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A PROPOSAL TO DETERMINE PROPERTIES OF THE GRAVITROPIC RESPONSE OF PLANTS IN THE ABSENCE OF A COMPLICATING G-FORCE (GTHRES)

FINAL REPORT
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GTHRES FINAL REPORT TO NASA
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I. Abstract

Gravitropic responses of oat seedlings (*Avena* sativa L.) were measured on Earth and in microgravity (IML-1). The seedlings were grown at 1 *g* either on Earth or on 1 g centrifuges. They were challenged by centripetal accelerations for which the intensity and duration of the stimulations were varied. All stimulation intensities were in the hypogravity region from 0.1 to 1.0 *g* . All responses occurred either in Spacelab microgravity or during clinorotation on Earth. The experiments were carried out with the same apparatus in Spacelab and on Earth. The experiments addressed a series of scientific questions and useful data were obtained to provide answers to some but not all of those questions.

II. Operational Objectives of GTHRES

Briefly stated, the general operational objective of GTHRES was to describe quantitatively the kinematics of dark grown *Avena* (oat) seedling shoots' tropistic responses to a range of *g*-force stimulations of different intensities and durations without complications from a background *g*-force due to earth's gravity during the response phase. Such measurements of responses to *g*-stimulations can be done only in a μg environment.

Test conditions were chosen so that for most effects the data to be acquired could be plotted as a family of curves relating tropistic response characteristics to stimulus quantities. Best fitted mathematical functions, with their slopes, intercepts, and maxima were items of particular interest. Prior to flight we recognized seven different

scientific matters that we believed would be addressed by such relationships, if interpretable data could be obtained from the GTHRES experiment. Those seven items are explained below as quantities which, prior to flight, we thought it would be important to measure. For each test the stimulus, produced by centripetal acceleration, would be varied in duration and/or intensity as required in separate treatments. After each stimulus episode the responses would occur in microgravity. Each set of seedlings would be tested only once.

In most cases it was of interest to measure each kind of response not only in microgravity but also in clinostat simulated weightlessness during ground based experiments. Accordingly, before the flight hardware, called the Gravitational Plant Physiology Facility (GPPF), was integrated into Spacelab, we arranged to use GPPF to provide a clinostat simulated weightless environment for experiments performed on Earth. This was possible because we had designed the GPPF centrifuges to rotate on horizontal axes and, by turning them slowly (1/5 revolution per minute), they functioned as clinostats. Test subjects on Earth clinorotated on the centrifuges were gravity compensated, the term often used to describe this method of simulation of weightlessness.

The criteria to be used for evaluating degree of GTHRES experiment success would depend on how much interpretable data had been acquired. (The nature or importance of scientific conclusions that might devolve from interpretations of that data were not considered relevant for determining experiment success as we defined it.)

GTHRES was designed specifically to measure the following:

(A) Maximum response (R_{max}) to laterally directed *g*-acceleration. R_{max} is defined as the maximum tropistic curvature attained by the plant within 60 min after the end of the stimulation episode. R_{max} was determined over a range of stimulus quantities (*g*-doses) exclusively within the hypogravity range ($0 < g < 1$). The *g*-dose is defined as $g \times t$, the product of applied acceleration times the duration of its application.

In an experiment on Earth, after a seedling has received a laterally directed *g-stimulation* it responds by bending away from the plumb line. It will become increasingly gravity stimulated the farther it bends during that response to the test stimulus it had received earlier. The Earth-*g* stimulation, counteracting the response from the experimentally imposed stimulation, may decrease the total amount of bending that will occur. To know quantitatively how much suppression (if any) of the tropistic response actually occurs has theoretical interest because it can be

modelled and, based on certain assumptions about the response mechanism, some models might be put at risk by GTHRES results.

On the other hand, it seems not impossible that a shoot responding in weightlessness would continue to bend long after a control plant (responding in Earth's 1*g*) would have ceased to bend so that, for responses that occur in weightlessness, both the duration and the degree of curvature might be enhanced, not diminished. That too would have important theoretical consequences.

Plots of $\mathbf{R_{max}}$ values under different test conditions also provided information needed to address several of the following topics.

(B) Threshold *g*-stimulus for minimum detectable tropistic response (g_{th}). A threshold can be determined by extrapolating to the abscissa, (x axis), the best fitted line relating each of a graded sequence of intensities of centripetal acceleration or *g*-doses to the subsequent curvature of the responding plant organ, (y axis). One might argue that the least stimulus that can cause a just detectable tropistic response would be the most salient quantitative measurement that could be made by GTHRES. There are different theories that purport to explain components of the stimulus-response process (which includes Susception, Perception, Memory Storage, Transduction, and Growth Change). The lower limit of responsiveness, the *g*-threshold (g_{th}) is not the same for all such theories and, if we knew with confidence the g_{th} for the plant's responses at 1*g* and especially over the full range of hypogravity, we might be better able to put at risk one or more of those theories. The NASA code name chosen for our experiment, GTHRES, is an acronym for GRAVITY THRESHOLD which emphasizes the importance accorded this one objective.

There has been more than one way for plant physiologists to make threshold measurements. The literature makes it very clear that, for threshold determinations, two kinds of experimental procedures have been exploited by different investigators. The g-dose/response tests always consisted of a series of stimulations for which either (a) *g* remained unity and only time of application was varied or (b) *g* was varied and t (also varied) and the threshold was determined by how long it took for a tropistic response to be detected for stimulations by different intensities of *g*. The quality of the researches that used either method was beyond reproach. By simple theory both methods should yield the same threshold dose but they did not. With procedure (a) the lowest credible threshold dose values reported have been in the neighborhood of a few tens of *g*-seconds; with procedure (b) the threshold values were about 5 or 6 *g*-min. That large difference cannot

be attributed to experimental error. Nevertheless, it has not been explained.

(C) **Sensitivity** of the plant's response parameter. Qualitatively this can be described as the incremental tropistic response produced by an increment of *g*-stimulation. It is defined by Equation 1, a slightly

$$S = |(dR/dQ)|(1/D_s) \quad \text{. . . . . Eq. 1.}$$

modified version of a function proposed by Mandel and Stiehler in1954 (**Ref 1**). Those authors provided a precise technical meaning for the term, Sensitivity. S was defined mathematically, and it was suggested that it could serve as an "evaluation of merit" for comparing different assay procedures which may be used to determine a particular characteristic of a substance or process being analyzed. In their terms, S, is the the ratio of incremental increase in the analytical result, **R**, to the corresponding incremental increase of the quantity being analyzed, **Q**, and normalized by the standard deviation (D_s) of values of **R**.

Mandel and Stiehler were thinking chiefly of physical or chemical laboratory assays but we may extend the application of their concept to the case of a plant using its *g*-sensing mechanism to perform a "*g*-assay" on its gravitational environment. For example, in Equation1, let **Q** be the laterally imposed *g*-force (or acceleration) and let **R** be the tropistic curvature. If the value of S can be determined from GTHRES data over a good part of the curve that relates **Q** and **R**, we can employ Equation1 to describe quantitatively the plant's **Sensitivity** with respect to *g*-force over any range of stimulus values (in our case within the hypogravity range). S had been evaluated earlier for oat coleoptile responses in the *g*-range above 1 *g* using a centrifuge on Earth (**Ref 2**).

(**D**) Limits of the range over which the **Reciprocity Rule** is quantitatively obeyed. The "Reciprocity Rule", also referred to (perhaps egregiously) as the "Reciprocity Law, is a generalization based on the gratuitous concept that stimulations of the organism, in our case exposures to *g*-accelerations (perhaps also to other kinds of stimulation), ought to cause responses proportional to the product of intensity of stimulation (**I**) times the duration (t) of application of the stimulus. It also has been referred to briefly as the **I x t Law**.

Some experimenters have attempted to test the validity of the Reciprocity Rule--i.e. to determine whether it is obeyed over a range of **I** and t values when they were varied reciprocally (holding their product constant). Other experimenters, with different experiment

objectives, felt required to assume the Rule's validity (even without testing it) in order to justify their research objectives.

For responses to laterally applied *g*-stimuli and over a very small range (about a factor of 2- or 3-fold change of **I** and t) the Rule seemed to be obeyed in the very few cases in which that had been tested. It is not known (but for theoretical reasons it would be useful to know) how large can be the range of reciprocal variation of **I** and t before the Rule ceases to predict quantitatively plants' tropistic responses--i.e. over what *g*-dose range the Rule is obeyed. Prior to GTHRES no tests of the Reciprocity Rule had been made in a laboratory in orbit where accelerations between 0 and 1 *g* could be used. Therefore GTHRES provided an opportunity to test the Reciprocity Rule in a new way by taking advantage of two important innovations: (a) all tropistic stimulations were in the hypogravity range, $0 < g < 1$, and (b) the plants' responses all occurred in near weightlessness.

(**E**) Parameters of a **nutational component of tropistic responses** (if nutation could be observed). Nutation (circumnutation) is known to be influenced by *g*-stimulations (**Refs 3, 4, 5, 6, 7**) which, however, were not required for the nutational oscillations to proceed in the one case where convincing results were obtained from an earlier Spacelab experiment in microgravity (**Ref 8**).

(**F**) Parameters of **autotropism** (if it could be observed). Autotropism is a differential growth process displayed by plant organs (especially shoots and roots) when, after a tropistic stimulation, the organ at first bends as expected in response to the stimulus, then later begins bending spontaneously in the opposite direction (**Ref 9**). Therefore, autotropic curvature is a straightening, sometimes described as a counter reaction, following an initial tropistic curvature. The first use of the term, autotropism, of which we are aware was that of Simon (**Ref 10**) who even used it in the title of his 95 page paper on the subject eight decades ago. Others may have used the term even earlier.

Autotropism was first interpreted by Darwin (**Ref 11**) as a circumnutation superimposed on a tropistic response but that interpretation probably was an oversimplification. Autotropism can be confused with circumnutation and it may require careful kinematic analyses to distinguish between those two kinds of plant behavior.

It seems fair to say that the study of autotropism is still in the exploratory or descriptive stage; testable theoretical explanations are needed. Perhaps a tropistic stimulation sets off an oscillatory response but, if so, it does it without there being a generally accepted theory to

explain it. It should be helpful if GTHRES results could be used to address the question: Is autotropism *g*-dependent?

(G) Comparison of parameters of responses in simulated weightlessness on earth *vs* in real microgravity (**to test the validity of the simulation method**). Many plant scientists might consider this to be the most salient objective of the GTHRES experiment because, for well over a century, simulation by clinorotation has been used (with fingers crossed) as a surrogate for true weightlessness without enough evidence having been gathered to demonstrate convincingly that the clinostat truly is a valid substitute for zero *g*.

Additional Scientific Applications of GTHRES Data--After the IML-1 mission we realized that there were six other matters that might be addressed by GTHRES data; some (but not all) could have been anticipated but we did not happen to think of them prior the mission.

(H) **Response lag.** Some (usually short) time after a growing seedling has been laterally *g*-stimulated, it begins to respond. The lag time for response is temperature dependent (Q_{10} for different species varies from not much above 1 to 3 or more). For oat coleoptiles under our experimental conditions the lags were 15 - 30 min. During that time a perceived stimulus is being transduced into a biochemically controlled growth process the details of which are partly understood. (Understanding of a mechanism may be operationally defined as whatever explanation currently enjoys nearly complete agreement within the scientific community.)

Since GTHRES could be expected to provide more (i.e., different kinds) of information than could be obtained on Earth, a comparison of response lag measurements on Earth and in different levels of hypogravity might reveal differences that could identify *g*-dependence of some portion of the stimulus-response process from Perception to Growth Change. (Until about a decade or two ago it was assumed that only the Susception-Perception part of the stimulus-response process could be gravity dependent; now agreement on this point is not unanimous.)

(I) The *g*-dependence of the **time after beginning of stimulation that the response becomes maximal.** When a tropistic response curve is traced with good time resolution it often is not a smooth curve; it shows slope changes that might be considered evidence either of autotropism or nutation or of both. This may make it

difficult to establish a satisfactory definition of "maximum response". The quite arbitrary definition we used when analyzing GTHRES data was to record it as that curvature which was measured 1 hr after the end of stimulation. In some cases maximum curvature was attained before that time so we decided to consider as a response parameter also the exact time after stimulation that maximum tropistic curvature was attained.

(J) For tropistic responses proceeding on Earth some data have been reported from which small increments of shoot growth could be calculated for regions along the length of the coleoptile or of the hypocotyl. From such data "waves" of maximal rate of curvature have been reported as moving along the length of the responding organs. From GTHRES data we might be able to make similar measurements of **changes in the location of maximal response rate** over the time period after stimulation unaltered by a significant *g*-influence. Such results have some intrinsic interest.

(K) **Guttation** is an exudation of (mostly) water from hydathodes at specific locations in the epidermis of many plant leaves, coleoptiles, and some other organs. In most environments the humidity is relatively low and usually the water of guttation evaporates as fast as it is exuded and therefore goes unnoticed. (A familiar exception is the so-called "morning dew" that may appear on a lawn early in the day. It is not rain that falls on the leaves nor real dew that condenses on them; it is guttation water droplets exuded by the grass leaves which accumulated enough to be seen but only when the air is not far from saturation and when there is almost no wind.)

We observed droplets of guttation exudate in 88% of the plants whose images we measured. The shape of the droplets and their location on the convex *vs* concave sides of the coleoptiles were items to be quantified. We chose to examine the shapes of guttation liquid drops because our first impression was that not in all nor even in most cases were they of the shape we would have expected, given a model dominated only by surface tension parameters.

(L) **Precocious Development Syndrome (PDS).** We had designed the GPPF and the GTHRES experiment protocol to permit us to receive down-linked video data from our experiments during the IML-1 mission. From that routine monitoring of our seedlings we became aware that the lengths of our plants growing in orbit were significantly greater than the lengths we had observed for plants of the same age

that had been cultured on Earth in the GPPF apparatus during pre-flight tests. The tips of some of the seedlings had even reached the "roof" of the Cube in which they developed, which made them practically useless as test subjects.

By adhering to the original test protocol we would be challenging our plants with their planned *g*-stimulations at a chronological age of 81 hr at which time they would have grown much taller than had been the case during Earth control tests for seedlings of the same age.

We reasoned that comparing plants at the same development stage probably would be more appropriate than comparing plants at the same age but at different stages of development so we decided to advance by about 10 hr the times of test stimulations in the latter part of the IML-1 mission to challenge the seedlings when they would be at the same height (presumably the same stage of development) as our Earth controls. That decision meant that we would have two populations of flight test data: those for which plants were younger but the same size as the Earth controls, and those that were taller but at the same age as the Earth controls.

PDS had a major impact on the GTHRES experiment because it potentially threatened the usefulness of much of the flight data we would obtain. To make maximal use of the flight data, we would have to carry out an extended series of postflight measurements (especially on plant populations that would be tested at an older age when they would be at nearly the same height as the more rapidly developing flight plants).

III. Operation and Performance of GPPF During the IML-1 Mission

During the flight we had encountered some problems. The GPPF performed as planned with the exception of some minor anomalies. ("Minor" is defined as less than catastrophic.)

1. Data collection on one rotor did not switch off the camera when it was supposed to do so. This caused the other rotor to stop for one revolution (ca. 5 min) which resulted in the loss of one data collection.

2. The latch on the MSB was inoperable because the latch had been turned too far in one direction. A small amount of precious crew time was lost. The latch was readjusted and caused no further problems.

Table I shows the array of *g* and t values that were used to create the stimulating doses.

Table I. GTHRES G-PULSE VALUES

CUBE GROUP	LEFT ROTOR		RIGHT ROTOR	
	g Value	Time (min)	g Value	Time (min)
1	1	2	1	25
2	1	5	1	13
3	0.2	10	0.2	25
4	0.6	3	0.4	5
5	0.2	65	0.2	125
6	0.6	42	0.4	33
7	0.6	8	0.4	12
8	0.6	22	0.4	63
9	0.2	25	0.2	65
10	0.2	10	0.1	130
11	0.6	42	0.4	33

Groups 1 to 5 planted on the ground and grown in Spacelab
Groups 6 to 11 planted and grown in Spacelab

3. During the programming of a G-Stim Episode on MD 5, the PI team on the ground noticed that the PS had programmed the microprocessor to provide the 0.2 G-Stim at Mission Day Zero (MD 0) instead of MD 5. The computer (thinking that it had forgotten to initiate the Stim) immediately started the Stim Episode. The crew was notified and within 6 min the rotor had been stopped and the correct values were given to initiate the Stim. The error caused 18 plants on that rotor to receive an unscheduled 1.2 *g*-min stimulation. When the rotor was re-programmed the plants were observed for 1 hr at µg prior to their receiving the correct Stim (0.2 *g* for 65 min). After examination of the data we could not see any evidence that the plants had responded to that brief unscheduled stimulation. Any tropistic response that might have occurred would have been completed prior to the scheduled 1 hr test stimulus. We concluded that whatever response the plants may have made in response to the unplanned stimulus was lost in the noise.

4. A problem occurred while cubes were being loaded into the PCOC near the end of the mission. When they had been loaded by the MS all

went as planned. However the IMAX camera people didn't get a shot of the loading and asked PS 1 to unload and then reload while they photo-documented the whole operation. During that procedure the cubes refused to remain in their proper locations in the PCOC trays long enough for the PS to close the lid of the PCOC. To secure the cubes in their trays, duct tape was used to hold them down. We have no explanation for this anomaly.

5. Some problems that were noticed after the flight are listed: When the video tapes were examined after Shuttle landing at the Dryden facility we noticed some loss of data on the first tapes (Tapes F1 and G1) on both VTR F and VTR G. On some later tapes we noticed the same kind of garbled data on tape G9 from VTR G (but not on the tape from VTR F).

We had designed a redundant recording system to record simultaneously the same data on both tapes. Only FOTRAN data was lost on <u>both</u> tapes, G1 and F1.

After the flight, consultations with the manufacturer of the TEAC recorders led us to believe that the malfunctions were related to the configuration of the recorders. Tests conducted at ARC in the summer of 1987 revealed that the original protocol for use of the recorders (viz. turning them on and off between picture taking sessions to save power) caused tape jams. This was attributed to slack in the tape which occurred after the recorder was turned off between sessions. The solution to the tape jamming, suggested by TEAC at that time, was to keep the power on to the recorders between time-lapse recordings in order to maintain tension on the tapes. After consulting with the Marshall Space Flight Center regarding the extra power required to implement this change, the procedural modification was incorporated. That corrected the tape jamming problem we had experienced during initial tests at ARC. We conducted many hours of recording without a problem. This included the EVT test which simulated the exact timeline we expected to follow during IML-1.

After noting the problem experienced during the flight, we again contacted TEAC. We explained that in both instances where malfunctions were noticed the power to the recorder had been on for several hours prior to the start of recording. The first occasion was when the equipment was initially turned on which occurred at MD 0/06:30. The first recording was scheduled for MD 0/12:57. For the next 5 hr the time-lapse recording of FOTRAN data were unusable. The next occasion when we noticed a similar malfunction was on the third mission day. A gap in data collection existed because plants seeded after the start of IML-1 were not at the proper stage for testing. This

gap occurred between MD 2/21:00 and MD 3/20:26. Only the VTR G was affected; VTR F operated normally. The manufacturer suggested that the problem probably was caused by the tape drum spinning past the tape and ablating a portion of the tape which resulted in the material being deposited on the tape head. After several recordings the material was removed allowing proper recording of the pictures to be resumed. Examination of the tape indicated some material had been removed from the tape. Apparently some of that material found its way to the recording head causing the failure to record properly.

Our records indicate that we tested the flight hardware in ground tests using the same timeline which incorporated the same long gaps between recordings but we had not observed an anomaly at that time. The reason for the failure only during flight may be that during weightlessness ablated material might be more easily deposited preferentially on the recording heads.

The only loss of data was to the FOTRAN experiment. That loss was significant as it included all of the pre-stim and most of the post-stim data for FOTRAN Batch-1. Three down-linked video episodes were recorded for these FOTRAN plants (one pre-stim and two post-stim); thus, some of the data thus retrieved could be used to analyze data missing from the tapes.

Even though our testing protocol was not able to identify the problem we encountered in flight, two features of the GPPF design (redundant recorders and use of down-link data acquisition) reduced the loss of data in both GTHRES and FOTRAN experiments.

IV. Post Mission Tests

Gas Samples from Plant Cubes--Upon completion of the flight we received locked syringes which contained gas samples taken by crew members from seven plant cubes-- three from GTHRES cubes and four from FOTRAN cubes. The samples were analyzed for oxygen, carbon dioxide, and ethylene content by D. R. Dilley's laboratory at Michigan State Univ. The results, given in Table II, show nominal concentrations of oxygen and ethylene. The carbon dioxide concentrations were a little higher than the standard atmospheric level but not high for the Spacelab environment. None of the analyses could be considered abnormal in the sense that they could have affected the growth rate of dark grown plant seedlings.

Table II. Gas sample data from the GPPF plant cubes. The shaded data are from GTHRES cubes. FOTRAN data (unshaded are included for completeness.

CUBE ID	MET OF SAMPLE	COMPONENT		
		O_2 %	CO_2 %	C_2H_4 ppm
F1	1/01:51	21.6	0.2	0.02
F4	2/15:06	22.2	0.3	0.01
F6	1/13:54	19.9	0.2	0.01
F7	2/03:30	18.1	0.3	0.01
20	1/18:30	18.5	0.2	0.02
35	2/14:06	17.6	0.3	0.02
45	4/23:25	17.0	0.4	0.02

Temperature Sensors Recalibration and Testing of GPPF-- After the IML-1 mission our immediate concern was to learn why our flight plants displayed a PDS. A possible explanation was that something about the GPPF had changed between the time preflight data were collected and the date of the flight, an interval of nine months. One candidate for such an anomaly might have been an altered calibration of the temperature control systems. If the temperatures had been much higher than nominal during the flight, that kind of apparatus anomaly might have accounted for the PDS. That possibility required rechecking temperature calibrations and post-flight testing of seedlings according to the same protocols that had been used during IML-1.

We requested that the GPPF hardware be returned to our home laboratory where we could check its performance--especially that of its temperature control system. Ninety days after the Shuttle landed, the GPPF arrived at our laboratory where it was maintained under NASA monitoring and in compliance with quality control procedures. (This was required to maintain the GPPF as flight qualifiable equipment that NASA may want to fly on future scientific missions.)

The pre-flight vs post-flight comparison of the temperature control system showed only small differences (insufficient to explain the PDS) which confirmed that there had not been a significant change in the GPPF's thermal control systems that could have accounted for the PDS.

Post-flight Measurements of Plants of Different Ages and Sizes--Most of our GTHRES results were to be used to contrast some measurements made in space with measurements of the same kinds that had been made on Earth prior to the IML-1 mission in the same GPPF flight apparatus. We had anticipated a need for only two-member comparisons: flight results vs ground control results. However, because of the **PDS** we had advanced the times of testing for some plants that were challenged with tropistic stimulations in the latter part of the mission. We did not have ground control data for those flight plants that were "too tall" when stimulated early in the flight, so we needed results from more tests to be done on Earth using the GPPF but stimulating the seedlings when they were older and at about the same height as the "too tall" flight plants. We also wanted to determine whether the GPPF apparatus operated in the same manner as it had prior to being integrated into Spacelab in preparation for flight. We began a lengthy examination of GPPF performance characteristics. We repeated preflight ground control tests and in some of the tests we used older ("too tall") seedlings so we could compare those results with the results we had observed in flight plants both at the same advanced development stage thereby making it possible to make more useful comparisons between ground based and flight results.

Fortunately, by our extensive post flight tests, we were able to accomplish many of our original research objectives (even though those post-flight efforts increased substantially the total cost of the experiment).

V. Scientific Results of GTHRES

Useful Comparisons--Our original research plan was to make quantitative comparisons of certain measurements (mostly of tropistic responses of the IML-1 flight plants with those we had measured on Earth prior to launch using the same test protocols and the same GPPF apparatus. The important difference in treatments was that preflight tests employed clinorotation to simulate weightlessness during the plant's tropistic responses while during IML-1 we could exploit the near weightless condition of satellite orbit. For all comparisons we wished to make, we expected to be able to compare only two populations of test plant results, flight data vs ground control data. Because of the experiment protocol changes we made in flight (and in post flight control studies) we now have five populations of flight and ground

control data on plants of different ages and heights at the times they were given their tropistic stimulations. These data are from:

Preflight plants of nominal height, designated $\mathbf{PreF_{nom}}$

Postflight plants of nominal height, designated $\mathbf{PostF_{nom}}$

Postflight plants of excessive height, designated $\mathbf{PostF_{tall}}$

Flight plants of nominal height, designated $\mathbf{F_{nom}}$

Flight plants of excessive height, designated $\mathbf{F_{tall}}$

The height difference denoted by the difference in subscripts, **non** and **tall**, was on average about 35% (20 mm vs 27 mm).

Not all possible comparisons among the above five data populations are scientifically interesting. Six kinds of comparison of results were considered relevant. These are tabulated as follows:

Comparison **I**--Pre-flight nominal ($\mathbf{PreF_{nom}}$) vs Post-flight nominal ($\mathbf{PostF_{nom}}$) was important to demonstrate whether or not some change in the GPPF could have accounted for the **PDS**.

Comparison **II**--Flight-nominal ($\mathbf{F_{nom}}$) vs Flight tall ($\mathbf{F_{tall}}$) should demonstrate quantitatively the effect of using plants of different heights and ages. Both groups experienced the **PDS** but, during the last part of the mission, we had tested some $\mathbf{F_{nom}}$ plants at an earlier stage when they were about as tall as were the pre-flight plants ($\mathbf{PreF_{nom}}$) so we could make comparisons between test results from plants of the same size. We could not have predicted in advance whether plants of the <u>same nominal height</u> but of <u>different ages</u> would show equivalent responses to the same set of tropistic stimulations.

Comparison **III**--Flight-nominal ($\mathbf{F_{nom}}$) vs Post-Flight-nominal ($\mathbf{PostF_{nom}}$) shows the difference between data from plants <u>of the same height</u> (but different chronological ages) from in-flight and from post-flight tests.

Comparison **IV**--Post-flight-nominal ($\mathbf{PostF_{nom}}$) vs Post-Flight-tall ($\mathbf{PostF_{tall}}$) i.e. older plants. This comparison shows the difference between post-flight data from Earth tested plants of different sizes and of different ages. The $\mathbf{PostF_{tall}}$ plants were of the same height as the Flight-tall ($\mathbf{F_{tall}}$) plants measured during IML-1.

Comparison **V** is useful for determining whether responses of plants of the same size (taller than nominal) were significantly different when

responses occurred in μg ($\mathbf{F_{tall}}$) compared with responses that occurred during clinorotation ($\mathbf{PostF_{tall}}$).

Comparison **VI** permits us to compare responses of plants of the same size (nominal height) when some responded in flight ($\mathbf{F_{nom}}$) and others responded during clinorotation ($\mathbf{PreF_{nom}}$).

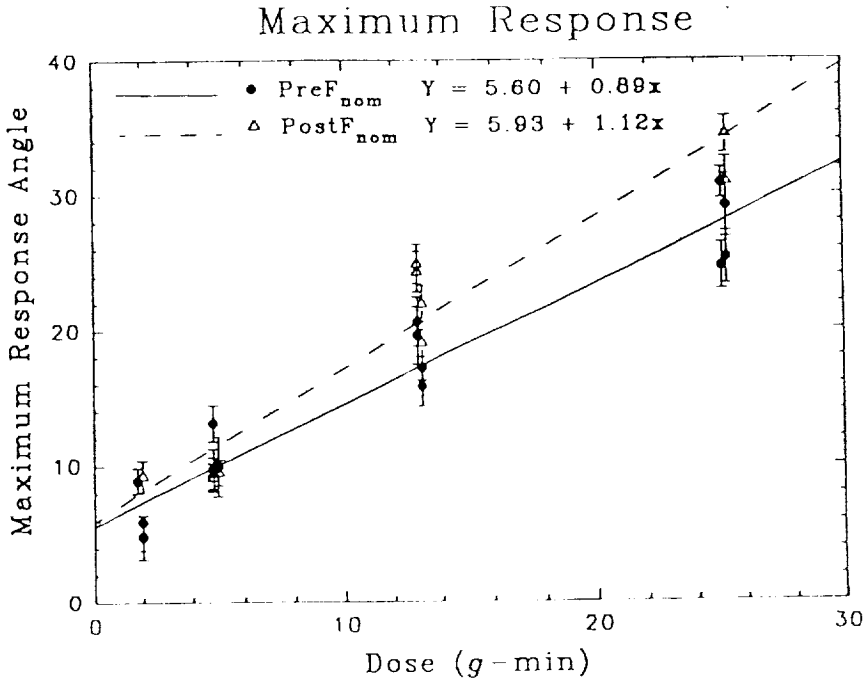

Fig. **1**--Maximum tropistic response of *Avena* coleoptiles (degrees curvature) after stimulations by different *g*-doses (*g* x t) where unit t is one minute. Data are from tests on Earth when responses took place under clinorotation simulated weightlessness. Solid regression line fits data obtained before the IML-1 mission. Broken regression line fits data obtained during post-flight experiments.

(A) Maximal Response--Figure**1** shows one example of pre-flight and post-flight measurements of plants' maximum responses to a range of g-doses. (This is **Comparison I**, mentioned above). All plants were stimulated at their nominal age 81 hr. The relationships between laterally directed g-stimulations and subsequent, maximum, tropistic curvatures for tests on Earth are shown for tests conducted

before and after the flight. Mean responses and slopes of the linear regression lines are nearly the same although the difference in the slopes is just statistically significant (p = 5%). Since there was little difference between plant responses measured before the flight and after the flight we conclude that no important change in the GPPF had occurred. Therefore, it should be fair to compare any measurements that were made during the flight with either pre-flight or post-flight control data.

Maximum response data are used for consideration of the following topics: **B, C, D, G,** and **I.**

(B) Threshold--Relevant data are shown in Figures 2 and 3. Figure 2 shows a set of flight data that relate maximum response to stimulus dose. The points are fitted with a linear regression line. Note that whenever such a response curve extrapolates to a negative stimulus value, that can be explained only: (a) by true nonlinearity of the real function or (b) by statistical uncertainty of the data when there is some reason to believe that the function really ought to be linear or (c) by the whimsical assumption that the seedling anticipates receipt of the test stimulus and responds significantly at zero time.

The linear regression line is a familiar convention although it has no compelling theoretical justification. Near the origin the experimental data necessarily become quite undependable (merely for statistical reasons). For data of Figure 2 the intercept on the abscissa has a negative value which seems unrealistic since a response function cannot have a negative value. That encouraged us to search for alternative regression equations that might fit the experimental data even better.

Figure 3 shows the same data fitted by a second order polynomial equation which gives a somewhat better fit than does a linear regression line and it shows only a small negative intercept (-8*g*-seconds), not significantly different from zero..

If we had a convincing theoretical requirement that the function must be a smooth curve beginning exactly at the origin, then in this case a second order polynomial will do the trick. But it is only a trick because, in spite of widespread wishful thinking, the requirement that the curve must extrapolate smoothly all the way to the origin has no firm conceptual justification. Some previous investigators have found it necessary to describe their response data frankly as a two-phase process; one slope for the region near the origin and another slope for the remainder of the data.

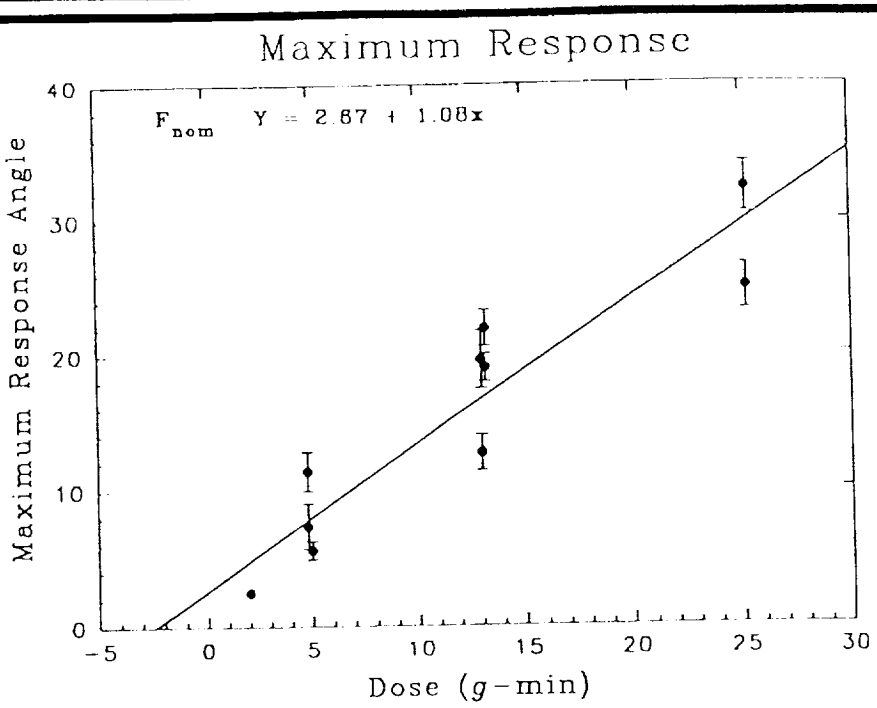

Fig. 2 --Maximum response data from tests in space. Ordinate, degrees curvature. Abscissa, *g*-dose. Points fitted by linear regression line.

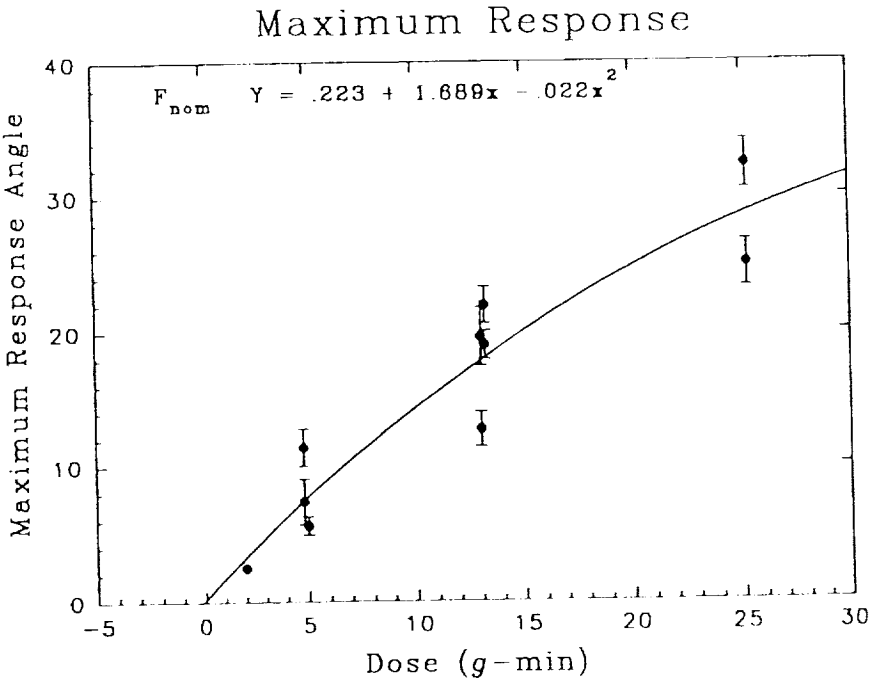

Fig. 3 --Same data as for Fig. 2 but fitted by a second order polynomial equation. Discussion in the text.

There is no generally accepted method of treating data that might establish a gravitropic threshold. We do not anticipate that future measurements of that quantity will be easily compared with ours' or others' data unless exactly the same protocols are followed.

We conclude from our observations of gravitropic responses in microgravity: (a) that the dose-response relationship probably was not truly linear with dose, (b) that the intercept was not far from the origin, and (c) that the tropistic response threshold value measured in μg was significantly lower than the 5 or 6 *g*-min reported by others who used oat seedlings for experiments in which responses were measured over a range of centripetal force stimulations or for experiments in which the plants' responses occurred during clinorotation (BIAXRO) to simulate weightlessness.

(C) Sensitivity--The graphic representation of S as a function of the response to *g*-stimulus is (approximately) the slope of the response curve. We have not yet completed our analyses of data over incremental regions of the response curves that provide a quantitative description of S over the range of our dose-response data (although it appears that the S function we measured in space may differ from ground based control data published earlier(**Ref 2**) in which the GPPF was used to describe such experimental results (when clinorotation substituted for microgravity during the response phases). This is one example that relates to **Comparison VI.**

A graphic representation of S should be (approximately) the slope of the response function as plotted in Figure **1.** Note: if the plot is a straight line (at any slope), then S has a constant value. If, by extrapolation, such a plot intercepts the y-axis (at x = zero stimulus) at a finite positive value, the reasonable interpretation is that, if precise data could be obtained all the way to zero stimulus, it would show a very steep climb from zero to the lower end of the data establishing the regression line. Thus, the value of S (near the origin) must have a very high initial value; then it must plunge to a lower constant value representing the slope of a linear regression curve. If that interpretation is true, the plant's mechanism for responding to very weak tropistic stimulation can be described as "trigger happy".

For the present, we cannot absolutely rule out the interpretation that the observed negative x-axis intercept of the tropistic response curve was merely statistical error, since the S function is uncertain very near the origin but, with increasing stimulus, it soon becomes a monotonic function of zero slope over the range of the data.

We cannot rely on precedent for predicting the shape of the S function for gravitational responses in general. S has been calculated for the effects on other biological processes by increasing stimulus quantities. For some environmental influences, S functions have proven to be far from monotonous. Since the *g* dose-response curve of Figure 1 (over the limited dose range covered by our data) seems to be linear (constant slope), that would be compatible with a constant S value for responses to hypogravity stimulations. If one prefers a curvilinear fit as in Figure 3, the S value would be highest a the low dose end of the data and would decline, about by half, at the high dose end, which we had found to be the case with sunflower nutation (unpublished observations). With so few points to establish the function, our IML-1 data were not sufficient to describe the dose function of S with great precision.

(D) Reciprocity--To test whether oat seedlings obeyed the Reciprocity Rule when stimulations were only in the hypogravity range ($0 < g < 1$) and when the responses occurred in microgravity, we needed data from experiments in which different test stimulations were of the same *g* x t dose and when *g* and t were varied reciprocally over the test series. If the Rule was observed, all tests at the same *g* x t dose should have produced the same tropistic curvature (give or take a divergence attributable to statistical variation).

The null hypothesis was that the Rule would be obeyed so all responses (as defined earlier) should be the same for all doses for which *g* and t values had been varied reciprocally. Obviously the more that *g* (and t) could be varied the better would be the test of reciprocity.

When we compared responses of flight plants of different heights ($\mathbf{F_{nom}}$ and $\mathbf{F_{tall}}$) and post-flight plants also of different heights ($\mathbf{PostF_{nom}}$ and $\mathbf{PostF_{tall}}$) to a range of stimulation doses (2 *g*-min to 25 *g*-min), within each group of plants that received the same *g*-dose the Reciprocity Rule predicts that all responses should be the same. Figure 4 compares the responses of flight and post-flight plants whose heights were either nominal (**nom**) or excessive (**tall**). This makes it possible to visualize **Comparisons II, III, IV, and V**, in each case at any of four *g* x t doses.

Figure **4** shows data from IML-1 tests that relate to the reciprocity functions measured in space. Since comparable Earth based measurements of reciprocity were accomplished and were published (**Ref 2**) prior to the IML-1 mission, these data also may be considered in relation to **Comparison VI.**

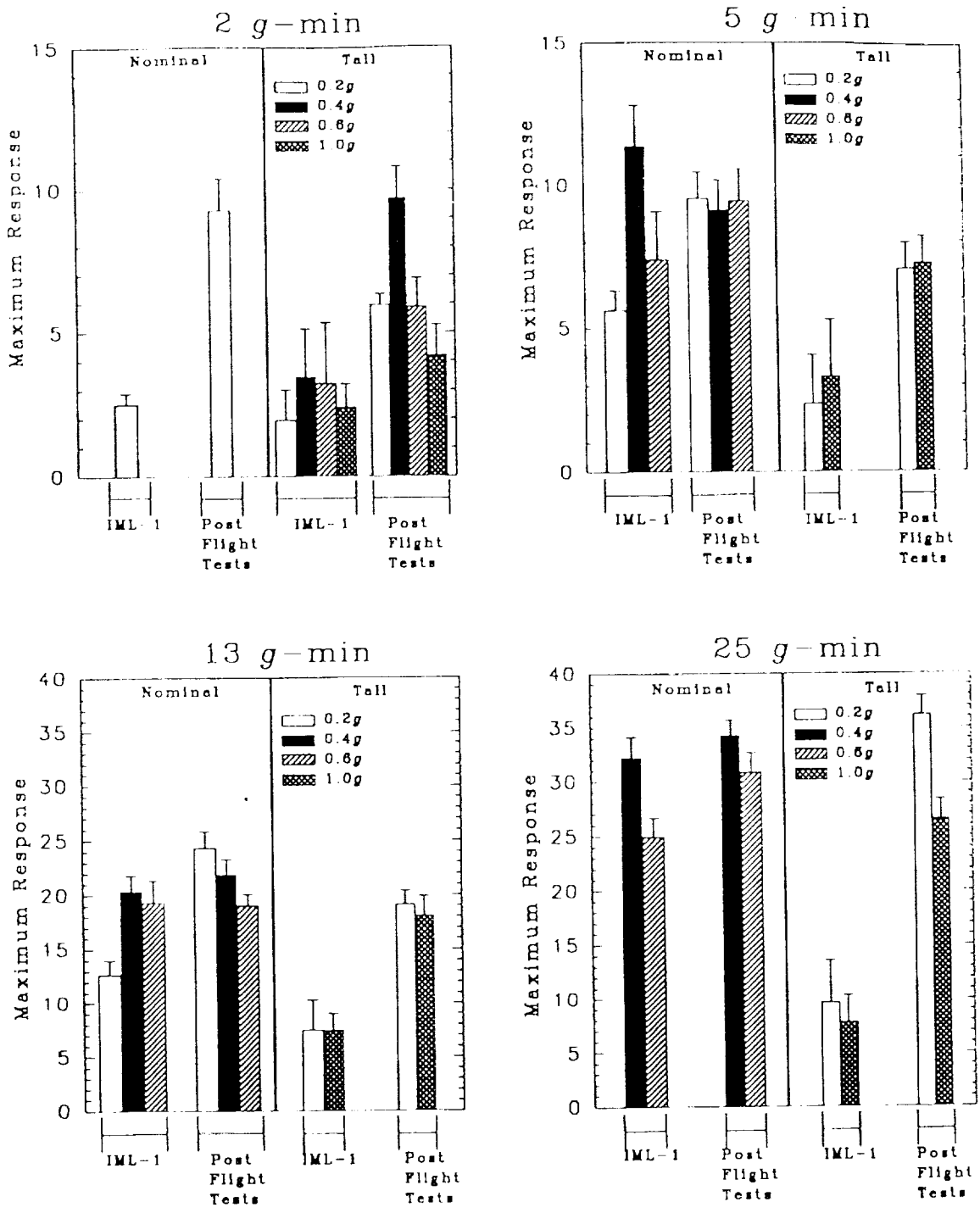

Fig. 4-- Response data from flight tests grouped by magnitude of tropistic stimulations. Responses by plants of nominal height and by tall plants are separated for comparison. Populations separated by *g*-doses (a) Stimulation, 2.0 *g*-min. (b) Stimulations: 5.0 *g*-min. (c) Stimulations, 13.0 *g*-min. (d) Stimulations, 25 *g*-min. Discussion in text.

The Reciprocity Rule can be operationally valid in any case only over a limited range of reciprocally varying *g* and t while holding *g* x t constant because there is limit to how large t can be made without the test plant accomplishing much of its response before the conclusion of the stimulus. For that reason alone we know that, in our case, the Rule cannot be tested unambiguously for response to stimulus durations beyond about 25 or 30 min. However, responses to longer stimulus times (in our data up to 125 min) are not without interest.

A more general statement of the Reciprocity Rule could be:

$$(g)^m \times (t)^n = \mathbf{R}$$

where *g* and t vary reciprocally, where the response, **R**, is constant and where it may be assumed that both exponents, **m** and **n**, are unity. That assumption is arbitrary; its only justification seems to be the law of parsimony. If the Rule is at fault because we gave equal weighting to *g* and t when nature intended that one or the other variable ought to carry more weight, then an attempt to demonstrate a range over which $(g)^1 \times (t)^1 = \mathbf{R}$ will fail because, as *g* and t are varied reciprocally, **R** will not remain constant but will show a trend either upwards or downwards (from which one might be able to determine with good precision what the exponents of the equation must be to keep **R** constant).

We do not have enough data to make that determination but it is one kind of experiment that can be done (on earth and in space) with more *g* x t combinations and more samples of each combination than could be allocated during IML-1.

Since the simple **I** x t Rule does not hold over the range of these data, we might have expected to see some trend (systematic departure from the mean value of the responses as *g* and t were varied). If we consider the four data sets of Figure **4** separately, within each data set no such trend is apparent. That might be taken as evidence that Reciprocity does not obtain. The error bars on the sets of mean response values are not unduly large. However, the variation from set to set is uncomfortably large. Also for the averages of the individual data sets there is no apparent trend in the departures from average values over the *g* x t dose range explored. Possibly, some unidentified uncontrolled variable may have been at work.

We conclude that the Reciprocity Rule (in which g and t are equally weighted) may be only a poor approximation. We could not accomplish our original objective of determining the range over which it applies and beyond which it fails. Chiefly because there are some large error

bars on data of Figure **10**, we cannot claim that GTHRES data provided convincing evidence that the I x t "Rule" applies consistently to gravitropic responses of oat coleoptiles either on Earth or in microgravity.

(E) Circumnutation--All our plant images were side views. If the major axis of a narrow circumnutational ellipse occurs in a plane which is in line with the camera's viewing direction, less than the full amplitude of nutational oscillation will be recorded. (If the ellipse was so oriented and was so narrow that it closely approximated a line, it would have been recorded as not showing any movement at all.) At the other extreme, if the plane of the ellipse was transverse to the viewing direction, the full amplitude of the nutational ellipse would have been recorded. If the orientation of the major axis of the ellipse changed with time in a regular fashion (as we often observed with sunflower hypocotyls: **Ref 12**), the amplitude of the excursions would appear to oscillate. However, for the GTHRES experiment, seed planting orientation was such that it was much more probable (based on the anatomy of the coleoptile) for any nutational oscillation to occur in the plane transverse to the camera's viewing direction, so it seems very likely that our data would show any oscillations that occurred at nearly full amplitude.

Prior to testing there was no way to predict whether or not we should expect to observe circumnutational behavior during IML-1 although it had not been observed in tests with oat seedlings on Earth. During GTHRES we observed no oat coleoptile growth movements that we could confidently identify as circumnutations. Those results add to the very small (single digit) number of tests designed to observe circumnutational behavior in the absence of a significant g-force. The first such test had been carried out on sunflower hypocotyls during the Spacelab-1 mission. In that case circumnutations were prominent (**Ref 8**). We are unaware of any previous attempt to observe nutational behavior of oat seedlings in μg.

We did not obtain convincing evidence of circumnutation by <u>oat</u> coleoptiles during IML-1 nor during ground control studies on clinostats either prior to launch or in post-flight tests. This is consistent with observations from other laboratories and it contrasts with observations by ourselves and by others when the experimental material was <u>sunflower</u> hypocotyls which did circumnutate both on Earth and in μg (Ref 8). It also contrasts with results of another IML-1 experiment, FOTRAN, during which many circumnutations by <u>wheat</u> coleoptiles were observed. We conclude that circumnutations in microgravity may or

may not occur in different plant species which defies simple generalization.

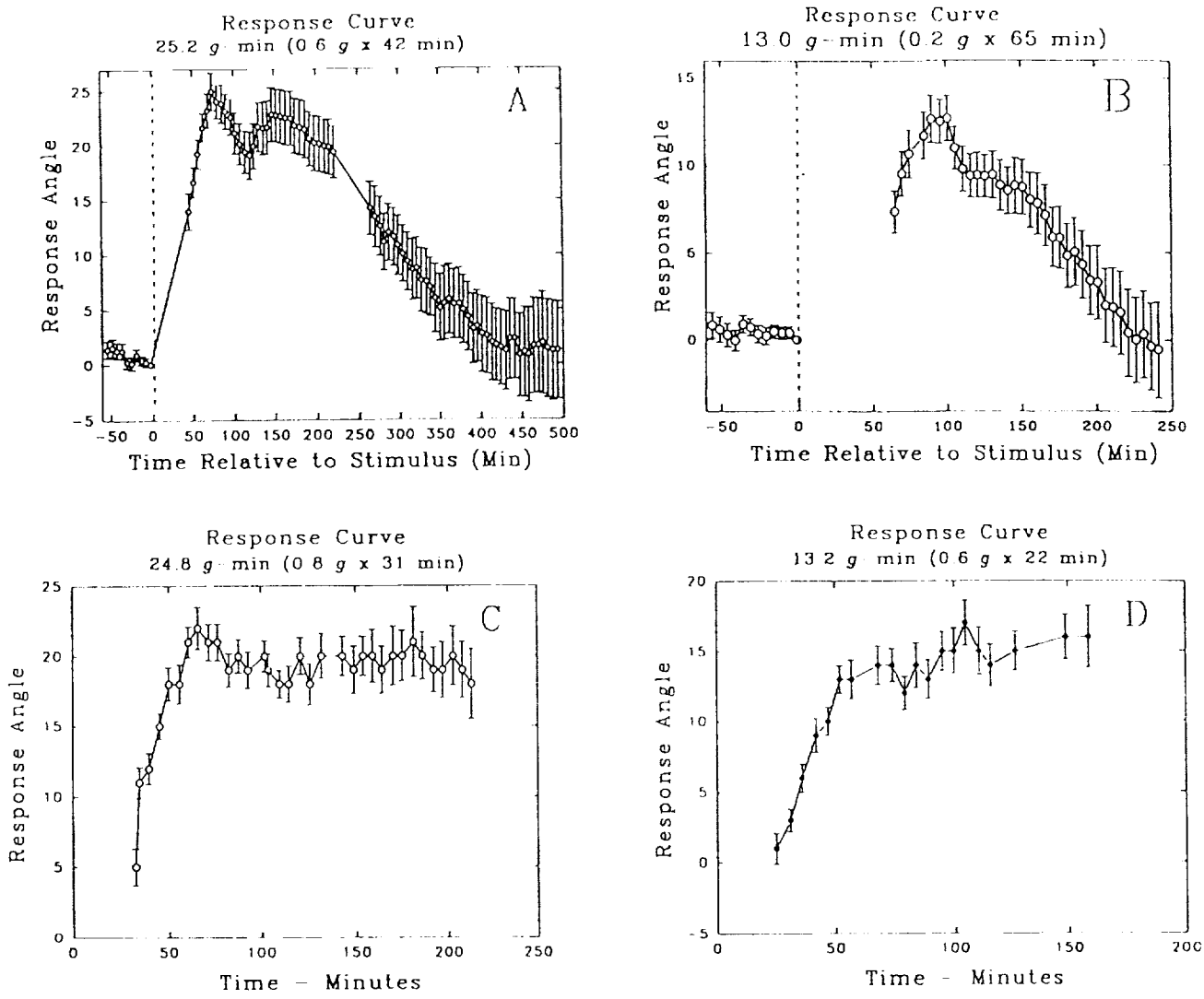

Fig. 5--Upper curves (A and B) show the time courses of response of two sets of flight plants that received different stimulations, 13.0 and 25.2 g-min. Lower curves (C and D) show the time courses of response of another two sets of clinostatted plants that received similar stimulations in pre-flight test. In each case the stimulations began at zero time. Zero response angle was established from 1 hr of prestimulation monitoring. Data were not recorded during the stimulations. The responses occurred in μg in the case of the flight data, during clinorotation in the case of the ground data. Discussion in the text.

(F) Autotropism--After g-stimulation, when oat coleoptiles' tropistic responses were recorded by time lapse video imagery, autotropism was prominent only in the case of flight plants. Figure (5) shows examples of autotropic behavior observed during IML-1.

Autotropism which occurred in spaceflight could not have been influenced by Earth's gravity. These results relate to **Comparison VI**. This will be further documented; our analyses of the data bearing on this topic is not yet completed. We probably shall be able to demonstrate that autotropic responses occurred consistently in true weightlessness but not in simulated weightlessness.

Clinorotation effectively reduced autotropic behavior. The same kind of inhibitory effect had been observed when the vigor of circumnutation by sunflower hypocotyls was tested both on the clinostat and in spaceflight (**Ref 8**), where it was found that clinostatting suppressed nutational activity. GTHRES results support the contention that clinorotation is not necessarily the equivalent of weightlessness.

(G) Space vs Simulated Weightlessness--In several cases we observed differences between plant responses that occurred in weightlessness vs those that occurred under clinorotation on Earth.

(1) In above section **F** we noted that autotropism was quantitatively different in μg as compared with what was observed using clinorotation on Earth.

(2) During the flight we measured both "nominal" and "tall" plants responses to different g-doses as shown in Figure **6**. These results apply to **Comparison II**.

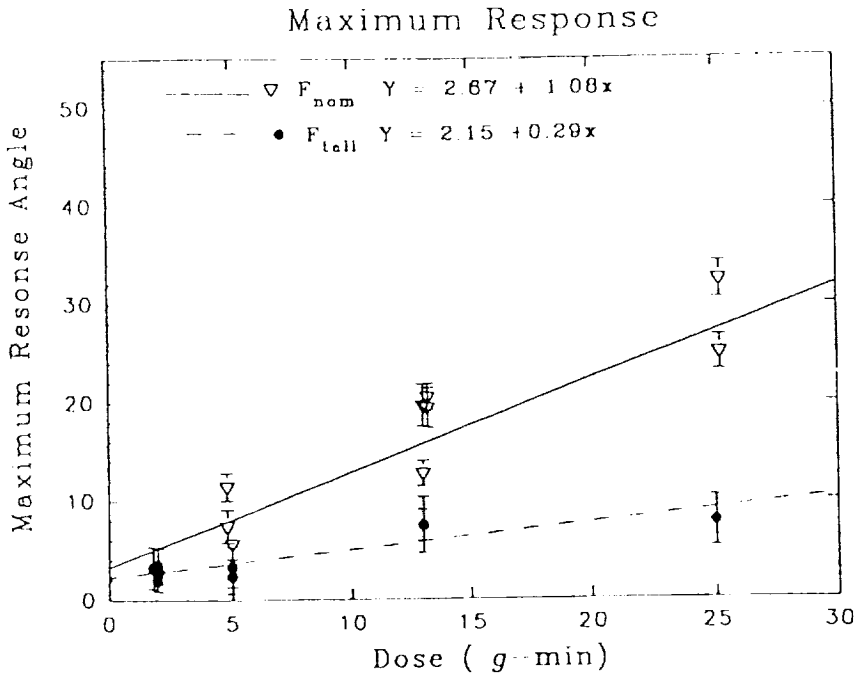

Fig. 6--Data on responses of flight plants of nominal height ($\mathbf{F_{nom}}$) compared with those of flight plants of excessive height ($\mathbf{F_{tall}}$). Discussion in the text.

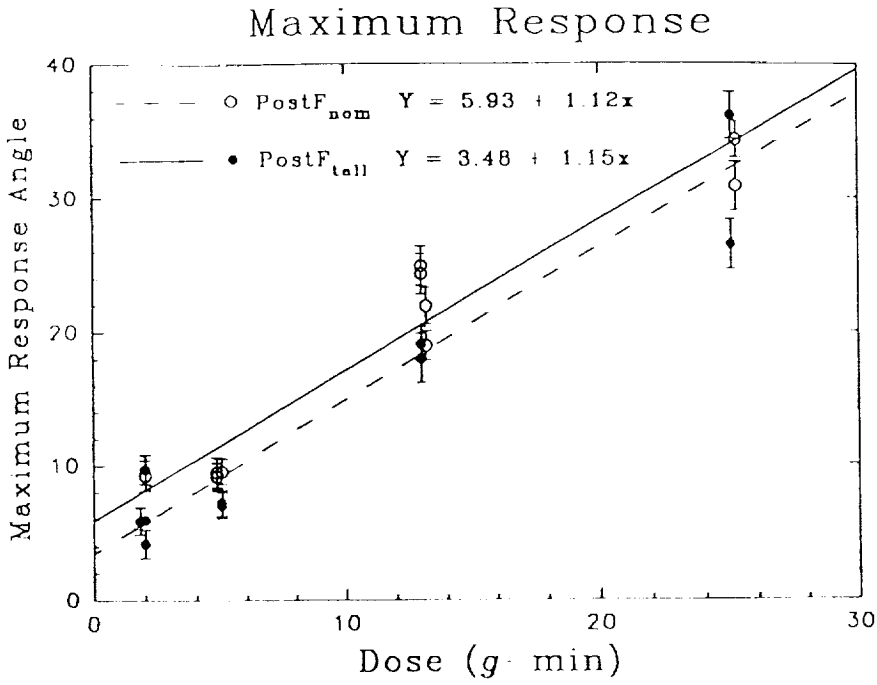

Fig. 7--Results from post-flight tests whereby comparison is made between responses of populations of plants of different heights and different ages--*viz*. $\mathbf{PostF_{nom}}$ vs $\mathbf{PostF_{tall}}$. Discussion in the text.

After the flight we measured tropistic responses of plants that were of nominal height ($\mathbf{PostF_{nom}}$) and responses of those that were older and taller ($\mathbf{PostF_{tall}}$), which we called **Comparison IV**. All responses occurred during clinorotation on Earth. The data are shown in Figure 7. A large difference is apparent between results of tests in space and of those that used clinorotation.

As shown by Figure 8, the $\mathbf{F_{nom}}$ and $\mathbf{PostF_{nom}}$ data were nearly the same. Thus, for "nom" plants, the difference between responding on the clinostat and responding in space were not significant.

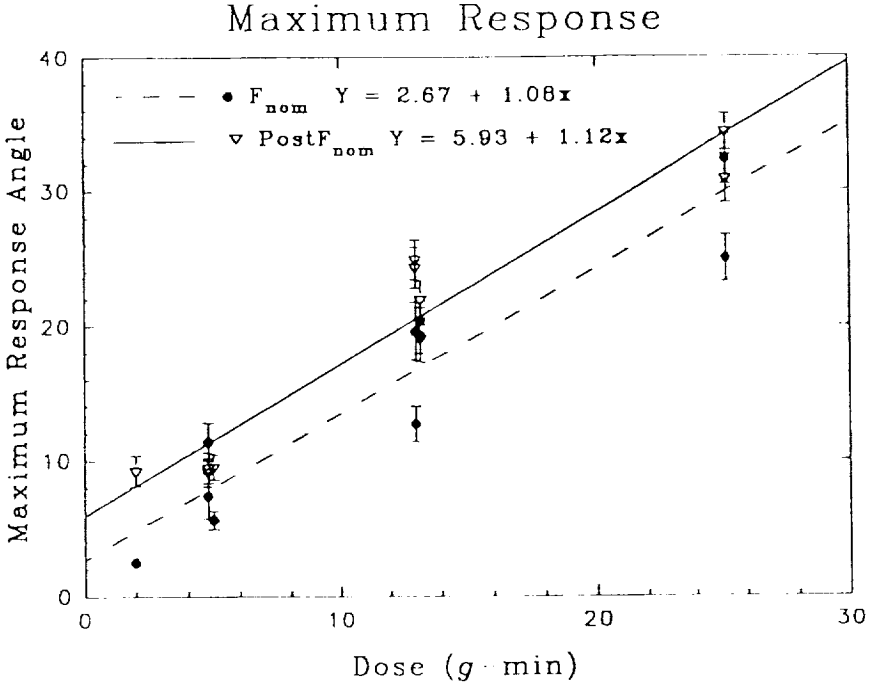

Fig. 8--Results from flight and post-flight experiments whereby the comparison is made between responses of populations of plants of different ages but nearly the same height at time of testing--*viz.* $\mathbf{F_{nom}}$ vs $\mathbf{PostF_{nom}}$. Discussion in the text.

Figure 9 shows data for Comparison V by which $\mathbf{F_{tall}}$ data differs significantly from $\mathbf{PostF_{tall}}$ data. Such a large difference was not evident when post-flight "nom" and "tall" plant data were compared (Fig. 7).

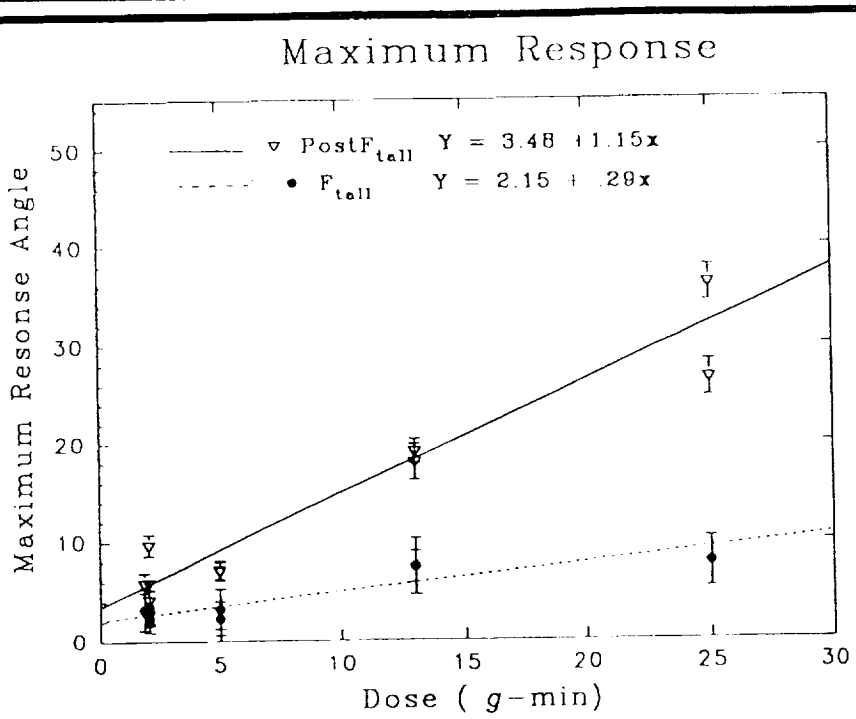

Fig. 9--Comparison of responses of flight and post-flight plants of the same height and different ages--*viz.* $\mathbf{PostF_{tall}}$ vs $\mathbf{F_{tall}}$. Discussion in the text.

For "nom" plants pre-flight ($\mathbf{PreF_{nom}}$) and post-flight ($\mathbf{PostF_{nom}}$)data were not significantly different as shown in Figure 10.

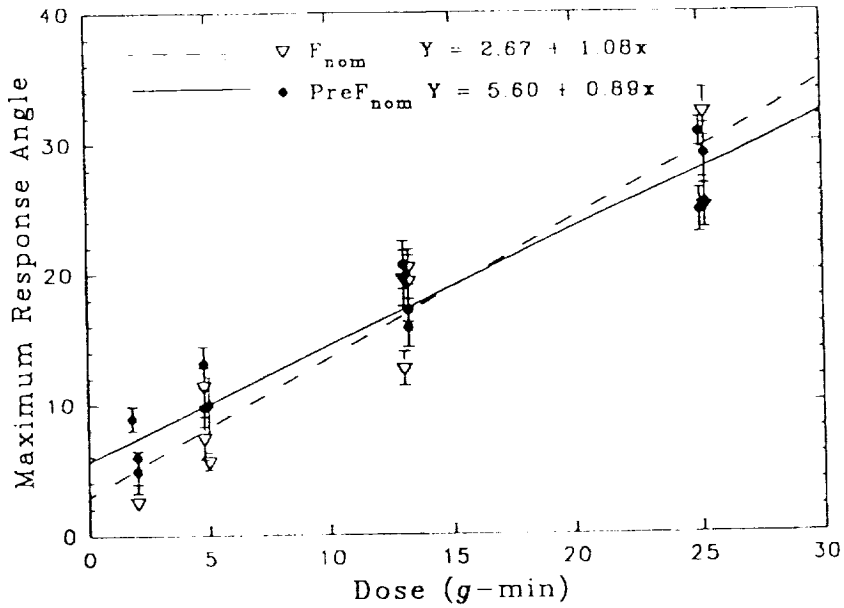

Fig. 10--Comparison of responses of flight and pre-flight plants of the same height but of different ages--*viz.* $\mathbf{F_{nom}}$ vs $\mathbf{PreF_{nom}}$. Discussion in the text.

Another way of making the comparisons is to focus on that set of dose/response data for which we acquired the most measurements which was for stimulations of 13 *g*-min. Those comparisons are shown in Figure 11 which includes 13 *g*-min data that allow us to make both Comparisons II and IV at the same time.

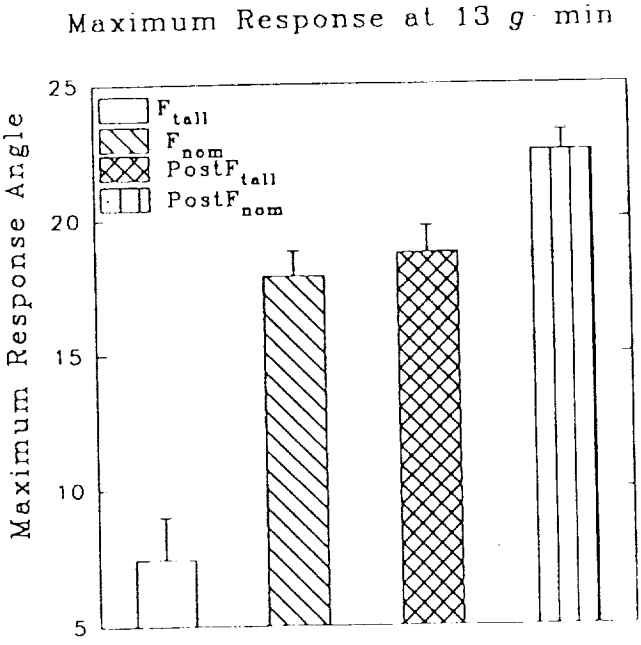

Fig. 11--Tropistic responses of flight and post-flight plants, in each case for plants of different heights. Data apply to equivalent stimulus doses (13 *g*-min). Bars show standard errors. The difference between F_{tall} plant data and the others is noteworthy and is discussed in the text.

Table III. Matrix showing the statistical significance of the differences between the regression lines (Max Response vs. Dose) of different experiments.

	F_{tall}	$PreF_{nom}$	F_{nom}	$PostF_{tall}$
F_{nom}	Fig. 6 p < 0.001	Fig. 10 NS		
$PostF_{tall}$	Fig. 9 p < 0.001	p < .01	NS	
$PostF_{nom}$	p < 0.001	Fig. 1 p < 0.05	Fig. 8 NS	Fig. 7 NS

Table III shows the statistical significance of the significance of differences between regression lines referred to above (Figs. 1, 6, 7, 8, 9, and 10) that relate tropistic responses to stimulating g-doses.

(II) Response Lags--The time from the start of a centripetal acceleration stimulus and the first detection of the plant's tropistic response was measured in flight and in Earth control experiments. The IR images were recorded at 5 min intervals which set a time resolution limit for measurements of the time for initiation of plants' responses after stimulation--also a lower limit on the precision with which the starting time of a tropistic response could be determined. Also, to avoid being misled by plants responding within the time of application of the stimulus, the only cases that were considered were those when the first post-stimulation datum was the same as the last pre-stimulation datum. Figure 12 is a plot of all such measurements we obtained on this topic. The measured time lags varied from about 10 to 35 min. There was no obvious trend relating to *g*-dose. Also there was no significant difference between flight results and Earth based results. We conclude that whatever the stimulated seedling is doing in the post-stimulation pre-response interval is not much influenced by gravity.

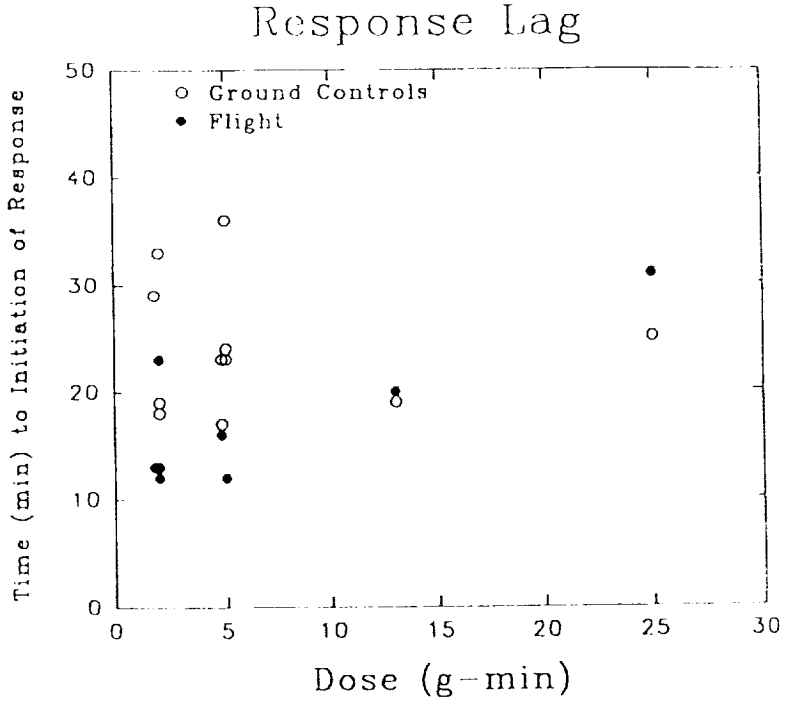

Fig. 12--Response lag, the interval between the end of the stimulus application and the start of the response, measured for different stimulation doses. Discussion in the text.

(I) Time to Attain Maximal Curvatures--We have not completed the analysis of relevant data.

(J) Spacial Localization of Curvatures--We have not finished (have not even begun) the analysis of data relevant to this topic. We have to develop a procedure for plant by plant analysies of samples of video images (which must be done carefully because, after we start the tedious job of data reduction by a chosen procedure, we surely do not want to change our minds about the methodology).

(K) Guttation--In 100% of the data image frames (87% of the plants) droplets of guttation water were observed. Analysis of droplet shape (in μg) promises to be of some interest. At the time of this writing data reduction and analyses are not yet completed.

(L) Precocious Development Syndrome--Evidence for differences in development of oat seedlings is shown in Figure 13 which displays graphically differences in development (prior to tropistic stimulations) for four sets of growth data. Plant age was defined as hours after seeds were planted in wet soil mixture.

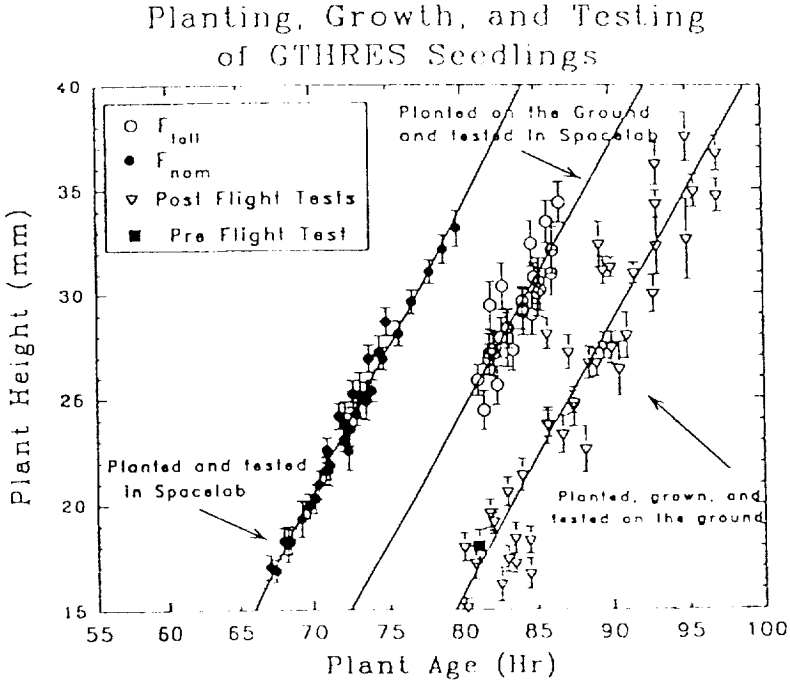

Fig. 13--Planting, Growth, and Testing of GTHRES plants. Seedling height plotted against plant age in hours. (Zero age defined as time of planting in wet soil mixture) Discussion in the text.

The populations are as follows:

(a) Flight plants that were planted on the ground and tested in Spacelab at the originally scheduled age ($\mathbf{F_{tall}}$); these plants were taller than had been expected when they were challenged with their test stimulations.

(b) Flight plants that were planted and tested in Spacelab at a younger age ($\mathbf{F_{nom}}$); these plants, although younger, were at about the same size as pre- or post-flight Earth (clinorotation) controls. Data from the Earth controls ($\mathbf{PreF_{nom}}$ and $\mathbf{PostF_{nom}}$) were nearly the same as expected.

(c) Earth control plants ($\mathbf{PreF_{nom}}$) that were planted, grown, and tested on Earth prior to launch. The regression lines all show very similar slopes (growth rates) but have quite different intercepts (times of emergence)

(d) Pre-flight test plants (that had not been monitored frequently over the time before stimulation at 81 hr). Only the one average $\mathbf{PreF_{nom}}$ point is plotted; it falls almost exactly on the regression line of the $\mathbf{PostF_{nom}}$ population (as would be expected). In addition, data comparing tropistic responses of preflight and postflight control plants ($\mathbf{PreF_{nom}}$ and $\mathbf{PostF_{nom}}$) showed that their tropistic responses also were very similar (*vide* Fig, 1).

The magnitude of the **PDS** was related to the time our test plants were growing in satellite orbit. Compared with the Earth controls which had been planted and tested preflight, the plants that were started on Earth but tested in space ($\mathbf{F_{tall}}$) appear to have germinated on average about 7.1 hr earlier than the Earth controls and the plants that had been planted and tested in Spacelab appear to have germinated on average about 12.6 hr earlier than the Earth controls. Symbolically, the emergence times show the following ordering:

$$\mathbf{F_{nom}} < \mathbf{F_{tall}} < (\mathbf{PreF_{nom}} = \mathbf{PostF_{nom}})$$

The **PDS** occurred both in oats (GTHRES) and in wheat (FOTRAN) experiments. In both species it was due almost entirely to earlier germination; shoot growth after emergence proceeded at about the same rate in all cases.

It is important to remember that **PDS** probably cannot be attributed to a mysterious effect of microgravity *per se* because, for most of the time from the initiation of germination until time for testing, all GTHRES flight plants were grown either at 1 *g* on Earth (for seeds planted prior to launch) or on 1 *g* centrifuges in space (for those

planted after launch) while awaiting their turn to be tested. That statement must be qualified by the fact that seeds planted on Earth (at different prelaunch times), and remained at 1 *g* until launch, were not put under 1 *g* centrifugation immediately after launch. They were in μ*g* for 6 hr 30 min until they were placed on the1 *g* centrifuges again at different times. At the end of those exposures to μ*g*, different batches of seedlings were at ages ranging from 25 and 50 hr after planting in moist soil medium to initiate germination. During that period the plants had begun to germinate but all were still in the pre-emergent phase. We doubt that the **PDS** could have been caused by an experience of weightlessness of such short duration (even though it occurred during the pre-emergence development stage) but we have no data to rule that out. (It could be tested only by an entirely separate flight experiment.)

We looked for **PDS** symptoms by following the preflight test scenarios because, if we could not reproduce preflight Earth-based test results, that would tell us that something about the apparatus had changed (presumably after prelaunch ground tests but before flight).

Why **PDS**?--Our initial focus was on calibration of the temperature control systems which we had not been able to recalibrated since nine months prior to IML-1 launch. If some change had occurred (preflight) and GPPF temperatures in-flight had been much higher than nominal, that might have explained the PDS. However, when we found only small departures from nominal calibration values (calibration variations mostly within spec limits and in any case much too small to account for the **PDS**), we were forced to reject out-of-spec temperature as the culprit and so far we have no other testable explanation for the **PDS**.

Not impossibly **PDS** might be reproduced by "flying" the Shuttle/Spacelab/GPPF in a realistic ground simulation--not merely a mock-up exercise as is used for crew training. However, even should that test show a **PDS**, it would not pinpoint its cause; it would only demonstrate that **PDS** was not an effect of μ*g*. The cost of attempting such an exercise would be utterly prohibitive.

Neither simple logic nor intuition allows us to assign a cause for the **PDS**. Reviews of biological literature on space flight results list some examples of individual organisms and of populations growing either faster or slower in space than on Earth (**Refs 13, 14**). Some of those observations seem quite convincing. It is tempting to attribute growth differences observed during IML-1 to an influence of the μ*g* environment. Nevertheless much of the supporting data has been unconvincing chiefly because experimental conditions could not have been all that well controlled--also because no testable theoretical explanation has been offered. In some cases the evidence also was questionable on statistical

grounds. In the more recent (and often better designed and better controlled experiments) differences in results often have been interpreted as demonstrating an inhibitory influence related to spaceflight. Any given result may well be correct but an observation of an inhibition (or stimulation) of any biological property observed in an experiment in orbit cannot be confidently attributed to weightlessness unless all other reasonable causes have been eliminated by proper controls which can be very difficult--even impossible.

At this writing we cannot absolutely rule out the possibility that the **PDS** we observed was, for some unknown reason, related to the μg condition. Our results should alert plant physiologists planning space flight experiments to consider at least the possibility of encountering a **PDS** during their projected in-flight experiments. That concern should properly influence research designers to monitor closely the development of test organisms in orbit--a feature we are very glad we were able to use during IML-1.

For an experiment in which the biological material was exposed to even slightly different habitat conditions in space and on Earth, or was not well sealed from the cabin atmosphere, or was not soft mounted so that vibration would not seriously affect it, or was not repeated often enough for the data to be statistically valid, it may not be possible to be confident that an observed zero *g* vs unit *g* difference in whatever biological property was under scrutiny was indicative of an effect of microgravity *per se*. That is especially true in those cases for which there is (as yet) no theory or even a good suggestion of a possible mechanism for predicting or explaining that effect.

We were well aware of this when we designed, fabricated and tested the flight hardware and provided support for the GTHRES experiment. We think it was a well controlled experiment. We did not anticipate that in flight there would be significantly altered rates of shoot emergence and seedling development (as a prophet once said, "you can't think of everything"). We do not as yet have a credible theoretical explanation for the **PDS** and we cannot promise that we shall ever be able to attribute it to a particular factor that was operative in orbit but not on Earth.

VI. SUMMARY

ITEM 1. Among the "nom" seedlings, all comparisons showed nearly the same responses which included those responding in μg and those responding on clinostats. Among the "tall" seedlings, two comparisons showed quite significant differences:

(a) Responses in flight were greatly different for "nom" and "tall" plants ($\mathbf{F_{nom}}$ and $\mathbf{F_{tall}}$),

(b) Tall flight plants ($\mathbf{F_{tall}}$) were greatly different from post-flight clinorotated controls ($\mathbf{PostF_{tall}}$),

(c) Tall flight plants ($\mathbf{F_{tall}}$) differed from clinorotated plants on Earth ($\mathbf{Post\ F_{nom}}$).

These data document one of the largest differences we observed between flight plants and the clinorotated controls. We conclude from these comparisons that clinorotation is not always the equivalent of μg--or, as some might have it, with tall seedlings there was a "500% clinostat effect" that was not reproduced by microgravity.

ITEM 2. We measured the threshold stimulus for gravitropic response in μg, whereby stimuli were confined to the hypogravity range of g-doses. The threshold was determined by extrapolation of the linear regression line to zero stimulus. The line intersected the ordinate at a positive response value and intersected the abscissa at -2.47 min ($\mathbf{F_{nom}}$) and -7.41. min ($\mathbf{F_{tall}}$). Since negative times are unreasonable and since we may allow for some statistical variation, we conclude that the threshold must be very close to the origin--consistent with some previous determinations by others using a different method--viz. not greater than about 15 or 20 *g*-seconds. Our result was <u>not</u> consistent with that obtained by use of another method for measuring the threshold--one that made use of clinorotation on two axes which gave threshold values of 5-6 *g*-minutes. When making comparisons between our results and those in the literature, it may be important to keep in mind that no quite comparable data exist for responses that occurred in microgravity.

ITEM 3. We were unable to confirm that the Reciprocity Rule was obeyed by oat coleoptiles responding either in microgravity or on clinostats.

ITEM 4. Circumnutations of oat coleoptiles were not observed in microgravity nor on clinostats on Earth.

ITEM 5. As far as we are aware, ours are the first report of autotropism being observed in microgravity (This applies to both GTHRES and FOTRAN results). We found that a gravity force was not a requirement for autotropism to occur in μg. For oat coleoptiles during clinorotation on Earth, autotropism did not occur or was at best only feeble. We interpret this as evidence of an inhibitory effect of clinorotation on Earth's *g* on the growth process of autotropism.

ITEM 6. Numerous comparisons were made between tropistic response data obtained in weightlessness and on clinostats on Earth. For most of those data the various comparisons showed no significant difference or only a small difference at best. However, for two such comparisons the differences were quite large. For those kinds of gravitropic responses that were compared the clinostat did not closely imitate the microgravity condition. These results advance only a little way the evaluation of clinorotation as an investigative tool. Our results do not greatly change our previous opinion of the usefulness of clinorotation experiments in plant physiology--namely: (a) Clinorotation cannot be depended upon to mimic perfectly the weightless condition; (b) Results of clinorotation experiments on Earth can be very useful as a guide for what may be expected, if and when an experimental question can be addressed by a spaceflight experiment in microgravity.

ITEM--7. The response lag (time after stimulation until beginning of response) was much the same for all tests in which plants responded in μg or under clinorotation on Earth.

ITEM--8. During IML-1 the oat seedlings exhibited a "precocious development syndrome" (**PDS**), characterized by shoot emergence up to 12.6 hr earlier than was the case with control seedlings cultured on Earth. The growth rate after emergence was not significantly different from that on Earth. At the time of testing 81 hr old plants in Spacelab they were about 1/3 taller than Earth controls.This precocious development occurred, not while the plants were in μg, but while they were growing for most of the time on 1*g* centrifuges in Spacelab prior to testing. Elaborate post mission tests with the GPPF in our home laboratory did not identify the cause of the **PDS.** However, we were able to reproduce our pre-flight ground controls and to make comparisons using older (taller) seedlings that were about the same height as the "over-achieving" flight plants. In that way for some kinds of plant responses we actually increased the number and kind of comparisons we could make that are scientifically interesting.

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