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TECHNICAL STATUS REPORT

RESEARCH ON GRAVITATIONAL PHYSIOLOGY

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A. GENERAL STATEMENT

The preceeding Technical Status Report on this project covered an activity period ending in February of this year. The present report covers the past 6-month interval ending 31 August 1974.

NASA support for this project is divided between two grants, one to the University of Pennsylvania and one to the University City Science Center. Although these cooperating institutions support different aspects of our work, on technical and scientific grounds it would not be appropriate to attempt to separate activities, progress, and problems which should pertain more to one grant than to the other. Accordingly, what we are reporting here relates both to grant NGR 39-010-149 and to grant NGR 39-030-010.

Our research has been on the broad topic of gravitational plant physiology. We have studied aspects of plant development (in Arabidopsis) and of behavior (in Helianthus)as these were affected by altered g-experience. We also examined the effect of increased g-levels on stem polarity (in Coleus).

B. ACCOMPLISHMENTS

1. Centrifuge Tests Completed -- During the current reporting period five centrifuge runs were completed. Table I shows relevant test parameters and experients which were on board.

TABLE I. CENTRIFUGE TESTS

Test No.	Completion Date	Duration in days	G-levels provided	Biological Materials	Research Task[a]
BCT-7	13 Mar '74	9	1;7.25;16.05	Arabidopsis	GXTRAP NULTST
				Helianthus	NUTFOG
BCT-8	28 April '74	6	1;7.5;16.6	Helianthus	NUTFOG
BCT-9	26 April '74	21	1;4;7.5	Arabidopsis	GXTRAP NULTST
			3;6.5	Helianthus	NUTFOG
BCT-10	24 May '74	9	1;4.1;9.1	Helianthus	NUTFOG
BCT-11	30 May '74	21	1;5.5;10.5	Arabidopsis	GXTRAP NULTST
			1;6.0;11.0	Helianthus	NUTFOG

[a] See Appendix I for definitions of research tasks referred to by code words in the body of the table.

The centrifuge has accumulated 5,206 hours of running time.

2. Tests on Polarity Alteration by Centrifugation (REVPOL)[a] -- Several experiments were conducted with stem cuttings of Coleus (and other species) to determine what effects centrifugation or inversion or both would have on the maintenance or reversal of polarity. These tests were preliminary and the following conclusions are only provisional.

(a) Polarity of root formation in Coleus stem sections was prominent even when the acceleration vector was imposed in the direction opposite from normal and at a level more than an order of magnitude greater than earth's gravity. There was no convincing evidence that polarity of root formation was significantly affected by centrifugation but there is as yet insufficient data to firmly, establish the absence of such an influence.

(b) Callus usually (but not always) formed at the cut ends of stem sections. Conventionally this has been assumed to be a polar phenomenon and is expected to be more prevalent on the morphologically lower end of the sections. Callus formation was found to be enhanced by inversion of the stem in the earth's normal 1 g field and also by centrifugation when the g-force was applied in the direction opposite from normal. This result was not consistent with what had been expected and, even though the same kind of result was obtained on two separate tests, no firm conclusions seems warranted without confirmation from additional experiments.

(c) Polarity of adventitious shoot formations was not as pronounced as that of root production. Results of preliminary tests have been variable and whether or not centrifugation has an affect on shoot production has not been well established.

The one confident claim that protracted centrifugation influenced polarity of new organ formation in a higher plant was that of Jones (1) who reported that inversion of pieces of Seakale root had a slight influence and centrifugation had a greater influence when continued for more than three days at 7 g. However upon recalculation of the g-force employed in those tests we discovered that Jones had made an error of more than an order

[a] See Appendix I.

of magnitude in reporting the centrifugal force which prevailed in his tests. From the information provided in his publication we determined that he probably had used 85 g instead of 7 g. Since our present centrifugation capability is limited to 20 g we are unable to reconfirm or extend the work of Jones on Seakale root.

3. Occurrence of epidermal trichomes and stomata on *Arabidopsis* leaves (CYTHST)[a] -- According to some authors (5,6) the differentiation of *Iris* leaves was modified by inversion with respect to the direction of the gravity vector. Specifically, the distribution of stomata was affected. This could be relevant to our research on *Arabidopsis* for it suggests that other g modifications such as clinostat rotation, centrifugation, or weightlessness might exert morphogenic influences on the differentiation of *Arabidopsis* leaf epidermis. We are interested especially in possible effects on trichome frequency and on number and distribution of stomata. While collecting data on various "earth controls" which may be compared at a later time with data from an *Arabidopsis* space experiment, we obtained trichome and stomata frequencies from leaves of plants grown on horizontal clinostats and those grown vertically with or without rotation about the vertical axis. From such data it should be possible to determine whether vertical rotation alone produced effects different from the results with stationary upright controls and also to determine whether any clinostat effects which were revealed should be attributed to the horizontal position or the rotatory motion or to both. These data are still in the process of being analyzed.

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[a] See Appendix I.

4. Data analysis from tests on Biosatellite Centrifuge at NASA Ames Research Center -- Three 21-day tests of the effects of chronic centrifugation were carried out on populations of *Arabidopsis thaliana* at A.R.C. In addition to 1 g the resultant g-forces tested were 2,4,6, 8,16, and 20 g. Observed endpoints included gross morphological characters such as size of certain plant organs and subcellular structures. From the data obtained it was possible in some cases to determine the g-function of specific developmental characteristics, to extrapolate those functions to 0 g for the purpose of predicting the morphology of a plant grown in satellite orbit, to describe morphological consequences of clinostat treatment, and to determine whether such effects of clinostating were influenced by the prevailing g-force.

Provisional conclusions from the data collected at A.R.C. were presented in a Laboratory Report (7). Further experimentation (on the NASA-U.C.S.C. Botanical Centrifuge in Philadelphia) will be helpful in confirming or correcting those preliminary conclusions and in extending our studies on the gravitational physiology of *Arabidopsis*.

With the completion of the report on our A.R.C. experiments (7) we have completed research task, ARCENT[a].

5. Influence of g-level on the clinostat effect -- In our preliminary studies on the A.R.C. centrifuge mentioned in the preceeding section we noted that for some plant characters the clinostat did not appear to compensate fully for the g-force since clinostated plants on the centrifuge exhibited some evidence of g-dependence. With *Arabidopsis* the hypocotyl length, the number of rosette leaves, and the mean petiole length were of particular interest in this connection. Two of the centrifuge tests carried

[a] See Appendix I.

out during the period covered by this report provided supplementary information on the degree to which clinostated plant on the centrifuge were affected by the magnitude of g. These results contributed to a research task designated locally as NULTST.[a]

Figure 1 shows results from tests BCT-9 and BCT-11 which related the average number of rosette leaves to the prevailing g-force. Figure 2 shows the evident decrease in hypocotyl length with increasing g-force. We note that the principal effect was achieved at relatively small values of g which is consistent with what had been observed in earlier experiments. Figure 3 shows the downward trend in mean petiole length with increasing g-level. All values plotted in the three figures are dimensionless ratios of measurements on plants rotating on "horizontal" clinostats compared with measurements of plants grown with their long axes parallel with the g-force vector.

6. Experiments on Helianthus seedling nutation (NUTFOG)[a] -- In our previous Technical Status Report we anticipated that a relatively limited series of experiments would be necessary to describe the effects of increased g on the nutation of sunflower hypocotyls. In theory (9) one could predict that the amplitude of nutational movement would be increased at elevated g-levels but the period of nutation would be unaffected. In our observations of 50 different plants at various g-levels from 1 to about 16 g, we have found that neither amplitude nor period were g-sensitive. Above 16 g both were increased. As yet we have no theoretical model which accounts satisfactorily for these results.

[a] See Appendix I.

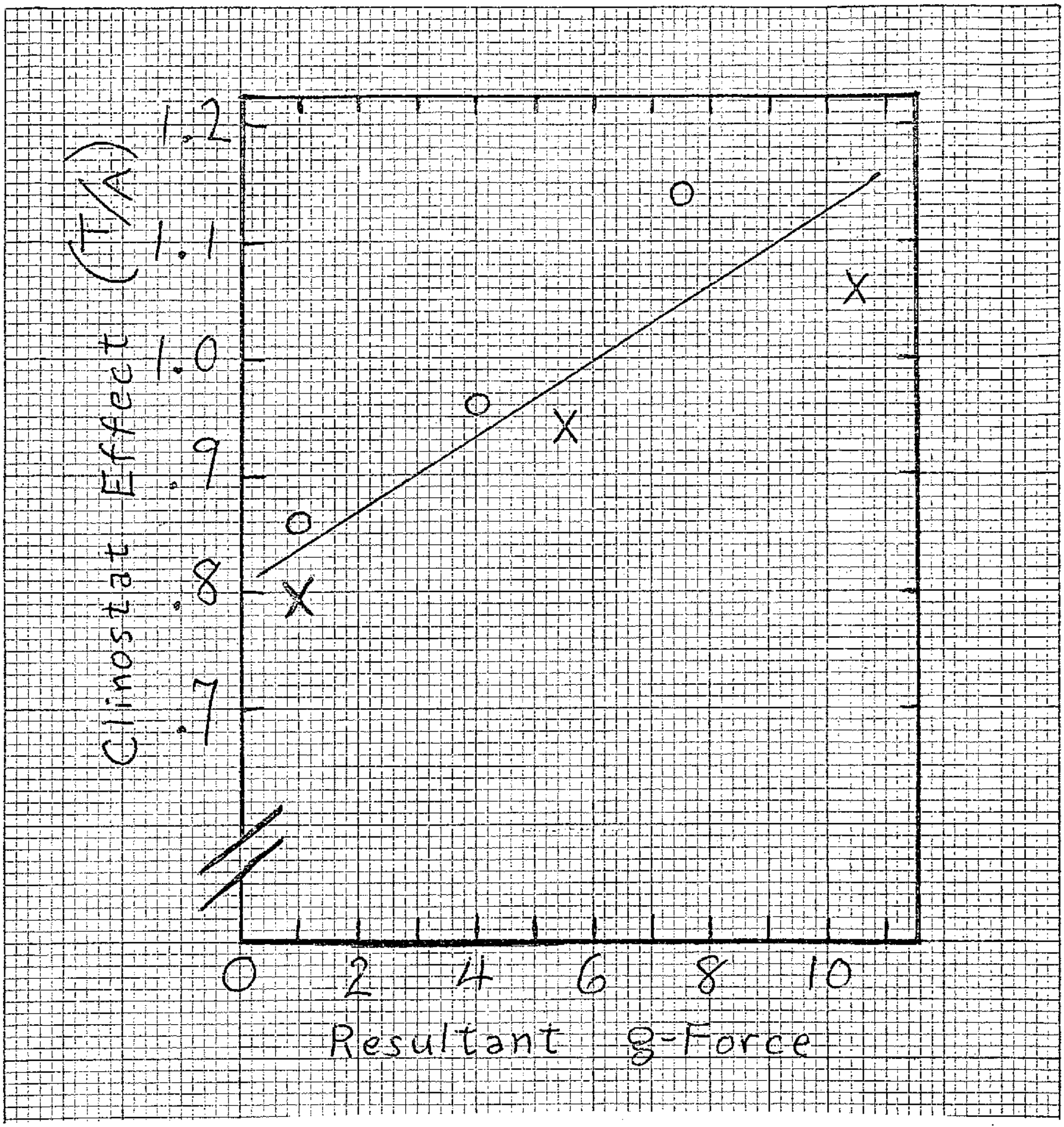

Fig. 1. Effect of the clinostat on the mean number of <u>Arabidopsis</u> rosette leaves at different acceleration levels. Ordinate: ratio of mean number of leaves for plants grown 21 days on "horizontal" clinostats (T) on board the centrifuge to mean number for plants grown with vertical axes parallel with g-force vector (A). Abscissa: chronic acceleration level in g-units. Circles designate values obtained in test BCT-9; crosses were obtained from test BCT-11.

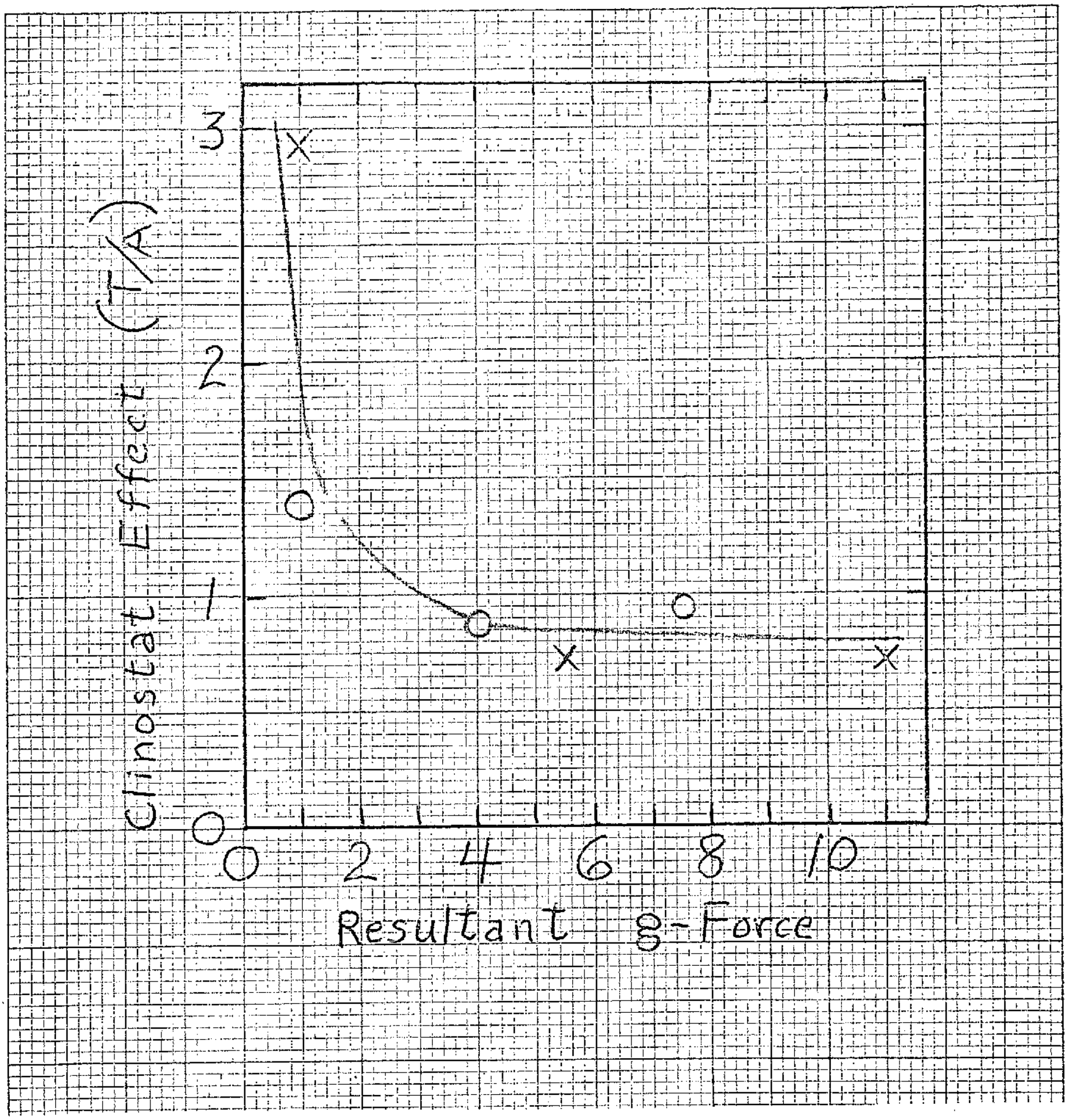

Fig. 2. Effect of the clinostat on the mean length of Arabidopsis hypocotyls at different acceleration levels. Ordinate: ratio of mean measurement for plants grown 21 days on "horizontal" clinostats (T) on board the centrifuge to mean measurement of same character for plants grown with vertical axes parallel with g-force vector (A). Abscissa: chronic acceleration level in g-units. Circles designate values obtained in test BCT-9; crosses were obtained from test BCT-11.

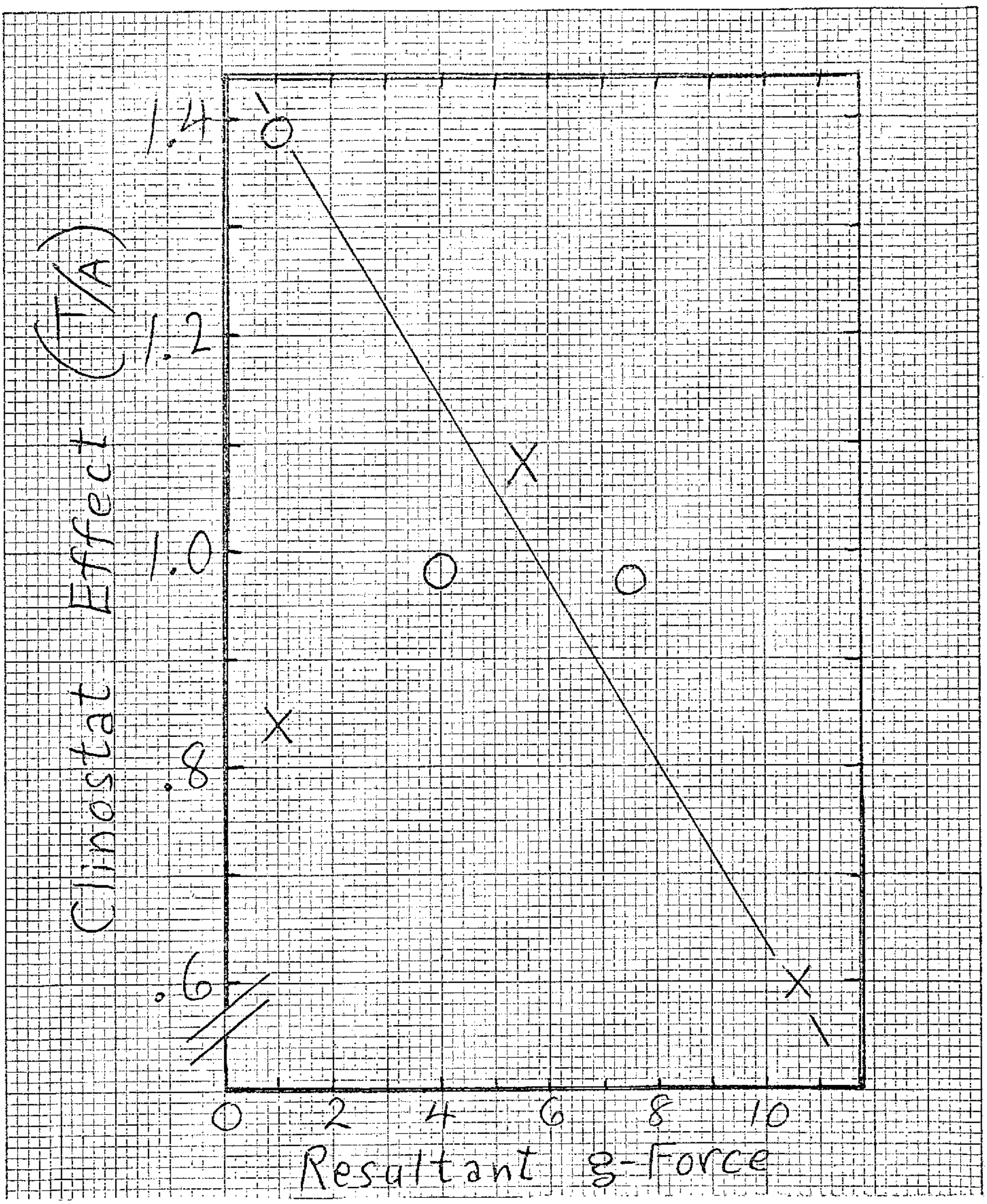

Fig. 3. Effect of the clinostat on the mean length of Arabidopsis petioles at different acceleration levels. Ordinate: ratio of mean petiole length for plants grown 21 days on "horizontal" clinostats (T) on board the centrifuge to mean petiole length for plants grown with vertical axes parallel with the g-force vector. Abscissa: chronic acceleration level in g-units. Circles designate values obtained in test BCT-9; crosses were obtained from test BCT-11.

7. Investigation of the vertical rotation effect on Arabidopsis development (VERTRO)[a] -- We have examined certain phenomena of Arabidopsis development on horizontal clinostats with upright controls either stationary or rotating about their vertical axes. In the literature there are reports that those two kinds of controls sometimes produced different effects (2,3,4) although in some cases such a difference was sought but not observed. Rather divers phenomena were involved.

The principal conclusion from the published results has been that, as vertical stationary and vertical rotating plants sometimes do (and always in a new situation might) yield different results, it seems desirable to employ both kinds of controls for comparison with the outcome of experiments on clinostated plants. However, a somewhat more interesting point is that the overall effect of exposure to clinostat conditions may be attributed partly to position (horizontal vs vertical) and partly to motion (rotation vs stationary). Although no one has proposed a very attractive theory which would account for a significant effect of rotatory motion alone, we must acknowledge that some of the data which document the putative vertical rotation effect have stood the test of rigorous statistical analysis and those results are sufficiently impressive to warrant serious consideration.

Early in our work with Arabidopsis we were not convinced by preliminary tests that vertically rotated seedlings developed differently from those which were stationary. Later results, however, caused us to question that preliminary judgement. Specifically, average leaf petiole length and also hypocotyl length may be affected appreciably by rotation of the test plants above the vertical axis. We have begun a series of more extensive tests to

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[a] See Appendix I.

acquire a larger population of results which may be expected to confirm or disprove the existence of a vertical rotation effect for Arabidopsis development.

8. Publication -- In our preceeding Technical Status Report for the period 1 Sept 1973 - 28 Feb 1974 we noted that work had been completed on task, BSATPP[a], and that a manuscript had been submitted for publication. The paper has been published (8).

[a] See Appendix I.

C. BIOLOGICAL MAINTENANCE ACTIVITIES

Seed sterilization -- We have been using *Arabidopsis* seed which we purchase from a commercial supplier.[b] Periodic tests of seed viability and or the morphology of seedling populations have persuaded us that seed may be considered useful for our experiments only up to five years after harvest and then only if proper storage conditions have prevailed. The last time our seed stock was replenished was in 1972 and we have discovered that this particular seed lot is not easily freed of viable microorganisms by our routine decontamination procedures which involve the use of 1% hydrogen peroxide applied in a standardized fashion.

By increasing the time of exposure or by using much more concentrated peroxide it has been possible to establish aseptic *Arabidopsis* cultures but such measure are contraindicated since it seems likely that they will produce morphological abnormalities in the test plants.

We have concluded that the commercial supply of *Arabidopsis* seed was very heavily contaminated with a mixed population of relatively resistent microorganisms and, since that supply is undependable, we now are undertaking to increase our seed stock locally. The cost of this seed production enterprise probably will exceed that of seed obtained from the commercial supplier but we feel we must have full control over and confidence in the quality of our seed supply.

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[b] Ferry Morse Seed Co.
Mountain View, Calif.

D. MECHANICAL AND ELECTRICAL MAINTENANCE ACTIVITIES

1. Rewiring connections to experiments on the centrifuge -- The NASA-UCSC Botanical Centrifuge has 32 slip rings which must accomodate all power and signal inputs and data outputs. A predictable development in the evolution of an experimental program using a centrifuge is that the need for slip ring communication continually increases and sooner or later exceeds the number of slip rings which are available. When we reached that point we had to choose between signal commutation and telemetry as a means of increasing our communication capacity with the moving centrifuge; we selected the former.

We took advantage of the opportunity to redesign and improve a number of aspects of the data read-out systems. The changes are essentially completed. Display and recording of on board environmental variable now is more convenient and in some cases more accurate. Also, alarm indication of non-nominal values for certain critical functions has been improved. That should facilitate trouble shooting during an experiment.

2. Environmental temperature control -- Maintenance of a prescribed temperature within narrow limits for all experimental packages riding on the centrifuge as well as in the 1 g controls has proven to be much more difficult than we had anticipated. There are several reasons for this but the problem relates primarily to two quantitatively important effects. (a) The large power consumption of the centrifuge with corresponding large refrigeration capacity necessarily introduces appreciable thermal gradients within the air recirculation system. Thus temperatures monitored at different points tend to show constant differences as they reflect the steady state gradients. (b) The experimental packages are mostly cubic Boxes within

which are lamps and other sources of heat. The Boxes are force ventilated by fans which is adequate to prevent uncontrolled temperature rise. When the centrifuge is running, however, the air within the Boxes is centrifuged toward the periphery and, depending upon Box orientation and position along the centrifuge radius, the effect may be to abet or to oppose the air stream generated by the fan. Unequal ventilation of the Boxes means unequal temperatures. For the most part these effects are not responsible for temperature differences greater than 1 centigrade degree between different Boxes, but even that is not always the case.

We are studying several possible modification in the ventilation system for the Boxes which we think may improve our ability to maintain a more nearly uniform temperature among the experimental packages.

E. PLANS FOR FUTURE EFFORTS

In the near future we expect to devote a substantial effort to each of the following activities.

1. Increasing supply of *Arabidopsis* seed. This will be carried out by our own laboratory personnel.

2. Critical evaluation of the putative vertical rotation effect on particular aspects of *Arabidopsis* development. We intend to determine how reproducible the effect may be and, if confirmed, we shall study it as a function of rotation rate. Two existing banks of variable speed clinostats should be ideal for these experiments. This task is locally identified as VERTRO.[a]

3. Continuation of our studies on nutation of the sunflower hypocotyl. (task NUTFOG)[a] We expect to complete the analysis of all our data on the effect of centrifugation on the period of nutation. We are designing centrifuge qualified apparatus for viewing test plants by a camera aimed along the plant axis and we shall examined (a) the amplitude of nutation as a function of the g-level, (b) the pattern of onset of nutational movement in very young seedlings, (c) the pattern of nutation which may develop in a plant grown on a horizontal clinostat, and (d) the pattern of nutation damping which may be expected of a nutating plant which is shifted from the vertical upright position to a horizontal clinostat. Each of these experiments has a bearing on the model (9) which was proposed by others to account for the kinetic properties of sunflower nutation.

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a See Appendix I.

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APPENDIX I. DEFINITIONS OF CODED RESEARCH TASKS

The following research tasks in gravitational plant biology are specifically relevant to this report.

1. ARCENT -- Complete the analyses of results from ARC centrifuge tests of 1967,8,9 and prepare summary report. (Task completed 31 July 1974)

2. BSATPP -- Analyze film data on petiole angle from Biosatellite II Capsicum experiment, P-1017, and prepare report. (Task completed 7 May 1974).

3. CYTHST -- Describe relevant aspects of the morphological development of Arabidopsis at the tissue and cellular levels for plants grown under different g-conditions.

4. GXTRAP -- Determine g-dependent morphological endpoints at several g-levels and extrapolate to g=0.

5. REVPOL -- Determine the influence of g-level on the reversal of polarity achieved by inversion of the plant axis inthe g-field.

6. NULTST -- Determine whether or not the results of "nullification" of g on the horizontal clinostat are in any way dependent on the prevailing g-level.

7. NUTFOG -- Determine whether nutational behavior is a function of g-level.

8. VERTRO -- Investigate the socalled "vertical rotation effect" in order to determine whether Arabidopsis seedlings develop differently when they are grown in the vertical stationary position as compared with the results of growth while being slowly rotated about the vertical axis (vertical clinostat).