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ROLE OF GRAVITATIONAL STRESS IN LAND PLANT EVOLUTION:
THE GRAVITATIONAL FACTOR IN LIGNIFICATION

by

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A. Gravity-Height-Lignin Relations in the Plant Body

1. Studies with cucumber.

The culture of cucumber seedlings from seed under experimental hypo-gravity and hypergravity for three weeks results in a distinctive overall pattern of lignification in the juvenile stem -- the hypocotyl (fig. 1). From the clinostat to normal g, lignin content increases ca. 2-fold, although seedling height increases by ca. 10%. The former is statistically significant; the latter is not. When g is increased in the centrifuge, height was reduced from a control level of ca. 15 cm to ca. 13.5 cm at 25 g (ca. 8% reduction) and ca. 12 cm at 50 g (ca. 20% reduction). Seedlings at 100 g were not shortened, but they were severely twisted and could not be accurately measured. Over the centrifuge range, lignin increases with g.

Lignification is obviously more sensitive to regular variation in g than is growth. If they are compared normalizing each parameter at the "0" g clinostat level we have

g	Axis height (cm)	Relative Lignin Content
"0"	100	100
1	90	220
25	82	371
50	72	507

The relation, although reflecting opposite trends, can hardly be considered as reciprocal in a formal sense. It is generally believed that extensive lignification is associated with cell senescence, although functionally it may also be considered part of tissue differentiation in the vascular plant. The onset of lignification from this viewpoint

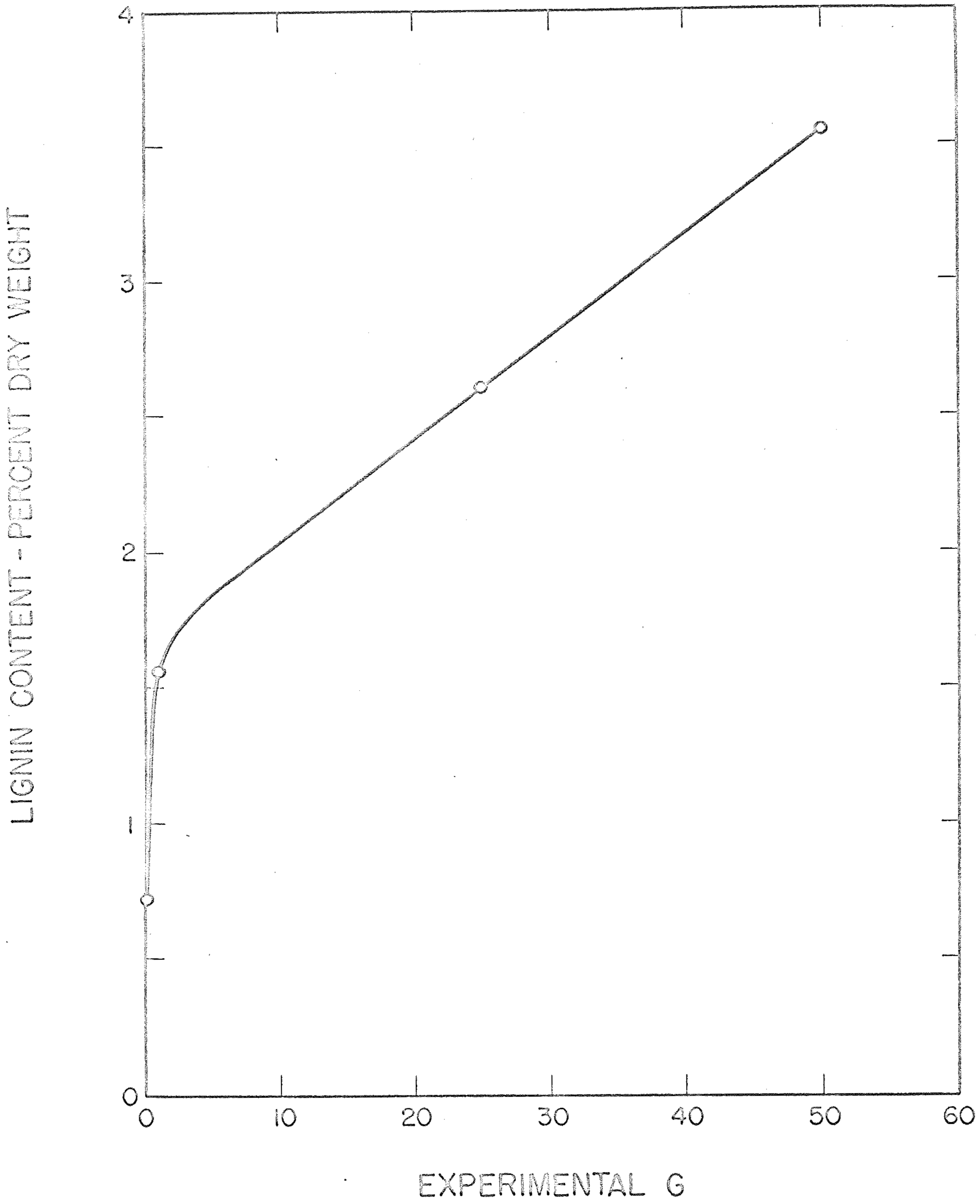

Fig. 1. Lignin content of three week old cucumber hypocotyl as a function of experimental gravity.

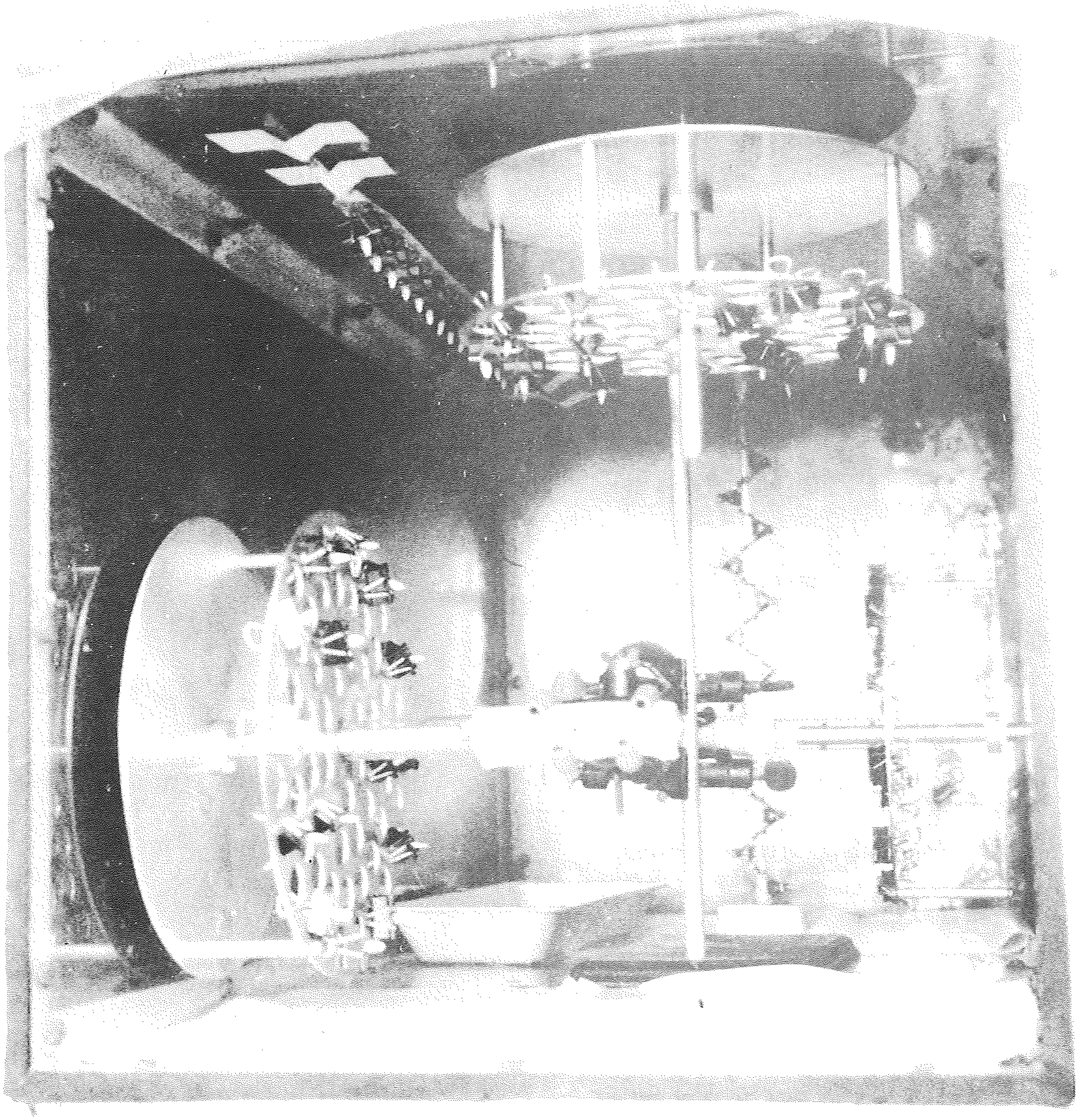

Fig. 2. One experimental clinostat constant humidity chamber under examination. Units in foreground are rotators with motors outside chamber to reduce HEAT load. Others are static controls.

should terminate active growth, or be at least correlated with termination. While this general concept may be correct in woody tissues, it obviously does not obtain here, using a herbaceous seedling, and under the experimental conditions applied.

Thus, within limits, it is possible to show by simulated gravitational conditions, an "uncoupling" of lignin from growth.

Earlier flotation studies also show no reciprocity between lignin and growth. Indeed, in some experiments, growth of the seedling and lignification were both reduced, although not in proportion.

When we consider the size-lignin relation in nature (below), with its strong positive correlation between lignin content and height of axis, an interesting paradox results:

If the concept of lignification (concomitant of senescence) as an "antigrowth" process is correct then what of the premise that ability to lignify supports aerial extension in evolution? Is, then, the growth-lignin relation "ontogenetic" (developmental) terms opposed to the phylogenetic (evolutionary) pattern. This problem will be given further consideration below.

In addition to relational gravi-simulation and flotation procedures described in earlier reports, several experiments have been addressed toward other supportive media (fig. 3) and combinations of viscous media and rotation (fig. 4).

Fig. 3. Extreme example of buoyant medium tested for its effects on orientation. Seen here are rye seedlings, but cucumber and radish can also be grown. Such media must remain tentative until toxicity has been evaluated.

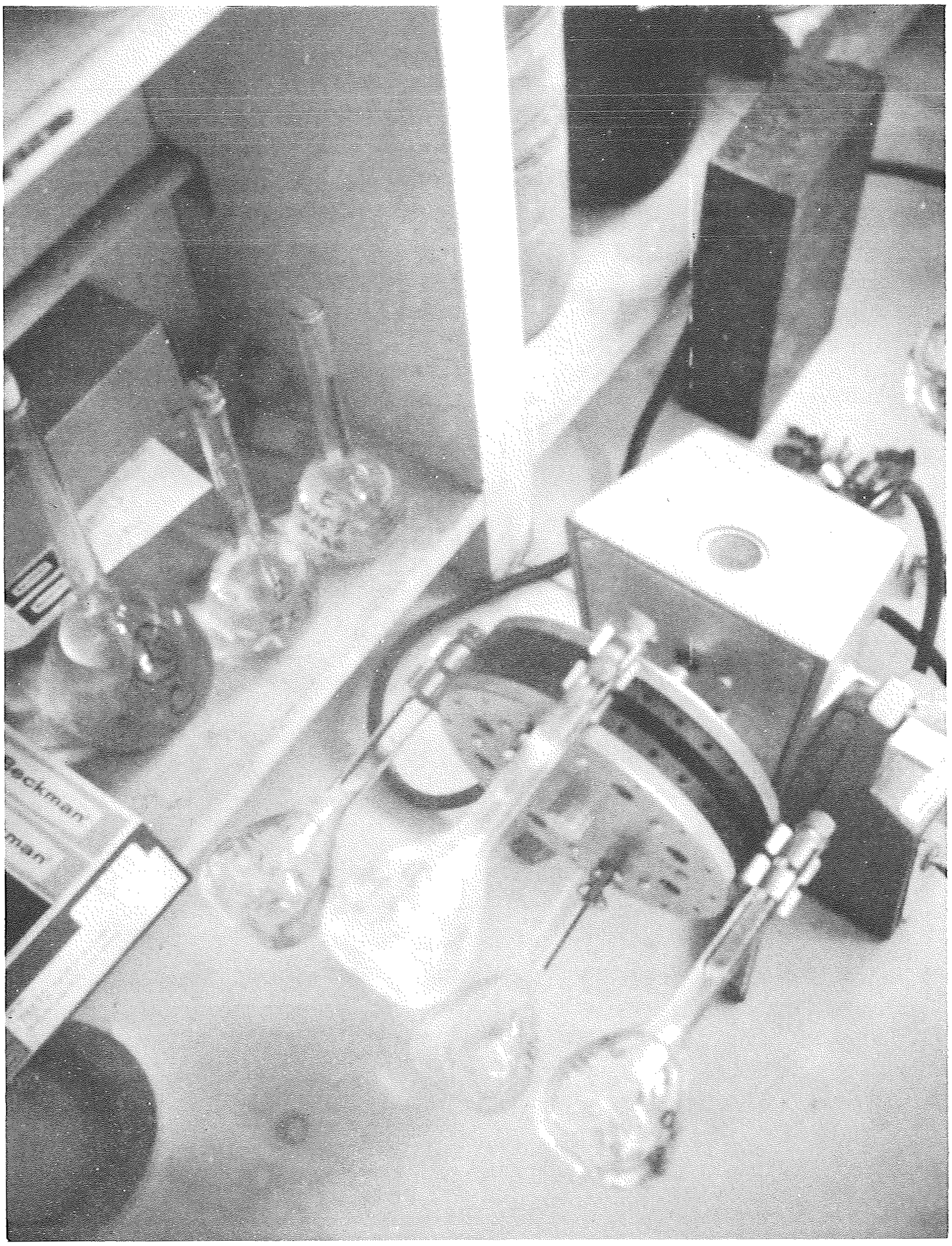

Fig. 4. Rotator modified by attached vertical volumetric flasks, each filled with agar or other suspending media to test medium-rotation interactions.

Even exotic, high "buoyancy" media such as mercury were tried as supports, and cucumber as well as other species showed little evidence of Hg toxicity from such intimate contact.

Low concentrations of pectin, agar, and polyvinyl alcohol have all been examined as semi-fluid supports. These experiments are still in progress, but they have shown several promising - and puzzling - results. First was the unexpected adverse effect of pectin. At all concentrations tested down to 0.5% (ca. 10^{-4}M), cucumber seeds failed completely to germinate. Other observations suggest unexpected physiological effects of other polymers in water at concentrations high enough to affect apparent buoyancy of the seeds. Some of these effects are indeed suggestive of a loss of geotropic sensitivity. Lignification has not yet been examined in any of these media pending further examination to eliminate possible complicating toxic response. In principle, however, a supportive medium superior to water should be a decided asset and this avenue will be kept open. When agar (0.25%) or polyvinyl alcohol (3%) were used as suspending media half-filling volumetric flasks affixed vertically to the rotator, after 7 days, seedlings in agar exhibited "confusion" in root direction and general development, but those supported in PVA were most profoundly abnormal in orientation and relative development (not size, however fig. 5).

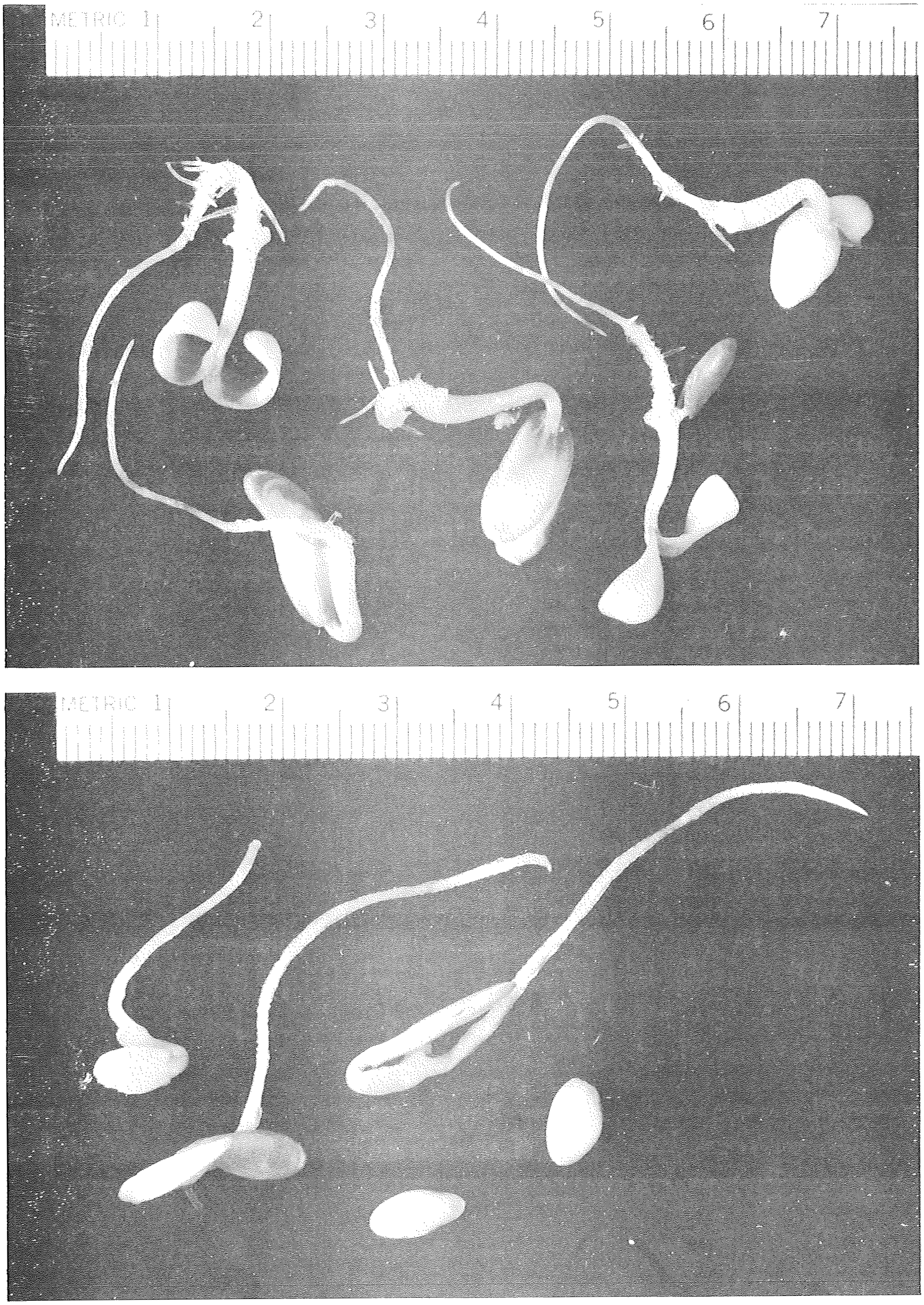

Fig. 5. Response of cucumber to clinostat-medium interaction. Upper I in 0.25% agar growth is normal, but orientation is disturbed. Lower in 5% polyvinyl alcohol, unbranched roots grow well, but hypocotyl elongation is arrested.

2. Lignin content - Size relations under natural conditions

The survey of size-associated trends in lignin contents has now been expanded to include over 10 species and forms examples from small mosses (1 cm axis) to giant plantain (120 cm floral axis) representing over a one hundred-fold range of lignin content (table 1).

New additions include dwarf plantain, Bryum and other small mosses, and Equisetum, normal and dwarf (figs. 6 and 7).

Upon examination of data gathered so far, a relationship is evident when lignin content is plotted on a logarithmic scale against axial height (fig. 8). The pattern suggests that each group -- mosses, Plantago, Equisetum -- has its own trend, however the vascular forms fall fairly closely together. A more accurate -- and meaningful correlative factor than axial height would be the ratio;

axial height/area at midpoint

This parameter can be related to pressure of the plant body upon itself, and when plotted (fig. 9), regroups the samples in a highly meaningful manner.

Table 1. Lignin content and size in nature

Plant form	Axial Height cm	Lignin % Dry wt.	Source of Plant
Moss Gametophytes			
Bryum, others	1-2	<0.1	New York, Illinois
Polytrichum	10-15	<0.3	New York, Illinois
Pogonatum	40-60	5-6	Mexico (>2000M)
Dendroligotrichum	60-80	10-12	New Zealand
Dawsonia	75-90	10-12	New Zealand
Equisetum Sporophytes			
"Dwarf"	5-6	0.16	Morraine Lake, Alberta (>2000M)
Normal	88-100	0.50	Alberta, British Columbia (<500M)
Plantago flower stalk			
P. lanceolata normal	30-40	0.47	Kauai, <1000M
P. lanceolata giant	100-120	1.1	Waimea Canyon, Kauai (>1300M)
P. major, normal	20-35	0.57	Manoa Area, Honolulu in compacted, constantly wet red clay at sea level.
P. major, dwarf	4-7	0.1	

Fig. 6. Equisetum of "normal" and dwarf stature. Right: widespread Equisetum arvensis. Left: Equisetum sp. collected in August 1969 near Morraine Lake, British Columbia. Scale shown is 10 cm.

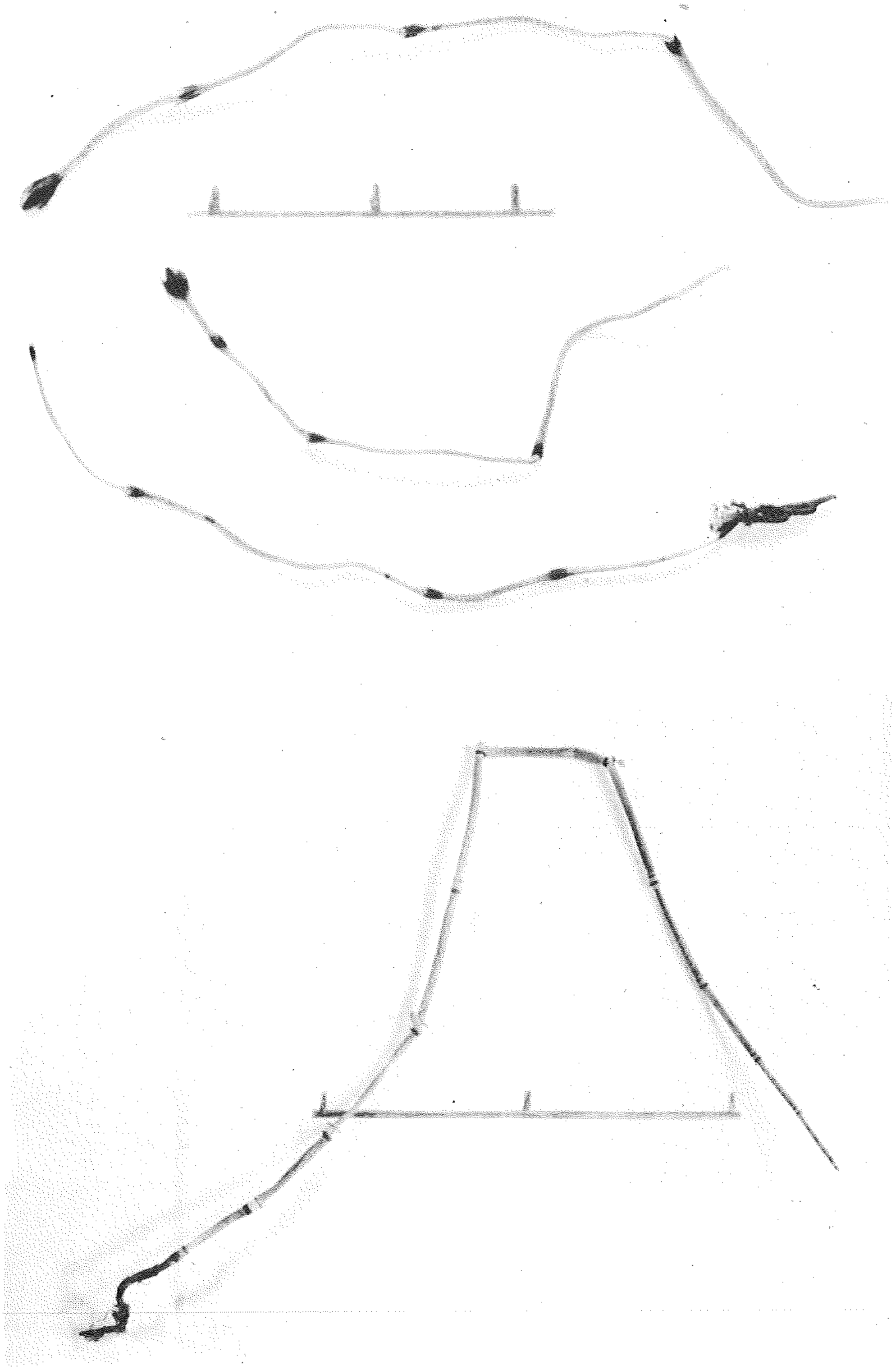

Fig. 7. Adjusted views of normal and dwarf *Equisetum*. Upper: Dwarf-scale division each 1 cm; normal-scale divisions each 10 cm.

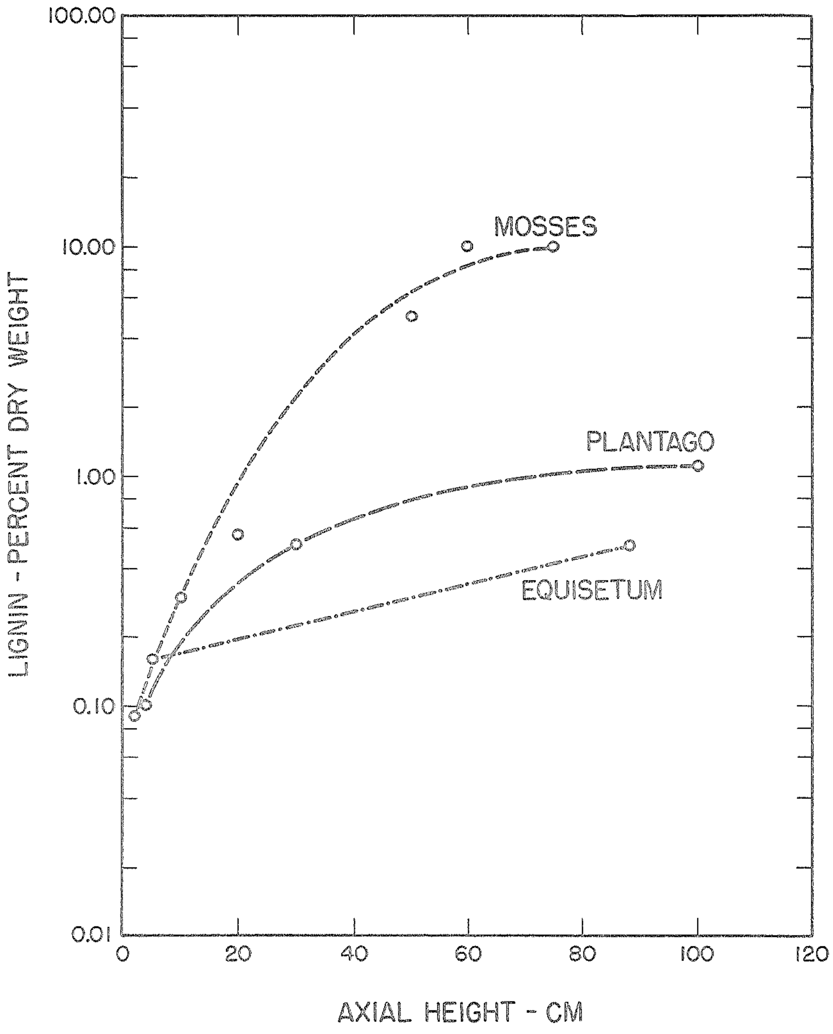

Fig. 8. Plot of lignin content against axial height.

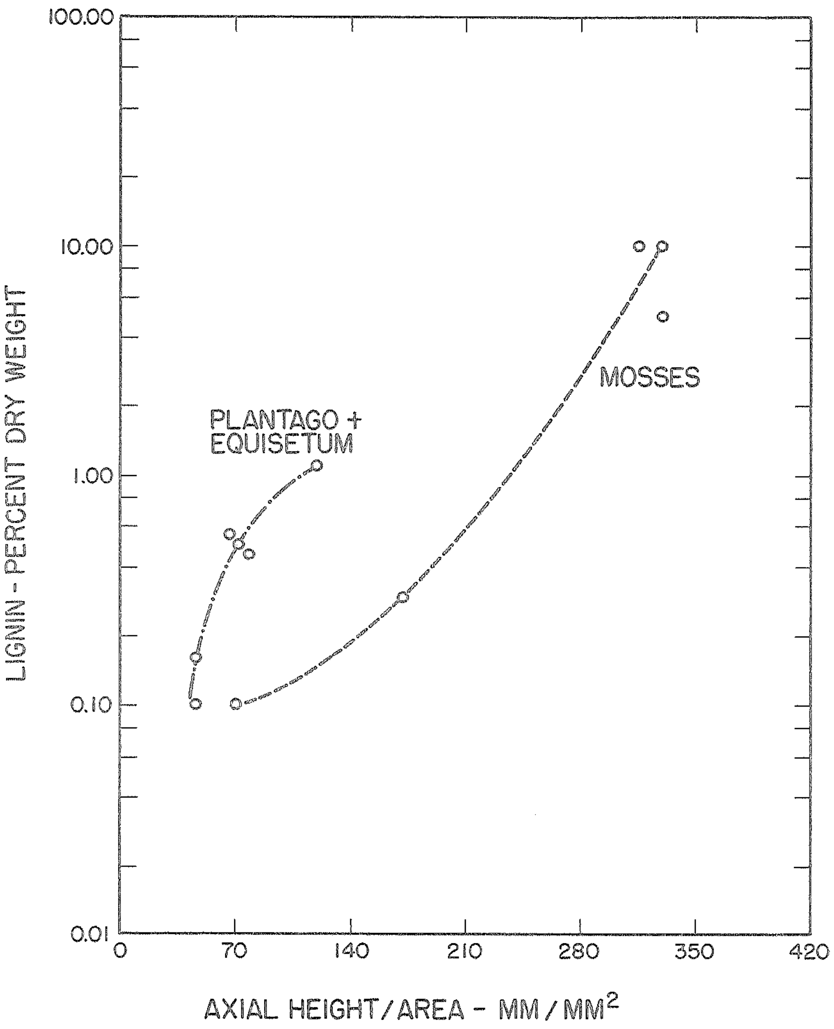

Fig. 9. Re Plot of lignin vs. axial hgt/axial area yielding effective plot of lignin vs. pressure.

B. Biomechanical Considerations

The principle of similitude had been admirably applied in a few clear instances by Lesage, a celebrated eighteenth-century physician, in an unfinished and unpublished work. Lesage argued, for example, that the larger ratio of surface to mass in a small animal would lead to excessive transpiration, were the skin as "porous" as our own; and that we may thus account for the hardened or thickened skins of insects and many other small terrestrial animals. Again, since the weight of a fruit increases as the cube of its linear dimensions, while the strength of the stalk increases as the square, it follows that the stalk must needs grow out of apparent due proportion to the fruit: or, alternatively, that tall trees should not bear large fruit on slender branches, and that melons and pumpkins must lie upon the ground. And yet again, that in quadrupeds a large head must be supported on a neck which is either excessively thick and strong like a bull's, or very short like an elephant's.

But it was Galileo who, wellnigh three hundred years ago, had first laid down this general principle of similitude; and he did so with the utmost possible clearness, and with a great wealth of illustration drawn from structures living and dead. He said that if we tried building ships, palaces or temples of enormous size, yards, beams and bolts would cease to hold together; nor can Nature grow a tree nor construct an animal beyond a certain size, while retaining the proportions and employing the materials which suffice in the case of a smaller structure.

The thing will fall to pieces of its own weight unless we either change its relative proportions, which will at length cause it to become clumsy, monstrous and inefficient, or else we must find new material, harder and stronger than was used before. Both processes are familiar to us in Nature and in Art, and practical applications, undreamed of by Galileo, meet us at every turn in this modern age of cement and steel.

Again, as Galileo was also careful to explain, besides the questions of pure stress and strain, or the strength of muscles to lift an increasing weight or of bones to resist its crushing stress, we have the important question of bending moments. This enters, more or less, into our whole range of problems; it affects the whole form of the skeleton, and sets a limit to the height of a tall tree.

We learn in elementary mechanics the simple case of two similar beams, supported at both ends and carrying no other weight than their own. Within the limits of their elasticity they tend to be deflected, or to sag downwards, in proportion to the squares of their linear dimensions; if a match-stick be two inches long and a similar beam six feet (or 36 times as long), the latter will sag under its own weight thirteen hundred times as much as the other. To counteract this tendency, as the size of an animal increases, the limbs tend to become thicker and shorter and the whole skeleton bulkier and heavier; bones make up some 8 per cent of the body of mouse or wren, 13 or 14 per cent of goose or dog, and 17 or 18 per cent of the body of a man. Elephant and hippopotamus have grown clumsy as well as big, and the elk is of necessity less graceful than the gazelle. It is of high interest, on the other hand, to observe how little the skeletal proportions differ in a little porpoise and a great whale, even in the limbs and limb-bones; for the whole influence of gravity has become negligible, or nearly so, in both of these.

In the problem of the tall tree we have to determine the point at which the tree will begin to bend under its own weight if it be ever so little displaced from the perpendicular. In such an investigation we have to make certain assumptions--for instance that the trunk tapers uniformly, and the sectional area of the branches varies according to some definite law, or (as Ruskin assumed) tends to be constant in any horizontal plane; and the mathematical treatment is apt to be somewhat difficult. But Greenhill showed, on such assumptions as the above, that a certain British Columbian pine-tree, of which the Kew flag-staff, which is 221 ft. high and 21 inches in diameter at the base, was made, could not possibly, by theory, have grown to more than about 300 ft. It is very curious that Galileo had suggested precisely the same height (*ducento braccie alta*) as the utmost limit of the altitude of a tree. In general, as Greenhill showed, the diameter of a tall homogeneous body must increase as the power 3/2 of its height, which accounts for the slender proportions of young trees compared with the squat or stunted appearance of old and large ones. In short, as Goethe says in *Dichtung und Wahrheit*, "Es ist dafür gesorgt dass die Bäume nicht in den Himmel wachsen."

But the tapering pine-tree is but a special case of a wider problem. The oak does not grow so tall as the pine-tree, but it carries a heavier load, and its boll, broad-based upon its spreading roots, shows a different contour. Smeaton took it for the pattern of his lighthouse, and Eiffel built his great tree of steel, a thousand feet high, to a similar but a stricter plan. Here the profile of tower or tree follows, or tends to follow, a logarithmic curve, giving equal strength throughout, according to a principle which we shall have occasion to discuss later on, when we come to treat of form and mechanical efficiency in the skeletons of animals. In the tree, moreover, anchoring roots form powerful wind-struts, and are most developed opposite to the direction of the prevailing winds; for the lifetime of a tree is affected by the frequency of storms, and its strength is related to the wind-pressure which it must needs withstand.

Thus, D'Arcy Thompson assumed that a geometric argument was sufficient to explain the upright character of the tall tree. That is, the trunk tapers as one proceeds base to apex so that the increase in cross section offsets the load generated by an increasing weight above. Stated otherwise (remembering that weight = force, and that force/area = pressure), the pressure exerted upon the plant axis by its own mass remains constant because diameter increases from apex to base. Assuming the material constitution of the plant axis remains constant, hence its bulk modulus or crush-resistance, such an argument represents the only reasonable solution. Conceivably the specific gravity of a plant might decrease acropetally, but this is not in fact the case.

When a semi-woody stem of moderate height is examined in the light of the above considerations, a plot of height vs. pressure from tip to base reveals a regular relationship of pronounced sigmoid character (fig. 10). While examination suggests an exponential or logarithmic relationship, the form of the logarithmic spiral $r = e^{ao}$ or $\log^{r} = ao$ suggested by Thompson does not in fact fit. When this figure is rotated 90°, its nature is more obviously that of a growth or probability curve, possibly of the form $y = Ke \frac{-X^2}{2}$ where y = pressure and X = stem length.

When seen graphically in a semi-logarithmic plot, the spiral retains its curvilinearity to a high degree, whereas the plot of $\log y$ vs X^2 is largely rectilinear (fig 11), and the best fit yet obtained.

Disregarding stem length, a comparison of calculated pressures with analyzed lignin contents, revealed a definite, linear regression. Thus $\log y \propto -X^2$ applies to lignin content as well as pressure correlation.

Lignin content is thus an alternate variable to cross-sectional area as a means for dealing with the upright plant axis, and one that D'Arcy Thompson failed to recognize.

The role of lignin in compressive strength of the axis (bulk modulus $B = F/_{a} .\ 1/_{l_o}$) is shown by comparing wood at ca. 10 Kg/mm^2 with cotton fibres at only 5 Kg/mm^2.

The packing properties of lignins within the cellulosic frame obviously contribute significantly to the bulking characteristics but the details of such interstitial reinforcement of walls warrants novel experimentation.

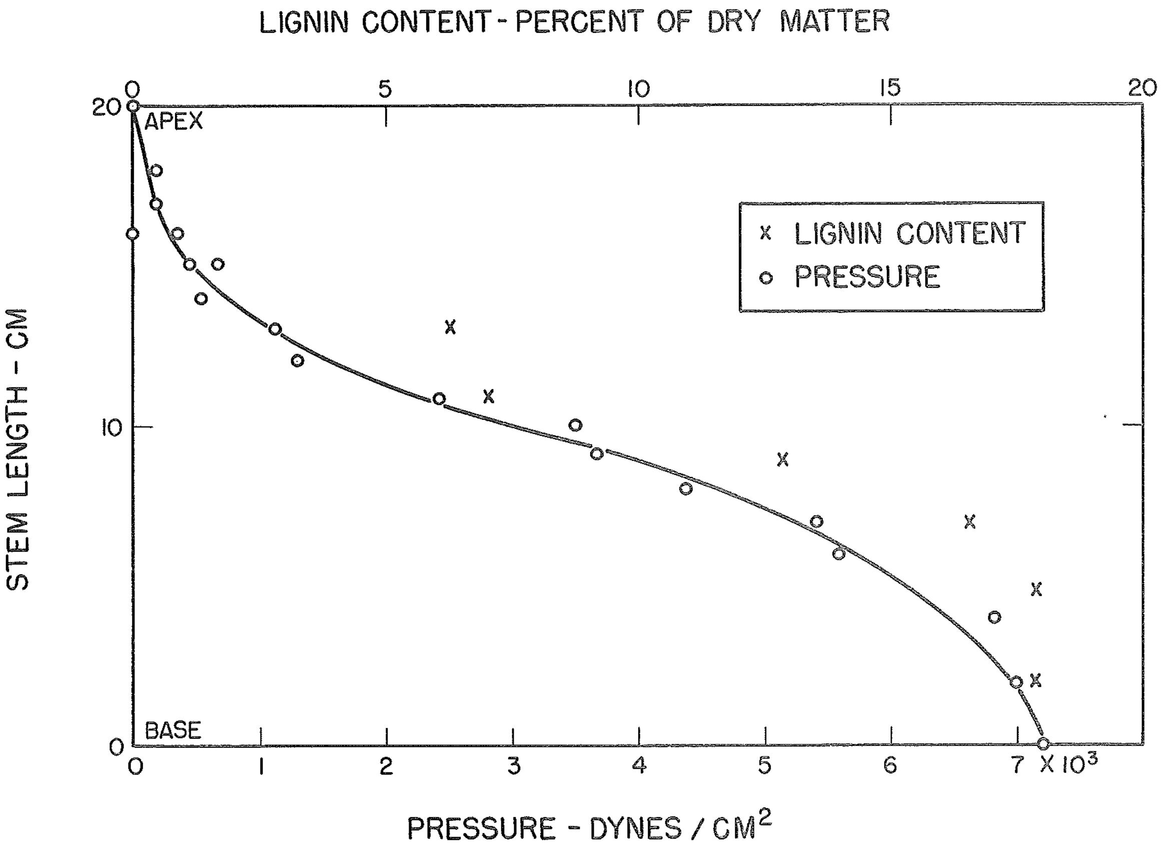

Fig. 10. Pressure lignification diagram for Euphorbia sp.

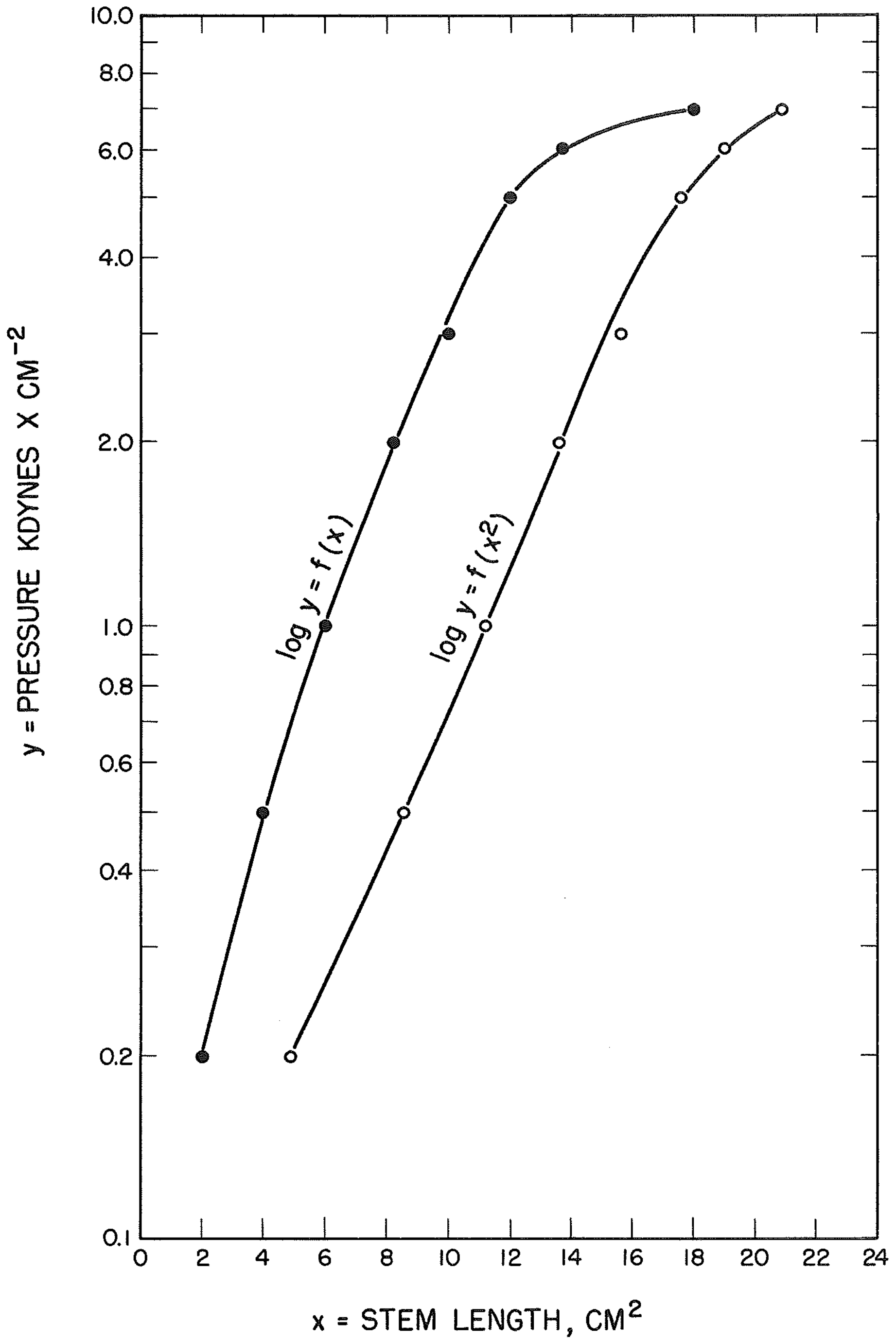

Fig. 11. Test plots of y functions in x and x^2.