EFFECTS OF PROLONGED ACCELERATION WITH OR WITHOUT CLINOSTAT ROTATION ON SEEDLINGS OF *Arabidopsis thaliana* (L.) Heynh.

31 July 1974

by: A.H. Brown, A.O. Dahl, and Lars Loercher

with the technical or administrative assistance of:

Mr. D.K. Chapman, U. of Penna.
Mr. Tom Edwards, Ames Research Center
Mr. R.P. Kuniewicz, U. of Penna.
Mrs. C. McCoy, Univ. City Science Center
Mr. P. O'Dowd, U. of Penna.
Mrs. S.L. Phelts, Univ. City Science Center
Mr. Bert Rock, Ames Research Center

ABSTRACT

Three 21-day tests of the effects of chronic centrifugation were carried out on populations of Arabidopsis thaliana at the NASA Ames Research Center. In addition to 1 g the resultant g-forces tested were: 2,4,6,8,16, and 20 g. Observed end points included gross morphological characters such as size of plant organs and, at the other extreme, features of sub-cellular structure and ultrastructure. The present report concerns only the gross morphology of test plants.

Plants were grown on banks of clinostats. The acceleration vector was directed either parallel with the plants' axes or transverse to the axes. Plant responses to chronic axial acceleration and to transverse acceleration with clinostated plants were determined. From the data obtained it was possible in some cases: (a) to determine the g-functions of specific plant developmental characters, (b) to extrapolate those functions to the hypothetical value at zero g in order to predict (tentatively) the morphology of a plant grown in space, (c) to describe morphological effects of clinostat rotation, (d) to determine which of those effects was influenced by the prevailing g-force, and (e) to put to direct test the assumption that clinostat rotation nullifies or compensates for the influence of gravity.

Results are reported as provisional since follow-on tests using centrifugation are still under way.

CONTENTS

INTRODUCTION

Under development in this laboratory is an experiment to be carried in an artificial satellite. The experiment is designed to observe the effect of weightlessness on the development of plant populations which will have grown from seed to maturity in earth orbit. How the experience of prolonged weightlessness will affect the various morphological end points of plant development cannot be confidently predicted. There are, nevertheless, certain experimental treatments (sometimes referred to as "earth-based controls") which on conceptual grounds ought to be compared with satellite conditions. These include: (a) chronic acceleration on a centrifuge at one or more levels above 1 g, (b) rotation on a horizontal clinostat at 1 g, and (c) a combination of exposure to prolonged acceleration on a centrifuge with simultaneous slow rotation by a clinostat about the test plant's longitudinal axis. Each of these treatments has its own rationale.

Plant development at g-levels above unity--Here one can examine the effect of the g-parameter above 1 g so that, for purely descriptive purposes, we shall have made g our experimental variable over a range from essentially zero (in satellite orbit) to whatever is the upper limit of our centrifugation capability -- in this case, 20 g.

In addition, if we can determine the effect of g-level on any given morphological end point over a g-range above 1 g, then extrapolation to zero g should establish one kind of prediction of what one can expect to observe under the condition of weightlessness.

Plant development on a clinostat -- It has become conventional to refer to the condition of plants grown on a horizontal clinostat as "gravity compensation" or "gravity nullification". Although this condition patently is not the same as weightlessness nevertheless, encouraged partly by the terminology itself and partly by the evident lack of recognizable geotropic response of plants on a clinostat, a number of plant physiologists have suggested that essentially all important features of development and behavior under weightlessness should duplicate those which plants exhibit on a horizontal clinostat. To test that suggestion we needed to establish quantitative measures of morphological end points for growth under socalled gravity compensation on the clinostat so as to compare them with corresponding measurements to be acquired from an experiment in earth orbit.

Moreover, the quantitative characterization of end points of plant growth on the clinostat might be compared with the extrapolated "zero g" predictions from experiments on board the centrifuge as described above.

Gravity nullification -- If rotation on a horizontal clinostat effectively nullifies the influence of gravity on plant development all morphological end points of plant populations grown on clinostats should be independent of the magnitude of the force vector. Therefore, with clinostats mounted on a centrifuge in such a way that the resultant acceleration vector lies normal to the axis of clinostat rotation, we should be able to determine whether the developmental process was really

independent of the g-level to which clinostated test plant populations were exposed. If the effect of g-force can be nullified by rotation on the clinostat, as has been claimed, plant responses should be the same over a range of different g-forces.

The experiments reported here supplied information applicable to each of the three objectives described above. The test plant species was the same as that selected for the space flight experiment.

MATERIALS AND METHODS

Our experimental organism was Arabidopsis thaliana (L.) Heynh., a member of the Cruciferae (mustard family). This plant has no widely recognized common name although it is sometimes called European wall cress. Grown aseptically on nutrient agar under our culture conditions the life cycle of this species is about 3 weeks. Of particular advantage is the absence of seed dormancy and the remarkably good germination which can approach 100%. The embryo within the seed is not well developed in advance of germination so that fundamental processes of shoot development (apex differentiation, formation of foliar primordia, and floral initiation) are maximally subjected to environmental influences which prevail during ontogeny.

Seeds used in these tests were from the same seed harvest. The seed lot was stored at 2° C until used. Periodic germination tests confirmed seed viability for up to 6 years. No evidence of seed lot deterioration was observed over the period in which these tests were conducted.

The test plants were grown in pyrex glass modules (Corning #6900, "cloud and pour test jar") about 36mm diameter x 130mm tall. Approximately 20 ml. of nutrient agar medium was added to each module. The medium was prepared as follows:

Ingredient	g/liter dist. H_2O in stock solution	used per liter of medium
A. $Ca(NO_3)_2 \cdot 4H_2O$	72.0 g	6.7 ml
B. $MgSO_4 \cdot 7H_2O$	18.9 g	6.7 ml
C. NH_4NO_3	12.0 g	6.7 ml
D. Glutamic acid		20 mg
E. Complex solution		6.7 ml
1. KNO_3	12.6 g	
2. KCL	6.4 g	
3. K_2HPO_4	12.6 g	
F. Micronutrient solution		1.0 ml
1. H_3BO_3	2.86 g	
2. $MnSO_4$	1.80 g	
3. $ZnCl_2$	0.22 g	
4. $CuSO_4$	0.08 g	
5. $(NH_4)_6Mo_7O_{24}$	0.08 g	
G. Complex solution		1.0 ml
1. $Fe_2(SO_4)_3$	3.26 g	
2. EDTA	.78 g	
H. Distilled water		to make 1 liter
I. Adjust pH with .1 normal HCL to pH 6.1		
J. Add Sucrose (Reagent Grade)		20 gms
K. Agar (Difco, Bacto-Agar) (thus, giving a medium of 1% agar)		10 gms

Seeds were surface sterilized before planting by the following procedure.

Dry seeds taken from storage at 4^{o} C were brought to a hood ventilated with sterile air as noted below. The seeds were placed in a sterile beaker and were wet with .5 ml 95% ethanol in distilled water. Immediately, 4.5 ml of 1.0% hydrogen peroxide was added. After 3.0 minutes the peroxide solution was decanted and the seeds were rinsed three times with 8 ml sterile distilled water.

One seed was placed in each module near the center of the agar surface. The open end of the module was then covered with a sheet of sterile Saran Wrap (0.5 mil.) which was pressed tightly over the module rim and retained by rubber bands. These manipulations were performed within a ventilated hood equipped with a 0.3 micrometer filter (Microvoid, model 11-750-70A, Air Control Inc., Narberth, Pa.) which proved adequate to insure a negligible incidence of contamination.

The Saran Wrap seal carefully applied was essentially impermeable to water. According to manufacturer's data, the diffusional transmission rates of water vapor through Saran Wrap filters should amount to about 0.5 mg. per day per cm^2 of film area in the case of 0.5 mil. film thickness. This nominal transmission rate was confirmed experimentally. Autoclaved Saran Wrap was found to be slightly more permeable but it, as well as native Saran Wrap film, gave very low water vapor transmission rates not markedly different from values predicted from the nominal permeability value. Very roughly, water loss from a planted module by diffusion through the film seal was about one mg. per day. In practice such a loss rate was negligible. The fact that rates of water loss

were not substantially greater than could be accounted for by diffusion through the film was evidence that the film adhered closely to the glass permitting no sensible passage of water vapor between the glass module wall and the adpressed film.

A satisfactory method of culturing Arabidopsis heterotrophically in darkness was not discovered. Our test plants were grown under 150 f.c. illumination from GroLux/Wide Spectrum fluorescent lamps (Sylvania).

Because the "Daylight" or "Cool White" fluorescent lamps in general use have low outputs in the far red spectral region, it has been common practice when growing plants under fluorescent lights to provide supplementary tungsten illumination. The "standard" GroLux fluroescent bulbs widely recommended as an illumination source for plant culture also have inadequate emission in the far red. However, GroLux/Wide Spectrum lamps have sufficient far red output so that supplemental tungsten illumination was not required for normal development of Arabidopsis.

Commencing with an early stage of seed germination Arabidopsis development proved to be light dependent. The specification of 150 f.c. was established empirically. It represents a compromise. Growth is healthy--even vigorous--yet, as shown in Figure 1, the 150 f.c. values lies on the steep part of a light intensity vs growth response curve. Much higher light intensities which might saturate the plants' growth response appeared to offer no definite advantages. Larger plants and a greater heat load associated with higher incident illumination would have been disadvantageous.

Light intensity was measured either by a G.E. Photographic Exposure Meter (Model 213) or by a Clairex photoconductive cell (CL 605) referred by

appropriate calibration to the G.E. 213 Meter. The Exposure Meter was itself calibrated at the G.E. Standards Laboratory. G.E. Exposure Meter readings were in foot candle units. However, since the relevant comparisons were made with a GroLux Wide Spectrum fluorescent light source, designated foot candle values were relative. An absolute calibration was achieved by measurement of total radiation using a YSI-Kettering Radiometer, Model 65. When the distance between the probe and the GroLux Wide Spectrum light source was varied to provide 150 f.c. on the probe, the total radiation received was $6.1 \pm 0.5 \times 10^3$ erg cm^{-2} sec^{-1}.

Choice of an experimental temperature also was a compromise. Under our culture conditions satisfactory growth obtained at temperatures between 16° C and 28° C. The character of the plants and particularly the rate at which they developed were temperature dependent throughout this range. Above 28°C growth was unsatisfactory. Chlorosis, etiolation, poor germination, and stunting of mature plants were the most prominent pathological symptoms. As Figure 2 shows, development of plant organs tended to proceed more rapidly the higher the temperature within the range where plants were considered normal and useful for experimental purposes. At the lower portion of the acceptable range of temperatures growth proceeded slowly and the time required for test plants to mature was undesirably extended. Avoiding either extreme, we selected 24° C as the design temperature for our tests.

From Figure 2 it is determined that mature leaf length, for instance, increases with temperature at the rate of 3% per degree. Thus, it was important to maintain the plant temperature within about $\pm 1^{\circ}$ C in order to reduce temperature dependent variation well below the biological variation

expected among sets of replicate experiments (i.e., statistical "noise").

Accelerations above one g were obtained using a centrifuge of 8.8 meter maximal radius located at the NASA Ames Research Center, Mountain View, California. This apparatus, capable of continuous operation at least for several weeks, provided mounting space on two arms or rotating platforms ample for a pair of test plant assemblies anchored side by side (at the same radius). At different distances from the center of rotation (between about 2 meters and 8.5 meters radius) several pairs of test plant assemblies could be secured. As many as three radial locations were employed simultaneously in some tests. The conservative design limit on the speed of this centrifuge corresponded to a force of 20 g at the ends of the arms. This set the upper limit of centrifugal force applied in our chronic acceleration experiments.

The plant test assemblies were either on a swinging platform or were secured at a fixed angle to the plane of centrifuge rotation calculated so that the resultant of gravitational and centrifugal forces would be appropriately aligned with the axes of the test plants for the chosen centrifuge rotation rate. In this report all g levels designated as greater than unity were vector quantities equated to the resultant of the two forces mentioned.

In each chronic centrifugation experiment the rotation was continuous with probably minor exceptions when the centrifuge was stopped briefly for technical reasons. When such stoppages occurred within a few hours of initiation of a test the results three weeks later could hardly be influenced by such an experience of the recently planted seeds. In one experiment

the centrifuge had to be stopped for a few minutes on two occasions near the end of a three week test. Here too we feel that a detectable influence on plant development must have been extremely unlikely.

In this connection it should be noted that although the developmental consequences of brief interruptions of chronic stress ought not to be significant, the reverse, one or more brief periods of high stress interjected into a regimen of essentially zero stress, could serve as an environmental signal providing directional information. This could enable the test organism to orient its development as it otherwise might not do or it might function as a trigger setting off a developmental sequence which otherwise would have proceeded differently or not at all.

In our experiments we were limited to the range 1 to 20 g and we attempted to cover this range in a series of three tests with plant populations subjected to 1, 2, 4, 6, 8, 16, and 20 g. Except for g=1 these were nominal values but the excellent speed regulation of the centrifuge made for quite negligible g-error from speed fluctuations. The rotation rate was constant over the entire duration of the experiments within $\pm$ 1.5%. The length of the centrifuge arms was great enough so that g-error from lack of precision in placement of the test plants along the radius was below $\pm$ 1%.

During each chronic centrifugation test our experiments were monitored for the following environmental variables:

1. Ambient temperature of air in the centrifuge rotunda.
2. Air temperature within the test plant assemblies (measured by Fenwall UUA 41J1 thermisters).
3. Incident light intensity on the plant modules (measured by Clairex CL605 photoconductive cells).

4. Centrifuge speed (Checked manually by counting revolutions using a stopwatch).

5. Performance of special apparatus (e.g., cameras, clinostats, etc.) as required.

Monitoring was accomplished routinely and recorded by the operator on duty at 30 minute intervals throughout the 3 week tests. Electrical access to the measuring instruments on board the centrifuge was through slip rings in each case.

We were concerned with the possibility that our test plants might be receiving vibrational inputs during centrifuge operation which could influence their development. Therefore, a test * was carried out using calibrated Statham Accelerometers mounted on all 3 axes near the end of one arm of the centrifuge. Vibration at about 50 cps was detected when the centrifuge was not operating; this amounted to approximately 0.012 g (peak-to-peak). This could have originated from heavy machinery operating at a remote location, the vibration being transmitted through the earth and the centrifuge mountings. In operation at peripheral g levels of 5 and also 10 g (in separate tests) the radial vibration was measured at 0.075 g, the vertical component was 0.036 g, and the horizontal component (in plane of rotation transverse to the centrifuge radius) was 0.016 g. While we have no proof that vibrational inputs of the magnitude observed (all below 10^{-1} g) are without effect on plant development neither have we reason from theory or experiment to anticipate that our test plants were significantly affected by apparatus vibration. For what it is worth, we may gain

---

Footnote: * We are grateful to Mr. Bert Rock for conducting this test.

consolation from the observation that the chronic g stresses deliberately imposed by our experiments were from 25 to 250 times greater than the highest vibrational levels (peak-to-peak) measured in the same units.

The plant test assemblies were chambers approximately 60 cm. X 60 cm. X 90 cm. At the top of each chamber was a bank of GroLux Wide Spectrum lamps 2 ft. long. * An exhaust fan (Sentinel 747, Rotron Mfg. Co., Woodstock, N.Y.) was located in the top of each unit to insure rapid air turnover and thus to avoid a significant temperature rise within the unit. Each inner wall was painted white to several decimeters below the lights and below that black for the remainder of the panel. This was to make light intensity on the plants more uniform across the floor area of the unit and was arrived at by trial and error. Edge effects were minimal over the 160 cm^2 area. on which 24 plant modules were arrayed. The incident light intensity over this area was 150 f.c. $\pm$ 6 f.c. in terms of our method of measurement as described above.

Each plant module was inserted into a holder or cup attached to the end of a shaft. The 24 shafts were geared together and driven by an electric motor at 0.5 rpm. The apparatus therefore was essentially a bank of clinostats, all turning together, with the longitudinal axes of the plants within the modules all parallel. By mounting the unit on the centrifuge at an appropriate angle to the horizontal it could be arranged that the resultant force vector was either parallel with the plant axes

---

Footnote: * We are indebted to Mr. C.C. Mpelkas, Sylvania Electric Products, Inc., for expediting the fabrication of these lamps in non-standard lengths for our experiments.

(designated "A") or was at 90° thereto (designated "T"). Consequently, during chronic centrifugation the g-force was imposed on the growing plants in either the longitudinal (axial) or the transverse direction.

When the acceleration vector was aligned transverse to the plant axis, viz. category "T", (as for a "horizontal clinostat") the individual plants were always rotated by the drive motor at 0.5 rpm. The "vertical" control could be similarly rotated or not depending on what was considered most appropriate. This choice was unintentionally simplified by the performance of the clinostat drive trains which unfortunately were unable to operate in "A" orientation for long periods at $g > 4$. This shortcoming was imposed by an inadequacy of clinostat design which had not been corrected at the time of these tests. Therefore, except at 1, 2 and 4 g, plant modules in the "A" position were not rotated for most of the duration of the chronic acceleration tests. In all cases plants in the "T" orientation were rotated at 0.5 rpm. during the entire course of their development.

The individual clinostats in each bank of 24--i.e., within a given unit--were geared together in such a way that some rotated clockwise, others counter clockwise. Some of our results strongly suggested that plant root growth was influenced by the direction of clinostat rotation and this quite unexpected indication would not have been evident had all clinostats rotated in the same direction.

At the conclusion of three weeks of growth on the centrifuge the plants were observed and/or chemically fixed for subsequent cytological examination as rapidly as possible. Fixation was performed in most cases manually by

quickly flooding each plant module with one of several cytological fixative solutions. The time which elapsed between stopping the centrifuge and chemical fixation of subpopulations (replicates) was recorded to about ± 10 sec. Generally, all plants were fixed from 5 to 40 minutes after stopping the centrifuge.

In one test (at 20 g) introduction of fixative into several modules was accomplished automatically while the centrifuge was rotating. Therefore, fixation of the plants was accomplished during exposure to the chronic g stress. Apparatus * used to accomplish this consisted of a movable assembly containing a set of three fluid resevoirs each communicating to a hypodermic needle. The apparatus was positioned so that upon activation the assembly swung over the array of plant modules and plunged the needles through the Saran Wrap covers of three juxtaposed modules. Movement was achieved by a piston driven by Freon pressure which was released through a solenoid valve by remote command. As the needles reached the end of their travel another valve was opened automatically and the contents of the fluid resevoirs were driven by Freon pressure through the needles and into the plant modules.

Operation of this remote fixation device was tested for reliability in preliminary test runs on the centrifuge at several g levels. Performance was monitored by observation with a TV camera on board the centrifuge. With only minor adjustments for speed and distance of movement this apparatus functioned as satisfactorily at 20 g as it did at 1 g. In the one critical

---

Footnote: * The remote fixation apparatus was designed to our specifications by Mr. D.E. Keyt and was fabricated by General Technical Services, Inc., in Upper Darby, Pa.

application it remained in its "cocked" position during 21 days of continuous centrifugation at 20 g and then functioned effectively by fixing three mature plant specimens with three different cytological fixatives after which the centrifuge was decelerated.

Time lapse photography was used to observe a few selected plants during their growth on the centrifuge. Bolex or Milliken cameras modified to operate in time lapse mode were employed. Up to three cameras were operated on the centrifuge during an experiment. Only one plant (out of 24 replicates) in the array on a clinostat unit was positioned in view of a camera. The plant image filled about $\frac{1}{2}$ of the frame of 16 mm Ektachrome type 7241 movie film.

The purpose of the time lapse photography was to record the growth kinetics of plants growing under centrifugation since observations made at the end of an experiment could reveal only the end points of growth processes.

Whenever the film records warranted systematic analysis a Vanguard Motion Analyser was used to reduce coordinates on successive frames of the film of representative plant parts to a series of numerical values which could be further processed by a computer.

Camera operations had to be synchronized with rotation of the clinostats and this was accomplished either by microswitches activated by cams attached to a moving part of the clinostat rotor assembly or by magnetically activated reed switches triggered (without mechanical contact) by small magnets riding on clinostat rotors.

The frame rate in all tests was designed to be 0.17 exposure min^{-1}. Several mechanical difficulties unfortunately limited the value of the time

lapse photographic records; nevertheless enough data was obtained by this means to indicate similar rates of plant development under chronic centrifugation up to 20 g and under the status (1 g) condition.

A uniform temperature for our experiments was provided by thermal regulation of the ambient air in the rotunda which housed the centrifuge. Control was barely adequate for the design requirement of 24 $\pm$ 1° C. Ventilation of each chamber unit on the centrifuge was sufficient to avoid more than a 0.2 to 0.3 degree temperature rise within the chambers.

Morphological end points of development which were evaluated in our test populations of Arabidopsis included gross form characters, internal anatomy, and cytological details. It was of interest to know for control populations how much variability characterized these morphological measurements. For gross characters such as leaf blade length or width the coefficient of variability * usually was about 35%; for mature flower stem length in the early stages of bolting 50% was not uncommon. Thus, our ability to attribute significance to a difference between measurements of populations of 24 plants grown under different g-levels varied considerably with the character considered. To put it another way, for a given type of measurement a difference between experimental and control means could be judged significant (at the 5% level) if the difference between means was 10 to 15% in some cases but only if it was 25 or 30% in other cases. Whenever critical comparisons were warranted a t test was employed to determine what significance could be assigned to observed differences.

Footnote: * coefficient of variability = 100 x standard deviation / mean

III. RESULTS

This report is limited to the consideration of gross morphological end points obtained in three separate tests. Within a particular test all plants in all treatments were grown simultaneously but the three tests were separated by one year intervals.

Tables I, II, and III contain the means and standard errors of all gross morphological measurements made on 21-day old plants grown at several g-levels as indicated.

Within any one test-treatment the plant-to-plant reproducibility was good but for the same kind of treatment reproducibility between different tests was somewhat less satisfactory. An overview of the situation may be seen in Figure 3 which displays morphological data profiles for plant populations grown at 1 g in the three separate tests. This method of plotting makes it apparent that leaf characters were less variable from one test to the next than was the hypocotyl length or the flowering stem height.

A. Leaf characters -- The growth habit of A. thaliana involves the production of from 5 to 7 rosette leaves before the flowering stem begins its rapid elongation. The flowering stem bears bracts which are morphologically similar to but distinct from rosette leaves. Leaf size and leaf shape varied with leaf number. Leaf length in the mature plant generally increased acropetally as shown in Table IV. Also the effect of gravity compensation by the clinostat was more pronounced with the youngest leaves--i.e., those which developed when the seedling as a whole (rather than the particular leaf)

had been exposed for longer times to clinostat rotation.

By focusing, for example, only on leaf no. 5, we have a highly uniform organ for comparison. As Figure 4 shows, over the range from 1 to 20 g there seems to be no g-dependence. (It is unlikely that the high point at g=2 could be statistically significant.)

Combined measurements for all rosette leaves (Figure 5) confirmed the evidence obtained from fifth leaf measurements--there was no indication of dependence on the g-level which prevailed during seedling development.

A similar independence of g was observed for the mean leaf width (Figure 6) and for the mean number of rosette leaves (Figure 7) when those characters were examined over the range, 1 to 20 g.

Taken together these results on leaf characters revealed no significant g-dependence and, if leaf number or leaf development is influenced by g within the range tested, such effects must be very slight--surely less than 5% of the "controls" at 1 g.

B. Hypocotyl length -- The length of the mature hypocotyl of A. thaliana seems to be relatively more sensitive than most other morphological characters to slight differences in conditions from one experiment to the next. That was suggested by the finding that variation in hypocotyl length was small within any one test treatment but the differences between separate tests often were rather larger than had been expected. A consistent trend in hypocotyl length with increasing g was not supported by our test results.

Growth of seedlings on horizontal clinostats at 1 g usually evoked a substantial increase in hypocotyl length.

C. Flowering stem length -- The length of the flowering stem (essentially, plant height) proved to vary even more than did hypocotyl length from one test to the next. Measurements spanned a 3-fold range and, if clinostat grown plants are included, the range in plant height was 60-fold. Oddly enough, variation within a given test treatment was generally small. A consistent trend with increasing g-level could not be identified from our test results.

The lengths of flowering stems of clinostat grown plants always were less than that of the respective upright controls.

IV. DISCUSSION

In addition to a straight forward determination of the g-function of leaf ontogeny our results provided information on the following questions relevant to the gravitational physiology of plant organ development.

1. From data obtained over a range of g-values greater than unity extrapolation to zero-g might furnish a prediction of the value to be expected for development under weightlessness; what are those extrapolated values for each character examined?
2. How does exposure to the horizontal clinostat affect development and is there a clinostat effect which is dependent on the prevailing g-level?
3. If the clinostat should in fact achieve gravity compensation or nullification, it should do so regardless of the chronic g-force to which clinostated plants may be exposed. How effective was gravity compensation at the various g-levels tested?

Considering especially the leaf characters, in answer to the first question we may suggest from the nearly zero slope of the g-functions depicated in Figures 5,6, and 7 that weightlessness should yield values for length, width, and number of leaves which would not differ significantly from those grown at 1 g. That prediction of course could be tested only by a space experiment.

It is of further interest to compare the zero-g predictions, obtained by extrapolation, with the values obtained using horizontal clinostats which some authorities have suggested can simulate the condition of weightlessness.

With leaf number (Figure 7) the agreement was good. With leaf width (Figure6) the agreement was poor; extrapolation gave a value 12% greater than what was attained with the horizontal clinostat. For leaf length a still greater disparity was observed; extrapolation yielded a value about 29% greater than that found with the clinostat.

In some cases the difference between clinostated and control plants was influenced by g; in other cases not. We may define a "clinostat effect" as the ratio of a measurement made using the horizontal clinostat divided by the measurement of the same character when the seedling was growing upright. In terms of our local convention growth on a clinostat with the force vector acting transverse to the axis of plant and clinostat is called "T" and, if the force vector acts axially or parallel with the plant axis, it is called "A". Thus the T/A measurements ratio is a quantitative expression of the clinostat effect. (A ratio of unity of course indicates no effect.)

For mechanical reasons we were unable to obtain data on clinostat rotated plants at chronic acceleration levels above about 8 g. However, in the range from 1 to 8 g our results demonstrated that, for some characters, the T/A ratio or clinostat effect was indeed g-dependent.

Figure 8 shows that the influence of g was not the same for all characters studied. The clinostat effects for the several characters differed with respect to magnitude or sign. Moreover, the g-functions of the clinostat effects apparently could be distinctively nonlinear. For leaf length (Figure 8) the point scatter was large and it was not possible to determine whether the T/A value was significantly g-dependent.

With leaf number the clinostat effect was progressively enhanced with increases in g-level. With hypocotyl length the clinostat effect always observed at 1 g was abolished at elevated g-levels. The flowering stem length showed a T/A ratio consistently below unity with an abrupt drop at 8 g. (However, the 8-g point was based on only one experiment.) These examples serve to illustrate that the clinostat effect on organ development often was even more strongly influenced by the g-force than were the direct measurements of morphological characters. Thus, in answer to the second of the above questions, for some plant characters there was a g-dependent clinostat effect but that was not true for all characters studied.

Our results furnished only a preliminary answer to the third question --Was gravity nullification by the clinostat independent of the g-level? Since we obtained measurements of morphological end points on clinostated plants which were undergoing chronic centrifugation we could determine whether the imposed centrifugal acceleration influenced the plants' growth responses. If gravity compensation was complete, the results should have been the same at all g-levels employed in our tests.

For certain morphological characters no g-dependence was observed with clinostated plants although in some cases the point scatter was such that no definitive conclusion would be justified. With other characters the results were not so equivocal. Figure 9 , for example, displays evident effects of the g-force on three plant characters. In plotting these data the ordinate values were normalized by the respective values obtained with horizontal clinostats at 1 g.

It may be noted that the upward trend in leaf number with increasing g-force is established by data from only one experiment. The same applies to the downward trend in flowering stem length. On the other hand, the effect on hypocotyl length was established much more convincingly since all experiments yielded values below that for unity g, which was precisely established by many experiments. The data shown in Figure 9 may cast suspicion on the effectiveness of the clinostat as a gravity compensator but further experimentation with clinostated plants on a centrifuge will be required to decide the matter.

V. CONCLUSIONS AND RECOMMENDATIONS

A. The gross morphology of Arabidopsis thaliana was not strongly influenced by continuous centrifugation from seed germination to maturity in the range from 1 to 20 g.

B. Rotation on horizontal clinostats at 1 g had a pronounced effect on leaf length, plant height, number of leaves, and length of hypocotyl. Other characters were not influenced consistently.

C. Plants grown on clinostats possibly might resemble those grown under weightlessness. Another way of predicting the morphology of a plant grown in the weightless condition would be to extrapolate the g-functions of various morphological characters back to zero g. For some characters those two methods of predicting the form of plants grown under weightlessness yielded predictions which were not in agreement.

D. Some of the effects of growth on rotating clinostats were altered when the clinostated plants were exposed to chronic centrifugation; the most extreme case of a g-dependent clinostat effect was on the length of the hypocotyl.

E. It would be useful to examine the effects of growth under weightlessness and to compare the results with predicted values of plant characters.

F. The variability of our test results was attributable only in part to biological variation (which, of course, is subject to statistical control). Variation in the results from one test to the next in some instances was evidently caused by unrecognized (therefore uncontrolled) differences in test conditions. Accordingly, the significance of some

of the morphological differences or lack of difference which we observed were not as well established as would be desirable. Further experimentation with even more tightly controlled test conditions will be needed to confirm or correct our provisional conclusions.

G. Further tests should emphasize a more precise determination of the extrapolated g-functions which could be used for predicting the morphological consequences of growth in a satellite laboratory.

H. Further tests should attempt to resolve the question of how adequately a clinostat compensates for the effects of the prevailing g-force.

I. The pace of acquiring scientific results in centrifugation experiments should be increased. The tests on which this report was based were carried out during summers of three separate years. To acquire enough data to establish conclusively whether many of our preliminary findings are statistically valid will require many additional experiments. To accomplish this in a reasonable time will call for a program of more frequent centrifuge test runs.*

Footnote: * This already is being implemented. The 3 tests reported here were carried out over a 25 month period at the NASA Ames Research Center. Subsequently, a botanical centrifuge was installed in the authors' laboratories to facilitate continuation of these researches. In the first 13 months of operation 11 separate tests have been carried out. Those results will be described in a separate report.

TABLE I

Morphological End Points of Arabidopsis thaliana Determined in Test # 1. Tabular values are mean measurements ± S.E.

A. Resultant force, 1.0 g

Plant Character	Orientation of Plants	
	Upright (A)	Clinostat (T)
Total leaf length (mm)	10.81 ± .50	8.44 ± .65
Petiole length (mm)	5.59 ± .30	4.28 ± .39
Blade length (mm)	5.22 ± .24	4.17 ± .29
Blade width (mm)	2.99 ± .09	2.45 ± .14
No. of leaves	5.89 ± .11	5.20 ± .15
Hypocotyl length (mm)	4.44 ± .27	6.72 ± .25
Flower stem length (mm)	55.2 ± 4.2	37.3 ± 7.5

B. Resultant force, 2.0 g

Plant Character	Orientation of Plants	
	Upright (A)	Clinostat (T)
Total leaf length (mm)	10.89 ± .52	6.27 ± .26
Petiole length (mm)	6.09 ± .31	2.77 ± .17
Blade length (mm)	4.80 ± .25	3.59 ± .11
Blade width (mm)	2.81 ± .08	2.57 ± .07
No. of leaves	5.78 ± .15	5.22 ± .15
Hypocotyl length (mm)	5.78 ± .46	5.61 ± .42
Flower stem length (mm)	58.9 ± 4.9	36.8 ± 4.8

C. Resultant force, 4.0 g

Plant Character	Orientation of Plants	
	Upright (A)	Clinostat (T)
Total leaf length (mm)	10.29 ± .49	9.74 ± .40
Petiole length (mm)	5.38 ± .26	5.01 ± .21
Blade length (mm)	4.92 ± .26	4.72 ± .23
Blade width (mm)	2.75 ± .08	2.98 ± .06
No. of leaves	5.60 ± .16	5.25 ± .16
Hypocotyl length (mm)	4.25 ± .21	4.75 ± .37
Flower stem length (mm)	58.1 ± 2.3	36.1 ± 4.4

TABLE II.

Morphological End Points of Arabidopsis thaliana Determined in Test # 2. Tabular values are mean measurements ± S.E.

A. Resultant force, 1.0 g

Plant Character	Orientation of Plants	
	Upright (A)	Clinostat (T)
Total leaf length (mm)	9.54 ± .25	7.80 ± .25
Petiole length (mm)	5.04 ± .16	3.99 ± .16
Blade length (mm)	4.50 ± .12	3.81 ± .11
Blade width (mm)	2.93 ± .05	2.50 ± .06
No. of leaves	6.67 ± .27	5.87 ± .27
Hypocotyl length (mm)	3.22 ± .16	4.98 ± .29
Flower stem length (mm)	22.8 ± 2.7	16.1 ± 2.8

B. Resultant force, 8.0 g

Plant Character	Orientation of Plants	
	"Upright" (A)	Clinostat (T)
Total leaf length (mm)	9.88 ± .28	8.27 ± .43
Petiole length (mm)	5.31 ± .18	4.40 ± .27
Blade length (mm)	4.53 ± .14	3.87 ± .18
Blade width (mm)	2.72 ± .06	2.75 ± .12
No. of leaves	5.43 ± .20	7.89 ± .20
Hypocotyl length (mm)	4.71 ± .35	3.97 ± .23
Flower stem length (mm)	36.7 ± 3.7	3.03 ± .53

C. Resultant force, 16.0 g

Plant Character	Orientation of Plants
	"Upright" (A)
Total leaf length (mm)	9.46 ± .30
Petiole length (mm)	5.13 ± .18
Blade length (mm)	4.33 ± .14
Blade width (mm)	2.76 ± .06
No. of leaves	6.28 ± .22
Hypocotyl length (mm)	4.19 ± .22
Flower stem length (mm)	40.3 ± 10.7

TABLE III.

Morphological End Points of *Arabidopsis thaliana* Determined in Test # 3. Tabular values are mean measurements ± S.E.

A. Resultant force, 1.0 g

Plant Character	Orientation of Plants
	Upright (A)
Total leaf length (mm)	10.40 ± .29
Petiole length (mm)	5.64 ± .17
Blade length (mm)	4.76 ± .15
Blade width (mm)	2.68 ± .06
No. of leaves	5.29 ± .13
Hypocotyl length (mm)	5.38 ± .44
Flower stem length (mm)	61.25 ± 3.97

B. Resultant force, 6.0 g

Plant Character	Orientation of Plants	
	"Upright" (A)	Clinostat (T)
Total leaf length (mm)	10.19 ± .29	7.92 ± .26
Petiole length (mm)	5.72 ± .19	4.05 ± .15
Blade length (mm)	4.47 ± .14	3.87 ± .12
Blade width (mm)	2.55 ± .06	2.51 ± .07
No. of leaves	5.14 ± .14	5.46 ± .14
Hypocotyl length (mm)	7.07 ± .57	6.27 ± .34
Flower stem length (mm)	50.96 ± 5.90	30.58 ± 1.66

C. Resultant force, 20.0 g

Plant Character	Orientation of Plants
	"Upright" (A)
Total leaf length (mm)	10.31 ± .29
Petiole length (mm)	5.81 ± .18
Blade length (mm)	4.50 ± .14
Blade width (mm)	2.64 ± .07
No. of leaves	5.08 ± .14
Hypocotyl length (mm)	6.29 ± .64
Flower stem length (mm)	59.94 ± 5.22

TABLE IV.

Measurements of length of rosette leaf populations in Test # 1.

Data are arranged by leaf number and prevailing g-force. Tabular entries are mean measurements ± 1 S.E. Leaf numbers were assigned in order of their appearance or their position along the axis in the acropetal direction. All plants grown on clinostats. The symbol, A, refers to treatments in which the acceleration vector was parallel with the plant (and clinostat) axis. The symbol, T, refers to treatments in which the acceleration vector was transverse to those axes.

g-level	Leaf number				
	1	2	3	4	5
1 g A	8.3 ± .5	8.2 ± .5	11.2 ± .7	12.3 ± .8	14.1 ± 1.2
1 g T	7.2 ± .5	7.1 ± .5	9.2 ± 1.6	9.5 ± 1.8	9.3 ± 2.2
2 g A	7.9 ± .2	7.9 ± .1	11.3 ± .4	12.6 ± .7	14.8 ± 1.0
2 g T	6.1 ± .2	6.3 ± .2	6.2 ± .7	6.6 ± .7	6.1 ± .9
4 g A	8.0 ± .3	8.0 ± .2	10.7 ± .6	12.2 ± .8	12.9 ± 1.2
4 g T	7.7 ± .1	8.0 ± .3	10.3 ± .5	11.1 ± .4	11.6 ± .5

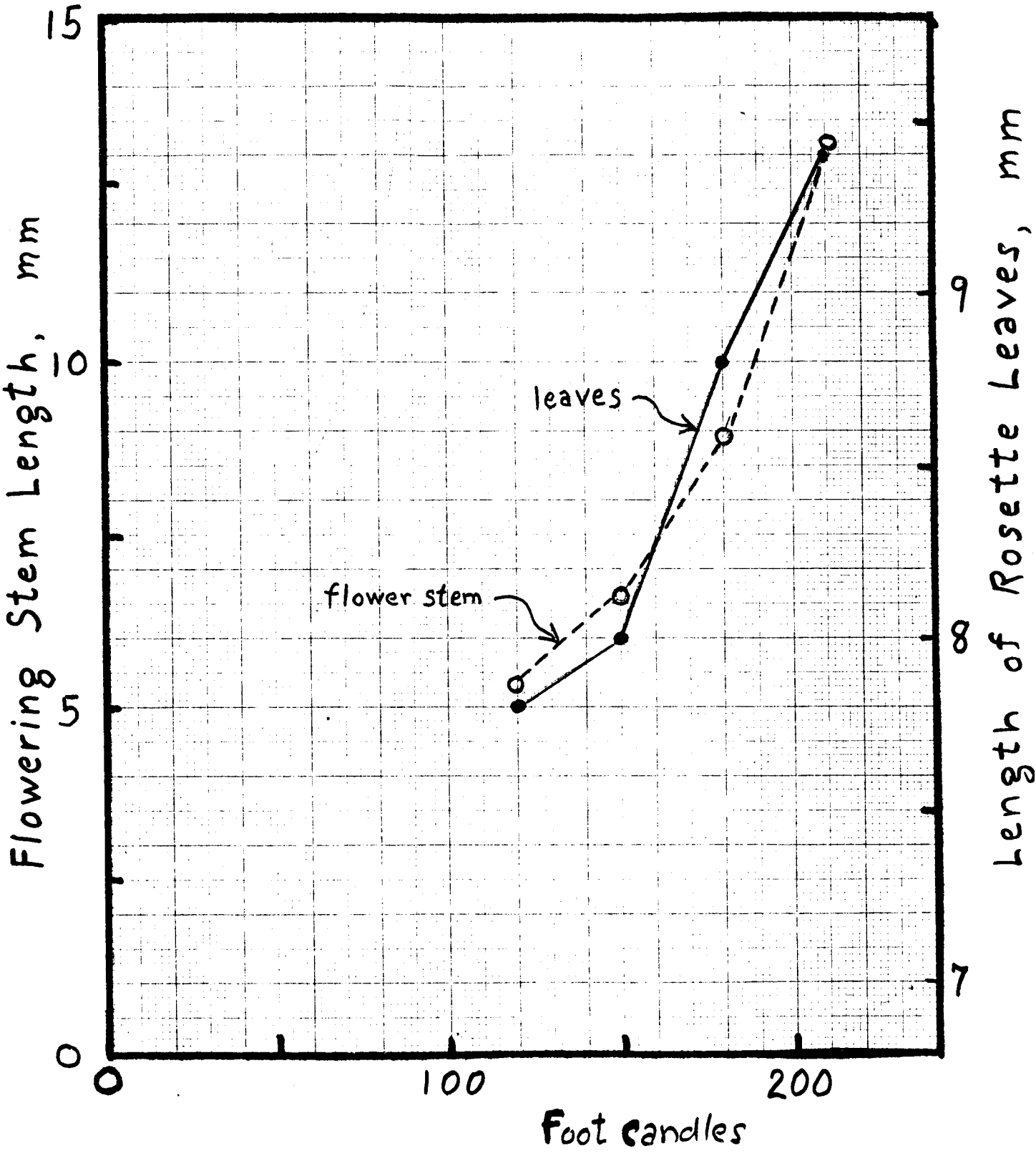

Fig. 1. Relation between average leaf length or flowering stem length of 16 day old Arabidopsis seedlings and light intensity.

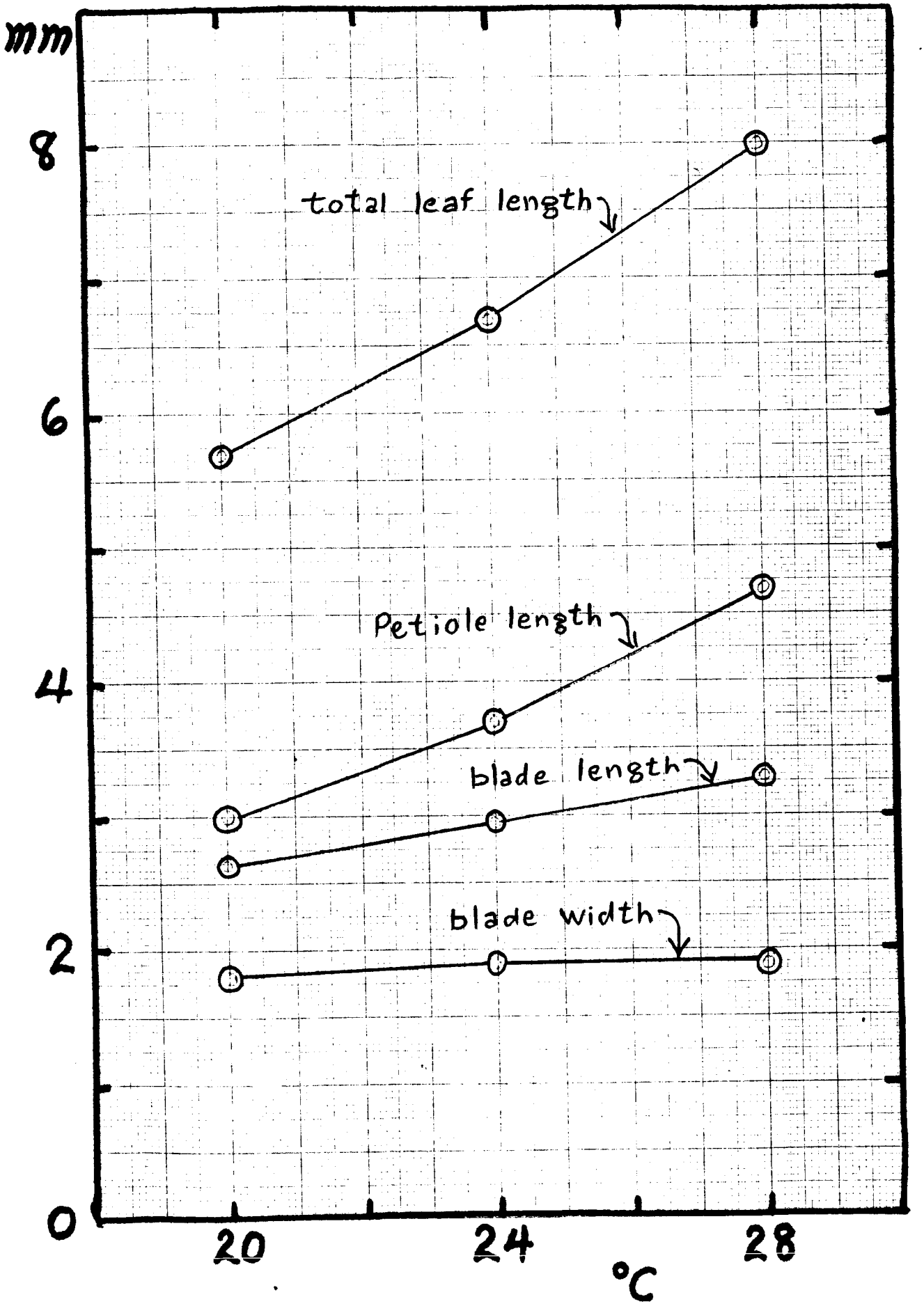

Fig. 2. Development of 21-day old rosette leaves in relation to temperature. Plotted points represent averages from six separate tests.

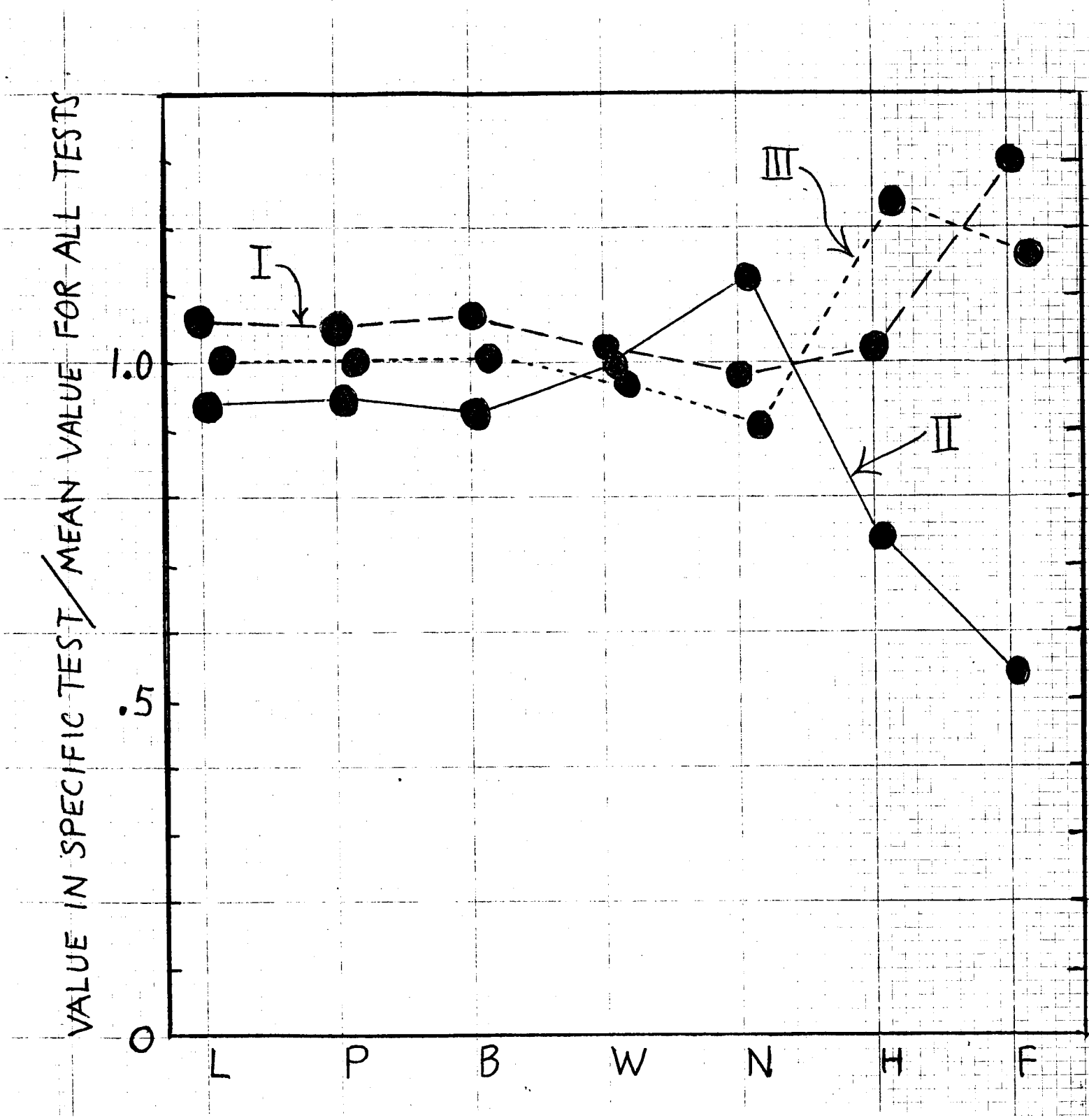

Fig. 3. Morphological "profiles" of 16-day old Arabidopsis seedlings grown at 1 g. At the bottom of the chart is an array of 7 characters: L = leaf length; P = petiole length; B = blade length; W = leaf width; N = number of rosette leaves; H = hypocotyl length; F = flowering stem length. Mean data points from each of three tests, conducted at different times, are connected to produce three superimposed "profiles". Values are normalized to the respective means of all three tests.

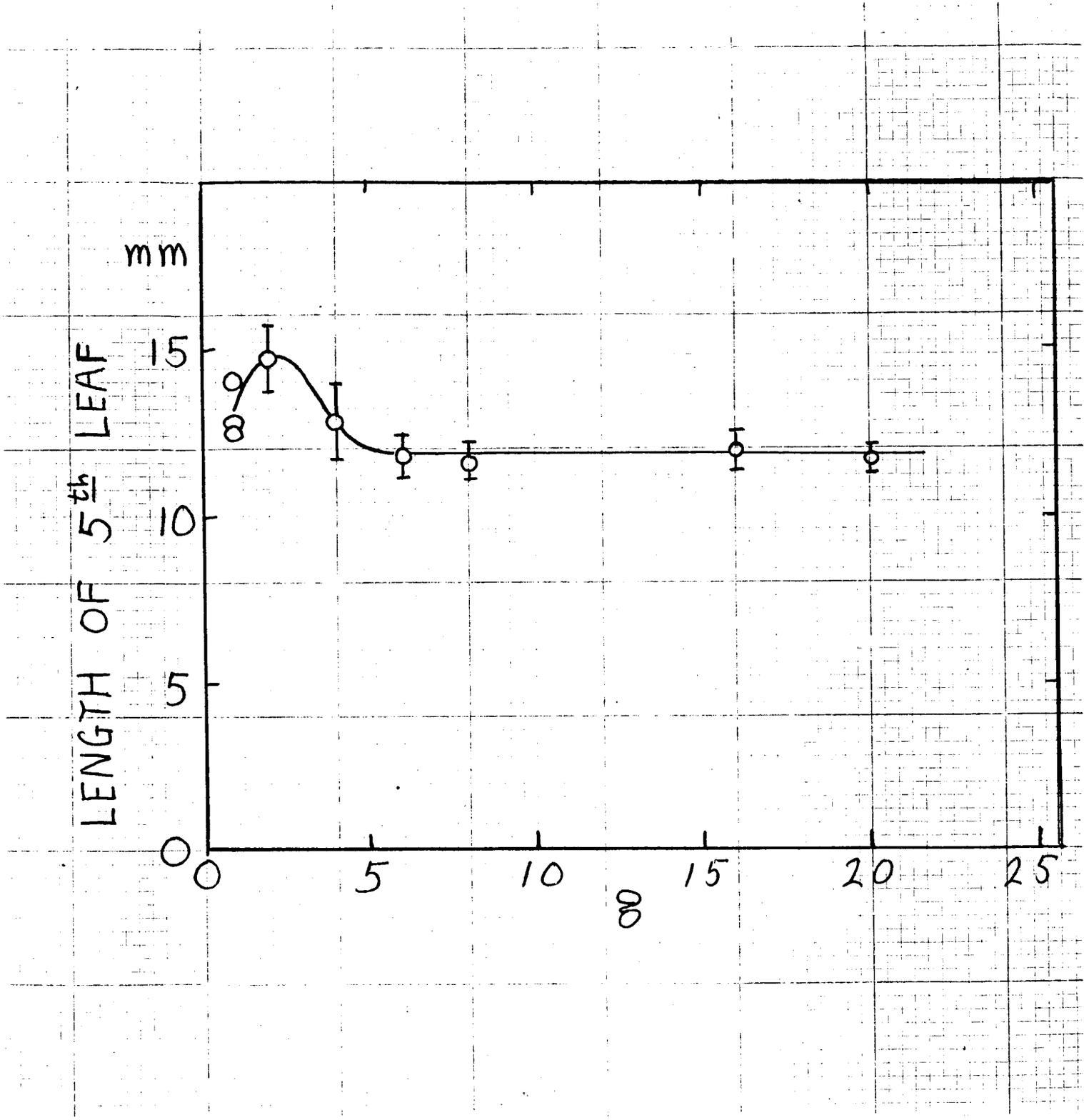

Fig. 4. Length of fifth rosette leaf in relation to prevailing g-force. Plotted points are averages; error bars represent ± 1 S.E. Data from three separate experiments.

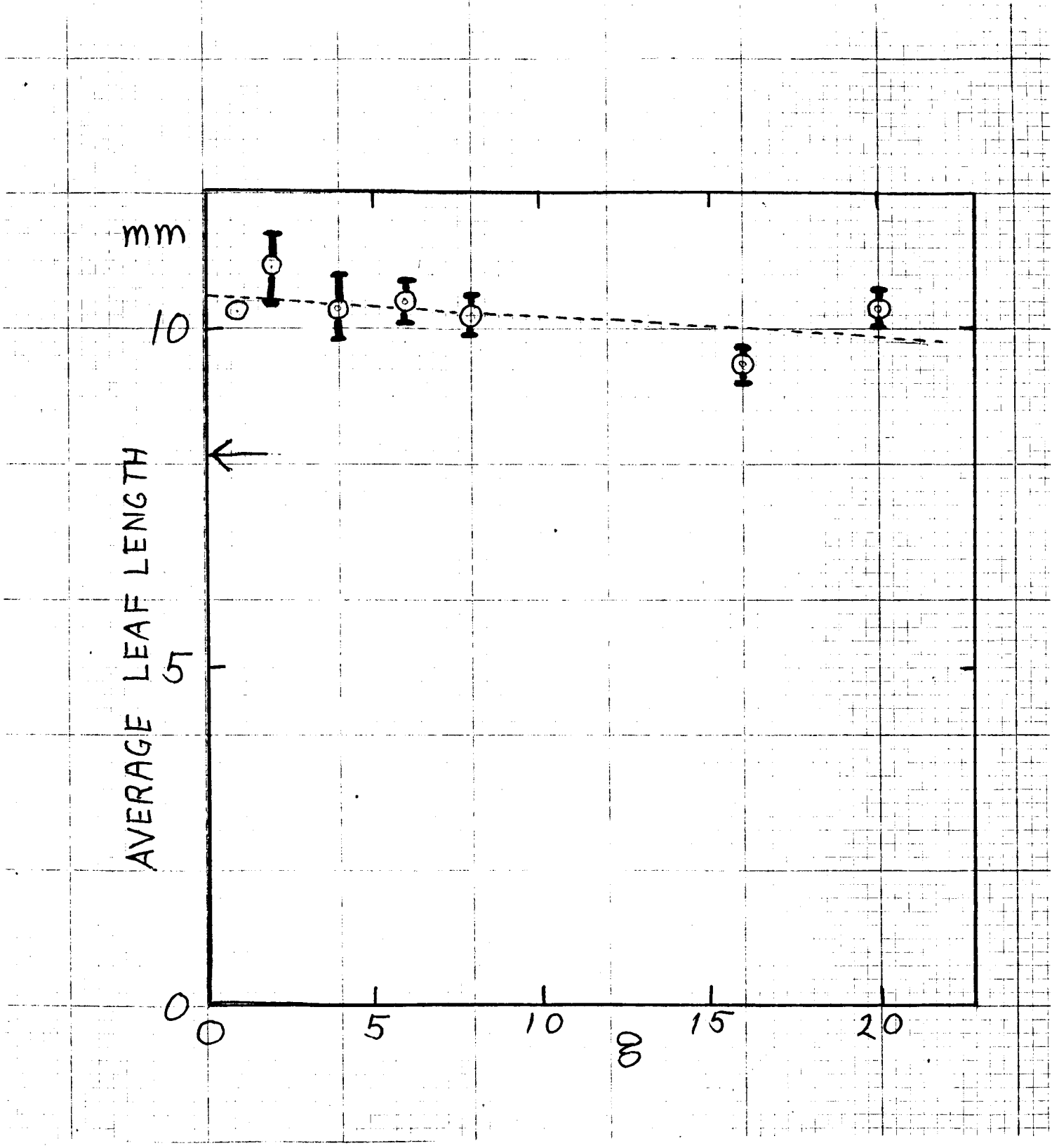

Fig. 5. The g-function of average leaf length. Plotted points are mean values and error bars show ± 1 S.E. which were measured on plant populations grown on the centrifuge at the indicated chronic g-levels. The arrow on the ordinate scale marks the mean value of leaf length in plants grown on a horizontal clinostat at unit g.

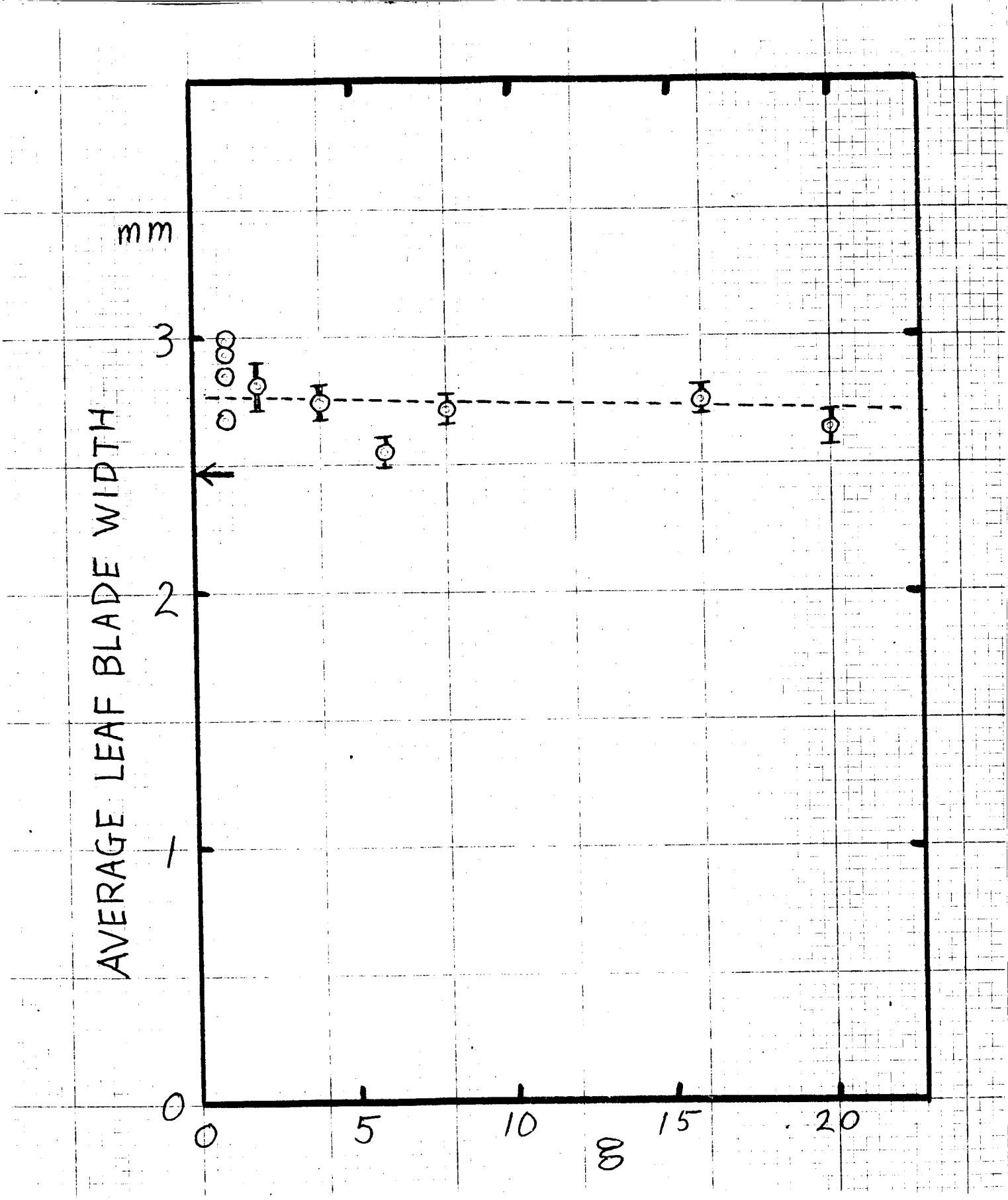

Fig. 6. The g-function of leaf blade width. Plotted points are mean values and error bars shown ± 1 S.E. measured on plant populations grown on the centrifuge at indicated chronic g-levels. The arrow marks the mean value of blade width in plants grown on a horizontal clinostat at unit g.

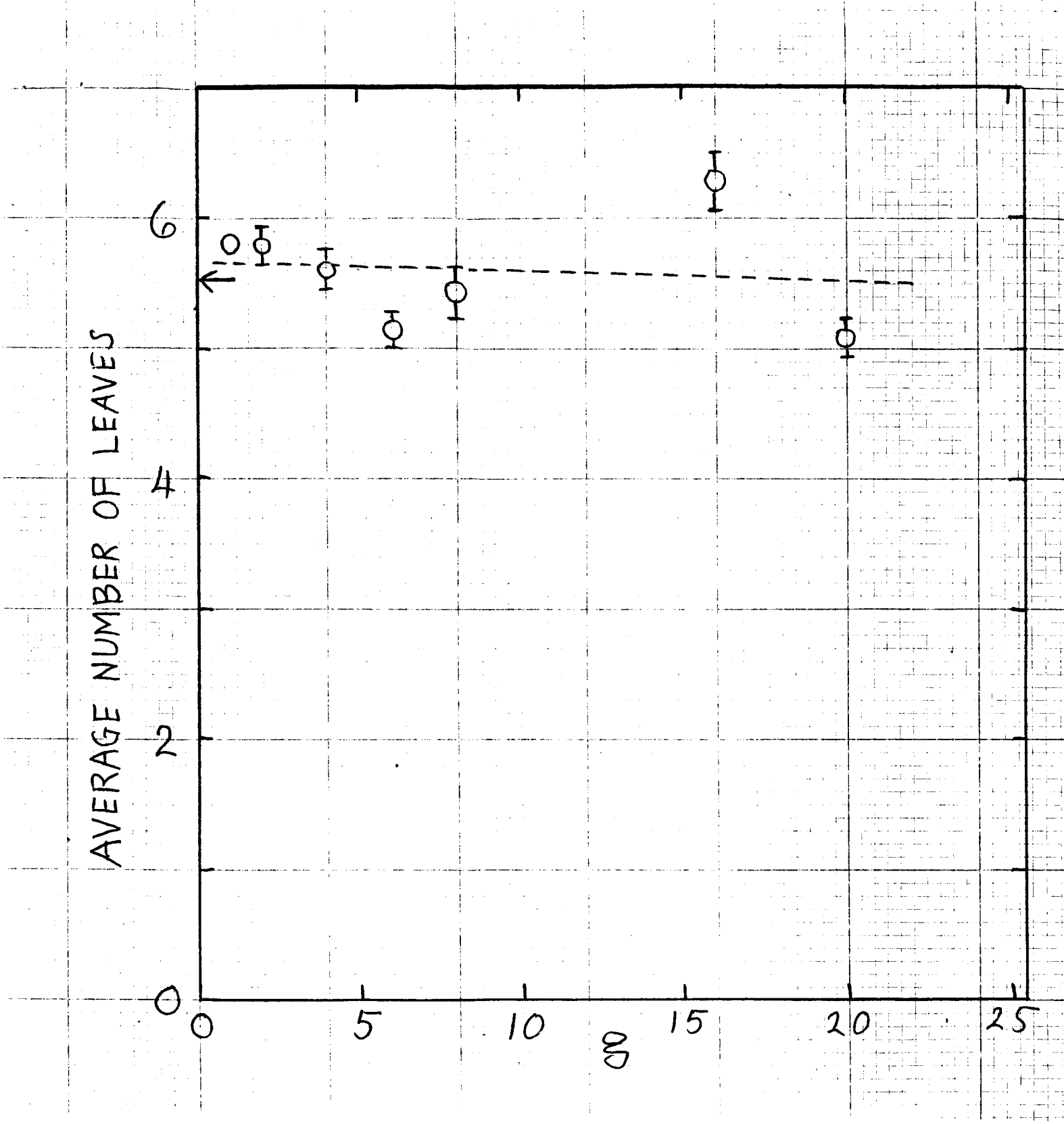

Fig. 7. The number of leaves as a function of the g-level. Plotted points are mean values and error bars show ± 1 S.E. for plants grown on the centrifuge. The arrow on the ordinate scale marks the mean number of leaves in plants grown on a horizontal clinostat at unit g.

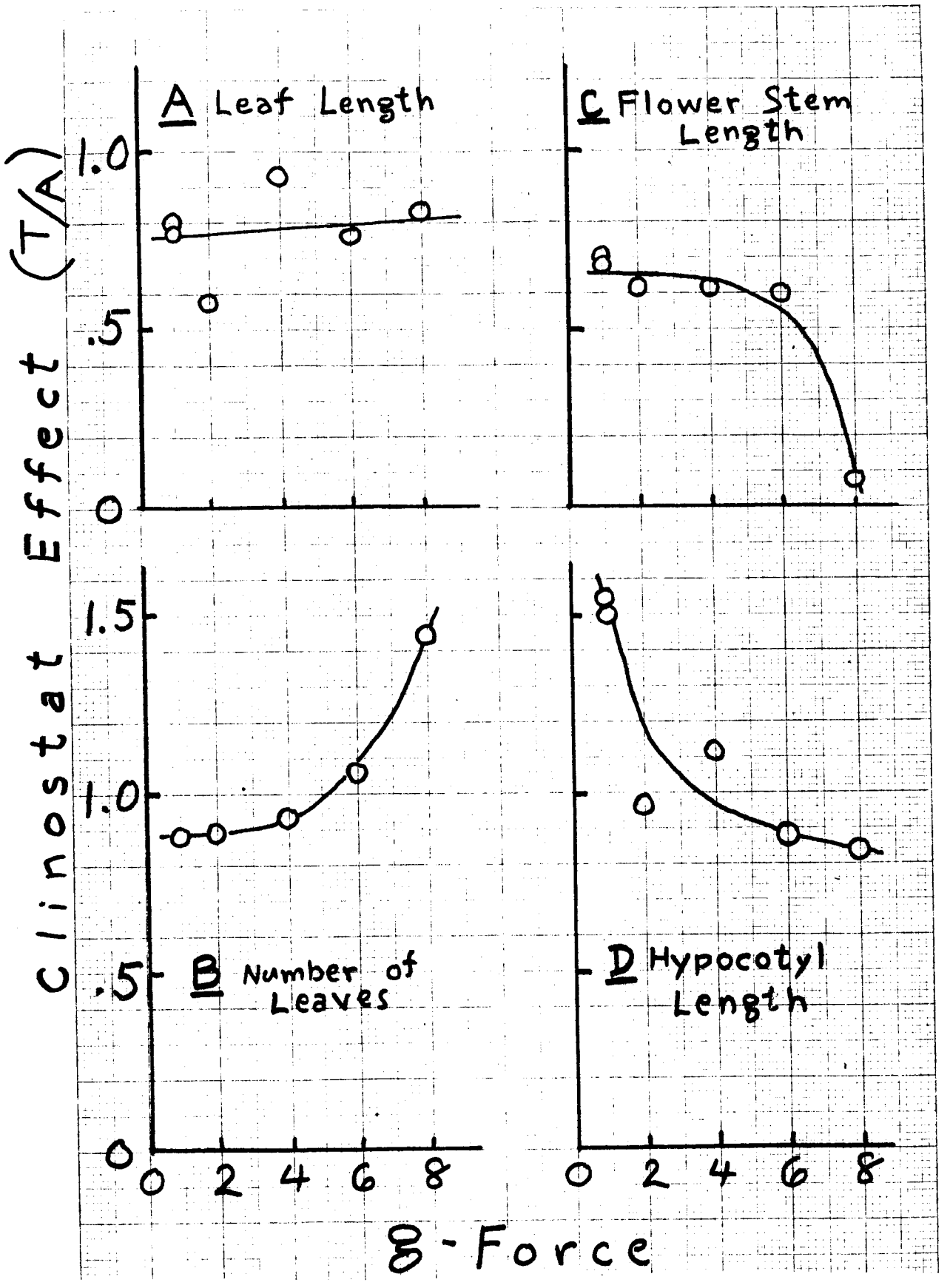

Fig. 8. Effect of the clinostat on four separate morphological characters at different chronic acceleration levels. Ordinates: ratio of mean measurement for plants grown 21 days on "horizontal" clinostat (T) to mean measurement of same character for plants grown with vertical axes parallel with g-force vector (A).

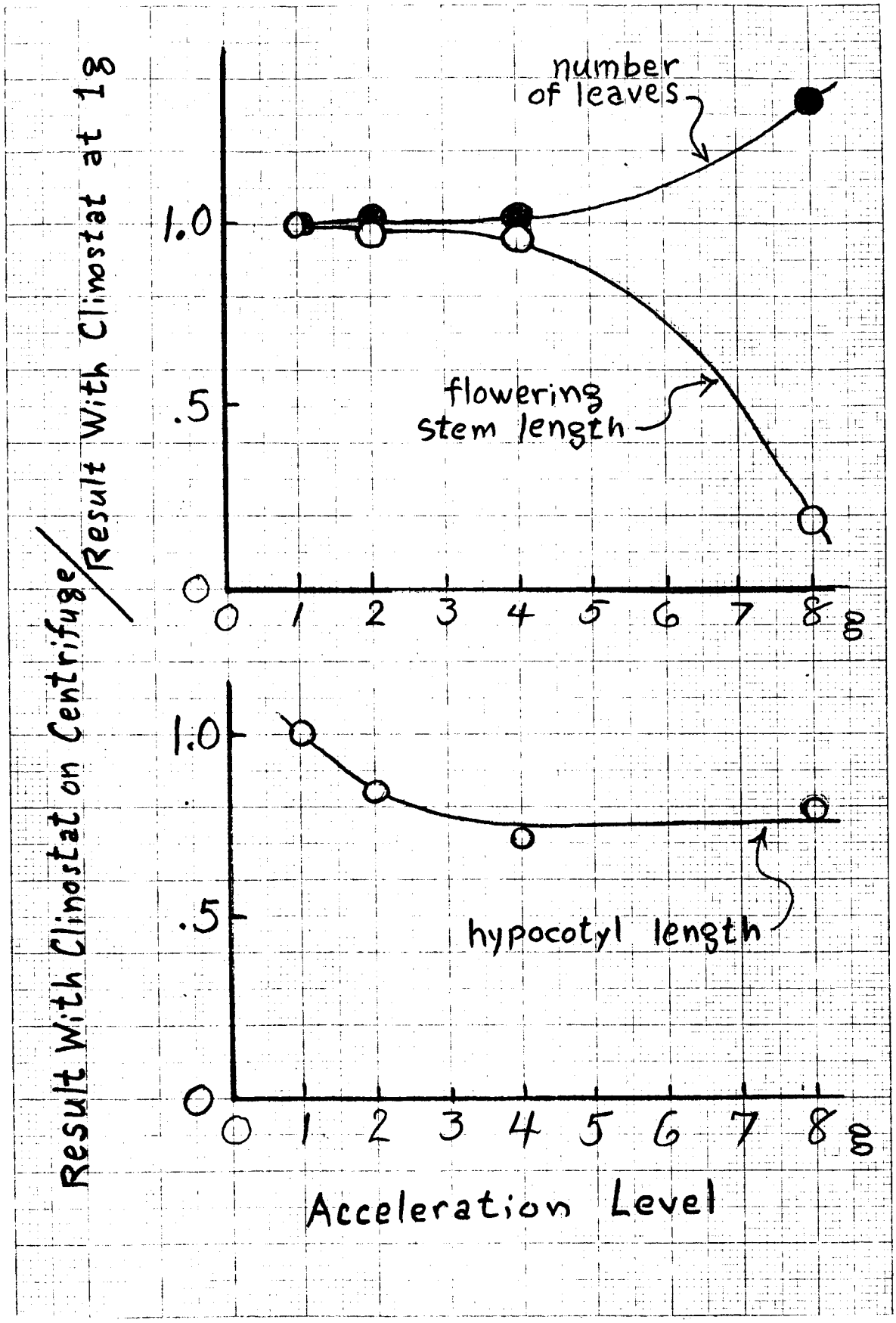

Fig. 9. The effect of chronic acceleration on the clinostat effect for three different morphological characteristics.