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LEGES SINE MORIBUS VANAE

UNIVERSITY of PENNSYLVANIA
Department of Biology
PHILADELPHIA, PENNSYLVANIA 19174

EFFECTS OF VERTICAL ROTATION ON ARABIDOPSIS DEVELOPMENT

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by

A.H. Brown, D.K. Chapman, and A.O. Dahl

with the technical assistance of

L. Goldstein, C. Kayser, A.L. Venditti, and F.T. Vogel

Plant Centrifuge Laboratory, University City Science Center
and Biology Department, University of Pennsylvania

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ABSTRACT

The clinostat imposes certain conditions on the test specimen which are different from those which apply to an organism which is allowed to develop in the vertical position without rotation. These effects include: rotation, displacement of the plant axis from the plumb line, centrifugal acceleration, and vibration (due to the clinostat drive mechanism). Although it has usually been assumed that the effects on the clinostated plant of all factors except displacement from the vertical may be neglected, there have been reports of certain effects of rotation in the vertical position.

The present study examined various gross morphological end points of *Arabidopsis* development in an attempt to separate the effects of growth on the horizontal clinostat into a component caused by rotation alone and another component caused by the altered position with respect to the direction of the g-vector. In a series of tests which involved comparisons between vertical stationary plants, vertical rotated plants, and plants rotated on clinostats, certain characters were consistently influenced by vertical rotation alone. The characters for which this effect was statistically significant were petiole length and leaf blade width.

We believe it is relevant that we found in each test that *Arabidopsis* hypocotyls grew longer when the vertical plants were stationary. However, in spite of the consistency of that effect the difference in any one test was not significant ($P > 0.05$).

The vertical rotation effects observed with *Arabidopsis* were not large. The mean petiole length of rotated plants was 8% less than that of stationary plants. Rotation increased leaf blade width by 6% over stationary controls. The total leaf length/width ratio was reduced 8% by rotation. The hypocotyls were 8% shorter when plants were rotated.

INTRODUCTION

Clinostats have been used by plant physiologists for about a century to provide test organisms with "compensation" for the directional influence of the gravitational vector when it acts at an angle to the plant's longitudinal axis. Most clinostats have been designed to rotate the plant about it own major axis and the most usual application has been to rotate the plant in horizontal orientation. Because plants require an appreciable presentation time in order to respond to a gravitational stimulus and show an even longer lag before a response becomes evident, a clinostat rotation period of about the same magnitude as the presentation time generally induces no geotropic responses even though the gravitational vector acts laterally on the plant at all times.

Plant growth on a clinostat is usually quite different from that of normal upright controls. This has often been attributed exclusively to a putative gravity "nullification". However the clinostat imposes several special conditions which might influence plant growth. The displacement of the plant axis away from the plumb line, the mechanical rotation around the axis, centrifugal acceleration caused by the rotation, and vibrations introduced from the clinostat drive mechanism are separate factors each of which possibly could have an influence of its own. Only a few reports have been published in which attempts were made to sort out effects of the several influences to determine whether any besides gravity compensation can be significant for plant development on clinostats.

Perhaps the influence of rotation alone (with the plant axis in coincidence with the plumb line) is the factor which most needs to be investigated. Many experimenters have used vertical stationary controls to compare with horizontally rotated plants on clinostats. But should the vertical controls be stationary or should they be rotated? In a few cases the experimenters have provided for both kinds of "controls" in their experimental designs. For example, S.A. Gordon et al. found that Avena coleoptiles were significantly more responsive to geostimulation if they were vertically rotated than if they were stationary (3). The authors identified "effects of rotation per se". Later from the same laboratory came a report that on average the phototropic responsiveness of horizontal clinostated plants was significantly different from that of both vertical stationary and vertically rotated controls (6). In that case vertical rotation substantially decreased the phototropic responsiveness as compared with that of stationary plants. In another investigation Hinchman and Shen-Miller found that the incidence of cells with multiple nucleoli in carrot root callus culture was increased nearly 4-fold by rotation on a horizontal clinostat (5). Two-thirds of the increase was attributable to the position effect, one-third to a motion effect.

In another case the morphological characters studied with the clinostat were amyloplast size and number (4). Control values for vertical stationary and vertically rotated plants did not differ significantly.

In some measurements we have carried out in this laboratory to study the effects of clinostating on Arabidopsis seedlings, we observed that vertical stationary controls and vertically rotated controls differed significantly for some characters we measured (1).

From results such as these we are warned that vertically rotated plants may or may not be morphologically or physiologically comparable to vertical stationary plants which further suggests that careful thought be given to exactly what kind of "controls" would be most appropriate for tests of the influence of the clinostat.

As a further consequence it seems possible in principle to separate a "motion effect", M, (attributable to rotation alone) from a "position effect", P, (due to displacement of the plant axis from the plumb line). The sum of the motion and position effects would constitute the overall "clinostat effect", C. Thus,

$$M + P = C \qquad (1)$$

where each term, if different from zero, may be either a plus or a minus quantity.

We define these quantities as follows:

$M = \left(\frac{r-s}{s} - 1\right) \times 100 =$	motion effect of vertical rotation
$P = \left(\frac{c-r}{s} - 1\right) \times 100 =$	position effect of horizontal orientation
$C = \left(\frac{c-s}{s} - 1\right) \times 100 =$	overall clinostat effect

r = measurement of a given character of test plants exposed to rotation in vertical orientation.

s = measurement of the given character of test plants grown vertically without rotation.

c = measurement of the given character of test plants grown on horizontal clinostat.

From these definitions it follows, that, if a vertical rotation effect is detected ($M \neq 0$), then C must consist of two components, M and P. However, M and P might be of different sign and C could turn out to be much smaller than either.

MATERIALS AND METHODS

The test species was Arabidopsis thaliana (L.) Heynh. The seed stock was traceable to Prof. G.P. Redi, Univ. of Missouri, - it was derived from a strain identified as 294-187-F. Plants were cultured asceptically by a method which has been standardized in this laboratory for all our work with Arabidopsis and which was reported elsewhere (2). Tests were set up with different seed lots and at different rotation rates. In some tests only the effect of vertical rotation was sought; in other tests effects of horizontal rotation also were sought. The tests were carried out at 24^{o} C under 162.5 F.C. continuous illumination by Sylvania WSGL fluroescent lamps. Table 1 lists relevant test conditions for five separate test populations carried out in different pieces of apparatus mostly at different times.

Table 1. Experimental Conditions Used in Different Tests

Test Designation	Date of Test Initiation	Rotation Rate Employed (R.P.M.)	Seed Lot Harvested
A	25 August 1970	0.5	1965
B	20 August 1974	2.18	1965
C	2 October 1974	2.18	1965
D	17 December 1974	2.0	1974
E	17 December 1974	2.0	1974

Plants were harvested after 21 days growth under constant conditions and the several morphological measurements were recorded for each plant.

In tests B and C the different treatments were furnished simultaneously to populations of about 20 seedlings in separate but presumably very similar culture chambers. In tests D and E only a vertical rotation effect was sought and the vertical stationary plants were in the same culture chamber as the vertical rotating plants arranged in alternating order within the chambers. This provision was considered to be effective in cancelling out any unrecognized differences that might prevail between the different culture chambers. In those tests (D and E) populations were from 10 to 12 plants per treatment.

Measurements were averaged and the standard error of the means of each measured value was calculated by the conventional formula, $\sqrt{\frac{\Sigma(X-\bar{X})^2}{n(n-1)}}$

RESULTS

Preliminary observations (test A) were made from an experiment set up for a different purpose. Populations were small; only 7 plants were represented in one treatment. Statistical errors were correspondingly larger than in subsequent tests. However, the results suggested several characters which seemed to warrant further investigation. In particular, the length of the hypocotyls and the mean length of all leaves (especially of petioles apart from that of the blades) were different for vertical stationary and vertical rotating plants. Those differences were significant at the 1% level. It was this finding that encouraged us to carry out further tests.

The results of Tests B and C are reported in Tables II and III. Calculation of M,R, and C as defined above, are shown in Figs. 1 and 2 in the form of "morphological profiles". It is evident that the results of these two tests were similar but not identical. The characters which showed significant vertical rotation effects (and therefore the greatest position effects) were petiole length and hypocotyl length. Visual inspection of Figs. 1 and 2 would suggest several characters for which vertical rotation effects were large and consequently for which position effects were prominent. Statistical analysis made the results less dramatic than the figures imply since the standard errors of measurements of some characters were rather large.

Tests D and E, which were set up as replicates but in apparatus which kept the seedlings of each test physically apart, were expected to yield the most reliable data from the standpoint of experimental design.

Fig.1. Calculated Motion Effects, Position Effects, and Overall Clinostat Effects on _Arabidopsis_ Morphology from Test B. The abbreviations which identify the groups of bars follow our local laboratory convention: HL = hypocotyl length; PL = petiole length; NL = number of rosette leaves; BL = leaf blade length; BW = leaf blade width; FL = flowering stem length. The first bar of each group represents the mean value of M, the effect of vertical rotation. The second bar of the group represents P, the effect of horizontal orientation. The third bar of the group represents C, the overall clinostat effect (which is the sum of the previous two bars). For the method of calculating M,P, and C, see text.

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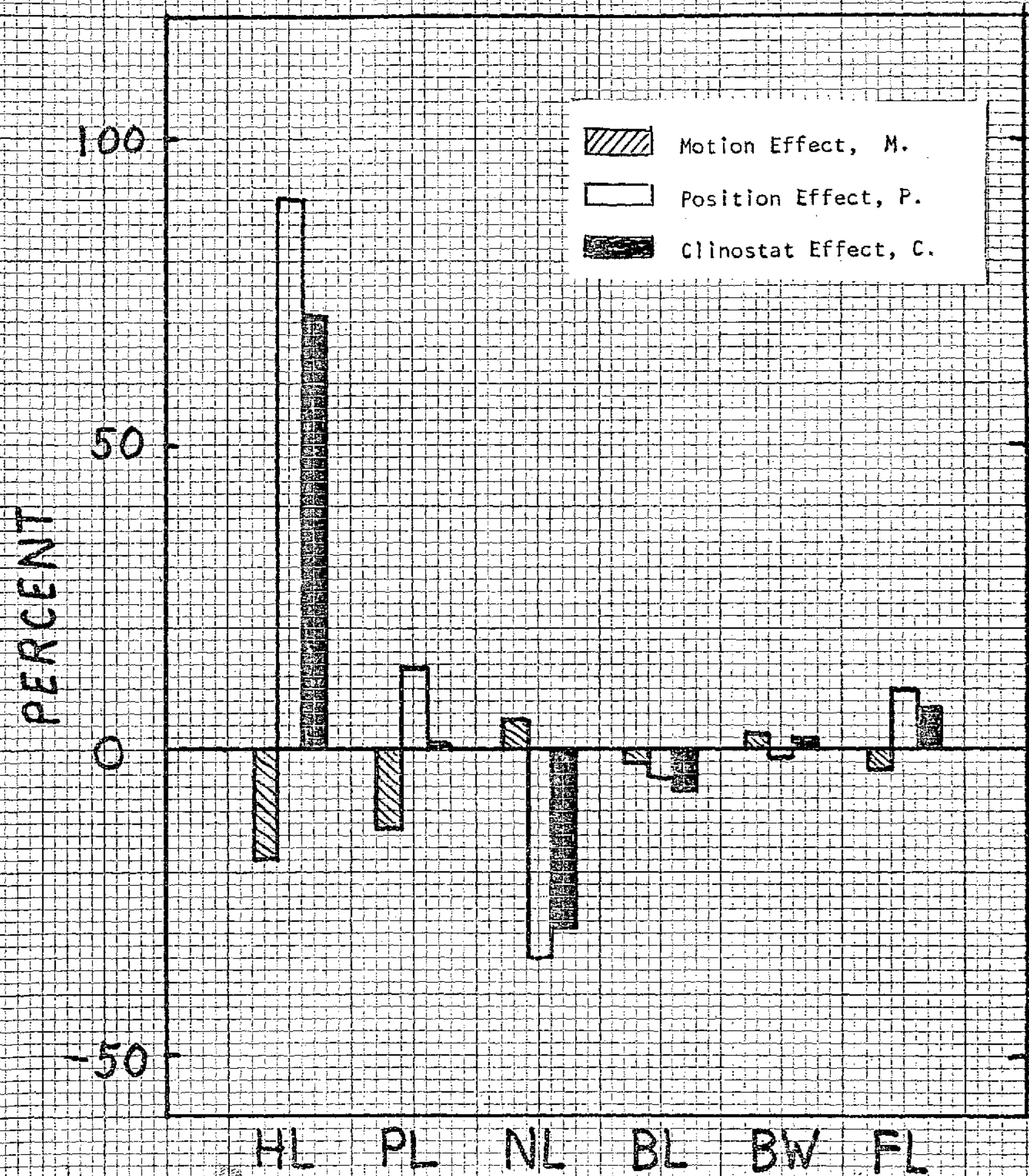

Motion Effect, M.
Position Effect, P.
Clinostat Effect, C.
PERCENT
100
50
0
-50
HL
PL
NL
BL
BW
FL

Fig. 2. Calculated Motion Effects, Position Effects, and Overall Clinostat Effects on <u>Arabidopsis</u> Morphology from Test C. Notation as in Fig. 1.

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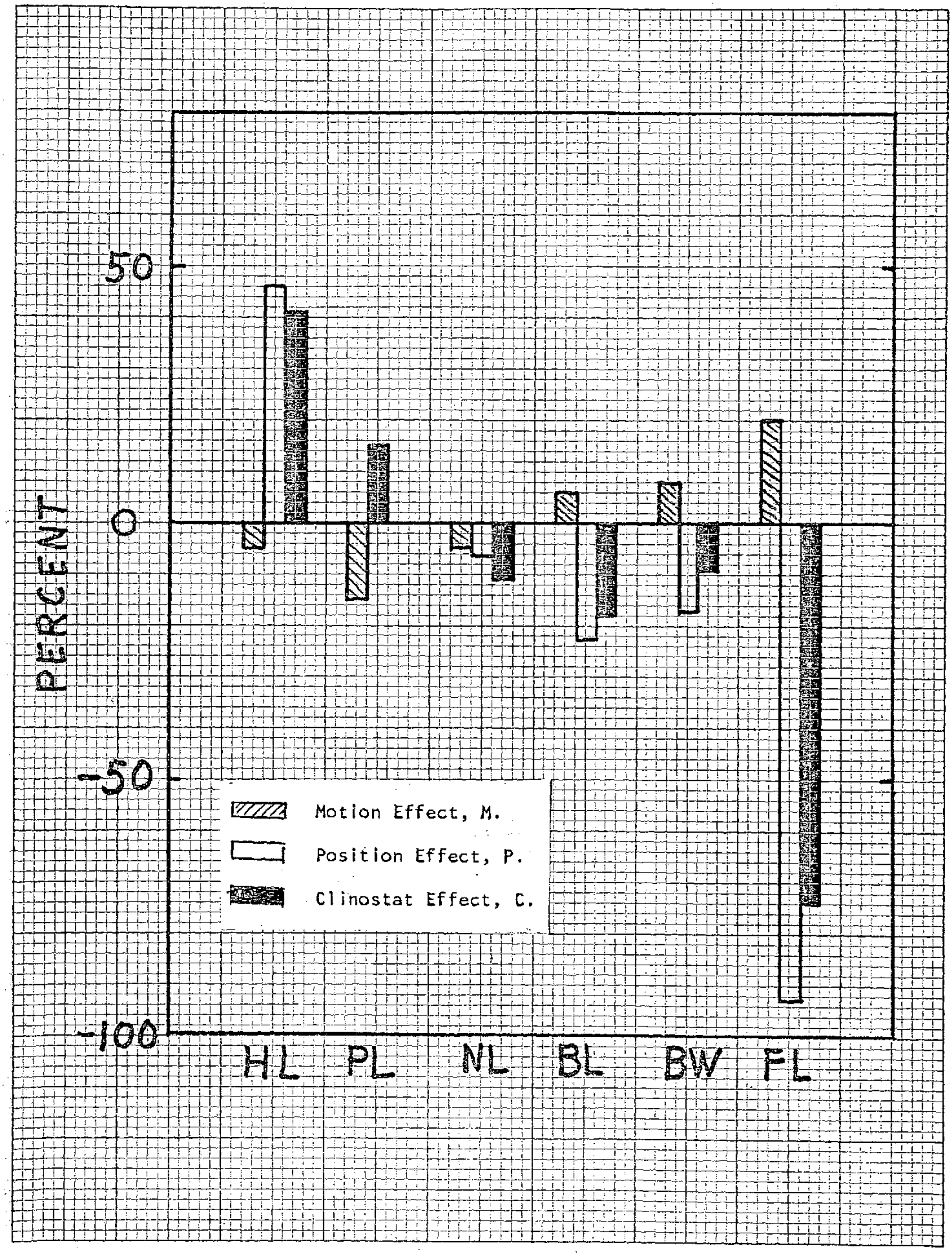

PERCENT
50
0
-50
-100
HL
PL
NL
BL
BW
FL
Motion Effect, M.
Position Effect, P.
Clinostat Effect, C.

Vertical stationary plants were located side by side alternating with vertically rotating plants. There were no horizontally oriented plants in Tests D and E so that the sole object of these two tests was to identify any effects of vertical rotation which might be revealed.

Fig. 3. shows a morphological profile of M values (vertical rotation effect) as derived from the pooled results of Tests D and E. It is evident from the figure that none of the measured characters displayed an effect of vertical rotation beyond the range of about $\pm$ 10% of the vertical stationary controls by which the numerical results were normalized. Only one character, leaf blade width, showed a motion effect significantly different from zero.

When the data from Tests B,C,D, and E were pooled to provide the maximum of reliable information by which to detect a motion effect, the results shown in Fig. 4. were obtained. For the characters, petiole length and leaf blade width, the M values differed significantly from zero at the 1% level of probability.

From inspection of Fig. 4. it may be suggested that further experiments which could increase the population sizes of all measurements being compared might very well reveal that other characters would display small but significant effects of vertical rotation. In that connection it should be noted that hypocotyl length showed an M value in most tests which was not significantly different from zero, yet in every test the value was negative. The consistency of this result is not taken into account by the statistical evaluation on a test by test basis and, even when all test results were pooled, the increase variability prevented ~~a~~ consistently negative results from being found numerically significant.

A somewhat weaker case could be made for the consistently increased value of M in the case of flower stem length.

Fig. 3. The Effects of Vertical Rotation on Morphological Endpoints of *Arabidopsis* Development from Tests D and E. Results of two replicate tests were pooled. Endpoint notations as in Fig. 1. Ordinate values are M, the motion effect, as defined in the test. Plotted points are mean values of M for the indicated endpoint characters. Vertical lines represent ± one standard error unit for each mean. Values above zero indicate that vertical rotation enhanced the measured character; those below zero indicate that vertical rotation was inhibitory. Only one M value, the leaf blade width, was significantly different from zero at the 1% level of probability.

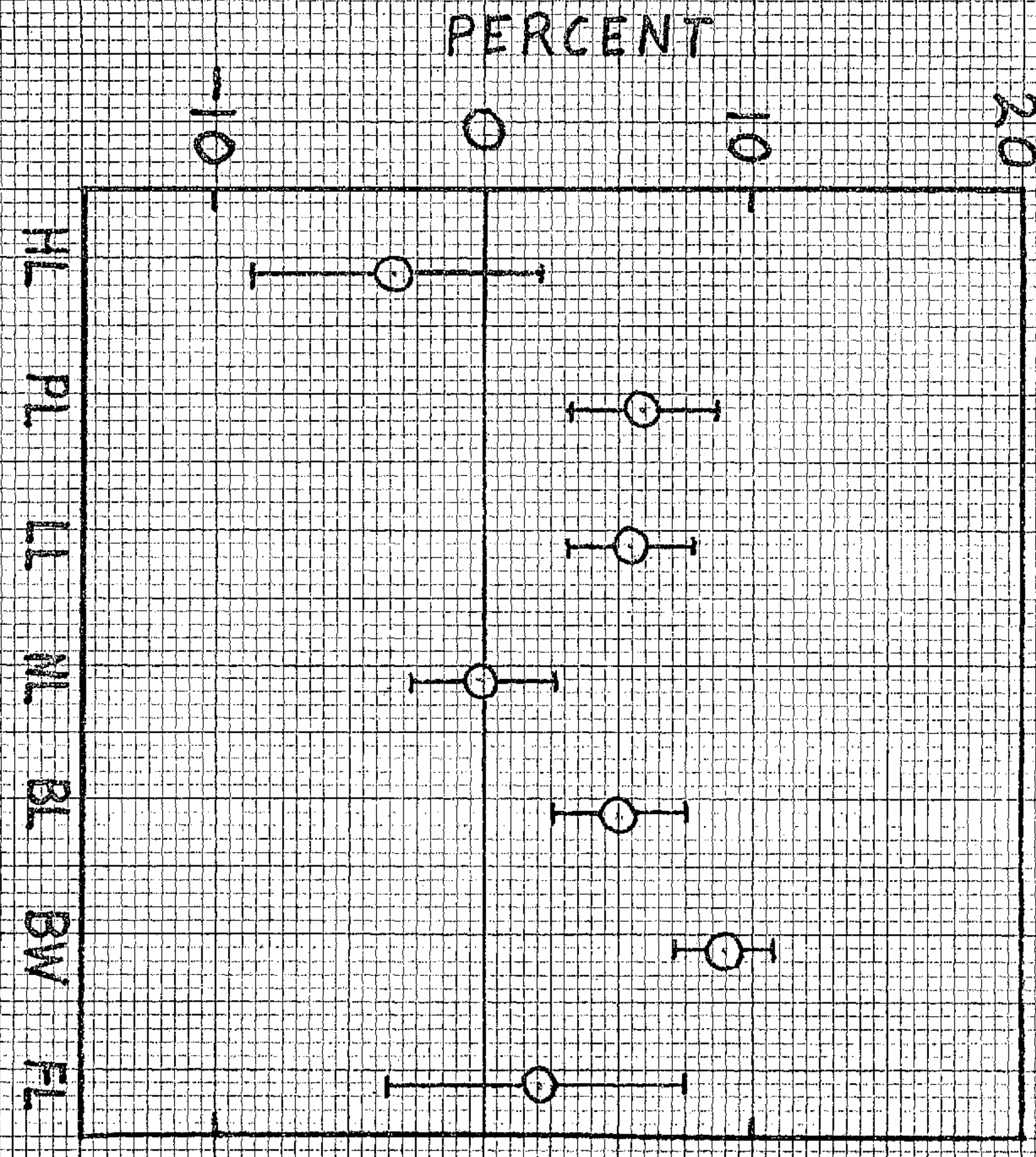
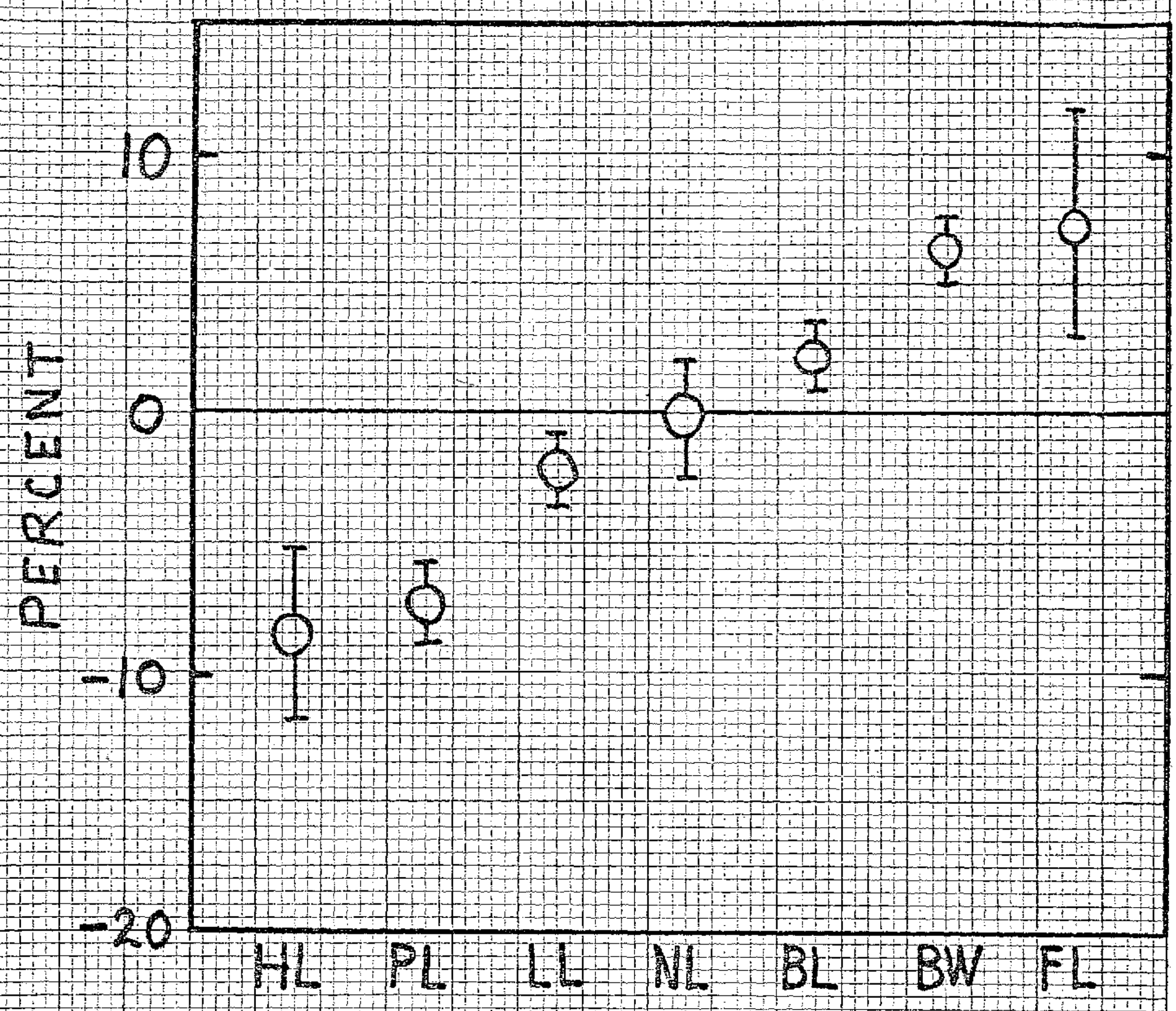

PERCENT
-10
0
10
20
HL
PL
LL
ML
BL
BW
FL

Fig. 4. Effects of Vertical Rotation on Morphological Endpoints of *Arabidopsis* Development Calculated from the Pooled Results of Tests B,C,D, and E. Characters are aligned as in earlier figures. (*Vide* legend of Fig. 1 for notation code.) Ordinate scale of calculated motion effect as in Fig. 3. Plotted points are mean values of M for the indicated endpoint character. Only two of the M values were significantly different from zero.


PERCENT
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DISCUSSION

It is important to note that the putative motion (vertical rotation) effect, M, on the characters we measured was in all cases small whether statistically significant or not. The largest negative effects we found were about 8% inhibition of petiole length, 8% reduction in leaf length/width ratio, and 8% shortening of the hypocotyl. The largest enhancement effects were on leaf blade width (6%) and flowering stem length (7%). Since clinostat effects range from about + 35% down to about - 25% for different characters measured, it is evident that the position effect (here called P) is the chief component of the overall clinostat effect (C in our present notation).

In some work reported on other plant systems the application of Equation 1 illustrates vertical rotation effects of a similar or even greater magnitude. In one case Shen-Miller and Gordon noted that vertical rotation alone decreased the phototropic responsiveness of Avena coleoptiles yet the horizontal position component of the clinostat treatment enhanced the phototropic response (6). From the data those authors presented, one may calculate an M value of + 5.1%, a P value of - 10.3% and an overall clinostat effect of - 5.2%. In another example Hinchman and Shen-Miller found the incidence of cells with multiple nucleoli in carrot root callus cultures was increased nearly 4-fold by rotation of the culture on the horizontal clinostat (5). Two-thirds of the increase was attributable to the horizontal position (P) and one-third to the rotary motion of the clinostat. In a third case Hinchman and Gordon found that certain clinostat effects on oat seedling amyloplasts were statistically significant.(4) All of the effect was attributable to the effect of horizontal position; effect of rotation in the vertical orientation was not observed. Thus, even though some vertical rotation effects evidently are far from negligible, it would be quite difficult to defend any sweeping generalizations at the present state of exploration of the subject.

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