hypogravity conditions. The axially directed test forces ranged from several orders of magnitude below unit-g to above unit-g. Test results (Figure 7) showed that the coleoptiles were responsive to the direction of application of the g stimulations, which may have been an indication of a direct effect on the polarity of auxin transport.

Early in the present century Knight's apparatus had become generally well known as "Knight's Wheel," and when James Small (1939) used a motor-driven centrifuge with four arms to carry out experiments similar to those of Knight, he described his device not as a homemade centrifuge but as "a new piece of apparatus known as Small's Knight's Wheel"—a name that was used chiefly by Small himself. Small used centrifugation much as
Knight had done. Small was concerned with the same scientific question but came to a different conclusion—viz., “The response to gravity is not necessarily identical with that to centrifugal force... The direction taken by the root is definitely not the direction of the diagonal of the parallelogram of forces constructed with gravity and centrifugal force as the components.” Small’s statements must stand on their own merits. His methods and results were well documented, and we have no explanation for why his experimental data supported a conclusion that now seems patently incorrect.

Some of the most careful (and tedious) measurements of the g-threshold for a gravitropic response were those of Gordon and Shen-Miller (1966; 1971) and Shen-Miller et al. (1968). One of their versions of BIAKRO is shown in figure 8. Although their results were reported to three significant figures (statistically well supported), they are usually cited conservatively only to one order of magnitude. *Avena* roots exhibited a threshold at about $10^{-4}$ g and shoots at about $10^{-3}$ g. Table II lists the threshold values that were reported and, as an interesting comparison, an approximate g-threshold for trained, male human subjects. Although the threshold was slightly lower for the oat plant, we chauvinistic plant scientists should not brag too loudly because the hominids took only seconds to detect their threshold exposure (threshold dose was 0.023 g-sec), whereas oat plants required days to accomplish the equivalent recognition of their threshold g-stimulus (dose between 5 and 6 g-min).

Table II. G-Thresholds for Least Detectable Response.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ORGAN</th>
<th>ACCELERATION THRESHOLD (g-units)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena sativa</em></td>
<td>root</td>
<td>$1.3 \times 10^{-4}$ g</td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>shoot</td>
<td>$1.4 \times 10^{-3}$ g</td>
</tr>
<tr>
<td>Hominids$^a$</td>
<td></td>
<td>$1.5 \times 10^{-4}$ g</td>
</tr>
</tbody>
</table>

$^a$Data of Jones and Young (1978)

**PLANT MEMORY**

How does the plant *remember* a g-stimulus? There is no doubt that it does, because any response that is dependent on the duration of application of the exciting factor has to involve information storage in some form. Moreover, the plant can perform simple arithmetic—addition, for example—on its stored information. A few experimenters have shown that when plants had been tropistically stimulated intermittently by several doses (1 $g \times t$) of the test signal the magnitude of their responses showed that their responses to the stimulations were fully additive, if the interval between stimulations was some tens of seconds, but extending the interval to hours caused additivity to fail completely. We may guess that this demonstrates another capacity of the plant—it can forget.

Some (probably most) investigators have assumed that a “Reciprocity Rule” should prevail, perhaps only because it seems reasonable that the plant would perceive a gravitropic stimulus as a g-dose (the *product* of the g-force and the duration of its application). If the force is made somewhat less and its application time is made somewhat more, the response could be the same (Günther-Massias, 1928).

Hiley (1923) expected that reciprocity would prevail; however, he was not able to demonstrate it experimentally. He wrote, “It is not easy to understand why, though a radicle grows straight under the influence of alternating stimuli of 10 mg for 1 min and 1 mg for 10 min in opposite directions, it does not grow straight under alternating and opposite stimuli of 10 mg for 30 min; yet such is...
undoubtedly the case.’’ If we include the concept that over longer intervals the plant ‘‘disremembers’’ some of its g-dose information, failure of reciprocity is not difficult to understand.

Experiments of the kind accomplished by Hiley and others (e.g., Günther-Massias, 1928, and Shen-Miller, 1970) could lead to a measurement of the rate of plant memory loss, and for that purpose a centrifuge is essential. Most experimenters who reported evidence of reciprocity did not focus on a precise determination of the range of g × t values over which the reciprocity rule applies. Beyond the limit in either direction (too large a g and too small a t or too small a g and too large a t) reciprocity failure occurred.

Plant responses to intermittent g-stimulations could become the basis for a series of tests in space to establish a plant’s forgetting function by relating some measure of survival of stored information to the interval between g × t doses, where both g and t and also the interval between doses would be made experimental variables. The limits of the range over which reciprocity occurs and the shape of the curve that may describe the course of the plant’s memory loss are likely to be very important for testing theories that will purport to explain these phenomena.

In one case, the plant’s ability to forget was the basis of a critical experiment involving the use of a centrifuge. To learn the kinematics of forgetting, the BIAKRO combination of centrifuge and clinostat was used to change abruptly sunflower seedlings’ environmental g information from 1 g to simulated zero g (see Figure 4). This was done to test a theory of the mechanism of circum- nutation (Israelsson and Jönsson, 1967) from which one could predict the kinematics of such a g-dependent transition. The expectation, based on the theory, was that nutational parameters would be altered and that transition between the two conditions would be completed within one period of nutational oscillation (ca. 114 min). However, the BIAKRO experiments showed (Figure 9) that the transition required ca. 1320 min, a time quite incompatible with theoretical expectation (Brown and Chapman, 1988).

At the time of publication those results were among the strongest bits of evidence against the exclusive role for gravity in driving and regulating parameters of circum- nutation. The BIAKRO measurements were made prior to a more definitive test of the theory, which was accomplished in space on the first Spacelab mission in 1983 (Brown et al., 1990). In that Spacelab experiment centrifuges also were used but only to provide 1 g during seedling growth so that, at the time of testing in μg, we could expect the plants to have been growing as uniformly straight as were test subjects that had been measured on Earth. The principal scientific question was not ‘‘How do seedlings’ nutational behaviors differ between a 1 g and a μg environment?’’; it was ‘‘Do seedlings nutate at all without guidance from significant environmental

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Time course of change in amplitude of circum- nutation in simulated unit g and simulated zero g. To left of broken line clinostatted plants were centrifuged to provide axial force of 1 g. After centrifuge was stopped (117 hr), plants continued to rotate on clinostats to simulate zero g. Error bars represent ± one standard error. (Reproduced with permission from American Journal of Botany, Vol. 75(8), page 1249, 1988.)}
\end{figure}

\textit{g information?}’’ The seedlings did nutate, which confirmed the need for modifying the theory of mechanism of circumnutation. Modifications have been suggested (Brown, 1991).

The plant not only can add but it also must be able to subtract from its memory since that is the basis for gravity compensation by clinorotation (at least in the case of the slowly rotating clinostat). Only a few papers have described plant responses to intermittent stimulations that can be adduced to describe properties of plant memory. Their authors were able to demonstrate convincingly that the phenomenon was real, but the kinetic measurements have not been sufficiently extensive to test critically any theory that might explain the mechanism of these intriguing examples of phyto-arithmetic. If more extensive experiments could be performed in the absence of complications from Earth’s gravitation, the results should be less ambiguous. This is an example of another not-yet-seized opportunity for measuring the plant’s responses to g-stimulations during spaceflight. It will not be a simple experiment because it will require numerous g-stimulation episodes extending over a wide range of stimulus intensities in combination with a wide range of stimulus

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durations for which a considerable amount of crew time may be needed (n.b., Crew time in space is a severely limited and precious resource). Some measurements of this kind were attempted during the IML-1 Space Shuttle mission in January, 1992.

PLANT SUSCESSION

Probably the least understood aspect of the plant’s g-response is what occurs very early in the stimulus/response sequence. This must begin as a physical process which is called susception. The susception of a stimulus must cause some kind of alteration of the plant’s macro- or micromorphology. Whatever the alteration, that must lead to subsequent changes at the biochemical level—changes that involve growth regulators and also their inhibitors and their transport mechanisms, about which we now have much empirical information, some would say more than we are smart enough to know what to do with. The fundamental details of how susception leads to perception remain elusive. Centrifugation, no doubt, will continue to play a major role both on Earth and in space in future efforts to probe what now seems to be one of nature’s best kept secrets: How susception of the physical stimulus is perceived by the plant.

CENTRIFUGATION FACILITIES IN SPACE

Long before NASA began its program of biological research in near-Earth orbit, potential experimenters were frustrated by lack of adequate planning to provide for centrifugation in space vehicles even though there was no lack of scientific questions that could be answered only in space. Three decades ago the most needed kind of experiment support equipment was an assortment of centrifugation facilities available for use on orbit (and, in “control” experiments, on Earth). Fortunately, that deficiency is being corrected.

It seems evident that future biological research on plants in space will require several different kinds of centrifugation facilities. No single design will be able to accommodate all types of experiments. Some small centrifuges have been flown (or are awaiting flight opportunities) with payload compartments ranging from about a deciliter to over a liter and rotor diameters of 15 to 60 cm. These were sized either to fit Spacelab racks or to fit Shuttle middeck lockers or to be carried in Spacehab.

Figure 10 illustrates one example, the Gravitational Plant Physiology Facility (GPPF), which was flown in Spacelab on the IML-1 Shuttle mission in January 1992. It contains four small, variable-g centrifuges which were used to implement two different spaceflight experiments. After GPPF was retrieved following completion of IML-1, it has been maintained under NASA monitoring and in compliance with NASA’s quality control procedures. Therefore, it is flight qualifyable and available for use by future investigators for various kinds of experiments on later missions.

Centrifuges are also available to fit the European Space Agency’s (ESA) Biorack. Two of them were flown first in Spacelab on STS 61-A, the D1 mission. These centrifuges are fixed-speed (1 g) and they were designed specifically only to provide 1 g in space for a number of different kinds of experiments so that, in the same mission, results of tests at zero g and at unit g can be compared. The payload compartments, called Type I containers, have 65 ml capacity. When accommodated in Biorack, centrifuges of this design can be flown on future Spacelab missions.

Another compact centrifugation facility called Variable Speed Middeck Locker Centrifuges (VSMDC), which is now in late development stage, was designed to fit a double locker in the Shuttle middeck. Fully self supporting (internal temperature control includes heating or cooling capability), it contains two centrifuges that can be programmed to operate independently over a range from $10^{-3}$ g to 1.10 g in up to 256 steps. Within that range software adjustments suitable for most uses conveniently limit the attainable stimulus accelerations to 0.05 g steps. Each payload compartment on either centrifuge can be viewed by one of two infrared (IR) video cameras, once each turn of the rotor. If required, images can be recorded while the rotor is running full speed or
Figure 11. Exploded view of Variable Speed Middeck Centrifuge (VSMDC) designed to fit standard double middeck locker of NASA Space Shuttle. Rotors operate independently. Specimens rotated in Specimen Container (SC) can receive accelerations in the range 0.0001 to 1.3 g. Minimal usable radius of SC = 95 mm; maximal = 190 mm. Rectangular volume of SC = ca. 90 × 90 × 85 mm³. Temperature control: 18 ± 37°C ± 0.2°C. Cameras (IR) monitor SCs on both rotors. Video data is stored on disks. Slip rings (16 on each rotor) enable electrical connections to any of 12 SCs. Separate rotor speeds and durations are programmable by Key Pad and LCD Display.

for viewing in time-lapse mode, the rotors run at 1 rev per 3 min (max acceleration = 3 × 10⁻⁵ g). A rectangular object 90 × 90 × 85 mm³ (ca. 650 ml) could fit within each of the six payload compartments on either centrifuge. See Figure 11.

Looking toward the more distant future, a larger centrifuge (diameter ca. 2 metres) is being designed for use on Space Station Freedom, which may become operational around the year 2000.

Each of these rotating machines was designed with the goal in mind of creating a versatile research tool with the widest possible capabilities. It is very important to note that in each case the critical specifications relevant to potential experimentation were supplied by working biologists, not by spacecraft engineers. We are, if not approaching, at least moving in the right direction toward a situation in which lack of centrifugation facilities no longer will be a bottleneck for accomplishing experiments in space at any desired g-level.

THE EARTH AS A CENTRIFUGE

We mentioned that we would consider a fourth kind of rotating machine, the Earth itself. Climbing a mountain, taking a plane trip, or riding the NASA Space Shuttle in orbit increases a person’s distance from the Earth’s center of mass; therefore, it affects (only by a small
amount) the weight (not the mass) of the subject. (Of course this is a very reliable and fully reversible weight reduction program.) Aside from that there is another (also reversible) weight effect caused specifically by the Earth’s rotation. Our planet is a slowly rotating machine of impressive dimensions. At the equator the Earth is moving toward tomorrow at a speed of just over a thousand mph. Gravity restrains surface objects from drifting off into space, which makes the Earth a centrifuge. The maximal centripetal acceleration (proportional to the Earth’s radius of rotation) is experienced at the equator, and it decreases to zero at either North or South Pole, as shown in Figure 12. The effect is small; its maximum is about $3.4 \times 10^{-3} \, g$.

Fortunately for us the Earth is not spinning very much faster because, if our 24-hour days could be reduced to about 1 hr 20 min, the equatorial region would be a dangerous place. The inhabitants of Nairobi would be weightless and probably would no longer respond to telephone calls. Then, if the spin rate were to increase still more, the only valuable real estate on Earth would be in the higher latitudes. However, there is no cause for alarm; our terrestrial centrifuge is slowing down and there seems to be no credible mechanism that would predict a reversal of that trend.

REFERENCES


