

Transgenic *Arabidopsis* Plants as the Subject of Magnetic Field Effects^{◇*}

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ABSTRACT

Problems like establishing a reliable food supply must be solved before humans can live in space. Specimens of *Arabidopsis thaliana* have been genetically engineered to contain a stress-response reporter gene system and are subjected to low homogeneous magnetic fields ranging from 0-9 Tesla. These plants contain the *alcohol dehydrogenase* promoter driven GUS gene, which produces the GUS enzyme under stressful environments such as cold temperatures, gravity changes, and hypoxia. A chemical reaction associated with the GUS enzyme results in a color change in stressed *Arabidopsis* tissue when incubated with a specific exogenous substrate. Through qualitative histochemical assays and quantitative fluorometric assays, we determine the level of GUS activity. We have found that the homogeneous magnetic fields of 0-9 T do not induce high levels of GUS activity in *Arabidopsis* until prolonged exposure.

In a separate but related experiment, we utilize mathematical modeling of the magnetization of *Arabidopsis* to estimate the gravitational forces that affect different parts of the plant (*i.e.* leaves/shoots, roots, whole) during magnetic levitation. Preliminary results show that forces acting on the plant sum to $\sim 6E-4$ Newtons. Future studies predict these models will help demonstrate that magnetic levitation can supply a long-term, low-g environment on Earth.

INTRODUCTION

The purpose of this paper is to report on the accomplishments of this summer's research and speculate about future studies. This summer we studied how various homogeneous magnetic fields affect genetically engineered plants to gather more knowledge about the space environment and its effect on plant metabolism. In addition, we attempted to simulate in a computer model the gravitational forces that act on different parts of the plant during magnetic levitation. The results from these

experiments support the hypothesis that exposure to homogeneous magnetic fields below 10 T for 2.5 h do not significantly stress plants and supports that magnetic levitation occurs in milligravity. These data provide more statistics for ongoing research in the field of biophysics and help in the preparation for experiments to be performed at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, Florida.

The human desire to colonize outer space has prompted experimental research in order to accomplish this goal. However, many problems such as the maintenance of life support or the production of reliable food supplies in a weightless environment exist. In the field of experimental biophysics, particularly space biology, many seek to develop plant nutrient delivery systems, optimized for space-flight application, that are critical for bio-regenerative life support systems that rely on the growth of higher plants for water purification, atmospheric conditioning, nutrient recycling, and food production (Porterfield, 1997).

Plants presumably rely on gravity to obtain nutrients and water through their roots. While the overall physiology of the root system on Earth lies mostly unsolved, studies suggest that the milligravity environment of space causes a disturbance in the movement of gases or liquids. This disruption may lead to zones of oxygen-depleted gases or liquids that induce hypoxia (oxygen deficiency) in the tissues of plants (Porterfield, 1997).

To study the effects of homogeneous magnetic fields on plants, researchers at the University of Florida (UF) use *Arabidopsis thaliana* (*Arabidopsis*) as a model (Meinke,

1998). A member of the mustard family, this simple angiosperm may grow in petri plates under fluorescent lights in the laboratory or in pots in a greenhouse (Meinke, 1998) and become mature enough for experiments after two weeks of planting. We use transgenic *Arabidopsis* containing the *alcohol dehydrogenase (Adh)* driven β -glucuronidase (GUS) gene to monitor the effects of low homogeneous magnetic fields on gene regulation of plant metabolism (Meisel, 1999). Under stressful environments like cold temperatures, gravity changes, and hypoxia, *Arabidopsis* produces the GUS enzyme, which reacts with X-Glucuronide (X-Gluc), an exogenous substrate, to produce a color change in the stressed plant tissue. After incubation with the X-Gluc, 70% ethanol leeches out the chlorophyll and other pigments in the plant tissue, leaving it white in color. A blue pigment, varying in intensity due to associated level of stress on the plant, indicates the presence of the GUS enzyme.

Besides the experimental aspect of this summer research, we pursued a related, theoretical approach to *Arabidopsis* during magnetic levitation to estimate separately the gravitational forces on the leaves and shoots of the plant. Since water (the primary constituent of plant tissue) is diamagnetic, *Arabidopsis* also behaves as a diamagnetic material.

Diamagnetic materials contain paired electrons of the opposite spin and no orbital currents. Both poles of a magnet repel diamagnetic substances even though the diamagnetic force of repulsion is very weak (Doherty, 1997). Diamagnetism occurs in all substances but its feeble effects are overshadowed in substances whose atoms have a net magnetic dipole moment (paramagnetic or ferromagnetic materials) (Halliday, 1960). A magnet near diamagnetic material will generate orbital electric currents in the atoms of the material,

and the magnetic fields associated with these orbital currents will be oriented so the approaching magnet repels them.

In the presence of a magnetic field, matter becomes magnetized, and we describe the state of magnetic polarization by the vector quantity Magnetization (M)= magnetic dipole moment per unit volume (Griffiths, 1999). H , known as the auxiliary field, deals with current created when material becomes magnetized, and is related to Magnetization by $M=\chi_m H$ where χ_m is the magnetic susceptibility, a dimensionless quantity that varies from one substance to another (negative for diamagnets) (Griffiths, 1999).

MATERIALS AND METHODS

The methodology we follow for the planting, harvesting, and analysis of *Arabidopsis* consists of various protocols for Space Biology provided by the Department of Horticultural Sciences at UF. We studied two different strains of *Arabidopsis*, one planted in regular test tubes, and the other in bigger, vertical centrifuge tubes and vertical plates. The seed strain for the regular tubes was originally used for NASA experiments and was adopted by the researchers at UF. The addition of the seed strain grown in vertical tubes and plates helps when reporting slighter stresses due to the environment since this strain is more sensitive to environmental changes than its regular tube counter part. Besides greater sensitivity, more of these smaller plants (ones used for the vertical plates and tubes) can fit into the bore for experiments; therefore, more results can be obtained easily. Since the plants are, to a certain extent, biohazards and are quite sensitive to their environments, we used sterile technique, proper fume-hood procedure, and safety precautions in the disposal of plant tissue.

First, we sterilized the seeds for the tubes and vertical plates using the method provided. All equipment such as the bio-transporters, tube racks, and plastic tubes were washed, bleached, and sterilized in the autoclave before use. Then, we made germination media and growth media and poured the plates and tubes. We planted the seeds for the regular tubes first. When the seeds germinated (about 6 days), we transplanted the seedlings to the bio-transporters where they continued growing on growth media. Next, after approximately 2 weeks, the plants in the regular tubes matured enough for experiments (shoots are tall but no sign of flowering has occurred). The plants on the vertical plates, however, needed only about 10 days to grow before the experiments. The plants on the vertical plates did not require transplantation, thus reducing the possibility for contamination.

We performed experiments on the Arabidopsis on the vertical plates first. However, procedures for the vertical plates and tubes were the same. Experiments lasted three days (totaling 6 days for all our samples), and each day, we collected certain plants for pre-experimental and post-experimental controls. Using a superconducting magnet, we subjected the plants on the plates to various homogeneous magnetic fields. Exposure time ranged from 2.5 h to 18 h, and the magnetic field ranged from 0-9 T.

We froze all plants used for quantitative analysis, whether they were subjected to magnetic fields or not, in the same manner, and logged their classifications (date, magnetic field, length of exposure). After extracting the plants from the agar growth medium, we separated the leaves from the roots. We quickly froze the plants in labeled microcentrifuge tubes using liquid nitrogen. We left other plants for qualitative analysis intact but placed them in plates filled with X-Gluc, which will

bind with GUS enzyme produced by the plant due to stress. After staining the plants, we used 70% ethanol to leech out chlorophyll and other pigments from the plants so a blue color of the GUS enzyme could easily be seen.

Finally, the frozen samples were placed in a -86°C freezer until they could be analyzed. Analysis consisted of weighing each frozen sample, grinding it, following the protocols for the 5-minute and 60-minute MUG assays, and finding the GUS activity in each sample using a protein spectrophotometer and a fluorometer. Previously, we flooded some plants in the vertical centrifuge tubes to produce definite stress on the plants as a control. We used the results of the biochemical assays on these plants to establish the induced level of GUS activity.

During the course of the program, we used a Superconducting Quantum Interference Device (SQUID) magnetometer to find the susceptibility of roots, leaves, agar medium, and distilled water. From these results, we then used the force equation (Geim, 1997) to approximate the forces on the plant during magnetic levitation using a simulation run on Maple mathematical software.

For the object to be floating in equilibrium, the total force $F(r)$ must vanish: $F(r) = -mge_z - (|\chi| * V * B(r) \nabla B(r)) / \mu_0 = 0$ (Geim, 1997)

The theoretical magnet used is located in the NHMFL laboratory in cell #5. From a set of data points that finds the magnetic field, field gradient, and homogeneity on axis with respect to the height, we derived an exponential equation to find the magnetic field gradient at any given point.

We also performed biochemical analysis on frozen samples that were collected before this summer when experiments were performed in Tallahassee,

Florida. These plants were exposed to homogeneous magnetic fields ranging from 0-25 T and magnetic field gradients. In addition, we photographed stained plants from this period and catalogued them in the computer.

RESULTS

Our findings mostly agree with previous experiments that suggest the Arabidopsis plants were differentially stressed by the high magnetic field environments (Meisel, 1999). As expected, since the superconducting magnet used at UF did not produce the high magnetic fields necessary to significantly stress the plants, results reported GUS activity, the ratio of GUS enzyme in nanomoles to the protein in the plant in micrograms per minute ($4\text{MU}/\mu\text{g protein/min}$), in the baseline level (below the induced GUS activity level established by the flooded plants). For example, the experimental results in Fig. 1 and Fig. 2 show that the GUS activity present in the leaves and roots after exposure to magnetic fields of 0-9 T for 2.5 h do not exceed 8 for the leaves and 20 for the roots for all the plants. These data suggest the plants can remain viable and feel little strain in these environments of homogeneous magnetic fields. The small variations of GUS activity found among the plants within each group (i.e. vertical plates, vertical tubes, and regular tubes) may be associated with slight fluctuating conditions that may affect individual samples. These factors can be air currents, the position of the plant with respect to light source, the stress associated with separating the leaves from the roots or the plant from the agar, and other considerations.

Furthermore, the research done this summer supplements results from prior research and provides more data points for the magnetic field range of 0-9 T. As

mentioned previously, the data collected this summer for the plants in vertical tubes, regular tubes, and vertical plates lie in the baseline of GUS activity and report negligible amounts of stress exhibited by the plant. Again, these data concur with the hypothesis that plants do not become stressed until subjected to high magnetic fields. Results for the experiments performed at the NHMFL lab indicate the induced GUS activity level occurs at fields higher than 15 T. Figures 3 and 4 display the results for all the magnetic fields and magnetic field gradients that have been analyzed. The student researcher did not help analyze the experiments performed in October 1999. In general, the roots appear to become more stressed than the leaves.

In addition, Fig. 5 exhibits the amount of GUS activity reported by plants grown in regular tubes that were subjected to the 9 T field from 0-16.5 h. Results show that, overall, the 0 T control plants did not experience more than baseline stress while the plants in a homogeneous 9 T field felt high levels of stress at 2.5 h and less stress as time progressed. The data for the 9 T plants are somewhat unexpected, since stress should increase with time when plants are subjected to a magnetic field. Fig.6 and Fig.7 depict the data for the plants (roots and leaves separately) in vertical tubes and plates. One should remember that the plant strain in the regular tubes is not as sensitive as the plants grown in the vertical plates or tubes. Unlike the plants in the regular tubes, the GUS activity level in these plants increased exponentially as time increased. Since the GUS activity ranges from 3 to 50 for leaves and 1 to 62 for the roots, one can see that roots become more stressed than the leaves, and that in time, the baseline GUS activity increases to high levels of GUS activity. So, the longer the plant is subjected to a magnetic field of 9T, the more stressed it becomes.

The preliminary experimental results for the magnetic susceptibility of agar, leaves, roots, distilled water, and plastic obtained from the SQUID are displayed in Table 1. The mass susceptibility has been calculated for each. We found the value for mass susceptibility of water to be $-1.01\text{E-}6$ (emu/gram), which is not very accurate when compared to the accepted theoretical value of $-7.20\text{E-}7$ (emu/gram). Therefore, the results for the magnetic mass susceptibility of the leaves and roots of the plant can be questioned. We hypothesize the error comes from either the calibration of the SQUID magnetometer or possible demagnetization occurring during the sample run.

After obtaining susceptibility data, we then used the equation in Geim's paper to calculate the appropriate forces on the plant. The model shows that the forces on a plant a distance of 128 mm above the center of the bore is on the order of 10^{-4} Newtons. The sum of the forces of the plant approximates $-.0006$ N. Other data have not yet been collected.

DISCUSSIONS

Our results indicate that with magnetic fields ranging from 0-9 T, Arabidopsis shows little stress qualitatively and quantitatively because of the absence or limited appearance of blue in the tissues of the stained plants and the small amounts of GUS activity reported when plants are in these fields for 2.5 hours. GUS activity for the plants studied in 2000 lies far below the induced GUS activity level so plants may be able to function normally for small amounts of time in those magnetic fields. However, the roots and leaves in the vertical tubes and plates that were placed in 9 T for longer than 5 hours exhibited great levels of GUS activity. These data suggest that exposure to even medium levels of homogeneous

magnetic fields can stress a plant after long stretches of time. Conclusions for the theoretical part of our experiment have not been yet reached. We will continue our experiments in finding the effects of magnetic fields on plant metabolism as well as show that magnetic levitation occurs in milligravity.

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REFERENCES

- Berry, M.V. and Geim, A. K. 1997. Of flying frogs and levitrons. *Eur. J. Phys.* 18: 307-313
- Doherty, Paul. 1997. *The Exploratorium Science Snacks*. Retrieved 29 June 2000. (http://www.exploratorium.edu/snacks/diamagnetism_www/index.html).
- Griffiths, D. J. 1999. *Introduction to Electrodynamics*. New Jersey: Prentice Hall.
- Halliday, D. and Resnick, R. 1960. *Physics (Part Two)*. 3rd Ed. New York: John Wiley and Sons.
- Meinke, D. W. 1998. Arabidopsis thaliana: Model Plant for Genome Analysis. *Science*. 282: 662-682.

Meisel, M. W. and Ferl, R. J. 1999. Plant Growth and Development under Magnetic Levitation Conditions. *NHMFL Reports*. 6:3-6.

Porterfield, D. M. and Matthews, S. W. 1997. Spaceflight Exposure Effects on Transcription, Activity, and Localization of Alcohol Dehydrogenase in the Roots of *Arabidopsis thaliana*. *Plant Physiol.* 113: 685-693.

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FIGURES

FIG. 1. The amount of GUS activity exhibited by leaves of plants with 2.5 h of exposure time to varying homogeneous magnetic fields of 0-9 T. The symbols denote the type of container the plants have been grown in. GUS activity is the ratio of GUS enzyme in nanomoles to the protein in the plant in micrograms per minute.

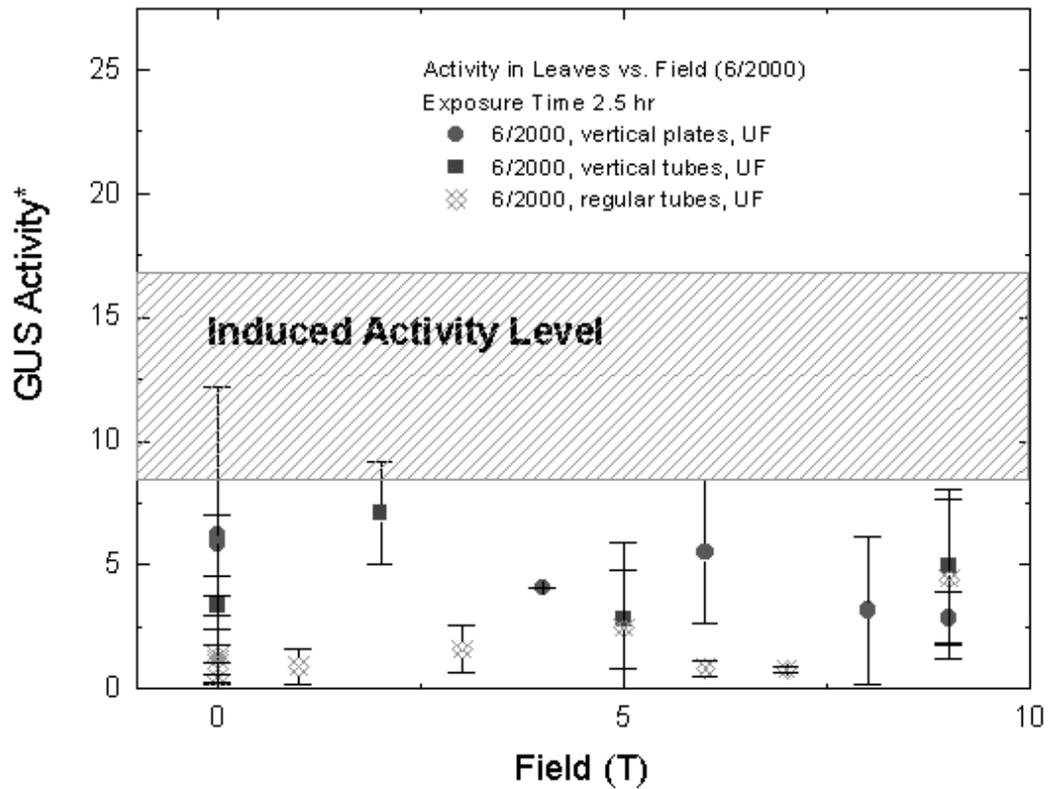


FIG. 2. The amount of GUS activity exhibited by roots of plants with 2.5 h of exposure time to varying homogeneous magnetic fields of 0-9 T. The symbols denote the type of container the plants have been grown in.

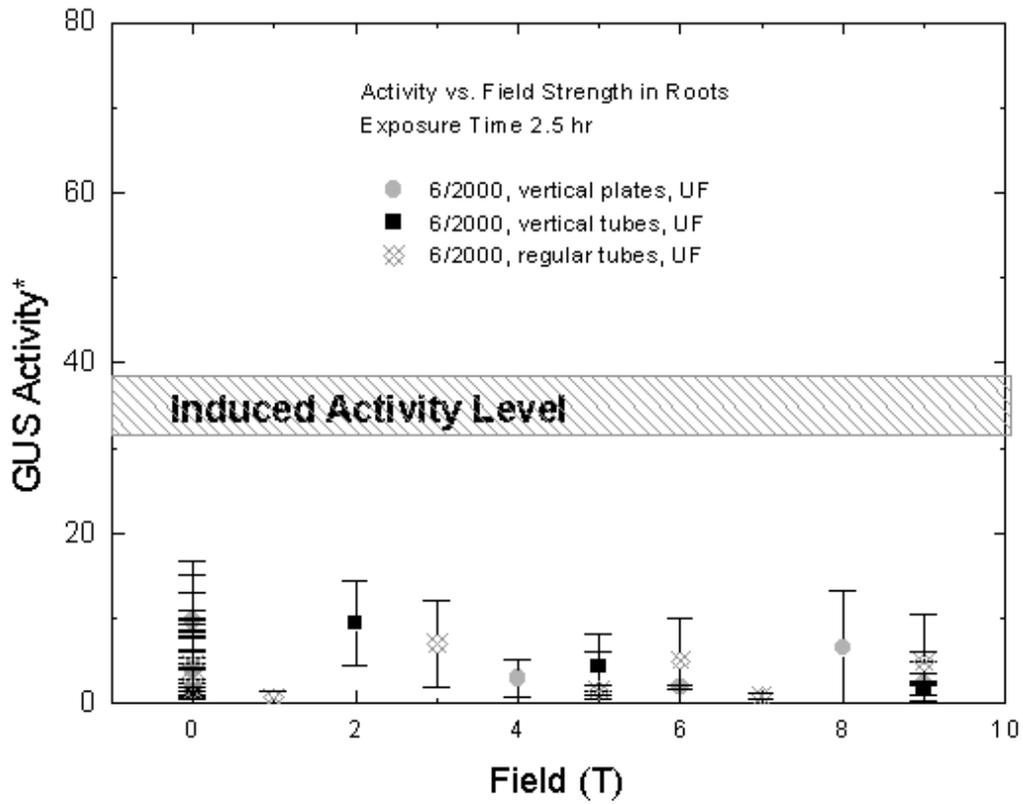


FIG. 3. The amount of GUS activity exhibited by leaves of plants with 2.5 h of exposure time to varying homogeneous magnetic fields and magnetic field gradients of 0-25 T. The symbols denote the date of exposure, type of container the plants have been grown in, and the research site of the experiment.

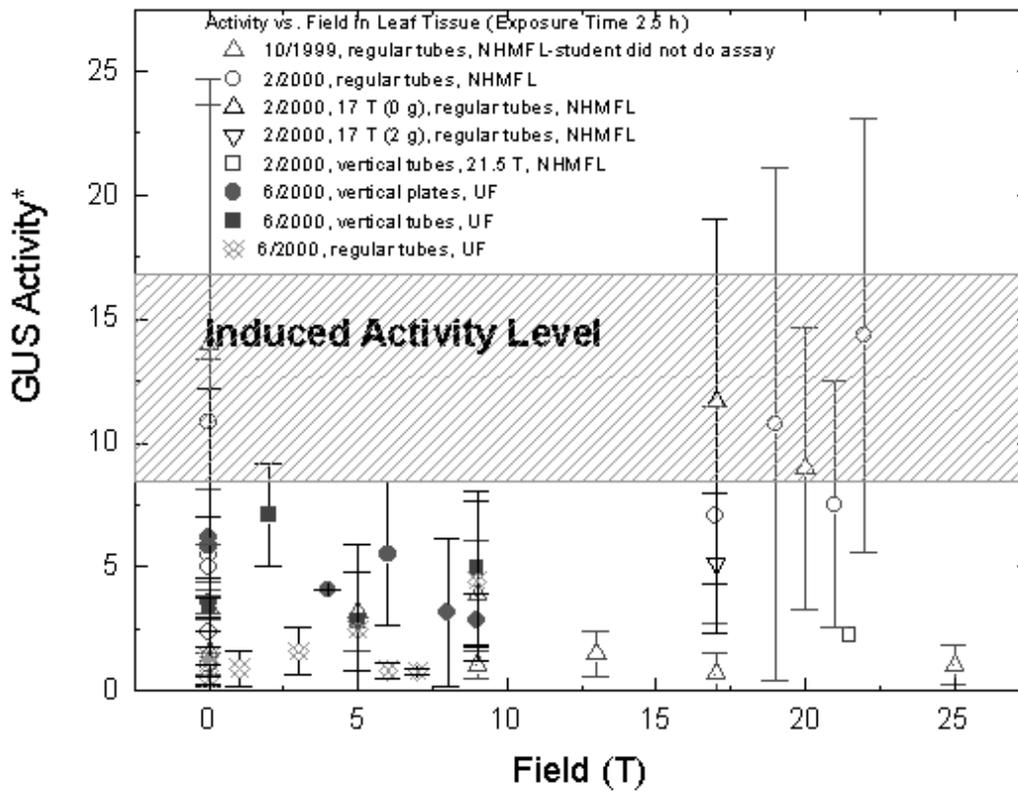


FIG. 4. The amount of GUS activity exhibited by the roots of plants with 2.5 h of exposure time to varying homogeneous magnetic fields and magnetic field gradients of 0-25 T. The symbols denote the date of exposure, type of container the plants have been grown in, and the research site of the experiment.

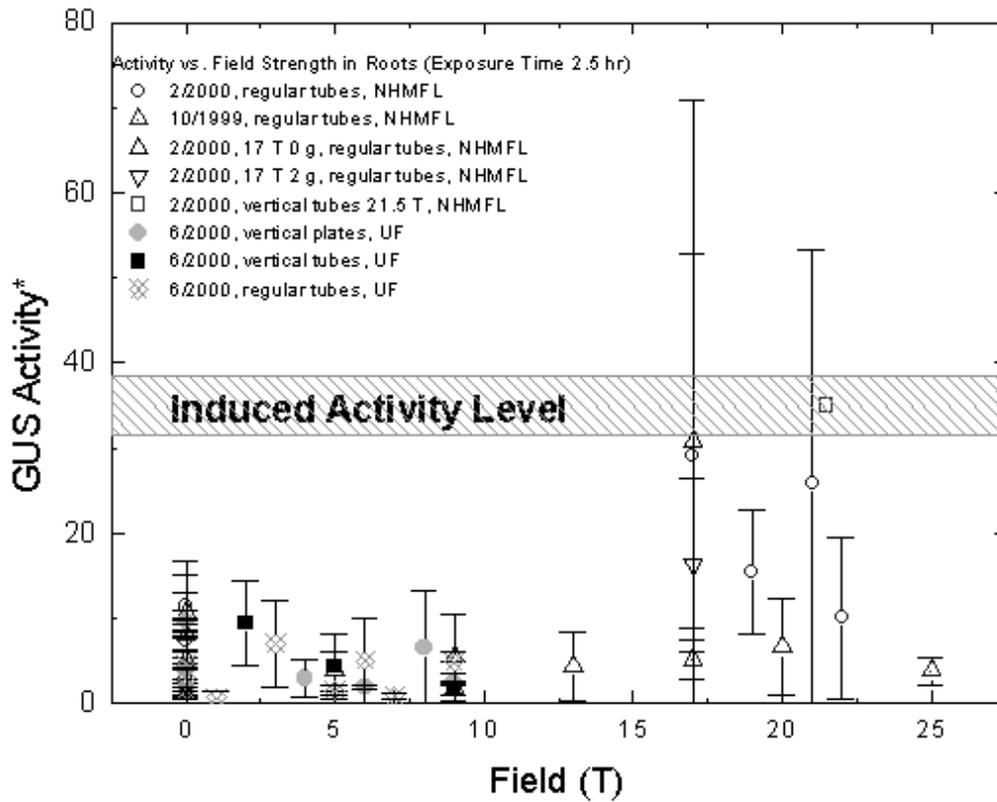


FIG. 5. The amount of GUS activity exhibited by the plants in the regular tubes with 0-16.5 h of exposure time to a homogeneous 9 T field.

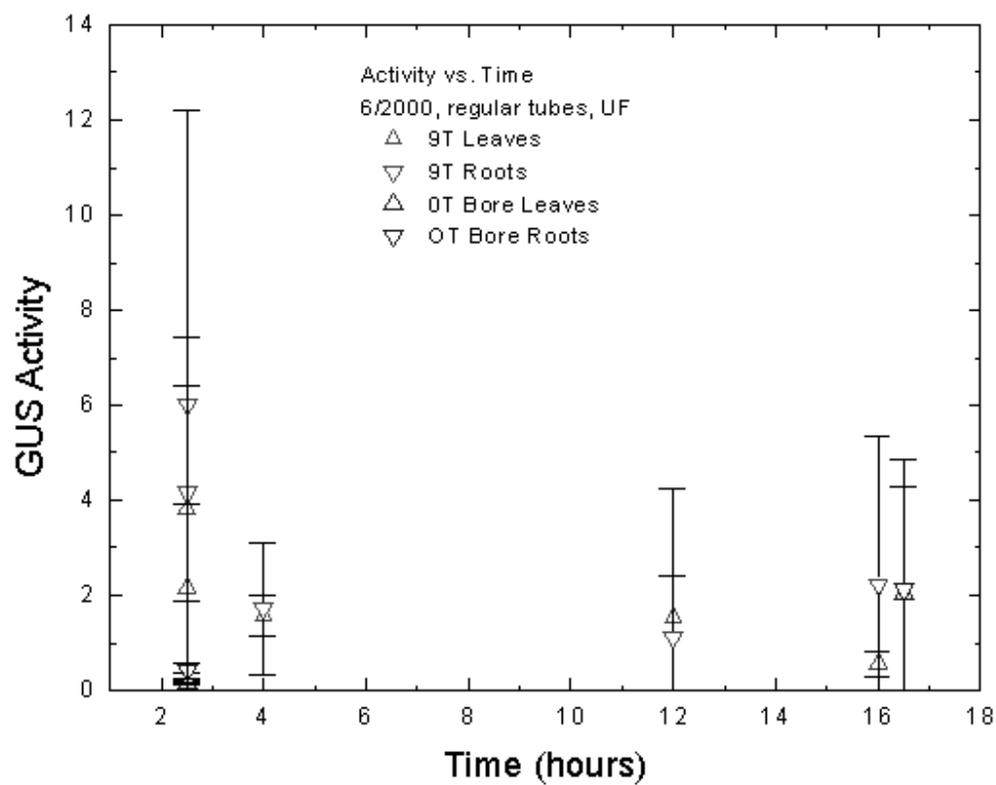


FIG. 6. The amount of GUS activity exhibited by the leaves of the plants in the vertical tubes and plates with 0-16.5 h of exposure time to a homogeneous 9 T field.

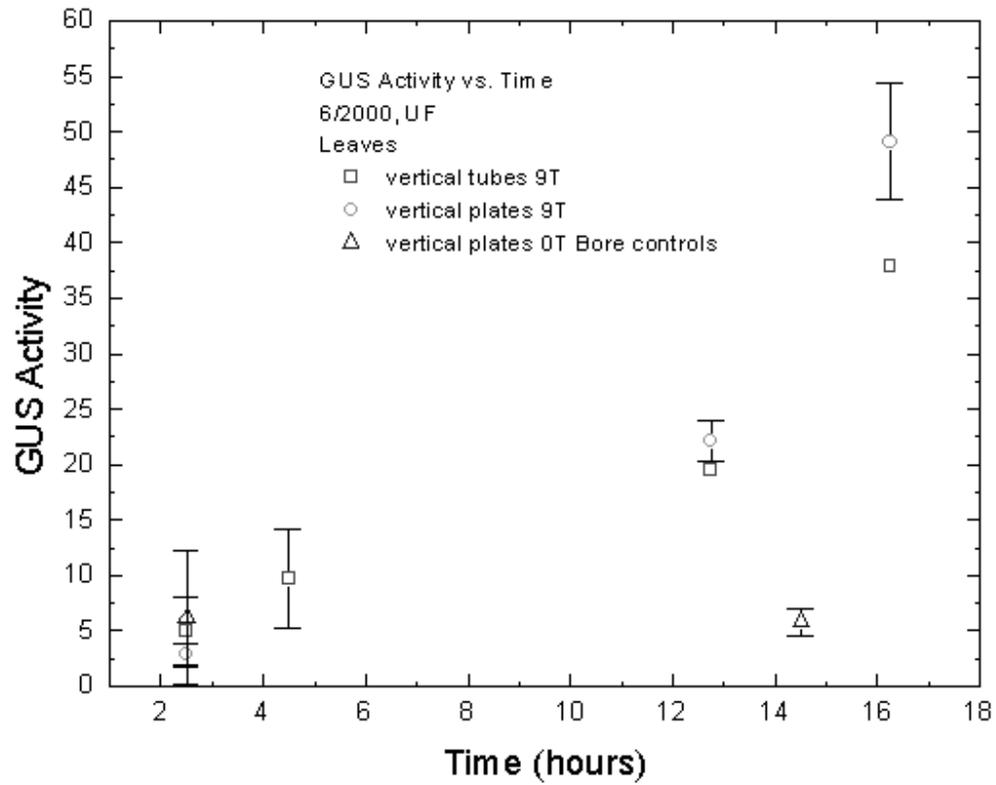


FIG. 7. The amount of GUS activity exhibited by the roots of the plants in the vertical tubes and plates with 0-16.5 h of exposure time to a homogeneous 9 T field.

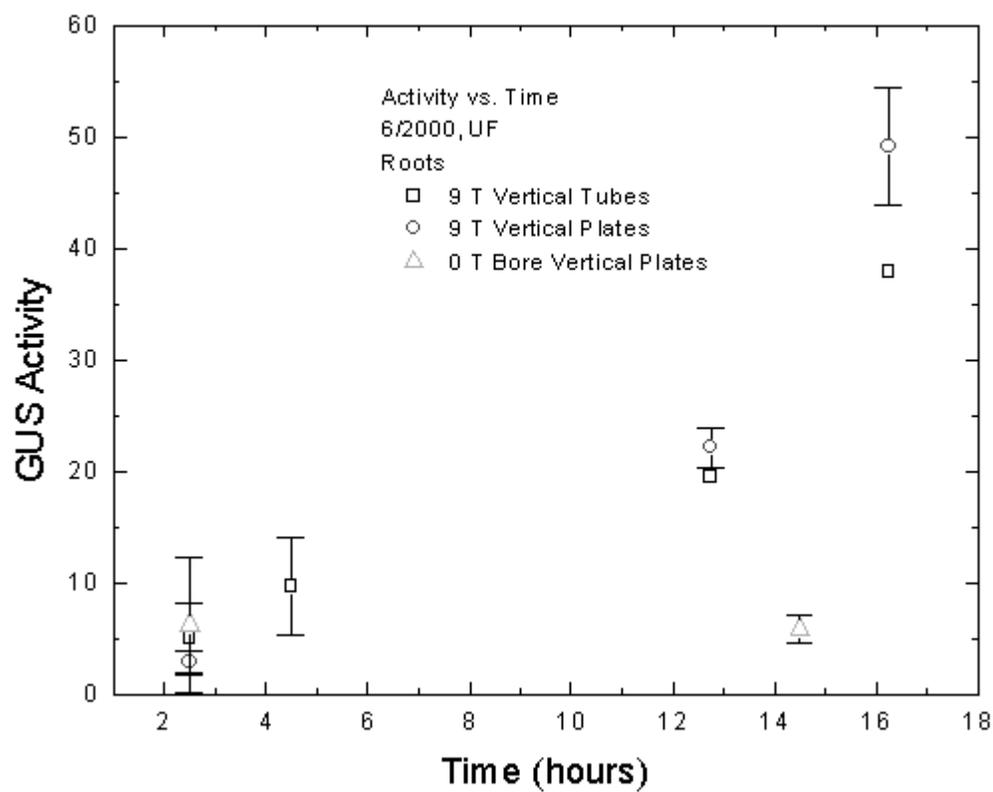


TABLE 1. The preliminary experimental data obtained from the SQUID magnetometer for the magnetic susceptibilities of water, leaves, and roots of a typical plant.

Subject (background subtracted)	Mass of Sample alone (grams)	Mass Susceptibility (emu/gram)
Distilled Water	.130	-1.01E-6
Arabidopsis Leaves	.200	-8.53E-7
Arabidopsis Roots	.047	-1.18E-6