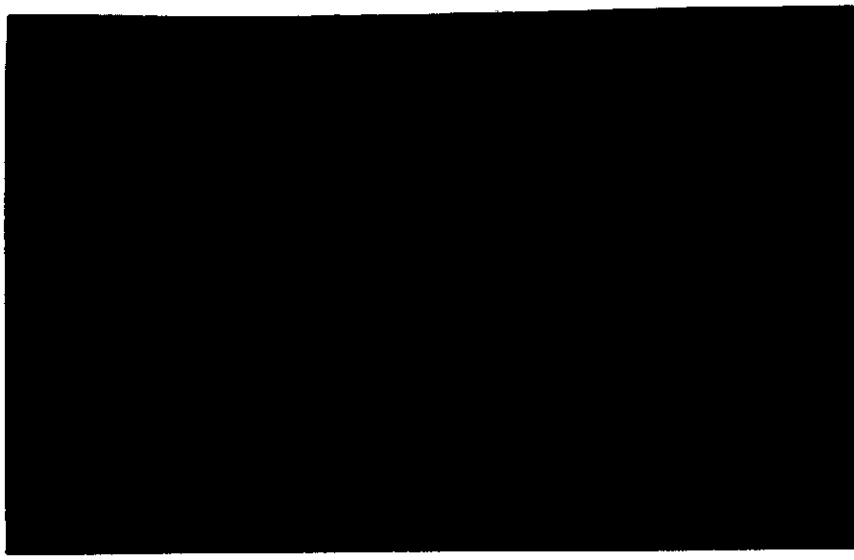

(NASA-CR-137420) RESEARCH ON THE EFFECTS OF ALTERED GRAVITY AND OTHER FACTORS ON THE GROWTH AND DEVELOPMENT OF HIGHER PLANTS Final Technical (University City Science Center) 13 p HC CSCL 06C

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FINAL TECHNICAL REPORT

Research on the Effects of Altered Gravity and Other Factors on the Growth and Development of Higher Plants

Contract No. NASW—2208

Report Prepared by: A.H. Brown

Date: 21 November 1973

CONTENTS

I. INTRODUCTION

The broad goals of this project have been: (a) to establish facilities for conducting experiments under conditions of sustained centrifugation, (b) to pursue research on the gravitational physiology of higher plants, (c) to develop experimental hardware suitable for studies of plant development in the weightless condition, and (d) to accomodate visiting investigators whose researches are of interest to the NASA Biomedical Program and who may require for some limited time, the use of a medium size centrifuge with associated facilities appropriate for plant physiological studies.

Since the termination of the present contract our project has received support from NASA grant, NGR 39-030-010. In that way the continuity of our efforts was ensured. As this is a continuing project it does not seem appropriate that we should attempt to sum up all our results in final form at this time. Accordingly this "Final Report" is more in the character of a progress report and as such it will emphasize progress we have made especially since our last Technical Status Report (1) of December 1972.

Our studies also have been supported by a contract between NASA and the University of Pennsylvania (NAS 2-2432). Research under that contract was designed to complement our efforts under the U.C.S.C. contract. The provision of two separate contracts has been justified chiefly as an administrative convenience. Some specific research tasks have been supported in part by both contracts. However, for reporting purposes we shall emphasize here those tasks which relate most directly to the establishment, maintenance, and use of the NASA-UCSC Botanical Centrifuge.

II. PROGRESS ON INDIVIDUAL RESEARCH AND DEVELOPMENT TASKS

A. Establishment of Research Facilities. The Botanical Centrifuge was installed early in 1972. However, to bring the machine to a condition of research readiness required many months. Considerable instrumentation was necessary and serious unforseen problems were encountered with the air conditioning system and with the operation of the centrifuge itself. Some of these difficulties were noted in an earlier report (1). The several problems were either solved or at least were under control and the accessory apparatus was at hand so that biological experimentation on the Centrifuge could begin on 17 May 1973.

B. Experiments on Gravitational Physiology of Higher Plants. Three tests of sustained acceleration effects were carried out prior to the termination of the subject contract. Two were designed mainly to observe the effects of chronic acceleration on the development of Arabidopsis seedlings over a range of g-levels. Both tests were 3 weeks in length. One test was initiated chiefly to examine nutational movements of sunflower hypocotyls at different g-levels. A major objective in all three tests was to evaluate equipment and procedures and to identify operational shortcomings so these could be rectified. In addition there were six different scientific tasks represented among the experiments in the three tests. (See section IV. A. for task descriptions).

The following table shows the scientific yield obtained from the biological experiments. Values less than 100% indicate partial test failures.

Expt. No.	g levels employed	Duration of test	Scientific Tasks Involved	Percent Data Yield
BCT - 1	1,5,10	21 days	GXTRAP	100%
			CNTCLN	50%
			CYTHST	50%
			NULTST	0
BCT - 2	1,8,15	21 days	GXTRAP	100%
			REVPOL	100%
			NUTFOG	0
			CYTHST	100%
BCT - 3	1,10	3 days	NUTFOG	100%
			CIRCAD	100%

Further information on the first two tests will be found in two memoranda (4,5); a similar concise summary of the third test is in preparation (6).

In these tests (and in those which have been performed since the termination of the subject contract) two or three different experimental payloads have been centrifuged simultaneously. It has not been difficult to schedule multiple purpose tests on the Centrifuge because of the relatively large payload capacity which is available and because the design of experiments and evaluation of results usually takes much less time than does the centrifugation exposure.

C. Development of Experimental Hardware for Space Flight Missions. An important but not urgent task before us is the further evaluation of the performance of the Advanced Development Hardware for the *Arabidopsis* flight experiment (coded ADHGSE). Only one biocompatibility and performance test was conducted soon after the hardware was delivered by the subcontractor (7). Lack of funds and technical assistance prevented a continuation of the testing program at that time. Making the Centrifuge research ready has consumed all the time of the available technically competent personnel who otherwise could have resumed work on ADHGSE.

III. SPECIAL PROBLEMS OF INSTRUMENTATION AND EQUIPMENT MAINTENANCE

A. Centrifuge Oil Leak. The Centrifuge main bearing is lubricated with oil of rather low viscosity. Originally the sealing gasket on the drive shaft was not tight enough to prevent unacceptable oil leakage. The subcontractor who fabricated and installed the Centrifuge attempted to correct the fault by several kinds of face seals because access to the faulty seal was extremely difficult. These attempts were unsuccessful and finally the machine was partly disassembled so that the shaft seal could be replaced. Thus the problem was corrected.

B. Air Conditioning. The system design calls for the compressor to operate continuously. Heat inputs to the system are chiefly the Centrifuge itself and a duct heater which is thermostatically controlled. The duct heater was found not to be large enough (with the Centrifuge running at low speed) to balance the cooling capacity of the refrigeration unit. The system has been improved by two expedients. Supplementary electric heaters (manually adjusted) can add more heat to the Centrifuge rotunda. Also a hot gas bypass line was installed in the refrigeration unit and this has the capability of reducing the effectiveness of cooling. The bypass is controlled by a valve whose opening can be adjusted manually. Thus, by forsaking fully automatic operation, we have been able to achieve the desired control of temperature in the Centrifuge rotunda over the full range of Centrifuge operating speeds.

C. Gas Communication to Payloads. The Centrifuge design included a rotary gas joint which can be used to communicate gas pressure to experiments in the payload cradles. So far our experiments have not required this facility. The gas pipe runs from the rotating joint element through the axis of centrifuge rotation inside the slip ring assembly. It was weakened by the threads which had been cut into the pipe in order to attach a fitting. The pipe broke at the threaded end during centrifugation. However, it was removed before the slip rings were damaged. This facility is not needed urgently but at some future time it will have to be replaced.

D. Vibration Mounting. The Centrifuge was anchored to the concrete floor at the time of installation. Communication of vibration across the Centrifuge base-floor interface proved to be excessive and undesirable. Improved vibration mounts were obtained and these were inserted between the Centrifuge base and the floor. The transmission of vibration was reduced substantially.

E. Centrifuge Protective Circuitry. Three kinds of warning or automatic protective devices are in use.

1. Bearing temperature protection device - A commercially available temperature indicator and safety power cutoff circuit was modified so that it can function as a protective circuit as well as display the Centrifuge bearing temperature. The sensor was mounted on the Centrifuge main bearing housing and the limiting temperature

is selected by the operator. In case of bearing overheating (as might results from loss of lubricant or mass asymetry) the Centrifuge will be shut down automatically and only manually can it be restarted. This protection device works as desired. It is required in order that the Centrifuge may be operated unattended for periods of time.

2. Vibration sensor and protective device - A commercially available vibration sensor with accompanying circuitry was installed on the base of the Centrifuge. The system responds to very low frequency vibrations. In the event of serious imbalance the oscillation (at the frequence of Centrifuge arm rotation) will be sensed and the Centrifuge will be shut down automatically. The sensitivity of this safety device is adjustable. The Centrifuge may be operated safely even when it is out of balance by several hundred g x lb but to reduce wear we keep the payloads balanced within 2 or 3 lb (static) or within about 50 g x lb at full speed. The protective device works as intended.

3. Power interruption - Occasional power outages occur. Usually these are very brief. The causes vary. We must accept the non-trivial probability that in any given experiment power to the laboratory may be interrupted. The Centrifuge is designed so that, should power go off, it will restart as soon as the power is restored. Some of the experimental equipment may malfunction if power is turned off even briefly. It is important that we should be made aware of such an event especially if it occurs when the Centrifuge is running unattended. At present, a warning siren (battery operated) is sounded whenever power is interrupted. It must be reset manually after power is restored. If the event occurs when the centrifuge is unattended, an indicator light establishes that fact even though the power has come on again. The duration of the power interruption can be determined from the time discrepancy shown by an electric clock.

F. Image Recording. We have not attempted to photograph events on film in cameras on board the Centrifuge although we do have this capability. Instead we have employed TV cameras and we inspect the images in real time or more frequently these images are recorded on audio tape. Instead of duplicating camera plus recording system for each of several experimental subjects under observation at the same time, we have recorded a sequence of up to three different camera images on the same tape. Our interests are almost exclusively related to time lapse records so every 10 min or 15 min or other chosen interval a programmed sequence of images is recorded. These images usually are transferred to film by using a camera to photograph the video monitor screen. By use of a tone discriminating circuit only one particular TV cameras image can be photographed in sequence since it was identified on the video tape by an audio tone at the moment of taping. For our purposes this system has been functioning satisfactorily.

G. Clinostat Drive Motors. We have constructed enclosures for certain kinds of biological test subjects (including *Arabidopsis* seedlings) which are essentially cubic boxes. Each such box is equipped with lights, a ventilating fan, a bank of clinostats geared to the same drive motor, a thermistor, and a photocell. The cubic shape permits the box to be installed in a Centrifuge payload cradle in any desired orientation. Four such Boxes were constructed locally to fit the four Centrifuge cradles. They are used routinely for experiments with Arabidopsis. In the course of experiments at the higher end of our attainable g-range we discovered that the clinostat drive motors in the Cubic Boxes would stall under g-loading. That problem was traced to the performance of the motor itself - not the clinostat array which is coupled to it. Until the problem is solved we cannot operate clinostats reliably above about 5 to 8 g. At the time of termination of the subject contract the problem had not been overcome.

H. Maintenance Provisions. Funding limitations on this project are severe. We have not found it possible to include a budget item for maintenance. Like all machines we can expect that the Centrifuge will require some repairs in the course of time. The ancillary equipment which is equally important for the successful conduct of research with the Centrifuge also cannot be expected to require zero maintenance. We have not purchased a maintenance contract from the manufacturer of the Centrifuge nor from the subcontractor who installed the air conditioning system. Both the Centrifuge and the air conditioning system components are beyond their warranty periods. We have been fortunate that repairs to equipment have not become a serious financial burden during the life of this contract. The problem of how to avoid future interruptions to our experimental program because we have failed to provide for contingencies has not been solved and has carried over into the current period of grant support.

IV. DIRECTIONS OF FUTURE RESEARCH AND DEVELOPMENT.

A. Scientific Tasks. There are 12 uncompleted tasks we now can identify as being relevant to our project which require the use of the Centrifuge to provide the appropriate g-levels demanded by experimental designs. These specific tasks are defined below. Each listing is preceeded by a code word which is our convenient local designation. Of course the alphabetical listing does not imply priority ordering.

(ADHCNT) Adapt the *Arabidopsis* Advanced Development Hardware for use on the Centrifuge.

(AMYSED) Measure amyloplast sedimentation rate as influenced by g-level.

(CIRCAD) Investigate the g-dependence of plant circadian rhythms.

(CNTCLN) Describe the morphological development of plants at different g-levels and on clinostats.

(CYTHST) Cytological and histological examination of plant materials grown under different g-conditions.

(EPIFOG) Describe epinasty as a function of prevailing g-level.

(GXTRAP) Determine g-dependent morphological end points at several g-levels and extrapolate to zero g.

(HYGCAP) With Biosatellite Pepper Plant hardware on the Centrifuge, extend the tests of Expt. P-1017 to g-levels beyond 1.

(HYGWTR) With wheat seedling hardware from Biosatellite on the Centrifuge, extend the tests of Expt. P-1096 to g-levels above 1.

(NULTST) Determine whether or not the physiology and development of plants on clinostats depend on the prevailing g-level.

(NUTFOG) Determine whether circumnutation is a function of g-level.

(REVPOL) Determine the influence of g-level on the reversal of polarity achieved by inversion of the plant axis in the g-field.

Much of the future research effort to be devoted to the above tasks will be supported mainly by a contract (NAS 2-7730) and a grant (NGR 39-010-149) to the University of Pennsylvania. Those aspects which relate specifically to the use of the U.C.S.C. facilities will be especially relevant to our continuing efforts under a grant to that institution (NGR 39-030-010).

B. <u>Accomodation of Visiting Investigators</u>. When the Centrifuge design and fabrication was authorized it was understood that the facility would be shared with extramural projects should scientists from other institutions wish to visit our laboratory for experiments which require the application of sustained acceleration to their test materials. Recently we became prepared to accomodate such guest investigators and we have prepared a short brochure which describes our facilities. Prior to the termination of the subject contract we have had one inquiry from a potential guest investigator.

V. REPORTS AND MEMORANDA

The following reports and intramural memoranda are among those generated by research on this project which have particular relevance to recent work supported by the subject contract.

(1) Brown, A.H., Technical Status Report, Contract No. NASW - 2208, 4 pp. 29 December 1972.

(2) Brown, A.H., Variability of _Arabidopsis_ 1 g A measurements of morphological end points. Intramural Memorandum, 5 pp. 23 May 1973.

(3) Brown, A.H., REVPOL observations from centrifuge test BCT-2/ 050773. Intramural Memorandum, 7 pp. 22 August 1973.

(4) Brown, A.H., Condensed summary of centrifuge test, BCT-2/ 050773. Intramural Memorandum, 5 pp. 30 August 1973.

(5) Brown, A.H., Condensed summary of centrifuge test, BCT-1/ 170573. Intramural Memorandum, 4 pp. 31 August 1973.

(6) Brown, A.H., Condensed summary of centrifuge test, BCT-3/ 280873. Intramural Memorandum (in preparation).

(7) Brown, A.H., Biocompatibility Test I — Advanced Development Hardware for _Arabidopsis_ satellite experiment, P-1003; 6-27 June 1972. Laboratory Report, 18 pp. 27 July 1972.

VI. PERSONNEL CONTRIBUTING DIRECTLY TO THIS RESEARCH AT SOME TIME DURING THE CONTRACT PERIOD

Dr. A.H. Brown, Coprincipal Investigator
Mr. D.K. Chapman, Research Specialist
Dr. A.O. Dahl, Coprincipal Investigator
Mr. W.C. Ellenbogen, Facilities Manager
Mrs. M.S. Falk, Secretary-Technician
Mr. D.E. Keyt, Engineer
Mr. S.W.W. Liu, Research Specialist
Mrs. C. McCoy, Laboratory Technician
Mr. A.L. Venditti, Electronic Shop Engineer