Gravitropism of plants is the most conspicuous response to the gravitational force and is essential for proper orientation of seedlings and stable balance of massive plants. Early detection of gravity ensures connection to important resources for an emerging seedling and therefore is detected before the primary root emerges (Ma and Hasenstein, 2006). In trees, proper gravity alignment requires suitable mass distribution, which affects branching patterns and canopy formation. These observations indicate that gravity sensing in plants is not a short-lived ability but vital throughout the entire life. Formal investigations of gravity effects started in the 18th century (Dodart, 1703) and were continued by Knight (1806), Ciesielski (1872), von Sachs (1879), and Charles and Francis Darwin (Darwin and Darwin, 1880).

Early studies on the response mechanism of plants postulated moving masses as gravity sensors (Haberlandt, 1903; Némec, 1901). Later studies demonstrated that not only dense statoliths, which in plants are typically starch-filled amyloplasts, but also protein bodies, at least in fungi, sediment (Eibel et al., 2000) and thus serve as gravisensors. Gravisensing is also achieved by buoyancy of material less dense than the cytoplasm such as lipid droplets (Grogli et al., 2006). Regardless of specific sensors, early events in gravitropism depend on mass interactions (Kiss, 2000).

The desire to investigate and manipulate the gravity receptor led to the development of ostensible gravi-compensators, also known as clinostats. The principle of clinostats is to rotate an experimental system such that the gravity vector constantly changes before a response by the tested organism becomes established. For most plants, the initiation of the response requires more than a few minutes; therefore, the typical angular velocity is between 1 and 2 revolutions per minute (~0.1–0.2 rad·s⁻¹). A strictly one-dimensional rotation results in epinasty (Palmer, 1973; Ursin and Bradford, 1989) and led to the development of the three-dimensional clinostat (Hasenstein et al., 1996) or random-positioning machines (RPM) (van Loon, 2007). Although the RPM mimics weightlessness remarkably well for some parameters (Kraft et al., 2000), mechanical devices induce unwanted side effects (Brown et al., 1976; John and Hasenstein, 2011).

The complexity of mechanical stimulation led to the application of a fundamentally different principle for studies of gravitational effects. This approach is based on the weak diamagnetic properties of biological material and was first studied by Audus (Audus, 1960; Audus and Whish, 1964) who named the response “magnetotropism” despite the fact that the magnetic force affects the same particles that respond to reorientation in the gravitational field. These studies are based on the equivalency of the (repelling) magnetic force that acts on diamagnetic bodies, which comprises essentially all biological material. The magnetic effect depends on a gradient that is described by the dynamic factor \( H_{\text{d}} \), where \( H \) is the magnetizing field intensity. The magnetic force depends on the difference of the magnetic susceptibilities between a particle and its surrounding medium just like weight depends on the difference of density between particle and medium. Therefore, a properly designed magnetic gradient can substitute for gravity.

Similar to Audus’ studies, an arrangement of a magnetic gradient perpendicular to gravity causes root curvature away from the denser zones of gradient (Kuznetsov and Hasenstein, 1996), and toward that zone in coleoptiles and inflorescences (Kuznetsov and Hasenstein, 1997; Weise et al., 2000). Based on the direction of the magnetic field gradient, the net force can act with or against gravity or at any desirable angle. Electromagnets can be stronger than permanent magnets and in vertically oriented bores
the gradient is directed parallel and antiparallel to the gravity vector (Geim, 1998; Pelrine, 2004). This configuration with appropriate magnetic fields (>16 T) and gradients (typically ~10^{10} Oe/cm) produces a setup where a diamagnetic body is levitated at the top equivalent point (i.e., the repelling force is equal to gravity and a body is levitating, i.e., experiences the equivalent of weightlessness). The center of the magnetic system has the highest magnetic field but no gradient and thus serves as control for magnetic effects. The bottom equivalent point generates 2 g, the sum of the normal gravity force and the added force obtained from the magnetic gradient. In addition to these specific points, other conditions can be selected to obtain gravity equivalents that correspond to e.g., Moon’s or Mars’ gravitational force, 0.17 and 0.39 g, respectively (Valles et al., 2005). Such configurations of magnetic gradients have been used in several studies (Guevorkian and Valles, 2004; Paul et al., 2006; Coleman et al., 2007).

Plant gravity sensing depends on starch-filled amyloplasts, but the precise mode of action is unclear and may include displacement (removal from original site), sedimentation (encounter with the new lower side of a statocyte wall), or continuous stimulation of the proximate wall (Hasenstein, 2009; Ma and Hasenstein, 2007). Magnetic gradients are therefore an important tool to study localized mass interactions independent of gravity effects on the entire organism. In addition, magnetic gradients have the potential to provide a gravity-like force to seedlings that grow in weightlessness, e.g., in orbit, or long-term space missions or other, reduced gravity environments, and therefore provide an inexpensive method to establish proper orientation of the primary root and developing shoot.

The following experiments describe attempts to characterize the forces generated by a magnetic system that should be sufficient to provide a directional stimulus (i.e., induce curvature) under weightlessness conditions. The data show that dependent on the distance, the magnetic gradient affects starch particles similar to gravity.

MATERIALS AND METHODS

Magnetic system—Two NdFeB magnets (50 × 50 × 12 mm, with a minimum magnetization of 11300 Oe, Magnet Sales & Manufacturing Culver City, California, USA) were mounted parallel to each other with a gap of 12 mm filled with acrylic plastic (Fig. 1). The acrylic material contained a groove (60°, equilateral) to accommodate a ferromagnetic wedge. Three differently shaped wedges were used. Type 1 had a triangular cross section (5.5 × 5.5 × 5.5 mm sides).

Fig. 1. Magnetic set-up used to measure displacement of starch grains under the influence of a high-gradient magnetic field. Two magnets (magnetized as indicated by N and S) are separated and surrounded by acrylic plastic. The center gap contained a groove to accommodate a ferromagnetic steel wedge, which distorts the magnetic field lines as shown. The shaded area was observed with a videomicroscope. The cuvette (white vertical zone) contained a starch suspension. Between parabolas, the system was inverted to redistribute the starch.

Fig. 2. The cross-sectional profile of the wedges used to generate a high-gradient magnetic field. Type two and three were approximated by the following functions, respectively: \( Y = 6.361 - \sqrt{4.8167x^2 - 26.492x + 40.461} \); \( Y = 6.0262 - \sqrt{4.24004x^2 - 23.3538x + 36.31538} \) for \( x = 0 \) to 5.5 mm, where \( x \) is the horizontal distance and \( y \) is the height (mm).
between particles and wedge were measured relative to the wedge tip using the program ImageJ (v. 1.46, National Institutes of Health, Bethesda, Maryland, USA) after correction for nonsquare pixel size (aspect ratio = 1.1). Distance calculations and all subsequent evaluations were done in Excel (Microsoft Office 2010). The average velocities for each second interval were plotted against the initial distance of each grain from the wedge tip. The data distribution was fitted to an exponential equation of the type $D = a \exp(-bx)$ using generalized reduced gradient algorithm (Solver plug-in, Excel). The use of this equation is based on the examined magnetic force being a function of $H^2/2$, a better fit than linear regression, using only two coefficients, and that all calculated values are positive.

**RESULTS**

**Sedimentation of starch grains in the gravitational field**—The three types of starch grains sedimented at different velocities (Table 1). Corn starch sedimented at two thirds the velocity of potato and wheat starch. This sedimentation difference is most likely the result of different density, surface, size and shape characteristics (Fig. 4). In contrast, the different size distributions for wheat starch did not produce a bimodal sedimentation velocity. These observations are indicative of variable starch composition. Corn starch is less spherical than wheat or potato and some particles appear heavily dimpled, possibly leading to greater drag or reduced sedimentation. However, the concentration of particles was low (1 mg/mL) such that particle interaction was minimal and particles generally sedimented uniformly. Importantly, the average size (Fig. 4) of the starch grains was considerably larger than generic amyloplasts (2–8 µm; Hinchman and Gordon, 1974) and corresponds with the variability in the movement of particles (Table 2), probably as a result of different density, cross section, and shapes.

**Movement under weightlessness**—Weightlessness was achieved when the sedimentation movement of starch particles stopped. The insertion of a wedge (induction of HGMF) caused

| Table 1. Sedimentation rates of starch grains under 1 g conditions in aqueous 1% NaN\textsubscript{3}. |
|---|---|
| Starch | Mean velocity ± SE (µm·s\textsuperscript{-1}) |
| Corn | 65.21 ± 1.9 |
| Wheat | 97.59 ± 1.6 |
| Potato | 95.39 ± 2.9 |

Sigma, St. Louis, Missouri, USA), wheat (Sigma S-5127), or potato (Sigma S-2630) at 1 mg/mL in 1% (w/v) NaN\textsubscript{3} and sealed with epoxy. The cuvettes were stored at 4°C until use.

**Experiment**—The experimental set-up consisted of the magnetic device (Fig. 1), a videomicroscope, and a recording device. The microscope used a 10× objective mounted to a Proximity series Infini Tube (Edmund Optics, Barrington, New Jersey, USA) that in turn was connected to a video camera (Panasonic WV-BP310) and a 14 cm monitor. The signal was recorded on a Sony DCR-TRV20 digital camera. The system was secured to the floor of NASA’s C9 aircraft but was free-floating during the weightlessness phase of flight parabolas. Each parabola resulted in about 20 s of free-fall (weightlessness). As the starch grains stabilized, a wedge was inserted, and the movement of the starch particles was recorded (Fig. 3). Of 160 parabolas that were flown, 81 produced useful data. Sedimentation of the starch grains under 1 g conditions was measured using the same suspension-filled cuvettes that were used for the flight experiment in the same device but without inserted wedge.

**Data evaluation**—Sequences from the videorecordings were selected and individual frames (720 × 540 pixels) were saved at 1-s intervals. Distances...
the starch particles to move away from the wedge (shown in video sequences as horizontal displacement). The movement in the presence of a HGMF depended on the type of starch, distance from the wedge, and the magnetic gradient (wedge shape, Table 2). Contrary to parallel sedimentation of grains, the magnetic gradient resulted in expanding trajectories (Fig. 5). The movement of individual particles varied because of interactions with the cuvette surface, clumping of particles, and perturbations (plane jitters) during the weightlessness phase. Despite these complications, the measurements successfully demonstrated that the

### Table 2. Response of starch grains from three plants in the presence of a high-gradient magnetic field during the weightlessness phase of parabolic flights.

The variables (averages ± SE as percentage of their nominal value) include starch and wedge and describe the velocity $v$ of grain movement in $\mu$m·s$^{-1}$ as a function of distance $d$ from the wedge in $\mu$m (compare with Fig. 5) according to $v = a \exp(bd)$.

<table>
<thead>
<tr>
<th>Wedge</th>
<th>Starch</th>
<th>$a$ ± SE (%)</th>
<th>$b$ ± SE (%)</th>
<th>$g$-equivalent *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>corn</td>
<td>26.57 ± 3.4</td>
<td>-0.0026 ± 2.3</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>wheat</td>
<td>79.77 ± 5.1</td>
<td>-0.0035 ± 4.1</td>
<td>1.22</td>
</tr>
<tr>
<td>1</td>
<td>corn</td>
<td>12.20 ± 2.6</td>
<td>-0.0034 ± 2.1</td>
<td>0.13</td>
</tr>
<tr>
<td>2</td>
<td>wheat</td>
<td>22.47 ± 2.0</td>
<td>-0.0019 ± 2.2</td>
<td>0.24</td>
</tr>
<tr>
<td>1</td>
<td>potato</td>
<td>21.38 ± 5.1</td>
<td>-0.0013 ± 3.5</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>potato</td>
<td>12.32 ± 3.9</td>
<td>-0.0033 ± 2.1</td>
<td>0.13</td>
</tr>
<tr>
<td>1, 2, 3</td>
<td>corn</td>
<td>11.27 ± 4.5</td>
<td>-0.0012 ± 4.6</td>
<td>0.12</td>
</tr>
<tr>
<td>1, 2, 3</td>
<td>wheat</td>
<td>33.78 ± 1.0</td>
<td>-0.0028 ± 0.5</td>
<td>0.35</td>
</tr>
<tr>
<td>1, 2, 3</td>
<td>potato</td>
<td>14.84 ± 1.0</td>
<td>-0.0021 ± 0.8</td>
<td>0.16</td>
</tr>
<tr>
<td>1</td>
<td>corn, wheat, potato</td>
<td>38.83 ± 1.0</td>
<td>-0.0024 ± 0.7</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>corn, wheat, potato</td>
<td>19.22 ± 1.3</td>
<td>-0.0028 ± 0.8</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>corn, wheat, potato</td>
<td>16.91 ± 0.6</td>
<td>-0.0021 ± 0.5</td>
<td>0.20</td>
</tr>
<tr>
<td>1, 2, 3</td>
<td>corn, wheat, potato</td>
<td>24.76 ± 0.2</td>
<td>-0.0024 ± 0.1</td>
<td>0.29</td>
</tr>
</tbody>
</table>

* Calculated by dividing maximal velocity ($a$) by sedimentation velocities (Table 1).

Fig. 5. Representative plot of corn starch particle movement during weightlessness in the presence of a high-gradient magnetic field (HGMF) as a result of inserting two types of wedges between two permanent NdFeB magnets. The position of the wedge tip is indicated by a circle (wedge type 1) or cross (type 2). Each sequence of points (* for wedge 1, + for wedge 2) was measured from individual frames. The distributions illustrate that grains in closer proximity to the wedge moved greater distances than more distant particles. The trajectories for particles exposed to HGMF based on wedge shape two (dashed lines) are more focused than for wedge one (solid lines). The standard deviation of the trajectory slopes (18 each) was 0.209 for wedge type 2 compared to 0.414 for wedge type 1.
type of starch and wedge shape affected particle movement (Table 2) and followed an exponential pattern (Fig. 6).

Using the sedimentation rate of the three starch varieties as 1 g equivalent, the experiments showed that, as expected, the sharp wedge (1) exerted the strongest force and generated about 0.6 g equivalents (Table 2). Wedges 2 and 3 generated substantially lower force but retained a more focused movement pattern and therefore better directionality (Fig. 5). Despite the less than 1 g force equivalent at the observation distance, the HGMF was able to move/repel starch grains reliably.

**DISCUSSION**

The displacement of the starch grains in water is comparable to the environment in a cell. The most important contributor to starch particle movement is the force acting upon them and the viscosity of the medium. Previous viscosity measurements have shown that cellular viscosity is anisotropic and, in the extreme case, up to 100-fold higher than water (Scherp and Hasenstein, 2007). Although the cytoplasmic viscosity for most statocytes is unknown, elevated viscosity should only delay, but not eliminate, gravitropic reaction based on amyloplast sedimentation, because viscous drag reduces velocity but does not prevent statolith movement. However, if gravitropic susceptibility depends on the frequency of statolith interaction with the cell wall or susceptible membranes, higher viscosity will reduce the gravitropic response, because of fewer interactions.

The data showed that particle movement was not uniform, and some particles moved at velocities greater than the sedimentation velocity. These different velocities may stem from changes in the crystalline structure of the grains, which translates into different magnetic susceptibilities (Kuznetsov et al., 2001). A further complication stems from the lack of access of the zone in close proximity to the magnetic wedge (see Fig. 3).

![Graph](image)

**Fig. 6.** Distribution of the velocity of individual grains (from Fig. 5, wedge 1) relative to the initial distance $d$ (in $\mu$m). Each circle represents the average velocity $v$ of all digitized frames ($N = 13$); $v$ depends on the distance from the wedge and is described by the function $v = 9.6525 \times \exp(-0.0022d)$, shown as solid line.

Data from this zone would render more precise estimates for the greatest velocity values. Thus, a conservative estimate of the HGMF-generated force is about 0.6 g.

Despite the variability of the estimated force, the value is well above the estimated sensitivity threshold of about 0.001 g (Shen-Miller et al., 1968) but is likely to produce a response with slower kinetics than reorientation in a 1 g environment. An additional complication stems from the fact that the magnetic gradient decreases rapidly. Based on the determined parameters (Table 2), a 1/100 g level stimulation (10-fold higher than the sensitivity threshold) extends to about 2 mm from the wedge. While this distance is sufficient if the positioning between root or shoot and HGMF is carefully controlled, routine applications of HGMF in, e.g., spacecraft or other low gravity environments, most likely require either stronger magnets or a better design of the gradient. For example, supplementing the wedge with a concavity on the opposite pole (Fig. 7) can alleviate this constraint and provide a greater effective range of the HGMF.

A combination of convex and concave poles would generate gradients that extend the effective range for amyloplast/statolith movements and roots or shoot curvature and provides a more homogeneous force field.

The properties of HGMF are uniquely suited to distinguish whether a constant stimulus is necessary for the generation of curvature (graviresponse) or whether a short-time stimulation (a pulse) is sufficient to induce curvature. Recent calculations suggest that roots stop producing a hormonal gradient as soon as the curvature reaches about 40% of the full response (Band et al., 2012). If HGMF generate a force that is sensed for the equivalent time, then a pulse-based model would be sufficient to explain gravitropism.

Another advantage of HGMFs is the ability to exert forces to small areas (Weise et al., 2000). The small spatial effectiveness is ideally suited to examine various regions of plants as to their ability to induce curvature rather than being just sensitive to gravity (Wolverton et al., 2002a, b). Most likely, a combination of a stimulus and the proximity of responsive tissue is required to induce differential curvature. This concept is supported by observations that instead of curvature being confined to the typical root elongation zone, roots of flax and brassica (and presumably other species) curve at the root–shoot junction if this region elongates substantially.

In addition to applying HGMF to gravitropism studies, the general problem of magnetoperception is a fascinating but rarely studied topic. Other than magnetotaxis in bacteria, which respond to a magnetic torque, the underlying principles of magnetoperception are not understood (Galland and Pazur, 2005; Pazur et al., 2007). The lack of a suitable and genetically accessible organism is hindering progress, but theoretical concepts are emerging. It is possible that magnetic fields alter metabolism or affect other sensory systems. Biological magnetoperception is linked to the ability to sense magnetic torque or dipole moment (similar to a compass needle). Such perception of magnetic fields is essential for magneto orientation and has been described for birds (Scherbakov and Winklhofer, 1999; Witschko et al., 2002; Heyers et al., 2010), fish (Eder et al., 2012) and insects (Wajnberg et al., 2010). These effects are different from the magnetic conditions required for ponderomotive forces and are more challenging to characterize. However, carefully designed magnetic conditions may help understand generic magnetic effects and magnetoperception. The movement of intracellular plastids (statoliths) relative to the immobile, but also diamagnetic tissue that contains these plastids is unique to the
HGMFs. Their effectiveness in causing curvature of roots and shoots in opposite directions (Kuznetsov and Hasenstein, 1996; Hasenstein and Kuznetsov, 1999) indicates that basic gravitropic response mechanisms remain intact.

It is important to point out that despite their seemingly static nature magnetic fields have profound effects that transcend the ponderomotive properties of a magnetic gradient. Although experiments showed that levitated insects and frogs were unaffected by their levitation experience (Geim, 1998; Geim et al., 1999; Simon and Geim, 2000), gene expression is significantly altered after exposure to HGMF (Paul et al., 2006). To date, it is unknown if these changes in gene expression affect curvature, but it is likely that the network-like nature of the gravireceptor (Niakamura et al., 2011; Rodrigo et al., 2011) is affected by magnetic and electric fields (Ishikawa and Evans, 1990; Stenz and Weisenseel, 1991; Wolverton et al., 2000).

Despite the intriguing properties of HGMF for biological systems, especially plant gravitropism, neither unidirectional fields, magnetic levitation, nor clinorotation are flawless substitutes for weightlessness. The unique flexibility and sensitivity of biological systems is capable of distinguishing subtle differences in mechano- and gravistimulation. It should not be surprising that the complexity and thus flexibility of plant responses to gravity and gravity-like forces will pose many challenges for future scientists. A combined approach based on biology and physics seems essential for such progress to become reality.

LITERATURE CITED


