ANNUAL REPORT

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"A STUDY OF THE MECHANISM OF THIGMOMORPHOGENESIS IN PLANTS, WITH SPECIAL REFERENCE TO THE ROLE OF ETHYLENE AND ITS SIGNIFICANCE TO RESEARCH WITH PLANTS IN SPACE"

PREPARED BY:

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I. Introduction: This grant was first activated on the 15th June, 1977 for 3 years, and hence, at the time of this annual report, research has been conducted for 1.5 years. During the past year, the following personnel have worked on various aspects of the project: 1) Mr. Raymond Hunt, undergraduate honors student; Mr. Kenneth Bridle, undergraduate student; Mr. Frank Telewski, graduate student; Mr. Ron Biro, graduate student; Miss Mary Ann Borch, technician; Dr. Yair Erner, visiting post-doctoral scientist; and Dr. Mordecai J. Jaffe, project director.

Because of the paucity of data concerning thigmomorphogenesis, all aspects of the phenomenon have been under study in this laboratory, although the role of ethylene mediation has been central to our approach. Although this report must necessarily point to a specific time in ongoing research, several aspects of this study have reached at least temporary stopping points and have therefore been written and submitted for publication. These will be identified in the report. Other papers which are currently in preparation, but not yet submitted, will also be identified.

As before, the phenomenon of thigmomorphogenesis will be divided into the sensory function, transduction step(s) and the response function.

II. The Sensory Function: In order to study the sensory function, it is first necessary to quantify and standardize the intensity of the stimulus. In the final analysis, this must mean reproducibly quantifying the amount of force applied to the stem. Derivative functions of this force can be applied by the excursion of a shaker or the speed of a fanning wind, but the actual amount of force applied to the plant by these methods cannot be known. Hence, a "thigmostimulation" was constructed which enabled us to duplicate the finger rubbing treatment, only with the added capability of varying the pressure on the rubbed stem with known amounts of force. The thigmostimulator, shown in Figure 1, positions a slightly concave teflon brace on one side of the stem and a vertically movable tygon sheathed roller on the other. The roller is fixed to a smoothly moving rack and pinion, which allows it to be rolled up and down a fixed distance along the stem. The teflon brace is devised as a loosely fitted sleeve over a long brass horizontal screw which passes through teflon bearings that hold the teflon brace level with the middle of thestimulated internode. A pulley of known radius is tightly fitted onto the screw behind the bearings, so that when the pully is turned, it causes the screw to turn until the teflon brace is hard against the internode. A thread is fastened to the pulley and wound around it with a small aluminum foil basket to hold weights at the other end of the thread. The weight of the basket is adjusted to just overcome the frictional inertia of the system between the screw and its bearings. In practice, the plant is positioned with the internode to be stimulated resting against the roller. Then the screw is rotated until the teflon brace just touches the other side of the internode. The desired weight is added to the basket which is pulled down by gravity, causing the brace to squeeze the internode between itself and the roller with the desired force. The roller is then moved up and down against the internode the desired number of times, and the plant is removed for incubation. The amount of force applied to the internode can be calculated by the equation:

$$\text{applied force} = F = AMA \times Wt \times 0.0098067,$$

where F = applied force in Newtons; 0.0098067 is a conversion factor from grams to Newtons; and the AMA is the actual mechanical advantage of the pulley acting as a lever. The AMA can be calculated from the equation:

$$AMA = \frac{2\pi L}{p} - f,$$

where L = the radius of the pulley in mm (i.e., the length of the lever arm); p = the pitch of the screw (i.e., 1/no of threads per mm); and f equals a factor related to the friction between the screw and its bearings. Since this friction is exactly compensated for by the weight of the basket, it is dropped from the equation. Thus, for example, if a 1.0 g weight is put in the basket, the force applied to the internode would be 1.94 Newtons, if a pulley of radius 35 mm and a screw of 1.11 pitch was used.

In order to determine the number of rubs necessary for saturation of the sensory function, the stimulus force was held constant, while the number of stimuli was varied. At a stimulus intensity of 2.91 Newtons, the sensory function seems to be saturated by 2 rubs (Figure 2). By using a combination of large and small pulleys and a uniform 5 rubs (where 1 rub consists of one up or down excursion of the roller), a dosage response of the force of mechanical stimulation versus net growth of the internode in 24 h can be plotted (Figure 3). It can be seen that the function of this line is curvilinear, and it can be shown that the relationship becomes linear when the force is plotted against the $\log_{10}$ of the net elongation (Figure 4). This log-linear relationship also seems to hold when dosage responses to derivative stimuli (such as number of rubs, wind speed, or degree of stem bending) are plotted (Figure 5).

We have done several experiments to see if there is a reciprocal relationship between the number of rubs and the force of each rub. This was done by analogy with the Bunsen-Roscoe Reciprocity rule of photochemistry, which holds that if the duration and intensity are varied, the response should always be the same as long as the product of frequency and intensity remain equal. As can be seen in Table I, reciprocity does not seem to hold for a high stimulus level equal to 0.97 Newtons x 24 rubs, but does for a low stimulus equal to 0.58 Newtons x 12 rubs.

This work, which is being done by Messrs. Biro and Bridle will enable us to establish the relationship between stimulus force and growth response on a mathematical basis. We shall then look for other, biochemical and biophysical correlates of these relationships to help elucidate the sensory function in thigmomorphogenesis.

III. The Transduction Step(s): In the past, I have established that there is a very rapid (in less than 1.0 second) drop in tissue electrical resistance immediately following mechanical stimulation. Further work on this aspect of the system has waited until the sensory relationships were worked out. As soon as the latter is finished, research on the transduction step(s) will continue.

IV. The Response Function: The response function is obviously very complex, involving both early, mid-term and long-term effects. Eventually, complete time courses of all measurable aspects of the response function must be studied. However, as a research strategy, I have chosen to observe the long term effects (i.e., greater than 24 h) first, the mid-term effects (between 5 min and 24 h) next, and finally the short term effects which start with the transduction step(s). In practice, we have reached the point where we have identified certain events that occur in these different phases, and they are being studied separately.

IV.A. Medium-term Effects of Mechanical Perturbation:

IV.A.1. The Ethylene Trigger: This work was done in part by Dr. Erner and mostly by Mr. Biro who is continuing it. We have voluminous data, all of which points to ethylene as an endogenous trigger which mediates thigmomorphogenesis (Table 2). The evidence is of two types. First, there is a burst of ethylene production in any internode which is mechanically perturbed, which peaks between 90 and 180 min after mechanical stimulation. This has been shown in 3 days: 1) By stimulating, removing 1 cm internode segments, and incubating them various lengths of time before assaying for ethylene production by gas liquid chromatography (Figure 6); 2) By stimulating and excising segments after incubation in situ for various lengths of time (Figure 7); and 3) by stimulating and taking a gas sample from the lumen of the hollow internode after various elapsed times. All of these methods demonstrate the "pulse" production of ethylene in the first 3 h.

The second type of evidence that ethylene is the endogenous trigger is the results of many experiments that demonstrate that exogenous ethylene or ethephon (ethrel) can mimic the effects of mechanical stimulation (Table 2). Most of these will be covered in detailed in other sections, but it should suffice to say that in the study of 13 different changes caused by mechanical perturbation, all of them could also be induced by exogenous ethylene or ethephon.

Finally, inhibitors or antagonists of ethylene have been used to block thigmomorphogenesis (Table 3). This part of the study is just beginning, but already indicates that compounds or treatments that are known to interfere with ethylene production or action, also interfere with thigmomorphogenesis.

IV.A.2. Anatomical Effects of Mechanical Perturbation: In order to provide a basis for understanding the physiological and biochemical changes that occur in response to mechanical perturbation, an anatomical study of thigmomorphogenesis was undertaken. Examination of first internodes of young bean plants which were subjected to mechanical perturbation, show decreased elongation and increased radial growth (Figures 8 and 9). The decreased elongation can be attributed to both reduced cell elongation of the epidermis and cortex (Table 4) and reduced cell division in the vascular and pith tissues. The increased radial enlargement is due to increased cortical cell diameter and increased secondary xylem production as the result of increased cambial activity (Table 5). All of these responses are observable within a few hours of a single mechanical perturbation (Figures 10 and 11). Treatment of plants with ethrel mimics all of these effects of mechanical perturbation (Figures 12 and 13). This work has been submitted for publication (Biro, Hunt, Erner and Jaffe, submitted).

IV.A.3. Evidence for a Translocatable Thigmomorphogenesis Factor (TTF): Whereas it is now well demonstrated that mechanical perturbation of an internode results in reduced length and increased diameter of that internode, the effect on other internodes of the same plant has not been documented. We now have evidence to show that perturbation of a single internode results in thigmomorphogenesis in that internode and all of the others as well (Figure 14 and Table 8). These data and others showing that more stimuli of the first internode are needed to produce as great an effect in the second and third internodes as in the first (Figure 15) suggest that there may be an acropetal translocatable thigmomorphogenetic factor (TTF) that moves upward from the stimulated internodes to the second and third internodes. To rule out the possibility of cessation of a translocatable growth promotor, grafting experiments were performed. The data, shown in Table 9, support the hypothesis that a TTF exists. Since we have proposed ethylene as an internal trigger, it was thought possible that ethylene might be the TTF. However, an experiment where the ethylene content of and production by

the second internode following mechanical stimulation of the first internode failed to support such an hypothesis. What the TTF might be, we do not as yet know, but we have evidence that it is not affected by the shoot tip or the cotyledons. A report of this work has been submitted for publication (Erner, Biro and Jaffe, submitted).

As part of our search for a TTF, we have begun to study the possible role of other hormones in thigmomorphogenesis. Table 10 shows that 10 μM exogenous auxin causes a decrease in elongation as does 1 μM ABA, but that neither cause an increase in diameter. Dr. Erner has done bioassays of chromatographed internode extracts, which indicate that either mechanical stimulation or exogenous ethrel on the 1st internode induce an increase in free auxin-like material in both the first internode (Figure 16) and the second internode. However, since the literature does not support the view that auxin is acropetally translocated, it is unlikely that IAA is the TTF.

IV.A.4. The Interaction of Mechanical Perturbation with Gravity: As an important part of the preparation for performing experiments with plants in the low gravity space environment, we have studied the interactions of mechanical perturbation with gravity. Two principal findings have come out of this research.

Clinostats have long been used to equally distribute the gravitic stimulus around a plant, in order to simulate the gravity-less condition. More recently the interpretation of clinostat experiments have come into question because of vectorial mechanical stresses set up by the turning plant. I have found that such stresses set up mechanical strains in the bean plants as the leaves flop over during horizontal clinostating, and that the strains induce nastic changes in the two pulvini associated with each primary leaf. Figure 17 shows that the sum of the adaxial stem-petiole angles increases in horizontally-held or in horizontally-clinostated plants. Furthermore, horizontally clinostated plants also displayed a decrease in the abaxial petiole-leaf angle (Figure 18). These effects, which could be detected at least as early as 2½ h after the beginning of clinostating, and could also be induced by treatment with ethrel, are shown diagramatically in Figure 19. Thus, it seems that the continual flopping of the leaves during clinostating, sets up a mechanical strain on the pulvini which is expressed as epinasty. In order to test this hypothesis, weights were added to the base of each primary leaf. The results show that when the weight of the leaves are increased, the epinastic response to clinostating is also increased (Table 11).

As can be seen in Figures 17 and 18, there are only very small effects of internode-rubbing on epinasty. However, a pronounced effect of mechanical perturbation on geotropism was found (Figure 20). Thus, if the first internode is rubbed just prior to presentation of the gravitic stimulus, there is much less geotropic bending, especially during the first 5 h. This effect can also be mimiced by application of ethrel.

The work on both of these interactions of gravity with mechanical perturbations, some of which has been presented here, is currently being written for publication. It is hoped that the effects of mechanical perturbation on plants in space will be able to be tested in the future.

IV.A.5. The Interaction of Mechanical Perturbation with Phototropic Light: In a manner similar to its interaction with gravity, the mechanical perturbation of the first internode also retards phototropism (Figure 21). This effect also

can be produced by exogenous ethrel, and the work is currently being written for publication.

IV.B. Long-Term Effects of Mechanical Stimulation:

IV.B.1. Field Experiments: Although we have had considerable experience working with thigmomorphogenesis of beans in the laboratory, we felt that it was necessary to demonstrate that this phenomenon also occurs in nature. Accordingly, field experiments were designed wherein adjacent plots were exposed to or sheltered from the wind. At the same time, wind speed and gusting, temperature, precipitation and light levels were measured.

Table 6 shows the results of 10 experiments run in the fall of 1977 and the spring of 1978. Net stem elongation and diameter were measured and it was found that statistically significant thigmomorphogenesis due to wind occurred in the field. Greater production of internodal secondary xylem was also observed in the wind exposed plants than in the sheltered ones. The experimental data were analyzed by multiple linear regression and the wind was found to be a statistically significant factor in the control of bean stem elongation and thickening. Laboratory wind experiments, done concurrently, show that as wind speed increases, thigmomorphogenesis increases in a log-linear fashion (Figure 5). Furthermore, it was found that temperatures below 16°C substantially reduced thigmomorphogenesis both in the field due to wind (Table 6) and in the laboratory due to rubbing (Table 7). There is a statistically significant interaction between mechanical perturbation and temperature for elongation. Thus, these experiments, done by Mr. Hunt, show that the wind does indeed cause thigmomorphogenesis in bean plants in the field. This work has been submitted for publication (Hunt and Jaffe, submitted).

IV.B.2. Effects of Mechanical Perturbation and Ethylene on the Membranes of the Endomembrane System: Since recent reports suggest that ethylene synthesis might be membrane-associated, and because cell membranes seem to control growth both by regulation of cell turgor and of cell wall changes, Dr. Erner has completed a study of the effects of both mechanical perturbation and exogenous ethrel on the protein and lipid components of the endomembrane system of cells of the first and second internode of bean plants. Endomembrane fractions show increased protein but decreased specific activities of the marker, membrane-associated enzymes succinic acid cytochrome-c oxidase (mitochondria), KCN-resistant NADPH cytochrome-c reductase (endoplasmic reticulum), and latent inosine diphosphatase (golgi apparatus) (Table 12). At present, the increase in endomembrane protein cannot be explained.

When endomembrane-associated lipids were studied it was seen that there were profound changes in both phospholipids and free fatty acids. Both mechanical perturbation and exogenous ethrel caused marked decreases in all the phospholipids of the first internode except phosphotydal inositol, which showed a decrease (Figure 22). Similar, but less pronounced effects, were seen in the second internode. Similarly, small but significant decreases in some of the fatty acids of the phospholipids were also seen (Table 13). When endomembrane free fatty acids were examined, lauric acid (16:1) was less in controls, but as the chain length increased, and as unsaturation increased, the fatty acid titers decreased after treatment with ethrel or mechanical perturbation (Table 14). This was particularly striking in the case of linolenic acid (18:3). Finally, endomembrane-associated sterols showed only small decreases due to treatment (Table 15). Thus, in summary, both mechanical perturbation and exogenous ethrel

cause an increase in the protein and a decrease in the lipids of the endomembrane system. It is as yet too early to be able to interpret these data, but it is interesting to note that when plant tissues are challenged with linolenic acid or its peroxide, ethylene synthesis can be induced. These experiments indicate that one of the ways that ethylene mediates thigmomorphogenesis is by inducing profound changes in the protein and lipid components of the membranes of the endomembrane system. This study is currently being written for publication.

Finally, I have intended to describe a model of thigmomorphogenesis in beans based on the data of this and other laboratories. Figure 23 shows that ethylene is the key endogenous trigger of thigmomorphogenesis, and sets off all of the other events which we have observed.

IV.B.3. Effects of Mechanical Perturbation on the Growth of Pine Seedlings: A project has been started by Mr. Telewski to study the possible effects of mechanical perturbation on pine seedlings. We have obtained from the Weyerhauser Tree Co. seeds of half-sib Loblolly Pine, some of which are known to produce trees with large trunks and some having small trunks. The plants were randomly distributed in "cone-tainers" with one-half of them mechanically stimulated (MS) simply by slowly waving the hand through them ten times, once each day. After three months, the net elongation of the plants was measured. The results, shown in Table 16, indicate that not only is thigmomorphogenesis easily demonstrable in pine seedlings, but there seems to be a correlation between the ability of a seedling to undergo thigmomorphogenesis and the kind of tree it will ultimately produce.

V. The Effects of Sound Vibration on the Growth and Development of Plants: As part of this project, we have been testing the effects of wind, rubbing and vibration on the growth of bean plants. Although sound is a vibration, it cannot specifically be considered a mechanical type of stimulus. Nevertheless, it was thought worthwhile to test it as a kind of environmental stress. There is another reason why this factor may be of interest to NASA. If fairly high levels of sound do affect plant growth or behavior, they will have to be taken into account in experiments involving plants to be performed in the orbiting space laboratory. This is because the ambient levels within the laboratory in orbit are expected to range from 50-65 db (personal communication from Thora Halstead). They will, of course, be even higher during take off and re-etnry.

Accordingly, we have performed preliminary experiments on bean plants, using a sound-proof chamber in the department of Speech and Hearing at Ohio University, and a warble tone generator which we built ourselves. Table 17 shows the results of our preliminary experiment. There were dramatic effects of 1200 hz at 90.5 db on the growth of both dark- and light-grown bean plants. In both cases, the elongation of the first true internode was dramatically changed.

With a special supplement to the grant, I have had 4 soundproofed chambers built of stressed concrete. These chambers are humidity and temperature controlled and without lights. Hence, for the present, only etiolated plants will be studied. However, in the future I intend to add light a control capability.

VI. Publications Supported by This Grant:

1. Jaffe, M.J. and Biro, R. Thigmomorphogenesis: The role of ethylene in wind induced growth retardation. In Proc. 4th Ann. Plant Growth Regulator Working Group, pp. 118-124 (1977).

2. Jaffe, M.J. and Biro, R. Thigmomorphogenesis: The effect of mechanical perturbation on the growth of plants with special reference to anatomical changes, the role of ethylene, and interaction with other environmental stresses. In press in: Proc. International Conference on Stress Physiology of Plants Useful for Food Production (1979).

3. Brio, R.L., Hunt, R.E., Erner, Y. and Jaffe, M.J. Thigmomorphogenesis: Decreased axial cell elongation and increased radial cell division in the internodes of mechanically perturbed or ethrel heated bean plants. Annals of Botany, submitted.

4. Hunt, E.R. and Jaffe, M.J. Thigmomorphogenesis: The interaction of wind and temperature in the field on thigmomorphogenesis in Phaseolus vulgaris L. Annals of Botany, submitted.

5. Erner, Y., Biro, R. and Jaffe, M.J. Thigmomorphogenesis: Evidence for a translocatable messenger of thigmomorphogenesis induced by mechanical perturbation of beans (Phaseolus vulgaris L.). Physiologia Plantarum, submitted.

Figure Headings

Figure 1. The thigmostimulator showing the roller (R), the teflon brace (B), the screw (S), the teflon bearings (TB), the pulley (P) and the weight basket (WB).

Figure 2. The effect of number of rubs (1-5) on elongation of the first internode when the force is held constant at 2.91 Newtons.

Figures 3 & 4. The effect of force amplitude (0.58-3.88 Newtons) on elongation of the first internode when the number of rubs is held constant at 5 (upper). The lower graph represents the same data with the growth values transformed to the $\log_{10}$. (Figure 3, upper; Figure 4, lower).

Figure 5. Log_{10}-linear curves of dosage responses to various kinds of mechanical perturbations (No. R = number of rubs with thigmostimulator, W.S. = wind speed, F.R. = number of rubs with the fingers, and D.B. = stem bending in degrees).

Figure 6. The time course of ethylene production following mechanical perturbation. One centimeter segments of the first internode were excised from control and MS plants and placed in sample vials. Ethylene samples were taken from the vials after different lengths of time and the difference in ethylene production between the two treatments was computed at each time interval.

Figure 7. The time course of ethylene production following mechanical perturbation. Segments were excised from control or M.S. plants at different intervals after treatment. The amount of ethylene produced due to M.S. was computed as the difference between the M.S. and control production.

Figure 8. The effect of wind (produced by an oscillating fan ½ hour daily for ten days) and M.S. (rubbing with fingers) on the appearance of 3 week old bean plants.

Figure 9. The appearance of excised first internode of controls and of plants which have been rubbed once daily for 10 days.

Figure 10. The time course of first internode epidermal cell elongation in control (o——o), and M.S. treated plants (●——●). Data represented are the averages of observations made from 2 sets of plants, each consisting of 18 plants.

Figure 11. The time courses of production of secondary xylem layers in bean first internodes. o——o, interfasicular region of controls; □——□, fasicular region of controls; o---o, interfasicular region of M.S. (rubbed daily) internodes; □---□, fasicular region of stimulated internodes. All data are averages of three experiments, each consisting of approximately 15 plants per treatment.

Figure 12. Effect of application of various concentrations of ethrel to the apical bud on the length (▲---▲) and width (o---o) of first internodes of beans as observed after 10 days of treatment. All data points are the average of three experiments each consisting of approximately 18 plants per treatment.

Figure 13. Effect of application of various concentrations of ethrel to the apical bud on the number of secondary xylem layers in bean first internodes, measured in the interfasicular (▲——▲), and fasicular (△- - -△) regions. All data are averages of three experiments, each consisting of approximately 18 plants per treatment.

Figure 14. The effect of mechanical perturbation of different internodes on the elongation of the stem. Treatments were begun when the internodes to be perturbed were one cm long, and continued daily for 10 days before measurements of length were taken. O——O = unperturbed controls, △——△ = first internodes perturbed, ■——■ = second internodes perturbed, ▲——▲ = perturbation of the first internode when the second internode was 1 cm long, □——□ = first and second internode perturbed when the internodes were 1 cm long, ●——● = first, second and third internodes perturbed as each internode became 1 cm long, and O- -O = application of 10 µl (1000 ppm) ethephon once daily to the first internode.

Figure 15. Response of internode I, II and III to various frequencies of perturbation (represented as 1 perturbation per the given number of days) of internode I. Treatments were begun when the first internodes were 1 cm long and continued over a 10 day period.

Figure 16. The effect of exogenous ethrel (E) or M.S. on IAA-like growth promoter activity and ABA-like growth inhibitor activity of the 1st internode as compared to untreated controls (C) after 10 days of treatment. IAA migrates to an rf of 5 and ABA to 7 and 8.

Figure 17. The time course of the effects of mechanical stimulation (MS), horizontal (HOR.) or vertical (VERT.) placement (top graph), and horizontal clinostating (HOR. CLINO.) or vertical clinostating (VERT. CLINO.) (bottom graph) on the petiole attitude of young bean plants. In those cases where a mechanical stimulus was given, the first internodes were given 10 rubs, one time, just before presentation of the gravitic stimulus or of the clinostat (0 hours). The vertical bars represent standard errors.

Figure 18. The time course of the effects of mechanical stimulation (MS), horizontal (HOR.) or vertical (VERT.) placement (top graph), and horizontal clinostating (HOR. CLINO.) or vertical clinostating (VERT. CLINO.) (bottom graph) on the leaf attitude of young bean plants. For details see caption to Figure 17.

Figure 19. Diagrammatic rendering of the leaf and petiole attitudes of epinasty caused by horizontal placement by horizontal placement or horizontal clinostating.

Figure 20. The time course of the effects of mechanical stimulation (MS), horizontal (HOR.) or vertical (VERT.) placement, and horizontal clinostating (HOR. CLINO.) or vertical clinostating (VERT. CLINO.) on the development of geotropic stem curvature. In those cases where a mechanical stimulus was given, the first internodes were given 10 rubs, once daily, for 5 days. In the graph, zero hours was when the gravitic stimulus or clinostating was begun, and the vertical bars represent standard errors.

Figure 21. The time course of the effect of mechanical perturbation on phototropism. Control = ( ), rubbed = ( ).

Figure 22. Effect of erogenous ethrel (E) and mechanical stimulation of the 1st internode (MS) on phospholipids of the 1st and 2nd internodes as compared to untreated controls (C). Plants were harvested after 10 days. PC = phosphatidyl choline, PS = phosphatidyl serine, PI = phosphatidyl inositol, PE = phosphatidyl ethanolamine, and PG = phosphatidyl glycerol.

Figure 23. Diagrammatic model of thigmomorphogeensis. Dashed lines indicate that no data currently exists in the bean system and hence is purely speculative.

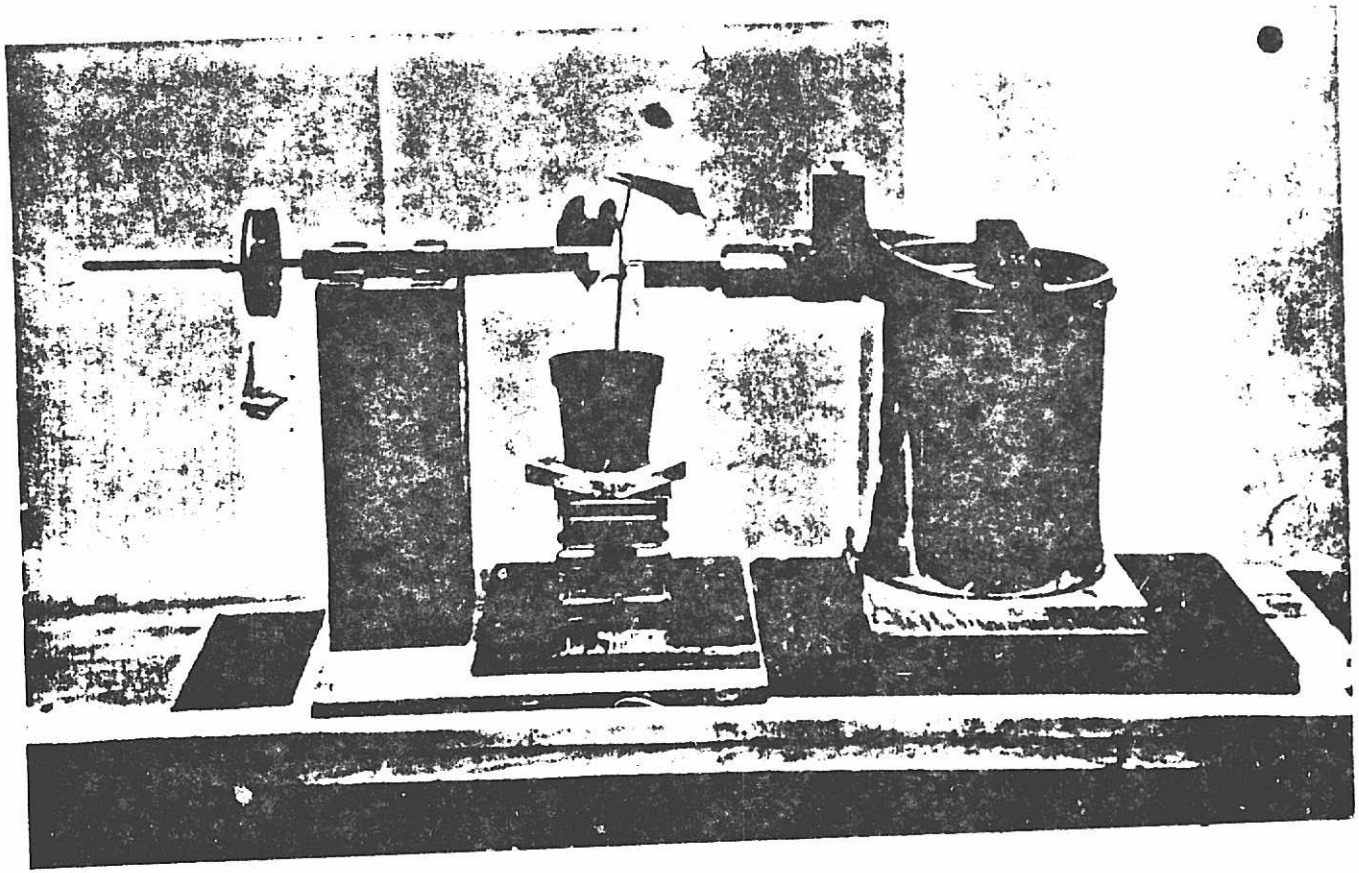


Figure 1

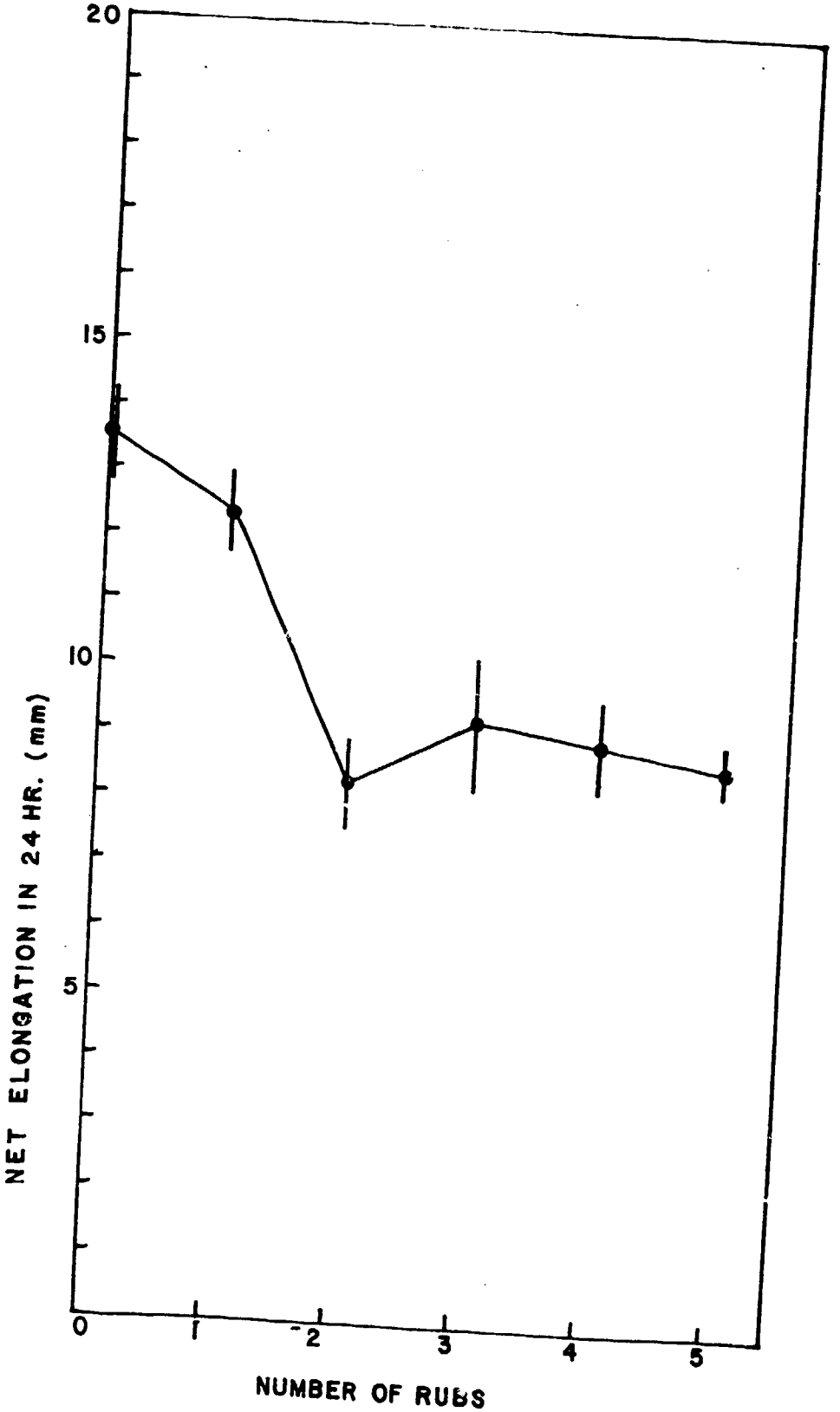
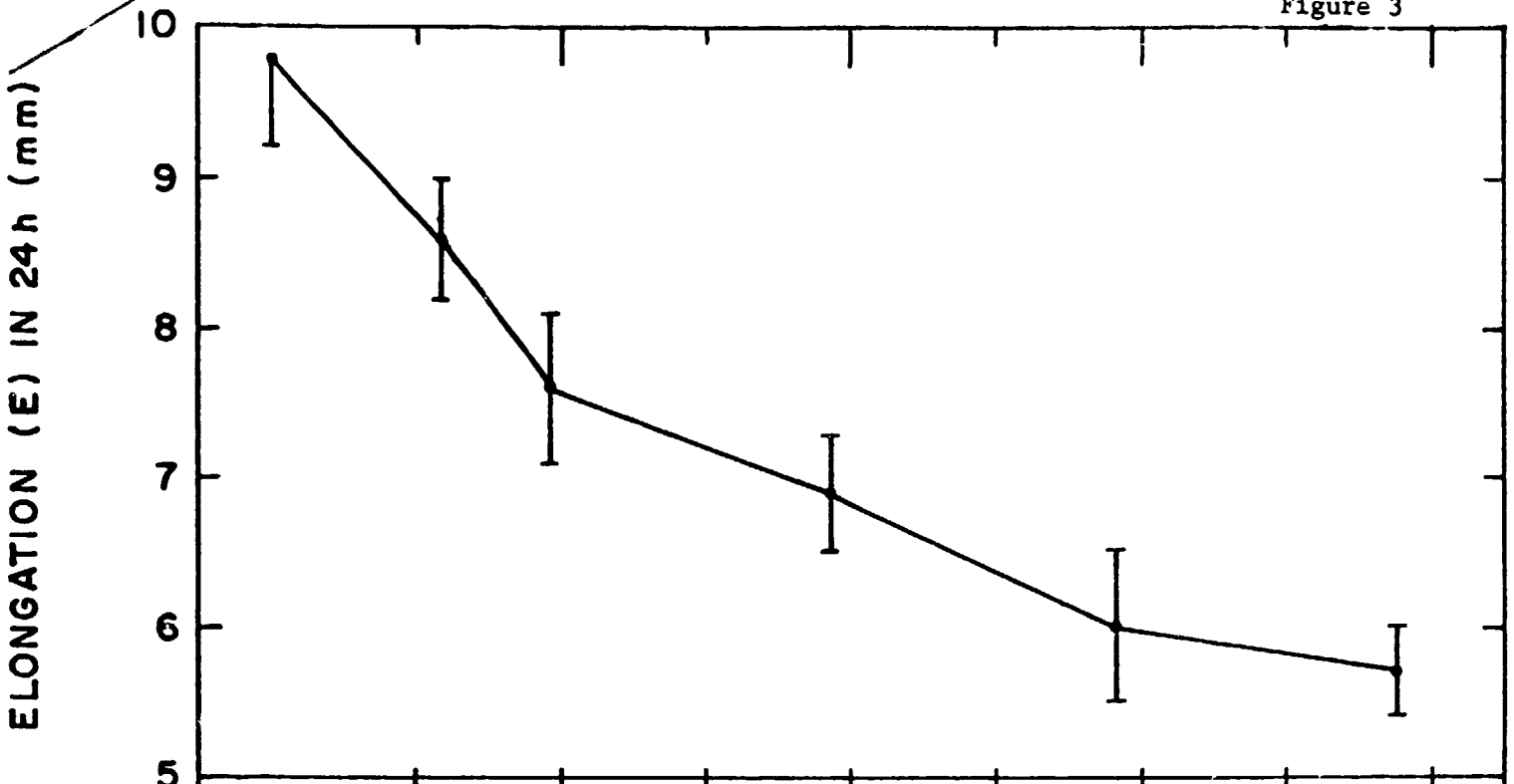


Figure 2

Figure 3



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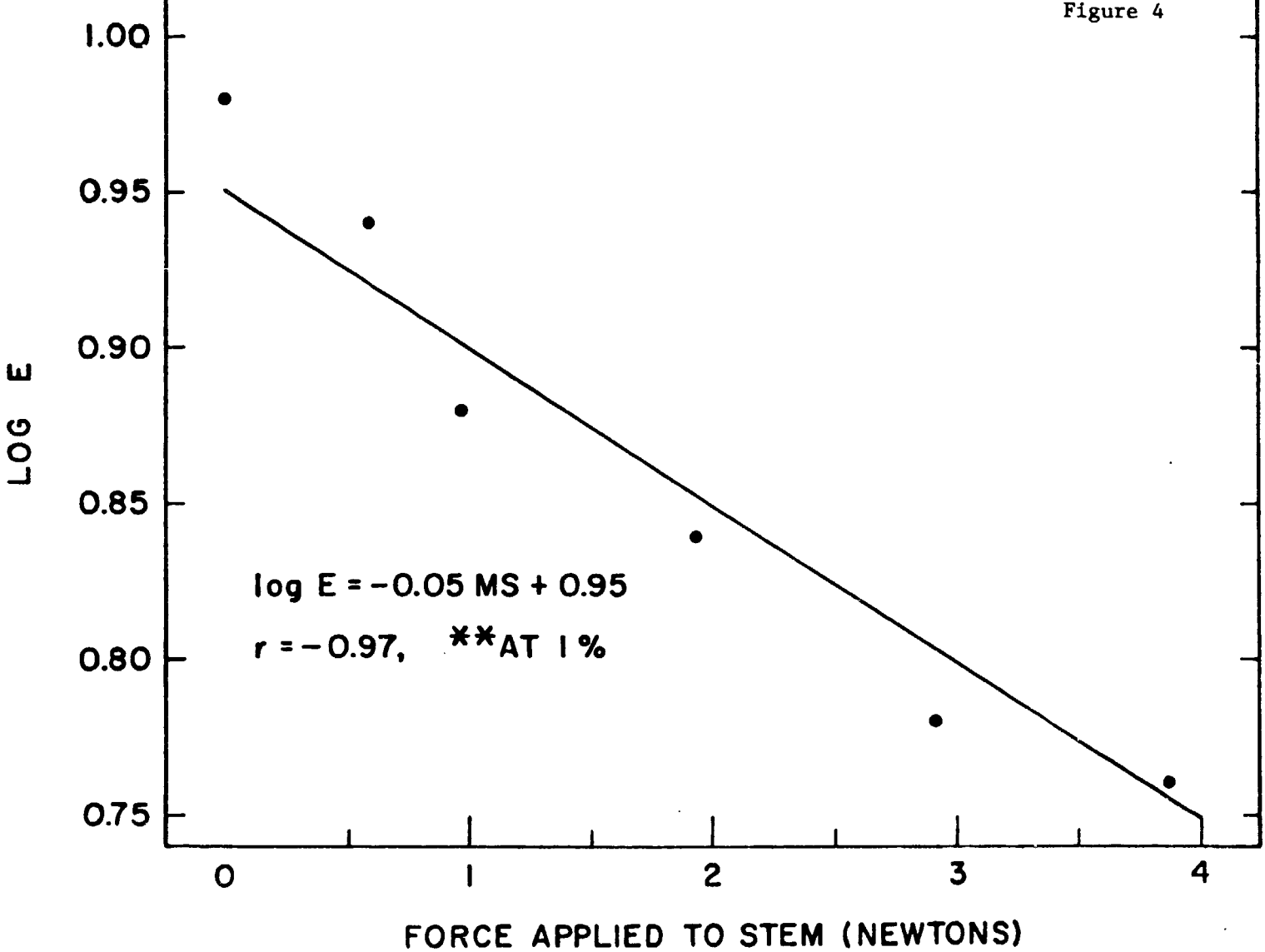

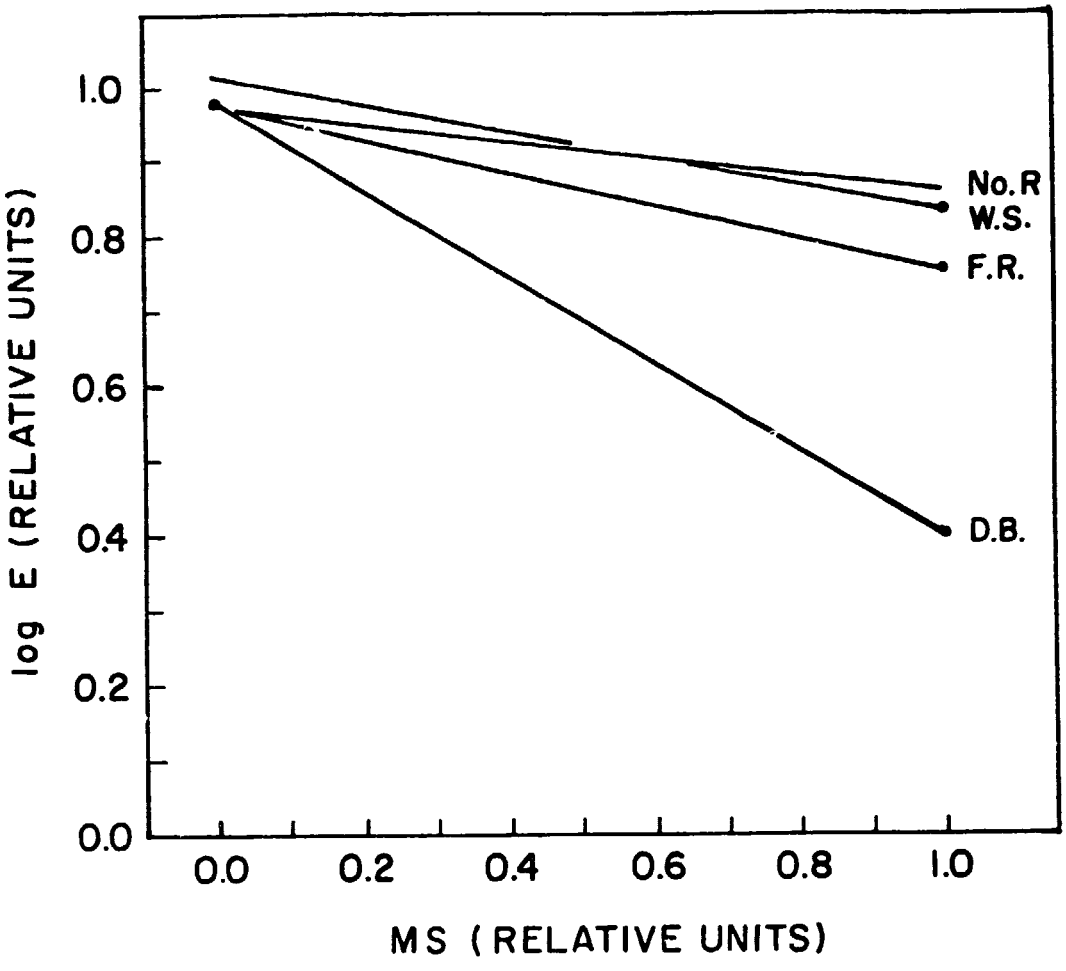
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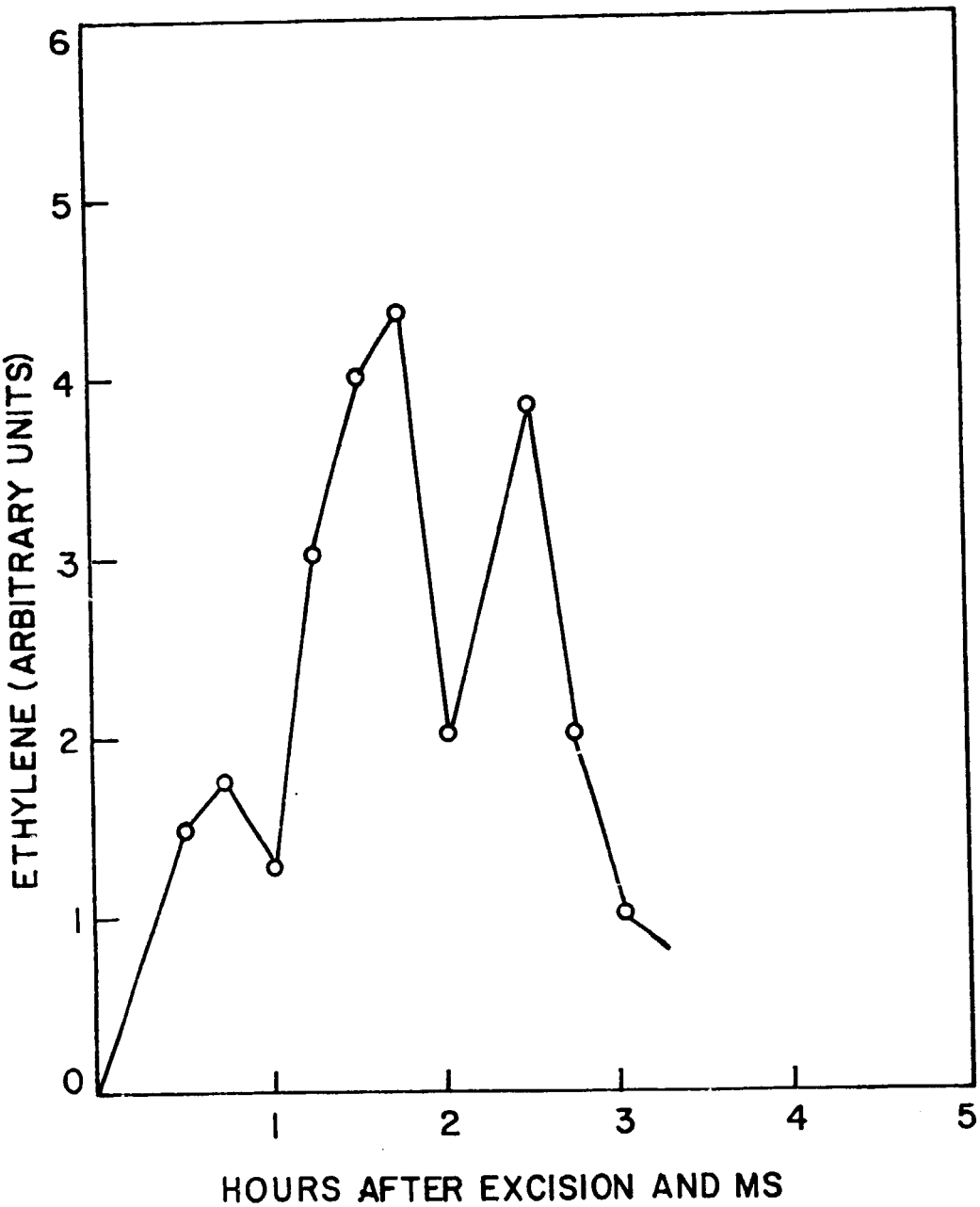
Figure 5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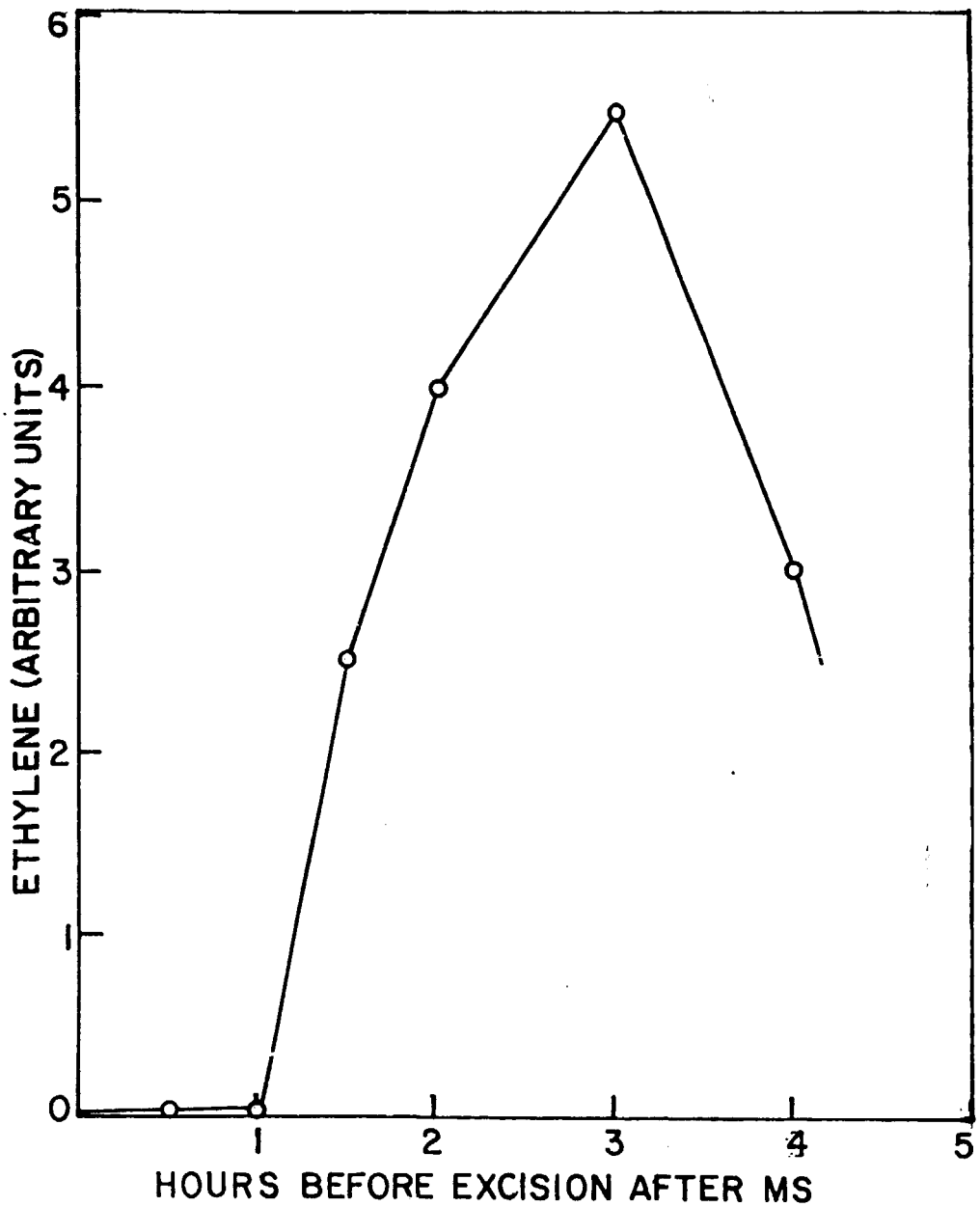
HOURS AFTER EXCISION AND MS

Figure 6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HOURS BEFORE EXCISION AFTER MS

Figure 7

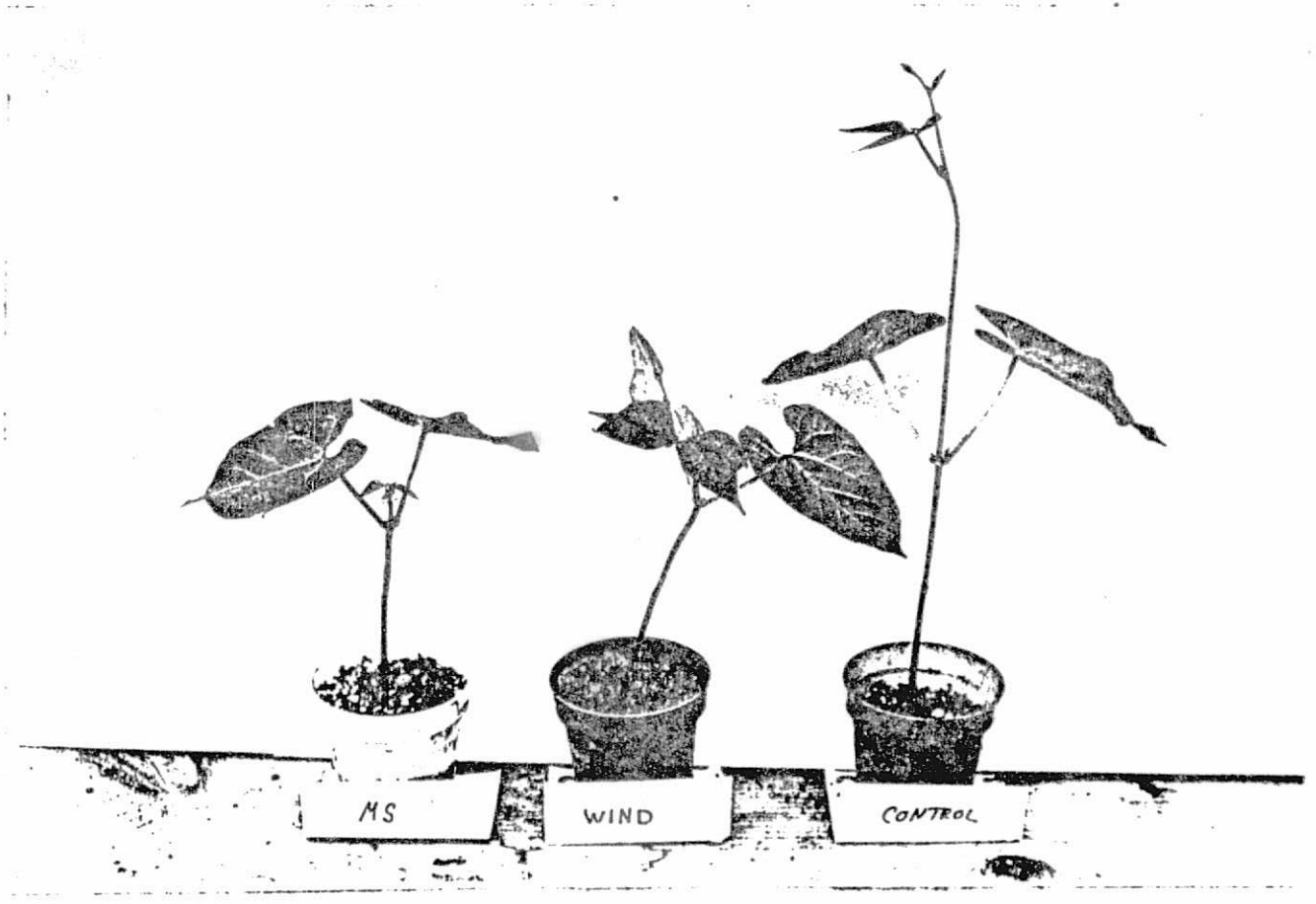


Figure 8

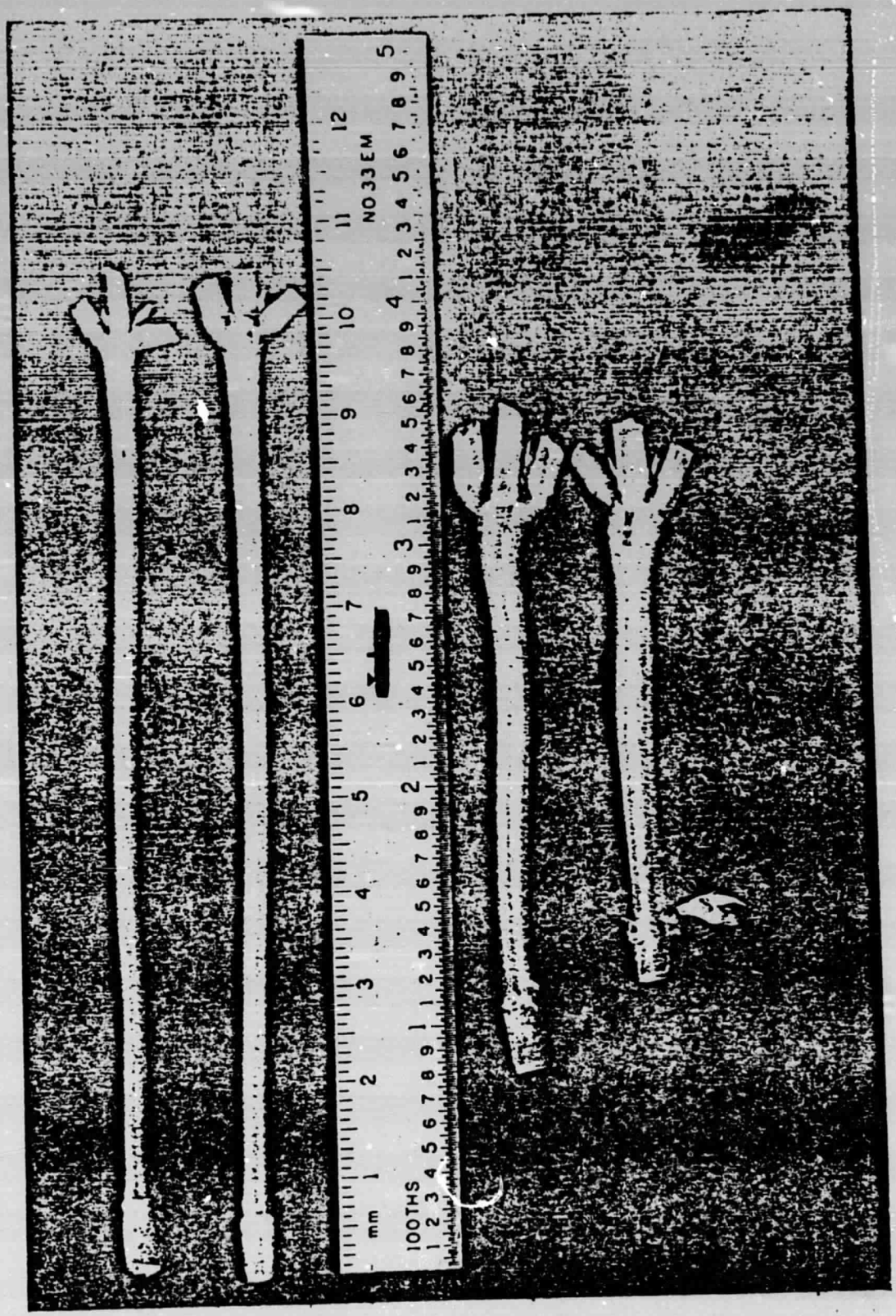
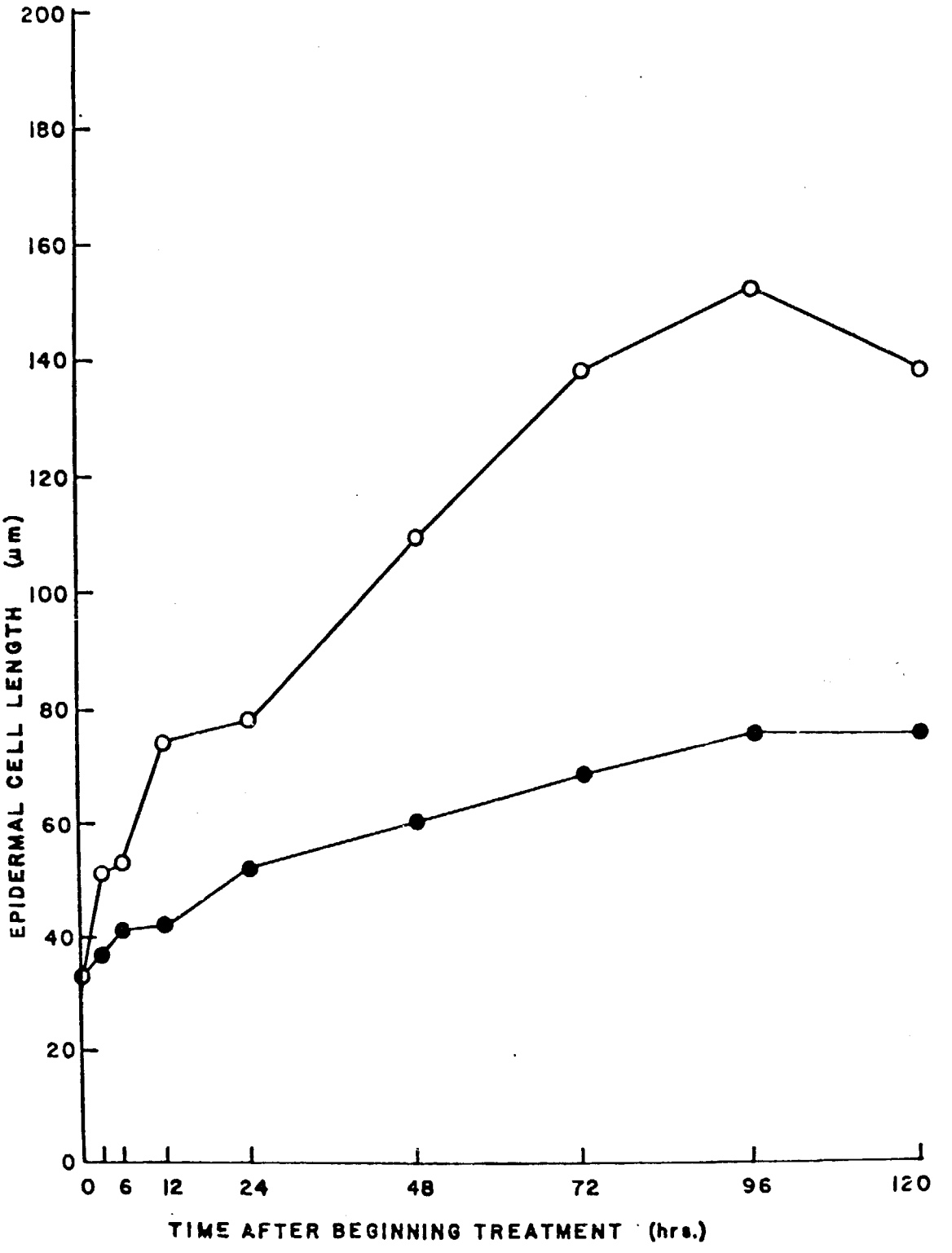


Figure 9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20
0
EPIDERMAL CELL LENGTH (μm)
0
6
12
24
48
72
96
120
TIME AFTER BEGINNING TREATMENT (hrs.)

Figure 10

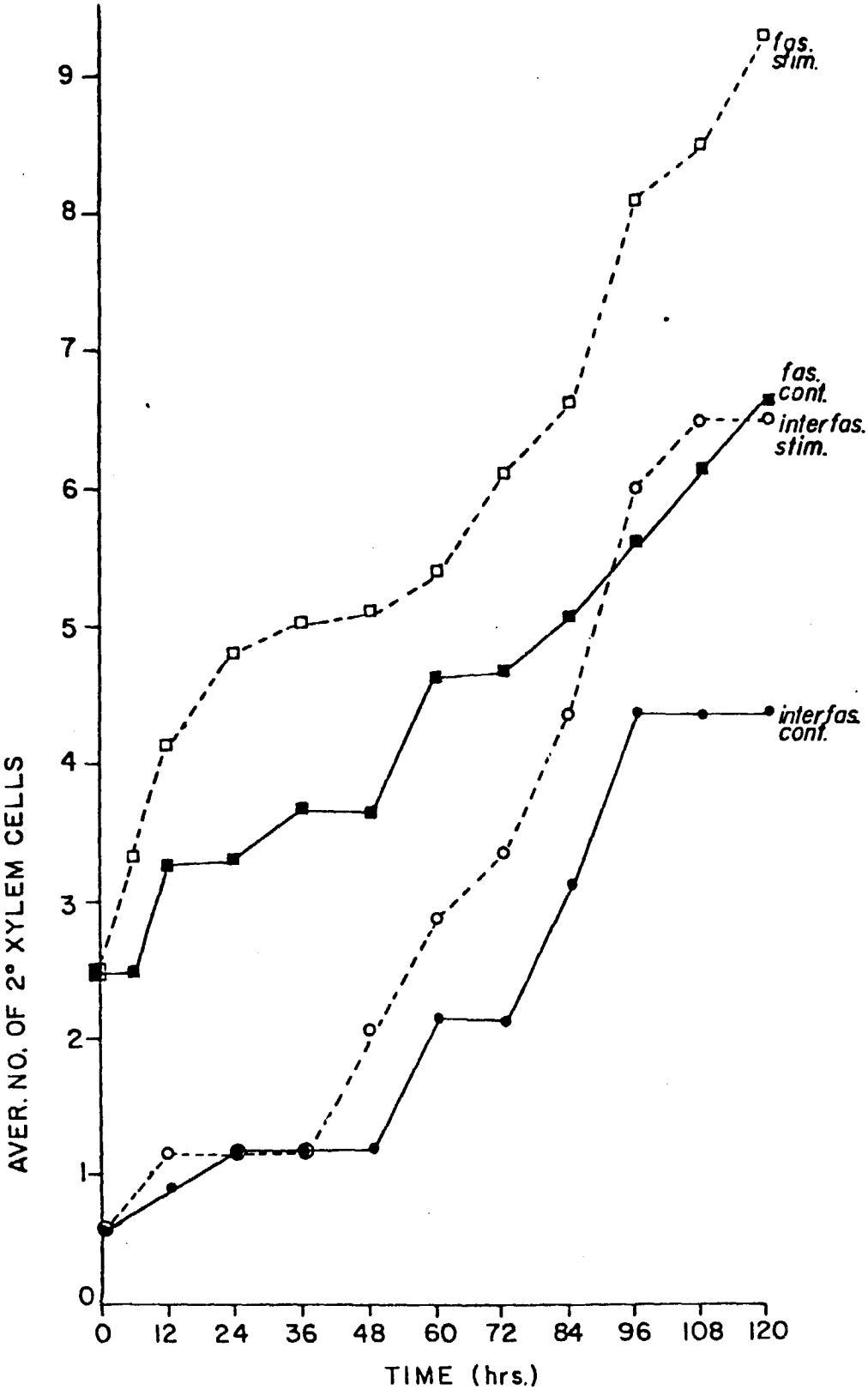

fas. stim.
fas. cont.
interfas. stim.
interfas. cont.
AVER. NO. OF 2° XYLEM CELLS
0
1
2
3
4
5
6
7
8
9
0
12
24
36
48
60
72
84
96
108
120
TIME (hrs.)

Figure 11

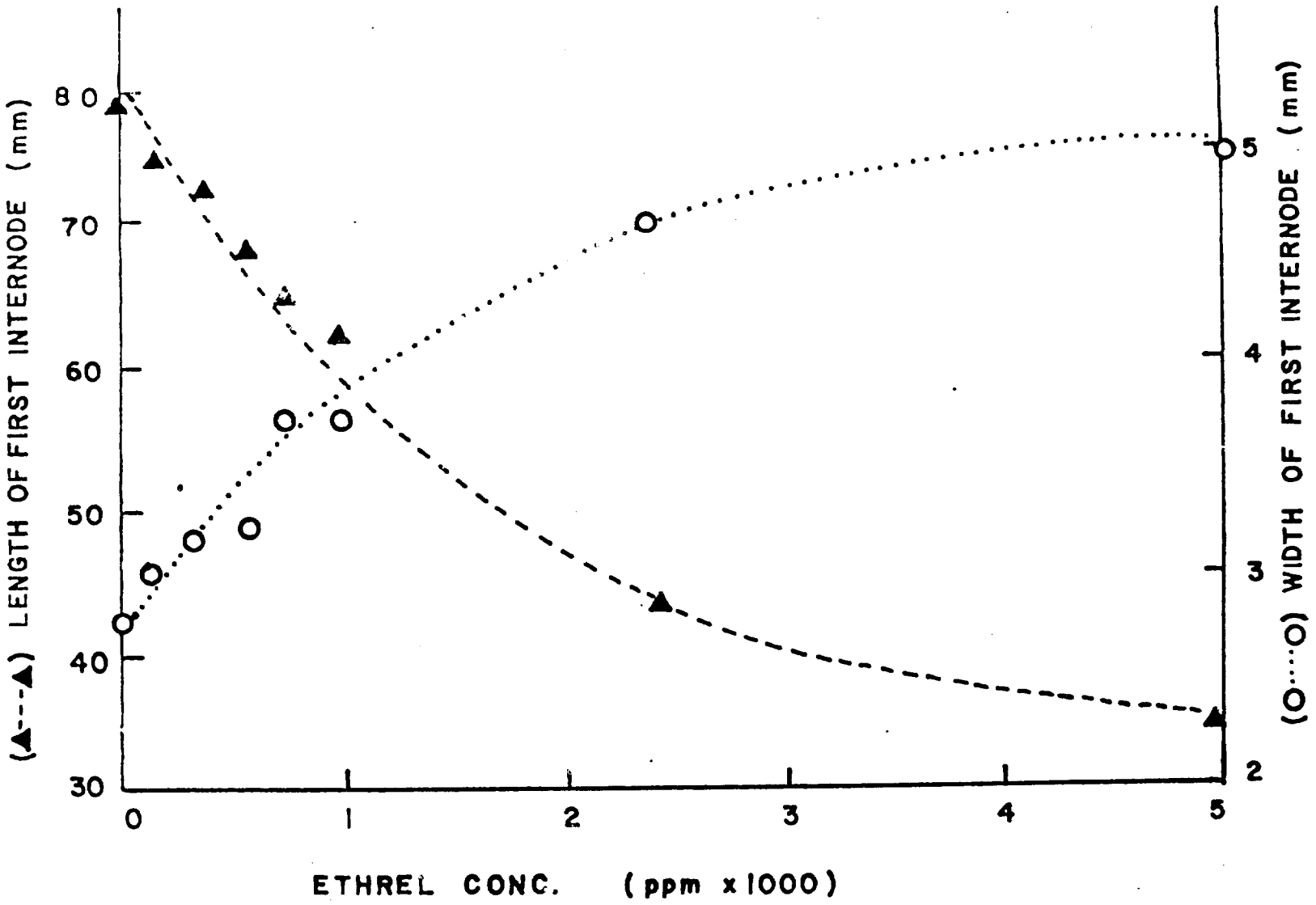

(▲---▲) LENGTH OF FIRST INTERNODE (mm)
(O....O) WIDTH OF FIRST INTERNODE (mm)
80
70
60
50
40
30
5
4
3
2
0
1
2
3
4
5
ETHREL CONC. (ppm x1000)

Figure 12

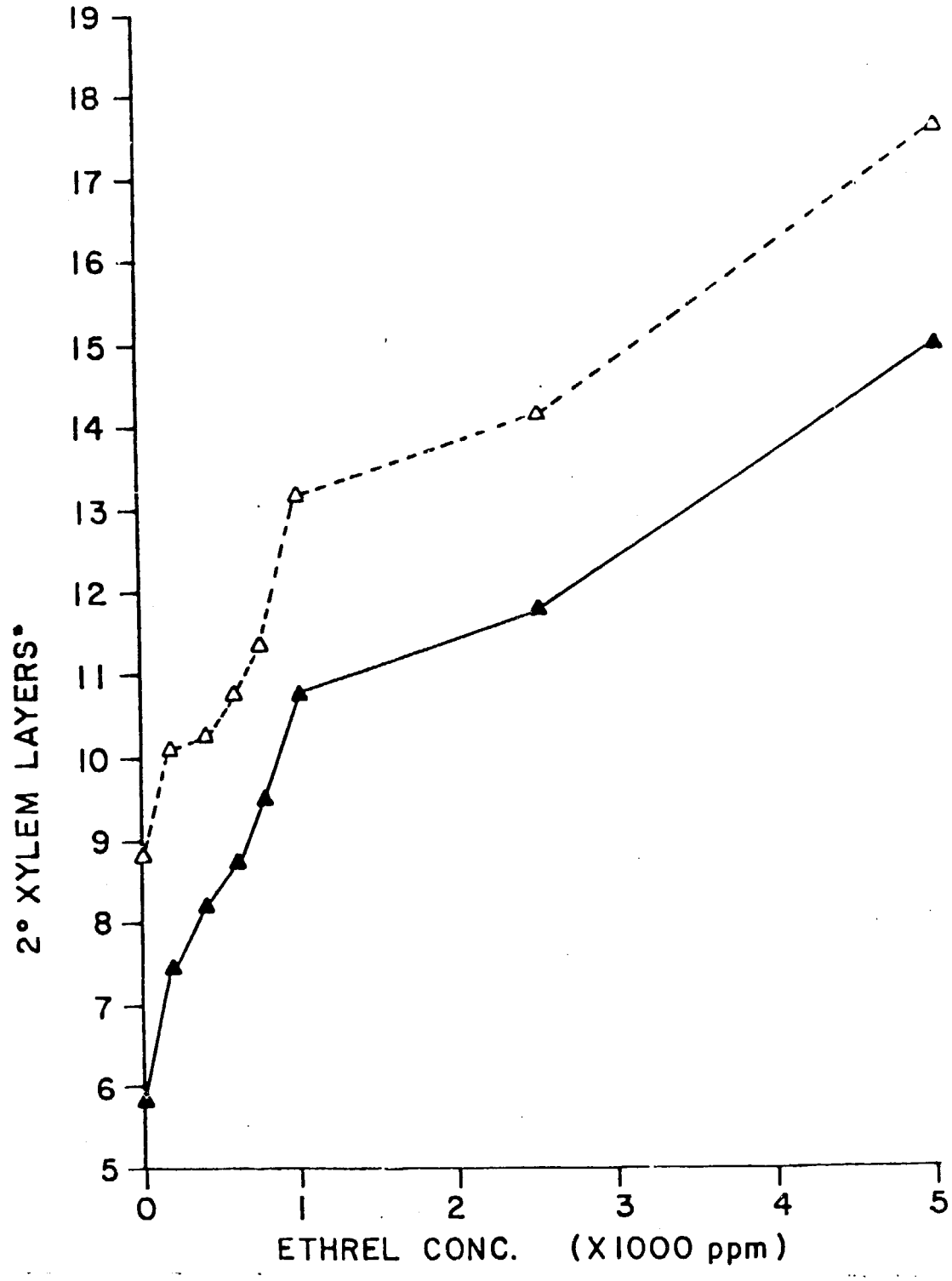

19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
2° XYLEM LAYERS
0
1
2
3
4
5
ETHREL CONC. (X1000 ppm)

Figure 13

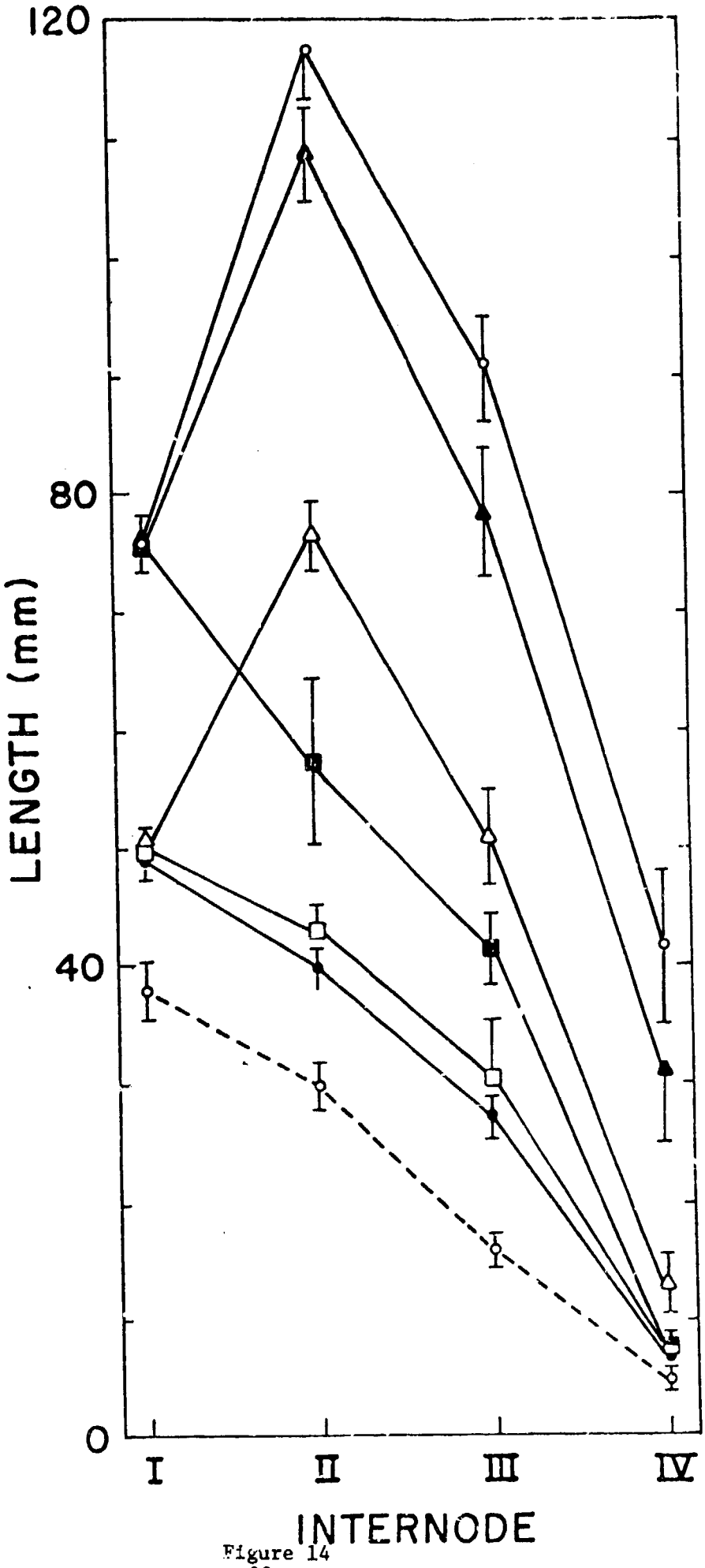


Figure 14

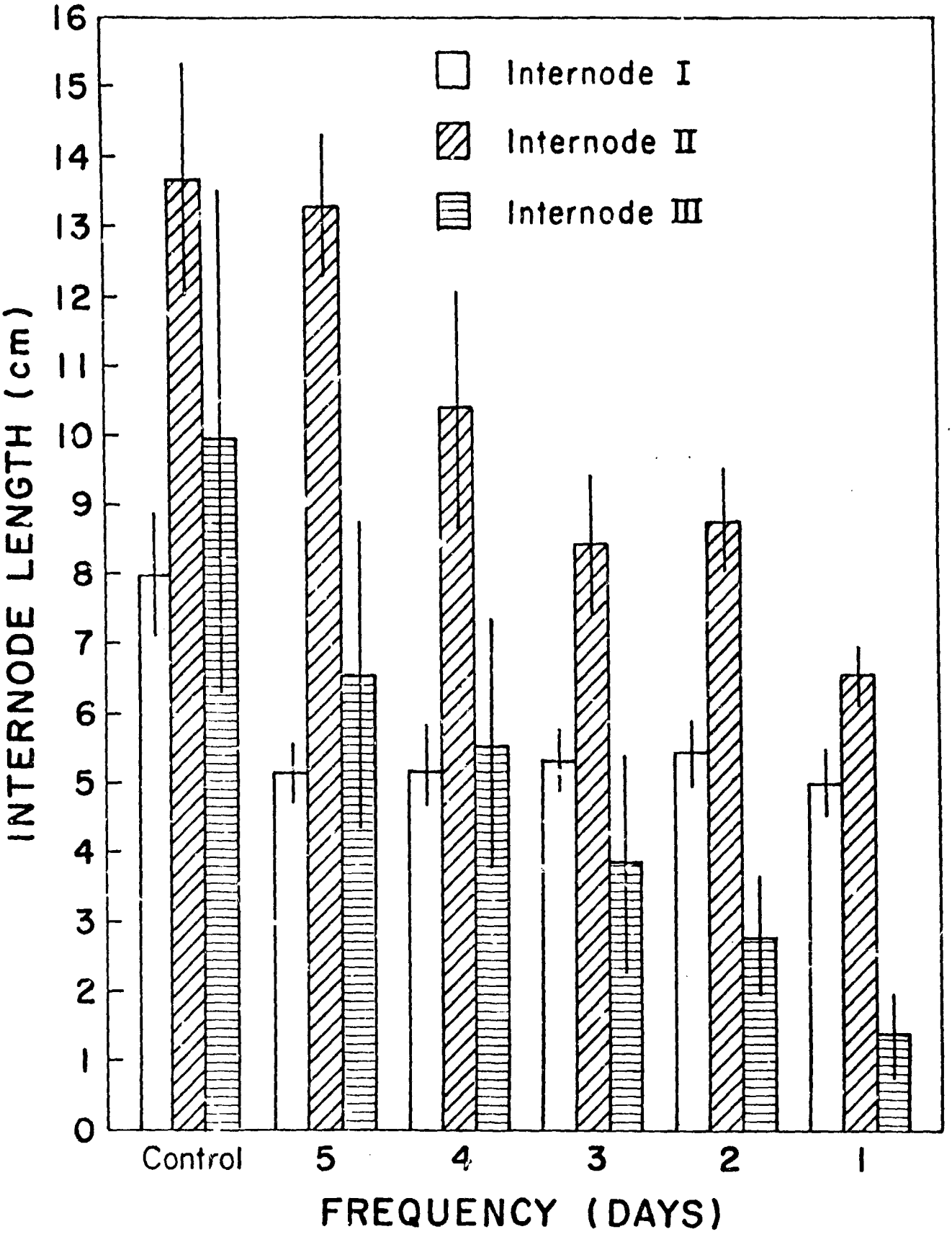

Internode I
Internode II
Internode III
INTERNODE LENGTH (cm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Control
5
4
3
2
1
FREQUENCY (DAYS)

Figure 15

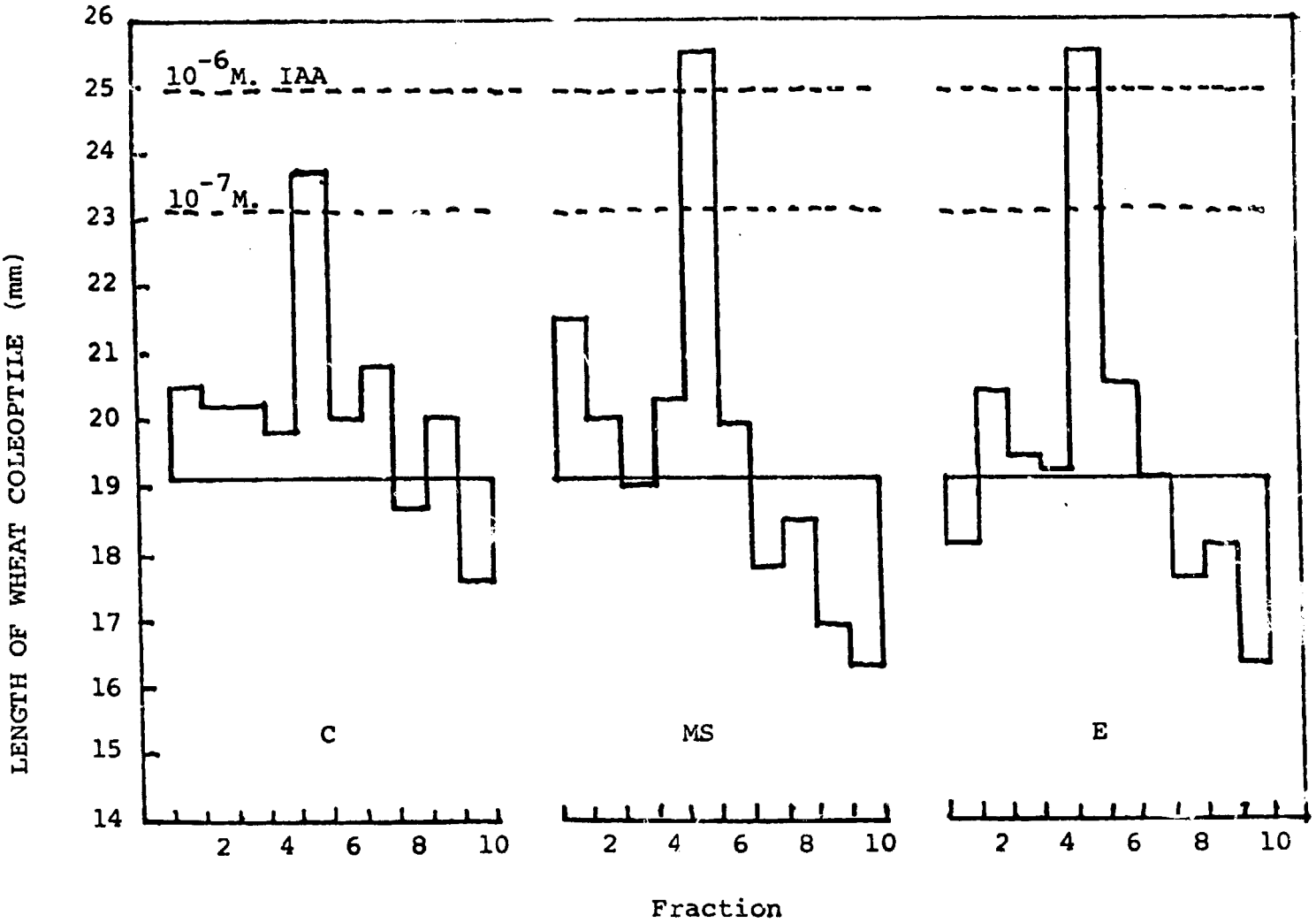

LENGTH OF WHEAT COLEOPTILE (mm)
26
25
24
23
22
21
20
19
18
17
16
15
14
10^{-6}M. IAA
10^{-7}M.
C
MS
E
2
4
6
8
10
Fraction

Figure 16

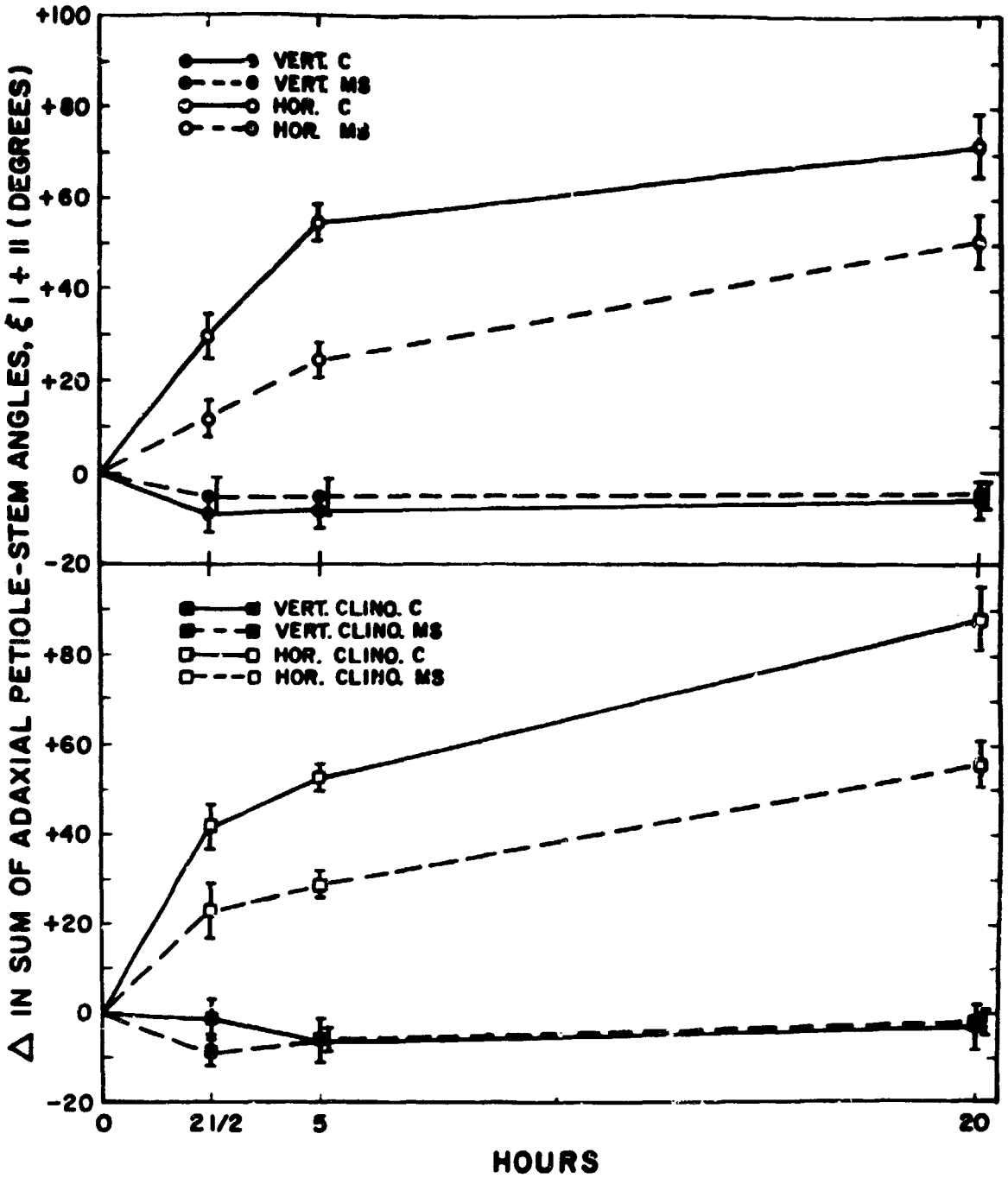

+100
+80
+60
+40
+20
0
-20
VERT. C
VERT. MS
HOR. C
HOR. MS
VERT. CLINO. C
VERT. CLINO. MS
HOR. CLINO. C
HOR. CLINO. MS
Δ IN SUM OF ADAXIAL PETIOLE-STEM ANGLES, Σ I + II (DEGREES)
0
2 1/2
5
20
HOURS

Figure 17

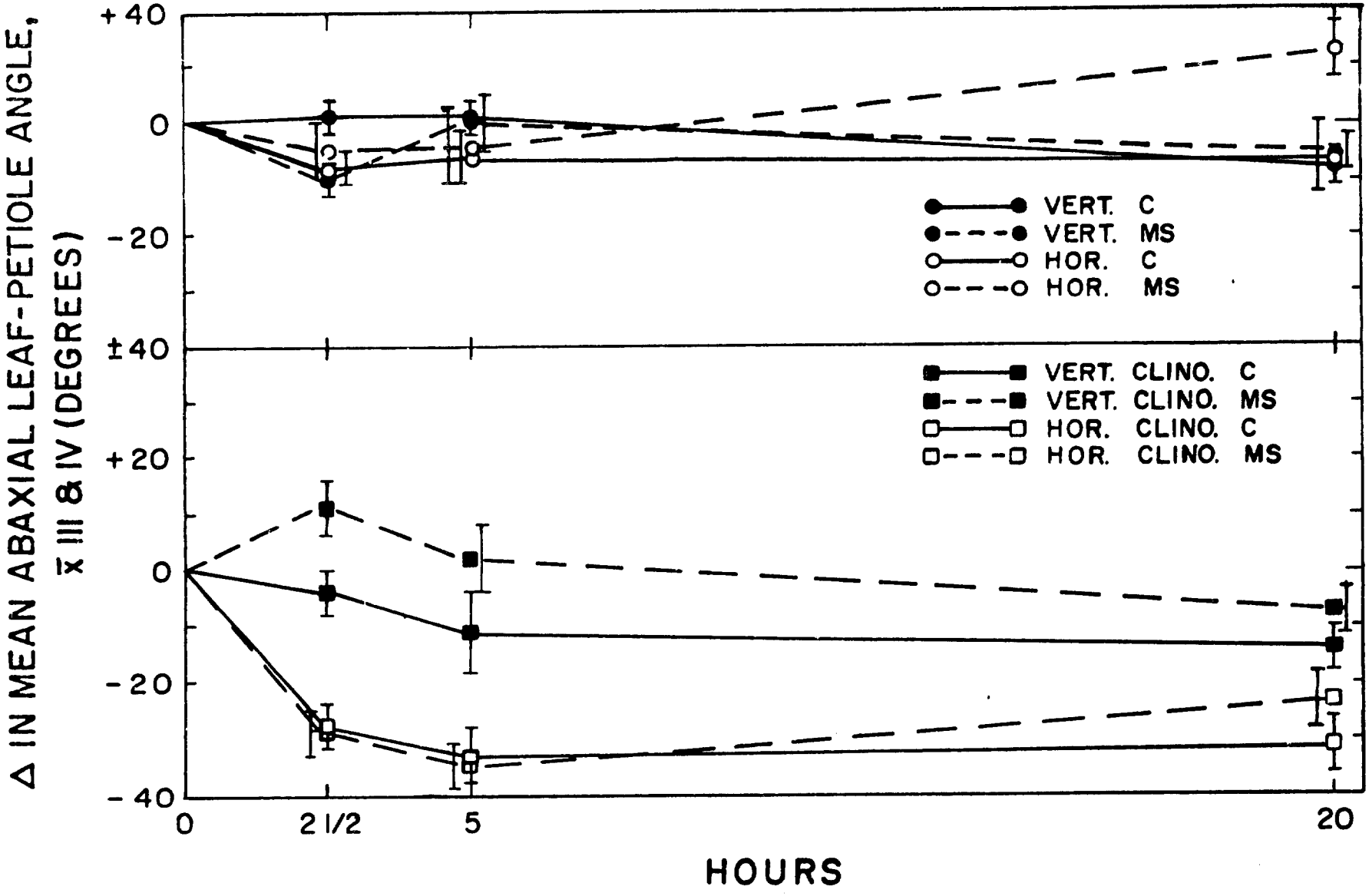
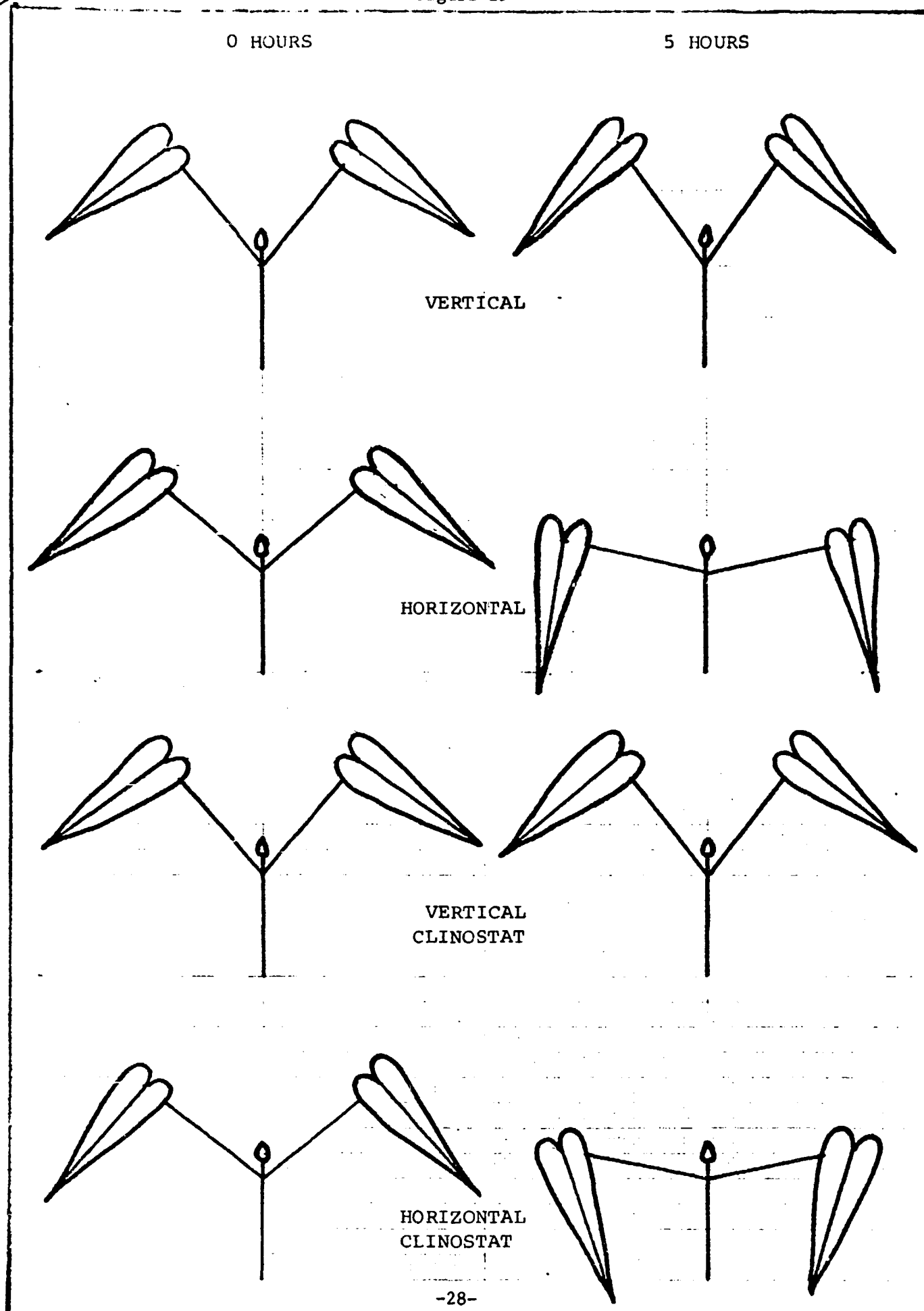

Δ IN MEAN ABAXIAL LEAF-PETIOLE ANGLE, x̄ III & IV (DEGREES)
+40
0
-20
±40
+20
0
-20
-40
0
2 1/2
5
20
HOURS
VERT. C
VERT. MS
HOR. C
HOR. MS
VERT. CLINO. C
VERT. CLINO. MS
HOR. CLINO. C
HOR. CLINO. MS

Figure 18

Figure 19


0 HOURS
5 HOURS
VERTICAL
HORIZONTAL
VERTICAL
CLINOSTAT
HORIZONTAL
CLINOSTAT

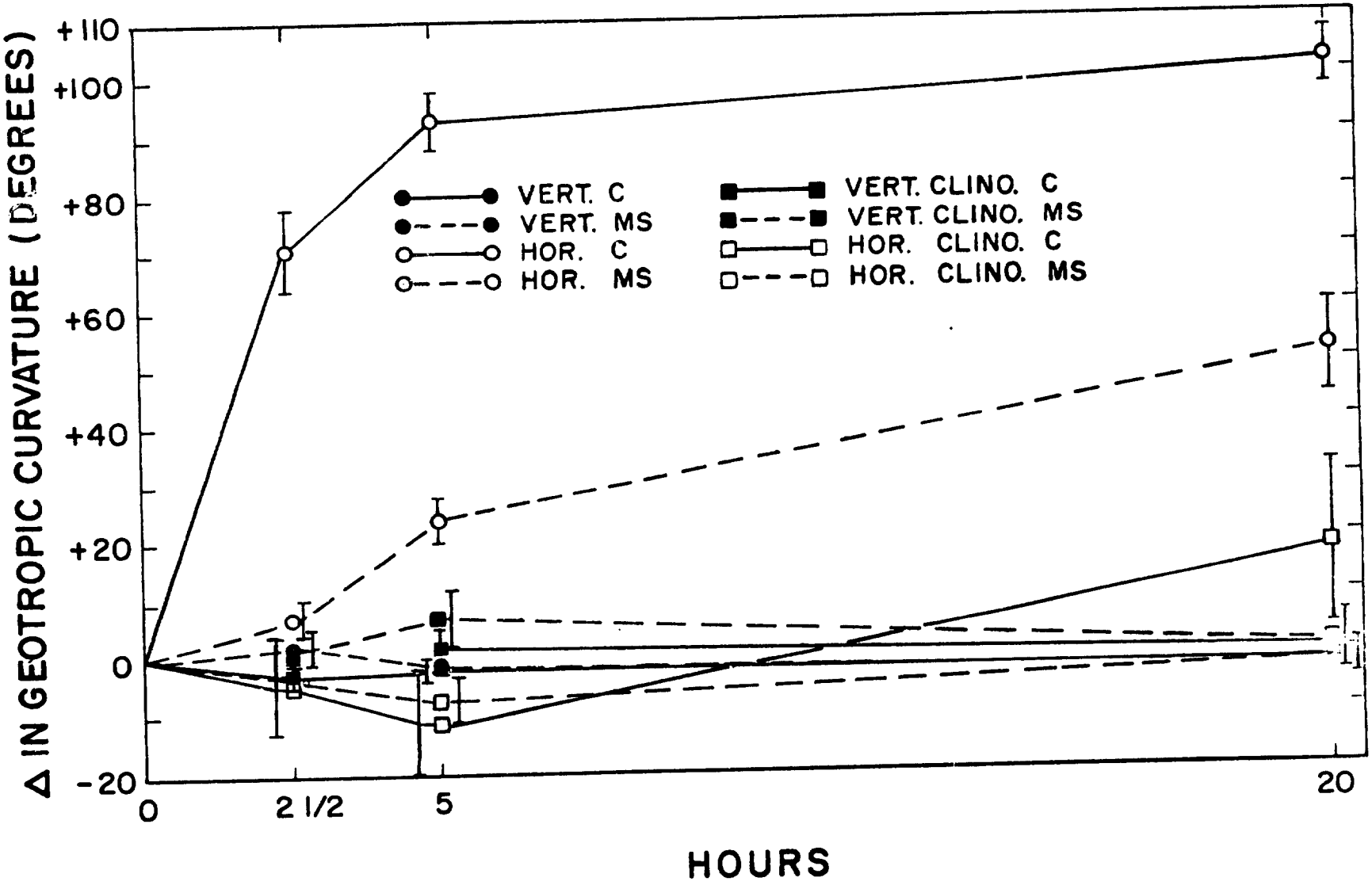

Δ IN GEOTROPIC CURVATURE (DEGREES)
+110
+100
+80
+60
+40
+20
0
-20
0
2 1/2
5
20
HOURS
VERT. C
VERT. MS
HOR. C
HOR. MS
VERT. CLINO. C
VERT. CLINO. MS
HOR. CLINO. C
HOR. CLINO. MS

Figure 20

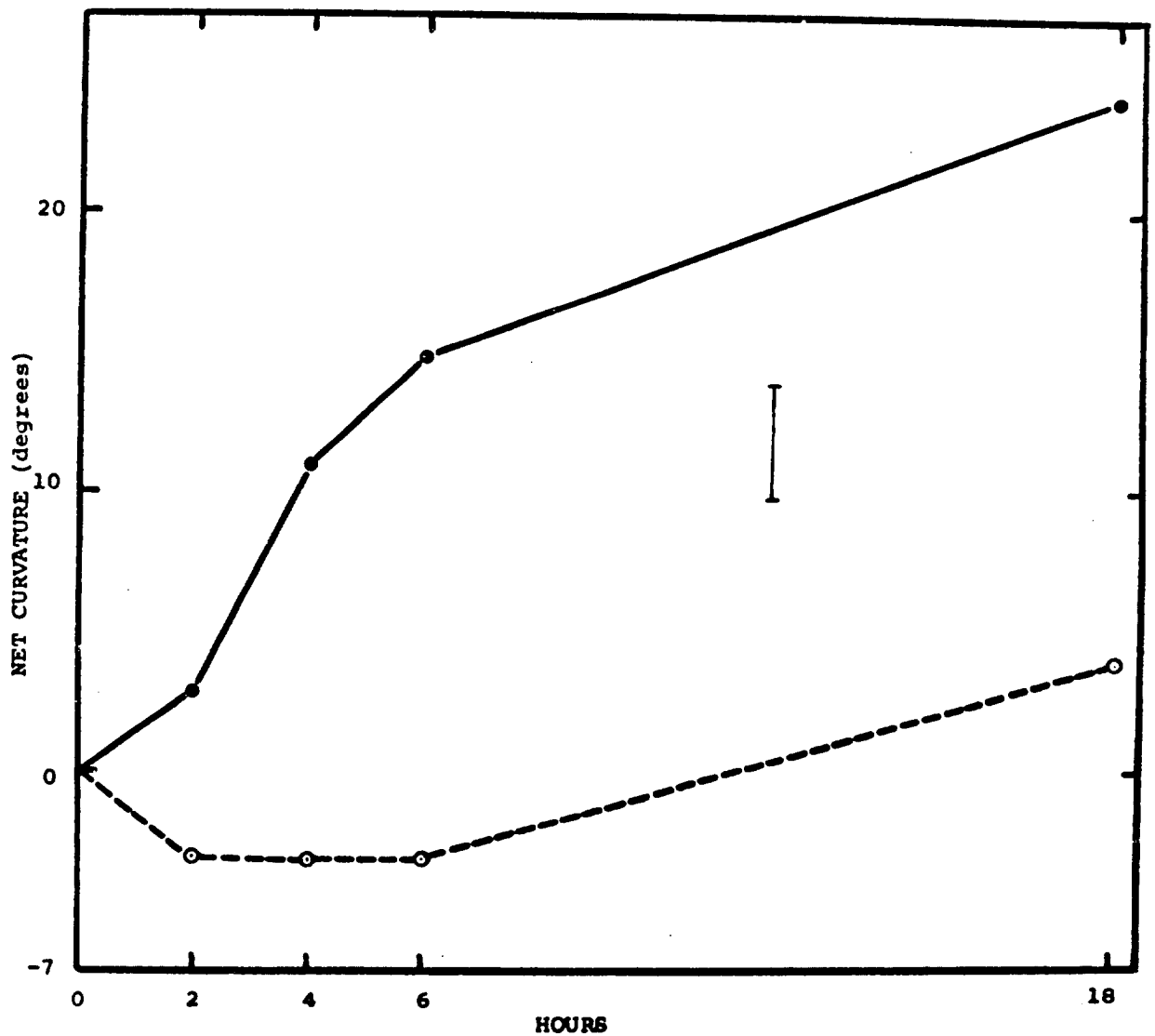


Figure 21

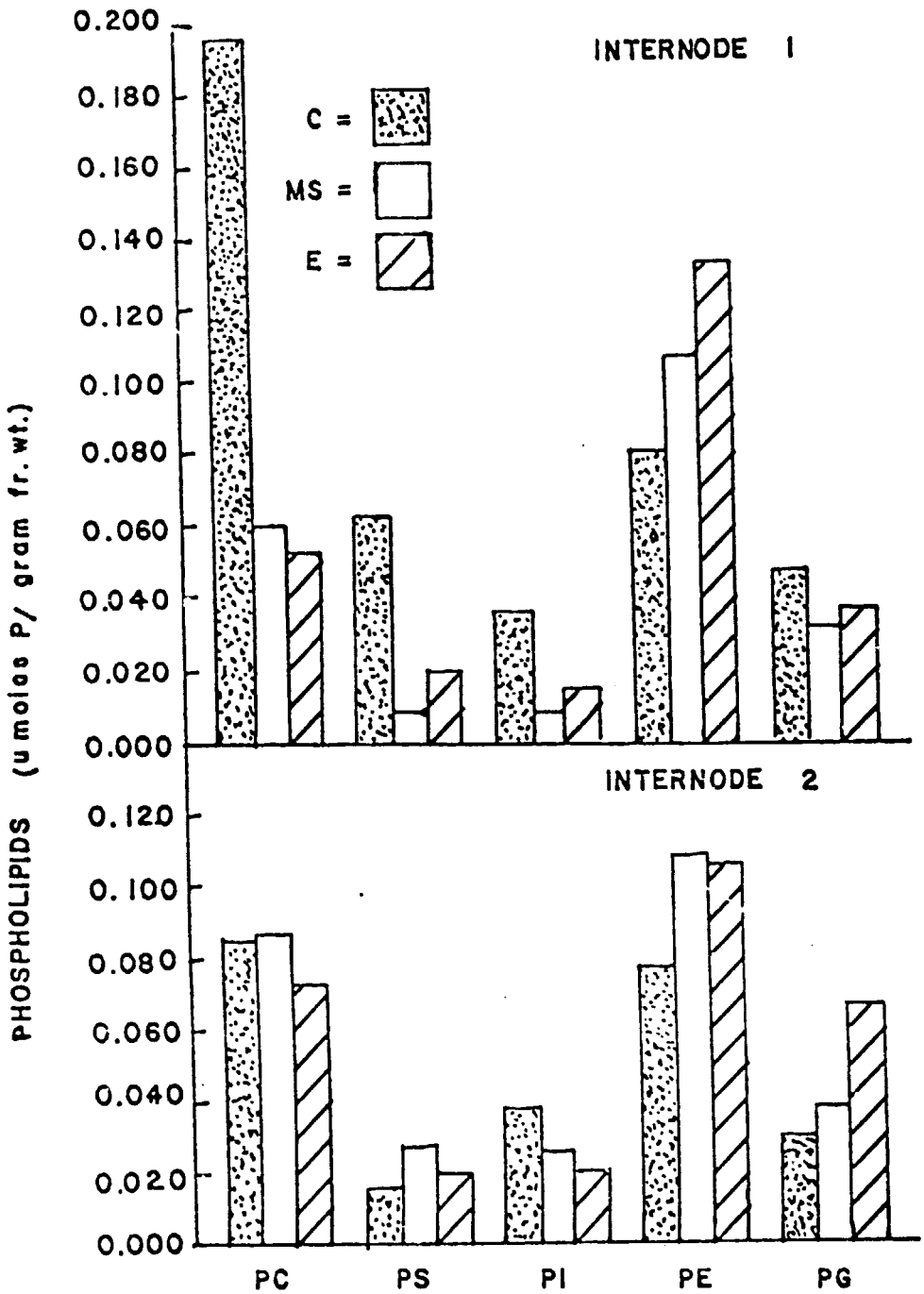

INTERNODE 1
C =
MS =
E =
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
INTERNODE 2
0.120
0.100
0.080
0.060
0.040
0.020
0.000
PHOSPHOLIPIDS (u moles P/ gram fr. wt.)
PC
PS
PI
PE
PG

Figure 22

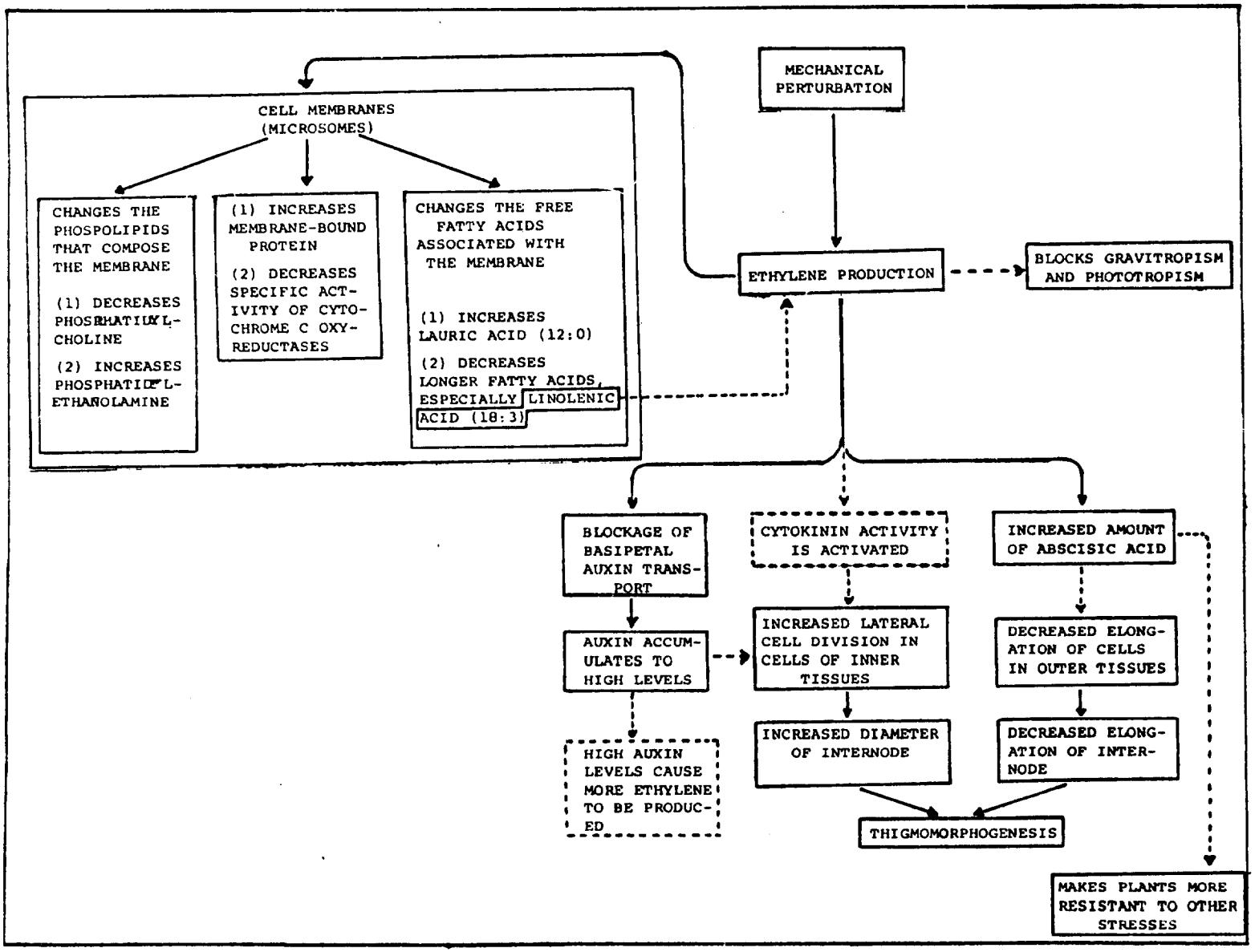

MECHANICAL PERTURBATION
CELL MEMBRANES (MICROSOMES)
CHANGES THE PHOSPOLIPIDS THAT COMPOSE THE MEMBRANE
(1) DECREASES PHOSPHATIDYL-CHOLINE
(2) INCREASES PHOSPHATIDYL-ETHANOLAMINE
(1) INCREASES MEMBRANE-BOUND PROTEIN
(2) DECREASES SPECIFIC ACTIVITY OF CYTOCHROME C OXYREDUCTASES
CHANGES THE FREE FATTY ACIDS ASSOCIATED WITH THE MEMBRANE
(1) INCREASES LAURIC ACID (12:0)
(2) DECREASES LONGER FATTY ACIDS, ESPECIALLY LINOLENIC ACID (18:3)
ETHYLENE PRODUCTION
BLOCKS GRAVITROPISM AND PHOTOTROPISM
BLOCKAGE OF BASIPETAL AUXIN TRANSPORT
CYTOKININ ACTIVITY IS ACTIVATED
INCREASED AMOUNT OF ABSCISIC ACID
AUXIN ACCUMULATES TO HIGH LEVELS
INCREASED LATERAL CELL DIVISION IN CELLS OF INNER TISSUES
DECREASED ELONGATION OF CELLS IN OUTER TISSUES
HIGH AUXIN LEVELS CAUSE MORE ETHYLENE TO BE PRODUCED
INCREASED DIAMETER OF INTERNODE
DECREASED ELONGATION OF INTERNODE
THIGMOMORPHOGENESIS
MAKES PLANTS MORE RESISTANT TO OTHER STRESSES

Figure 23

Table 1. Reciprocity between force and stimulus number at high and low stimulus forces. First internodes were stimulated the equivalent of 12 rubs at 0.58 Newtons in the low range or 12 rubbs at 1.94 Newtons, and the net elongation measured after 24 h. Each datum is followed by its standard error.

low force range			high force range		
Force Applied (Newtons)	No. of Rubs	Net Elongation (mm)	Force Applied (Newtons)	No. of Rubs	Net Elongation (mm)
0.58	12	16±1	0.97	24	9±1
1.17	6	15±1	1.94	12	9±1
1.75	4	16±2	2.91	8	6±1
2.33	3	14±1	3.88	6	6±0

Table 2. Summary of Evidence that Ethylene Acts as the Trigger for Thigmomorphogenesis.

1. M.S. induces E. production (Goeschel et al., Irvine and Osborne, Hiraki and Ota, Jaffe and Biro, Brown and Leopold)

2. Hypobaric condition blocks thigmomorphogenesis (Jaffe and Biro)

3. M.S. and E. both induce (Jaffe et al.):

   a) decreased axial cortical cell elongation and medullary cell division
   b) increased lateral cortical cell enlargement and axial medullary cell division
   c) increased proteins in endomembranes
   d) decreased phosphatydal choline and increased phosphatydal ethanolamine in endomembranes
   e) increased free lauric acid and decreased free linolenic acid in endomembranes
   f) similar patterns in fatty acids bound to phospholipids
   g) decreases in specific activities of endomembrane-associated enzymes
   h) petiole-stem epinasty in beans and tomatoes
   i) inhibition of geotropism
   j) inhibition of phototropism
   k) increase in auxin in internode
   l) resistance of plants to some other stresses

Table 3. Ethylene inhibitors that are also capable of partially or completely blocking thigmomorphogenesis of the first bean internode.

Factor	Net Elongation (mm)
control - MS	8.2±0.9
control + MS	4.9±0.6
2 h hypobaric pressure (0.1 Atmos.) + MS	7.2±0.5
1 m M EDTA + 0.1% DMSO	5.4±0.9
1 m M $CoCl_2$ + 0.1% DMSO	5.6±0.6

Table 4. The effect of mechanical perturbation on cell lengths as observed in longitudinal sections of bean first internodes. All cells were measured with an ocular micrometer calibrated with a stage micrometer. Each datum is the average of 30 cell measurements and is followed by its standard error.

	Length (mm)	
Cell Type	Control	M.S.
Epidermal Cells	110 ± 10	47 ± 6
Cortical Cells	102 ± 4	86 ± 10
Pith Cells	189 ± 12	202 ± 17

Table 5. A comparison of cell numbers in control and mechanically stimulated bean internodes. Analysis was performed as described in Table 1. Each datum is followed by its standard error.

Tissue	Number of cells along section radius		Radial thickness of tissue type (μm)	
	control	rubbed	control	rubbed
Pith	6.8 ± 0.5	8.8 ± 0.4	283.7 ± 2.6	313.0 ± 4.8
2° Xylem	5.1 ± 0.3	8.3 ± 0.3	70.8 ± 1.4	88.6 ± 1.7
2° Phloem	6.6 ± 0.4	6.8 ± 0.2	58.6 ± 0.9	57.7 ± 1.3
1° Phloem	3.0 ± 0.2	3.2 ± 0.2	23.2 ± 0.6	12.9 ± 0.8
Cortex	4.2 ± 0.3	4.7 ± 0.3	108.5 ± 1.0	131.7 ± 2.0

Table 6. Summary of Field Data for Multiple Linear Regression analysis for the ten field experiments during the fall of 1977 and the spring of 1978. The treatments were exposed to the wind (MS) and sheltered from the wind (C), for ten days for each experiment. In the third column, the number of plants (n) in each treatment is shown. Also shown is the average net elongation (E), difference in net elongation between controls and exposed plants (ΔE, $\Delta E = C - MS$), average plant diameter (D), and the difference (ΔD, $\Delta D = MS - C$) of the diameters between exposed and control plant diameters. The environmental factors included for regression analysis are wind speed (V), wind gusting frequency (F), irrigation (I), and the average daily high (H) and low (L) temperatures.

Experiment	Treatment	n	E (mm)	ΔE (mm)	D (mm)	ΔD (mm)	V (Km/hr)	F (Gust counts/hr)	H (°C)	L (°C)	I (cm)
1	C	24	86.1		2.6		0	0	26.1	15.0	0.13
1	MS	27	62.2	23.9	2.8	0.2	3.3	5.6	26.7	16.7	0.05
2	C	26	45.3		2.1		0	0	25.0	15.0	0.64
2	MS	24	50.8	-5.5	2.4	0.3	5.8	5.9	23.3	15.0	0.69
3	C	59	72.8		2.9		0	0	22.2	10.6	3.28
3	MS	60	72.3	0.5	3.2	0.3	6.5	6.7	22.2	10.6	3.30
4	C	16	42.5		2.3		0	0	16.7	2.2	2.21
4	MS	16	31.4	11.1	2.4	0.1	8.9	14.1	15.6	2.2	2.16
5	C	17	18.3		2.4		0	0	18.3	2.8	1.65
5	MS	17	10.5	7.8	2.7	0.3	7.7	11.7	18.3	3.3	1.52
6	C	27	28.8		2.8		0	0	12.8	6.1	4.95
6	MS	21	21.0	7.5	2.6	-0.2	7.1	12.8	12.8	6.1	5.33
7	C	23	107.6		3.4		0	0	28.9	13.3	3.81
7	MS	22	78.8	28.8	3.8	0.4	7.3	12.0	26.1	12.2	3.68
8	C	15	51.8		2.9		0	0	26.7	13.3	4.57
8	MS	10	28.1	23.7	3.6	0.7	1.6	5.5	24.4	12.2	4.57
9	C	12	44.8		2.7		0	0	27.8	14.4	3.18
9	MS	12	35.9	8.9	3.4	0.7	2.7	9.0	26.1	13.3	3.18
10	C	27	142.0		2.5		0	0	30.0	16.1	6.73
10	MS	27	108.1	33.9	3.0	0.5	2.2	8.2	28.9	14.4	6.73

Table 7. The effect of mechanical stimulation on the elongation of bean plants at various day/night ambient temperatures. These experiments were done in a controlled growth chamber for ten days. The mechanically stimulated plants were rubbed ten times each day (MS) and the controls (C) were not. The first two columns are the daily day (H)/night (L) temperatures (high/low, °C). The second two columns are the elongation of C and MS plants. The last column is the net retardation of elongation due to mechanical stimulation (C - MS). Experiments 6-9 are from Jaffe (1976a).

	Daily day/night temperatures (°C)		Elongation (mm)		Difference in Elongation
Experiment	H	L	C	MS	(C-MS)
1	15.6	15.6	33.6	27.0	6.6
2	18.3	12.8	26.2	13.2	13.0
3	18.3	15.6	80.0	28.2	51.8
4	18.3	18.3	183.8	60.4	123.4
5	21.1	15.6	73.1	29.3	43.8
6	21.1	21.1	163.0	60.0	103.0
7	23.9	23.9	292.0	110.0	182.0
8	26.7	26.7	286.0	156.0	130.0
9	30.0	30.0	185.0	145.0	40.0

Table 8. The effect of mechanical stimulation of different internodes, and application of ethephon solution (1000 ppm, 10 µl) on the diameter of bean internodes.

The Stimulated Internode	Diameter of Internode (mm) I	II	III
Control	2.14±0.05	1.34±0.03	1.00±0.02
I	2.56±0.08	1.67±0.06	1.11±0.04
I + II	2.58±0.07	2.20±0.07	1.22±0.05
II	2.27±0.04	2.05±0.06	1.12±0.04
I*	2.27±0.04	1.39±0.02	1.00±0.02
I + II + III	2.52±0.07	2.22±0.08	1.44±0.06
Ethephon**	3.80±0.06	2.42±0.04	1.86±0.04

*Stimulated at the same time as internode II

**Applied to the middle of the first internode

Table 9. Height of plants in 10 days (mm) of plants grafted to the first internode. In the MS plants, the left plant (donor) was stimulated.

Experiment	C		MS		Non-Grafted MS		t		
No.	Left	Right	Left	Right	Left	Right	value[1]	df	α
1	51.3±12.6	52.2±12.8	43.8±6.8	44.1±8.2	-	-	1.457	5	0.30
2	189.2±26.3	184.4±44.3	92.3±35	148.0±33.6	-	-	2.549	11	0.05*
3	113.0±44.9	137.0±61.5	50.0±24	76.0±34.2	56.1±18.7	105.3±36.8	4.565	12	0.001***

[1]The t-test was done between the right-hand plants (receivers) of control (C) and mechanically stimulated (MS) plants.

Table 10. The effect of mechanical stimulation and plant hormones on the growth of young bean plants, and on the thickness of the first internode. Hormones were applied to the bud once daily in a 50 µl drop. The first true internode of the stimulated plants was rubbed 10 times once each day. The duration of the experiment was 5 days.

	Change in total length (mm)		Change in diameter of internode #1 (mm)	
Additive	Control	Rubbed	Control	Rubbed
control (H_2O)	72 ± 5	39 ± 2	0.30 ± 0.08	0.92 ± 0.09
1µM. GA	99 ± 12	77 ± 6	0.28 ± 0.06	0.74 ± 0.16
1µM. ABA	42 ± 4	32 ± 2	0.21 ± 0.04	0.28 ± 0.06
10µM. IAA	46 ± 3	30 ± 1	0.19 ± 0.06	---
44 mM. Ethrel*	37 ± 1	13 ± 1	0.57 ± 0.05	0.93 ± 0.05

*to produce about 5 µl ethylene/gram/24 hrs. A series of concentrations of each hormone was tried, but only one is shown here.

Table 11. The effect of adding weights to the base of the 2 primary bean leaves just prior to horizontal clinostating, on the sum of the adaxial petiole-stem angles.

Clinostated	position of plant	0.34 g added to the base of each leaf	Δ in average sum of adaxial angles (degrees) in 2.5 hours
no	vertical	no	-11±4
no	horizontal	no	22±4
yes	horizontal	no	29±5
yes	horizontal	yes	46±7

Table 12. The effects of untreated control (C), mechanical stimulation (MS) and exogenous Ethrel (E) on microsomal protein and enzyme activity. Treatments were on the first internode for 10 days, and each datum is the average of 3 experiments.

Internode/ Treatment	Protein (µg/g fr wt)	Enzyme Specific Activity		
		Cyto-c Reductase* (OD/min/mg prot.)	Succinic Acid Cytochrome-c Oxidase** (OD/min/mg prot.)	IDPase*** (OD/mg prot)
1 / C	397	2.2	1.4	20.4×10^{-6}
MS	554	1.4	0.3	32.8×10^{-6}
E	466	1.3	0.3	20.3×10^{-6}
2 / C	543	1.8	1.0	21.0×10^{-6}
MS	593	1.0	0.0	36.5×10^{-6}
E	595	0.6	0.3	25.0×10^{-6}

*KCN-insensitive NADPH cytochrome-c reductase

**KCN-insensitive succinic acid cytochrome-c oxidase

***Latent IDPase.

Table 13. The effects of untreated control (C), mechanical stimulation (MS) and exogenous Ethrel (E) on the fatty acids hydrolyzed from endomembrane phospholipids. The treatments were on the first internode for 10 days, and each datum is the average of 3 experiments.

Fatty Acid	F.A. (mole % of phospholipids) C	MS	E
Palmitic	39.8	46.8	48.9
Stearic	4.2	4.0	3.1
Oleic	4.2	3.6	3.1
Linoleic	36.5	31.1	31.9
Linolenic	15.2	14.4	13.6

Table 14. The effects of untreated control (C), Mechanical stimulation (MS) and exogenous Ethrel (E) on endomembrane free fatty acids. The treatments were on the first internode for 10 days, and each datum is the average of 3 experiments.

Internode/ Treatment	Fatty Acids (mM/kg fr wt) Myristic (14:0)	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
1 / C	5	70	1	26	5	90	81
MS	28	42	3	14	5	19	40
E	20	25	0	7	2	12	21
2 / C	10	43	1	11	5	30	71
MS	16	54	0	15	5	53	49
E	18	47	0	16	4	19	20

Table 15. The effects of untreated control (C), mechanical stimulation (MS) and exogenous Ethrel (E) on endomembrane sterols (measured by gas-liquid chromatography). The treatments were on the first internode for 10 days, and each datum is the average of 3 experiments.

	% of Total Sterols		
	Control	MS	Ethrel
Cholestane	0.8 0.5	1.0 0.6	1.0 0.6
Cholesterol	trace	trace	trace
Campesterol	6.5 0.9	6.9 1.0	7.4 0.5
Stigmasterol	37.6 3	39.6 2	38.7 3
β-Sitosterol	54.0 3	56.0 3	55.5 4

Table 16. Thigmomorphogenesis in Loblolly pine seedlings. The plants were grown in "cone-tainers" with one-half of them mechanically stimulated (MS) simply by slowly waving the hand through them ten times, once each day. After 3 months, the net elongation of the plants was measured.

Ultimate type of tree	Weyerhaueser Code No.	Net growth in 3 months (mm) C	MS	C-MS	% change
large trunk	8-61	65.3	51.5	13.8**	-21
	8-43	50.0	35.0	15.0**	-30
small trunk	8-66	70.0	63.0	7.0^{NS}	-10
	8-73	56.0	51.5	4.5^{NS}	- 8

**Significant at the 1% level, according to t-test

^{NS}Not significant at the 1% or 5% level, according to t-test

Table 17. The effect of sound on the elongation of the first internode of young bean plants. Plants were exposed to 1200 hz at 90.5 decibels for two weeks starting at emergence.

Illumination	Sound	length (mm±S.E.)	diameter (mm±S.E.)
None (etiolated plants)	none (control)	94.6±4.9	2.48±0.05
	plus sound	221.3±3.3	2.37±0.04
Light (green plants)	none (control)	114.3±2.3	2.36±0.04
	plus sound	90.6±1.8	2.00±0.04