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STUDIES ON THE PHYSIOLOGY OF THE GEOTROPIC RESPONSE IN THE WHEAT CULM

A thesis submitted to the University of Glasgow
for the degree of Doctor of Philosophy
in the Faculty of Science

by

Ian George Bridges

March
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ACKNOWLEDGEMENTS

I am grateful to my supervisor, Professor M. B. Wilkins, for sound advice and encouragement, and to Dr. J. R. Millman for many helpful discussions.

My thanks are also due to Mr. T. N. Tait for his assistance with the photographic reproduction of plates and diagrams, and to Miss M. G. Cuthill for typing the manuscript.

I am indebted to E. Merck AG., Darmstadt, Germany who kindly provided samples of the morphactin chlorfluorenol methyl ester.

The work reported in this thesis was carried out at the Universities of Glasgow and Nottingham while the author was in receipt of a grant from the Science Research Council.

Errata

page 6, line 25	evoke a response.
Figure 2	cylinder quartered.
page 39, line 17	Hoffmann-La Roche.
page 48, line 17	and 100 ml acetone.
page 58, line 3	co-ordination between nodes.
Figure 24 abscissa	Log angular velocity (2 π RPH).
page 77, line 29	during a 2-h transport period is shown.

SUMMARY

Geotropic curvatures generally result from differential growth in the extending zones of root and shoot apices, but the shoots of plants in certain orders, notably the Gramineae, Caryophyllaceae and Commelinaceae, have the capacity to respond to gravity at the nodes. Curvature at the node provides the only means of correction in grass stems, when these are displaced from their preferred orientation after floral initiation, and the research reported in this thesis has been undertaken to elucidate the physiology of this specialised response. The experimental material is the spring wheat var. Kolibri.

There are typically four nodes per plant, and a response is possible at each. The motile tissues are found in the leaf sheath base, and curvature is brought about by expansion of the parenchymatous tissues in this region. Lignification is minimal in the basal regions of leaf sheaths and internodes, and the sclerenchymatous bundle sheaths of the leaf sheath are replaced by large, but unthickened, bundle caps in the leaf sheath base.

The initial stages of the gravi-perception mechanism appear to be similar to those in other organs; the threshold acceleration lies between $^{1}/_{10,000} \times g$ and $^{1}/_{1,000} \times g$, and there is reason to implicate starch grains as statoliths, but the response lacks the chemical co-ordination envisaged in the Cholodny-Went theory of geotropism. The responses at nodes on the same stem are not chemically co-ordinated, and evidence is presented to show that the polarised transport of growth regulators is not required for the individual responses. The magnitude of the response is a function of the sine of the angle of displacement from vertical, and a physical co-ordination system based on the quantity of stimulus perceived at each growth centre is envisaged.

Growth is induced in response to geotropic stimulation, and the ability to respond is dependent on the physiological age of the organ. This age

dependency appears to be connected with the capacity for growth, and not the capacity for gravi-perception.

The geotropic response is associated with changes in sugar metabolism, and a considerable increase in the molar concentration of reducing sugars is apparent in the lower halves of intact leaf sheath bases. The production of these sugars is controlled by the gravi-perception mechanism and not the subsequent growth response. The reducing sugars result from the inversion of sucrose. The function of the reducing sugars is not to satisfy a requirement for increased turgor, as has previously been suggested.

Growth is induced by exposure to buffers of low pH, whilst buffers of neutral pH inhibit the geo-induced response. The response to low pH develops immediately; it is optimal at pH3, and its development is not inhibited by anoxia or metabolic inhibitors. It is dependent on cell turgor and temperature, and its Q_{10} is comparable with the Q_{10} for the geotropically induced response. The data are interpreted as indicative of wall softening by hydrolysis of acid labile bonds, and the control of such a process by the gravi-perception mechanism is discussed.

INDEX

GENERAL INTRODUCTION

The term 'geotropism' is used to refer to the sequence of events which occurs when a plant organ is displaced from its preferred orientation in the gravitational field. The phenomenon was first documented by Dodart (1703) who noted that in all the plants he examined the roots grew towards the earth and the shoots away from it. The connection between geotropic curvature and growth was conclusively established by Frank (1868) who showed that roots and shoots could only respond to geotropic stimulation when in an active state of growth and Frank (1868) was also responsible for the introduction of the term geotropism (geo = earth).

Knight (1806) and Dutrochet (1824) showed that curvature could be induced by both gravitational and centrifugal forces and thereby established that gravity was the stimulus concerned. Knight was able to show that when the roots of Phaseolus seedlings were exposed to a centrifugal force of about 10 x g by rotation on a vertical wheel, they grew away from the axis in the plane of rotation, and Dutrochet showed that when subjected to a force of only 1 x g on a horizontal wheel, they grew obliquely along the path of the resultant between gravitational and centrifugal forces.

The liminal or preferred direction for growth in a particular organ may be in one of several directions. When it is parallel to the gravity vector the organ is said to be orthogeotropic, and the category is further divided into positive and negative conditions, depending on whether the direction of growth is into or out of the gravitational field. When the preferred direction is not parallel to the gravity vector but at an angle to it, the organ is said to exhibit plagiotropism, and the special condition in which the liminal direction is at right angles to the vector is known as diageotropism. The geotropic behaviour of a particular organ need not remain constant throughout the life of the organ but may change as various stages in development are reached. A classical example of this is found

in the flower stalks of Fritillaria meleagris, where the flower buds and fruits are both negatively orthogeotropic whilst the open flowers are positively orthogeotropic (Kaldewey, 1962). Changes may also occur in response to damage incurred by other plant parts, and such readjustments are often observed in laterals where the mode of development changes from plagiotropic to orthogeotropic following damage to the main axis.

The earliest attempts to explain geotropic curvature in shoots (Dodart, 1703) were based on the assumption that horizontal displacement would lead to desiccation on the upper side of the organ with consequent changes in water content throughout the organ, whilst subsequent explanations envisaged the displacement of dense 'nutrient juices' to the lower side of the displaced organ under the influence of gravity (Austruc, 1709; Knight, 1806). It was thought that roots would always bend downwards under their own weight, but this concept was dismissed by the demonstration that bending root tips were able to exert sufficient pressure to penetrate mercury with ease (Pinot, 1829).

It is now realised that gravity can affect the plant organ only by causing mass acceleration, but the exact identity of the mass involved has yet to be demonstrated conclusively. The observation that an organ will respond when supported horizontally along its entire length (Frank, 1868) precludes any connection between graviperception, and the initial, purely physical, bending of the organ under its own weight, and the action of gravity, must therefore be concerned with the displacement of something within the cell. The entire cell contents may be operative in this respect because a difference in hydrostatic pressure will develop between the upper and lower faces of the cell wall following horizontal exposure. This difference in pressure, which has been estimated by Audus (1962) to be in the region of 2.0 dyn cm^{-2}, is unlikely to form the basis of a graviperception mechanism because it is minute by comparison with the turgor pressure. Turgor pressure usually amounts to some 10^6 dyn cm^{-2}, and an

ability to perceive changes of the order of 1 in 10^9 would be required in order to explain the perception of forces as low as $\frac{1}{1,000} \times g$. Audus (1962) has used Stokes' law to estimate migration velocities for the various particulate components in the cytoplasm and he has concluded that starch grains, and possibly mitochondria, are the only inclusions which could sediment, and therefore function as statoliths within the presentation time. Smaller particles are unlikely to form the basis of a gravi-perception mechanism because of their slow migration rates and high susceptibility to hindrance from thermal agitation and cyclosis, and these latter mixing effects are also likely to prevent the development of a general density gradient throughout the cytoplasm.

The statolith theory for graviperception originated with the suggestion by Berthold (1886) that perception might be causally linked with the sedimentation of the mobile starch grains, and the concept was more specifically formulated by Haberlandt (1900) and Nemec (1900) who noted a close correlation between the presence of mobile starch grains and geosensitivity. Many experiments have been carried out to support or disprove the statolith theory, and these are well covered in review articles (Rawitscher, 1932; Brauner, 1962; Audus, 1962; Wilkins, 1966; Audus, 1969). The existence of moveable starch grains in gravi-sensitive organs is widespread and moveable starch grains are even found in species which do not otherwise store starch (Haberlandt, 1928). Certain organs retain a capacity for graviperception in the absence of starch grains (Fischler, 1905; Linsbauer, 1907), but this does not invalidate the theory unless it can also be established that other particles do not assume the rôle of statoliths.

Techniques designed to remove the starch grains from gravi-sensitive organs have given rise to some interesting anomalies. Early workers in this field used darkness (Zollikofer, 1913; Protic, 1928) or low temperature treatments (Haberlandt, 1903; Protic, 1928; Hawker, 1933) to remove

statolith starch, and they always found a close correlation between the decline in statolith starch and the loss of geo-sensitivity. Von Bismarck (1959), however, found that destarching by cold treatment did not eliminate the geotropic response in Sphagnum stems. The presentation time of 1¼ h for Sphagnum is rather long, and it may well be that the starch grains are not essential statoliths in this species. Starch was first removed chemically by Syre (1938), who found that sulphur dioxide treatment would remove starch without eliminating gravi-sensitivity. More recently, Gillespie-Pickard and Thimann (1966) have been able to destarch Triticum coleoptiles, by incubation in a solution of gibberellin and kinetin, without complete loss of geosensitivity. The development of the response was retarded, however, following destarching, and it may be that alternative statoliths can assume the rôle of perceptors in this organ. This view is supported by the work of Inverson (1969) and Pilet (1971) who have been able to eliminate geo-sensitivity in cress roots by destarching, using the gibberellin/kinetin technique.

Experiments designed to test the statolith theory by manoeuvring statoliths to selected regions in the statocytes, have met with mixed results. Zimmermann (1927) noted that roots which were briefly inverted following a period of horizontal exposure developed greater curvatures than did roots which were immediately returned to the normal vertical position, and Von Ubisch (1928) argued that, were this phenomenon attributable to the manipulation of statoliths, then an initial inversion ought to result in a reduction of the response when the roots were inverted for a second time after a period of horizontal stimulation. She found that the second inversion augmented the response and suggested that the statolith theory was erroneous. Von Ubisch has been strongly criticised for the poor documentation of her work, but the experiments of Larsen (1965) have fully confirmed the validity of her data, and it may be that a criticism of the experimental technique is more appropriate. Von Ubisch (1928) and Larsen

(1965) used initial inversion periods of only 20 min in their studies. Longer periods were found to induce spontaneous curvatures and were therefore deemed unsuitable for use in experiments of this type. It may be argued that a 20-min inversion period is not sufficiently long to permit full equilibrium and that the results are therefore complicated by stimulatory effects initiated during the first inversion period. This criticism is supported by the findings of Grahme and Hertz (1964) who were able to show, by measuring the intensity of the geo-electric effect, that the results obtained from the von Ubisch treatment were in agreement with the statolith theory when the initial inversion period lasted for 5 h but were not in agreement when it lasted for only 20 min or 1 h. It is unfortunate, however, that different measuring techniques were used for the longer 5-h inversion treatment and the shorter 20-min and 1-h inversion treatments.

Many workers have demonstrated correlations between geosensitivity and the distribution and degree of development of statolith tissues. Hawker (1932) measured the total volume of starch statenchyma in eight different species and showed a very good inverse correlation with presentation time in all cases. This approach has also been adopted by Hertel and his co-workers (Hertel, et al. 1969, Filner and Hertel 1970) who have found a similar inverse correlation between gravi-sensitivity and the size and abundance of starch grains in the statenchyma of certain amylomaize mutants.

Other lines of inquiry which implicate starch grains in gravi-perception involve the removal by surgery of tissues containing the starch grains and these experiments are most convincing when applied to roots. The starch statenchyma is confined to the root cap and experiments involving the removal of the root cap from maize roots show that the treatment will eliminate geo-sensitivity without affecting growth in the root (Juniper et al. 1966, Gibbons and Wilkins 1970).

The problems inherent in determining the effects of measured amounts of stimulation are additionally complicated for geotropic stimulation by the

fact that the gravitational force can never be eliminated in an earthbound laboratory. Space research has made it technically possible to maintain organisms in conditions of weightlessness for indefinite periods and this facility may prove invaluable in the years to come, but experiments carried out to date during short term space flights are subject to serious limitations. Although it is impossible to eliminate the gravitational force on earth, it is possible to compensate for it mechanically. Devices for gravity compensation are known as clinostats, a term first introduced by Sachs in 1882, and they involve the rotation of the plant about a horizontal axis at an angular velocity which is neither slow enough to permit gravitational stimulation or fast enough to cause centrifugal stimulation. The problem of optimising the speed of rotation has usually been tackled by trial and error, the effects of rotation at a range of speeds being determined for a given plant species before starting experimentation, but Dedolph and Dipert (1971) have recently computed the formula $\omega = 1.39 \times 10^{2} (Lot)^{-1/3}$ to express the optimal angular velocity (ω) in terms of the radius of rotation (Lo) and the duration of the experiment (t). Because it is dependent only on Lo and t a rate so calculated is applicable to any movable particle in any cell and values so computed (e.g. 2 RPM for 72-h rotation at a radius of 20 mm) are in close agreement with values determined experimentally. Using the clinostat it is possible to stimulate for a measured period of time simply by stopping rotation for that period, and it is therefore possible to start to define the perception phase of the geotropic response sequence.

The presentation time is the minimum length of time for which an organ must be stimulated in order to invoke a response. Factors affecting the rate of statolith sedimentation may be expected to affect the length of the presentation time and this is found to be the case. The presentation time is temperature dependent (Hawker 1933). It exhibits a steep negative coefficient with increasing temperature and the coefficient becomes positive at higher temperatures in keeping with the effects of temperature on cyto-

plasmic viscosity.

The force exerted by the statoliths will be governed by the acceleration to which they are subjected, and it is possible with the aid of the centrifuge to determine whether the statoliths must be in contact with the receptor for a fixed length of time (t) or whether a fixed quantity of stimulus (fxt) is required in order to initiate a response. Rutten-Pekelharing (1910) demonstrated, from her work with cress roots, that it was the product of force and time which was important, and her work has led to the formulation of the reciprocity rule for geotropism. Lundegårdh (1918, 1926) extended this work to include larger than minimal stimuli, and found that any combination of force and time which gave an equivalent quantity of stimulus would evoke a similar response. He also showed that both the velocity of curvature and the maximum curvature achieved were proportional to the intensity of stimulation.

Presentation time used to be calculated as the time required to yield a response which was just discernible in 50% of the sample. The decision as to whether an organ had responded was, of course, somewhat arbitrary because curvatures of less than 2° were not readily distinguishable. The more modern approach has been to measure the response to a series of known stimuli and to plot the curvatures developed against the log of stimulation time (Johnsson 1965). The procedure usually involves the application of a fixed g force for increasing periods of time, and the reciprocity rule requires that the intercept obtained on the time axis when the data are graphed shall give the presentation time. The superiority of this method is demonstrated by Johnsson, Rengman and Graham (1971) who compare the presentation times obtained for segments sampled at various distances from the apex of the Avena coleoptile with the values obtained by Dolk (1936) using the old 50% method. They show that, whilst the presentation time remains constant at all distances from the apex, the rate of the response declines as the basal regions are approached, and the stimulation periods

needed for the development of the 2° curvature required for a visible response are very similar to the presentation times reported by Dolk (1936).

The reciprocity rule has been shown to hold only over limited ranges of g and t and the assumption that g and t are never limiting remains unproven. There appear to be lower and upper limits to the acceleration which the organ can perceive and, whilst values cited for the lower threshold vary with the material and the method of investigation, they appear to lie in the region of 10^{-3}xg to 10^{-5}xg (Chance and Smith, 1946; Lyon, 1961; Gordon and Shen Miller, 1966; Shen Miller, Hinchman and Gordon, 1968). Values for the upper threshold are rather difficult to justify because of the time taken to reach the required angular velocity for a given centrifugal force, but there appears to be a saturation point above which increases in acceleration can no longer be perceived.

Although the presentation times calculated by extrapolation appear to correlate with the times for statolith sedimentation, evidence now available suggests that stimulation times considerably shorter than presentation time can still evoke a response. This point is well illustrated by Johnsson (1971), who has found the threshold time to evoke a geotropic response in the Avena coleoptile to be about 1/10 the presentation time obtained by extrapolation. A definite change in the response curve occurs when the stimulation time equals the extrapolated presentation time and two possible explanations are advanced by Johnsson to explain this change. The first explanation involves the assumption that perception is dependent on the statoliths reaching the statocyte wall. In this model stimulation will begin when the first statoliths reach the wall and become maximal when all are sedimented, but it may not be necessary for statoliths to reach the statocyte wall, and the second model is based on the assumption that the redistribution of cell constituents by moving statoliths may be sufficient to trigger the perception mechanism. In both models maximum stimulation will be achieved when all the statoliths are completely sedimented, and from

this time on the response will be logarithmic in agreement with the reciprocity rule. Johnsson (1971) has tested these two models with an experiment involving a period of geotropic stimulation in one direction followed by rotation through 180° about the long axis of the plant and a brief period of stimulation in the opposite direction, and he has shown that the curvature obtained following successive stimuli is never independent of the length of the second stimulation period. His experiment proves that sedimentation is not necessary for gravi-perception and supports the suggestion (Johnsson 1965,1971) that the threshold stimulus need only be sufficient to overcome the random brownian movements of particles. It is probable, however, that the effects of cyclosis will also have to be incorporated in this model.

Sachs (1882) suggested that it was the component of gravity acting at right angles to the axis of amorthotropic organ which determined the magnitude of the response, and he predicted that the response would be a function of the sine of the angle of displacement. Czapek (1895,1906) was first to dispute this hypothesis, maintaining that curvature was greatest at 135° displacement and Metzner (1929) formulated an 'extended sine rule' to explain the discrepancy. His formulation $C = gt \sin\theta (1-gk\cos\theta)$, where C = curvature developed, g = gravitational constant, θ = angle of exposure and t = duration of stimulus, takes account of the longitudinal component of gravity by introducing the term $\cos\theta$. Since the sign of cos changes from positive to negative when θ increases beyond 90°, the inclusion of this term reduces C when θ is less than 90° and increases C when θ is greater than 90°. The optimum angle for exposure varies with the value of k so that when $k = 1$, $\theta = 120°$ and when $k = 10$, $\theta = 133°$, but when values of k exceed 1 the theoretical curvatures for stimulation at angles less than 90° become negative. Thus the extended rule fails to provide a satisfactory explanation for the experimental results.

Larsen (1962) believed that the discrepancy with the sine rule, as well

as other discrepancies detected when inversion procedures were used to test the statolith theory, could be explained if the statoliths were somehow restricted in their freedom of movement, and he envisaged a system involving submicroscopic 'pendulum-like' particles which carried an electrical charge. The deviation from the sine rule could then be explained by the operation of a constant longitudinal force in the form of an electrical potential gradient. There is, however, no experimental evidence in support of this suggestion and the model may be criticised theoretically on grounds of the susceptibility of the tiny particles to randomization by thermal agitation and cyclosis.

An alternative hypothesis to explain the deviation from the sine rule has been advanced by Audus (1964), who assumed that starch grains were involved in gravi-perception and constructed a model system, based on a statocyte cell from the root cap of Vicia faba, to enable him to consider the effect of orientation on statolith sedimentation. He found that the relationship between the logarithm of the number of statolith contacts with the cell wall and the angle of displacement was very similar to the relationship between geotropic curvature and angle of displacement, and proposed an explanation based on the number of contacts between statoliths and the statocyte cell wall. It is, however, the intensity of stimulation which is thought to be of importance in gravi-perception, at least in instances involving minimal stimulation, and it may be argued, from the application of the reciprocity rule, that the effect of 2 statoliths ought to be the same whether they sediment side by side or one above the other.

If the deviation from the sine rule can be explained in terms of the number of contacts between statoliths and receptor, then stimulation at 45° displacement ought to be more effective than stimulation at 135° displacement, if the statoliths are manoeuvred to the top of the statocyte before stimulation. Initial attempts to substantiate this reasoning were unsuccessful (Larsen 1965,1969), but recently Iversen and Larsen (1971) have been able to show that the greatest initial response rate is obtained

at 45° displacement when cress roots are inverted for 16 min prior to stimulation. It is, however, interesting to note that as curvature is allowed to develop the optimum response shifts from the roots stimulated at 45° displacement to those stimulated at 135° displacement. The shift is connected with the duration of the response and cannot be explained in terms of statolith contacts. It is assumed by Larsen (1971) to represent the influence of tonic effects, but the effect could also be explained in terms of the quantity of stimulus perceived. Microscopic investigations suggest that the rate of starch grain sedimentation is not linear, but that it tends to slow down as sedimentation progresses (Iversen, Pedersen and Larsen 1969). If we now consider the statocyte and assume that geo-sensitivity increases as we approach the morphologically apical regions of the transverse wall, we see that the time spent by the statoliths alongside the more sensitive apical regions of the statocyte wall is greater following displacement at 135° when the statoliths are initially at equilibrium at the bottom of the cell, than it is when they are initially at equilibrium at the top of the cell.

Little is known about the nature of the gravi-receptor mechanism. Nemèc (1900,1901) proposed that gravi-perception resulted from an interaction between statolith starch and protoplasm, and he substantiated his hypothesis with what were, for the time, some rather remarkable observations. Using light microscopy on material fixed in chromacetic acid and stained in haematoxylin, he was able to discern densely staining protoplasmic bodies of lamellar or granular appearance. These bodies were confined to the wall where the starch grains were normally situated, and they became visible only when the organ was displaced. They slowly disappeared under the starch grains when the organ was returned to its liminal position. Audus (1962) has confirmed the existence of Nemèc's protoplasmic structures and identified them as masses of endoplasmic reticulum which are confined, in the roots of Vicia faba, to the morphologically lower cell wall. Although an interaction

on this wall between statolith and receptor would establish that a displacement from equilibrium had occurred, it is difficult to see how it could confer direction. To establish the direction of displacement, the migration of statoliths must be involved either directly in a system requiring contact between statolith and receptor, or indirectly in a system involving the displacement of other cell particles.

Observations on the electron microscope led Griffiths and Audus (1964) to the conclusion that, although organelles were displaced by the sedimentation of starch grains, the changes were too small to account for the response, at least in bean roots. They concluded that the amyloplasts themselves induced the transverse polarity but were unable to identify the receptors. Sievers and Volkmann (1971), working with the columella statocyst cells of the root cap have again found a region of multiple rough endoplasmic reticulum situated above the basal transverse cell walls. In normal vertical exposure the statoliths rest above this endoplasmic reticulum and do not touch the plasma membrane, but they may press against the endoplasmic reticulum and thereby establish geotropic equilibrium. The cells in each storey of the root cap are arranged in parabolic bands, the slopes of which become steeper as the root apex is approached and, whilst the plane of the E.R. complex forms a right angle with the longitddinal root axis for the cells in the centres of the paraboli, the angles become acute for the peripheral cells. Thus displacement through 90° will still allow some statoliths to press against the E.R. complex, and it is possible to envisage a system in which statolith displacement induces a response, the direction and intensity of which is determined by the amount of E.R. uncovered.

The geotropic response sequence is additionally complicated in organs in which the sites of perception and response are remote because the information obtained from the asymmetric redistribution of statoliths in individual cells must be translated into a transverse polarity across the receptor tissues as a whole, and this information must be transmitted to the

growing zone. This problem does not arise where the sites of perception and response are common, and in many lower plants and fungi where this situation exists the response sequences are relatively simple. Buder (1961) has studied one such system in the rhizoids of Chara and has found particles of unknown chemical composition which sediment in much the same way as amyloplasts. The rhizoids are constructed from tube like cells 30 μm in diameter and 300 μm long and the particles, known as 'Glanzkorper', normally lie some 20 μm behind the apex. They may be displaced to the basal part of the cell by centrifugation without altering the growth rate, but geo-sensitivity is abolished until new statoliths are synthesized in the apical regions of the cell. The rhizoid cells have been examined under the electron microscope by Sievers (1971) who has shown that new cell wall material, which is provided by golgi vesicles, is transported from the dictyosomes which lie behind the glanzkorper in the more basal regions of the growing apex. When the glanzkorper sediment they prevent the acropetal transport of golgi vesicles to the lower part of the cell and divert them to the upper side. The cell then grows asymmetrically until its tip is returned to vertical. This beautiful self-regulating system indicates just how significant the displacement of organelles by heavy statoliths could yet prove to be in the process of gravi-perception.

The classical studies of Darwin (1880), Boysen-Jenson (1910,1911,1913), Paál (1914,1919), Cholodny (1926) and Went (1926,1928) were all consistent with the hypothesis that the growth of shoots could be controlled by the influence of substances produced in the apex and transported to the growing zones in a polar fashion. Paál (1914,1919) suggested that the differential growth exhibited in the tropic responses might reflect an unequal distribution of growth regulatory substances, and Cholodny (1926) and Went (1926) suggested that such an unequal distribution might result from diversion of the polar transport stream towards the lower side of the geotropically stimulated shoot. This concept became known as the Cholodny-Went hypothesis,

a hypothesis which Cholodny (1926) maintained could also be applied to roots if root apices were to secrete a substance inhibitory to root growth. Support for this idea came when it was shown that root growth was promoted by the removal of the root apex (Cholodny 1924, Bünning 1928) and the possibility of a universal growth regulator was raised by the demonstration that the increase in growth rate could be suppressed completely by the application of a coleoptile tip to the cut stump (Cholodny 1926,1931; Koeble, Nelson and Snow 1931).

Evidence to support the Cholodny-Went theory was first provided by Dolk (1930) who collected the growth substances which diffused into agar blocks applied to the basal ends of Avena coleoptile segments. Using the Avena curvature test first introduced as a quantitative assay by Went (1928), Dolk was able to show that equivalent amounts of growth substance could be collected in blocks applied to vertical and horizontal segments. When split receiver blocks were applied to the basal ends of vertical segments the activities in the diffusates were again equivalent, but when this treatment was applied to horizontal segments activity in the diffusates from the lower halves of the coleoptiles was almost twice that from the upper halves. The concept of redistribution of a fixed amount of growth substance during transport from the apex was further supported by the work of Koch (1934) who bisected the apices of Avena coleoptile segments and removed one half from each before placing the segments horizontal. He found that segments bent upwards, even when orientated so that the lower part of the apex was absent, and he concluded that the growth substance must have migrated from the upper side to the lower side of the organ. Brauner and Appel (1960) were able to substantiate this work using small mica barriers which they inserted either horizontally or vertically into the apices of Zea coleoptile segments, and Shaw and Wilkins (1972) have obtained evidence to implicate the lateral transport of growth regulators in root geotropism using essentially similar methods.

The 'growth substance' became known as auxin and a unified theory for the involvement of auxin in all geotropic responses soon began to emerge. Schmitz (1933) used the diffusion method to observe an increase in 'auxin' levels in the nodes of several grasses and Hawker (1932) and Boysen-Jensen (1933) observed similar effects in the lower halves of horizontal bean roots, again using the diffusion technique.

The concept was made more convincing by the emergence of an immediate geoelectric effect in all geosensitive organs studied (Brauner 1927,1928). A potential difference between the upper and lower sides of a horizontal plant organ was first reported by Bose (1907) and the phenomenon was studied in detail by Brauner (1927,1928,1942,1959) and Clark (1937). The geoelectric effect provided a simple means of linking gravi-perception with the subsequent response but the link, which involved the lateral migration of the IAA anion across an induced electrical gradient, was never popular with physicists and was severely criticised when it was noted that geoelectric effects could also be induced in purely physical systems (Brauner 1942). The theory persisted, however, and was further substantiated by the demonstration that electrolytes could be used to inhibit the tropic responses, presumably by short-circuiting the induced potential (Wilks and Lund 1947; Schrank 1950,1953,1957). The demonstration that auxin gradients caused electrical gradients (Ramshorn 1934) seems to have been ignored, but with the demonstration that the immediate electrical effects could be attributed to artifacts associated with the electrode systems (Woodcock and Wilkins 1969a, 1969b) and that the effects of electrolytes could be explained in terms of their osmotic potentials (Bridges and Wilkins 1971), the significance of this finding becomes apparent. The true geoelectric effect which develops only after a lag period (Graham 1964; Graham and Hertz 1962,1964; Wilkins and Woodcock 1966, Woodcock and Wilkins 1969a) can be induced in vertical material by the asymmetric application of IAA (Graham 1964, Wilkins and

Woodcock 1965) and it appears to result from the asymmetric redistribution of IAA. It has only been measured in coleoptiles and hypocotyls and its development in both cases can be correlated with the establishment of an auxin asymmetry.

In order to explain the opposite effects of auxin in shoots and roots it was necessary to assume that auxin was present in roots at supra-optimal concentrations. The dosage response curves obtained for auxin induced growth in roots and shoots were in agreement with this possibility (Thimann 1937), but the observation that root growth could be promoted by the addition of IAA at very low concentrations (Audus and Brownbridge 1957) would seem to preclude its initial presence at supra-optimal concentrations.

The auxin involvement in geotropism came into question when early experiments with ^{14}C labelled IAA failed to demonstrate lateral redistribution in excised segments (Reisner 1957, Reisner and Simon 1960, Ching and Fang 1958). Gillespie and Thimann (1961) adapted the donor-tissue-split receiver technique of Dolk and found that the export from Avena coleoptile segments into the lower split receiver blocks was 50% greater than that into the upper receivers following symmetrical donation to the apical end of the segment. They were not able to demonstrate a redistribution in the coleoptile tissues, but in a subsequent investigation with Zea coleoptile segments they found an asymmetry in both tissues and receivers (Gillespie and Thimann 1963). They concluded that the geotropic response was mediated by a lateral redistribution of auxin but, as pointed out by Goldsmith and Wilkins (1964), their experimental technique was not sufficiently refined to enable this conclusion to be reached because the asymmetry could also have arisen from a differential effect on longitudinal transport. Subsequent investigations by Goldsmith and Wilkins (1962,1964), Gillespie and Thimann (1963) and Hertel and Leopold (1963) provided unequivocal evidence to support the existence of a lateral transport system. Goldsmith and Wilkins (1962, 1964) supplied labelled IAA asymmetrically to the apical ends of Zea

coleoptile segments and found that 10% of the total radioactivity in vertical segments was recovered in the half opposite the asymmetric source compared with 25% when the donor was applied to the upper half and 4% when the donor was applied to the lower half of the horizontal segment.

Although the distribution patterns recorded by Goldsmith and Wilkins (1962,1964) could only be explained in terms of the lateral transport system, subsequent work by Hertel and Leopold (1963b), Naqvi and Gordon (1966) and Little and Goldsmith (1967) led to the demonstration of a reduction in basipetal transport in Zea coleoptiles following inversion, and this suggested that the redistribution could also be implemented, at least in part, by an effect of gravity on the longitudinal transport system. Naqvi and Gordon (1966) showed the development of an increased capacity for basipetal transport in the lower halves of horizontal Zea coleoptile segments and connected the change with an increase in flux rather than transport velocity. This led them to believe that the primary effect of gravity was to alter the capacity for basipetal transport, and that lateral transport could largely be explained as a consequence of this alteration.

The problem was finally resolved by Cane and Wilkins (1969) who were able to open out Zea coleoptile segments to produce flat sheets of tissue which could then be used to study auxin transport in upper, lower and lateral tissue orientations. They found evidence of an increased capacity for basipetal transport in the 'lower' portions and polarized downward movement in side tissues, but downward polarity in side tissues could not be linked with the increased capacity for basipetal transport because the capacity for basipetal transport was constant throughout the side tissue.

Additional evidence to support the existence of an independent lateral transport system, and establish its connection with the statolith theory, has recently been provided by Hertel and his co-workers (1969,1970), who have been able to correlate the size and mobility of starch grains in normal and mutant maize coleoptiles with their capacity for lateral auxin transport.

The reduced amyloplast content of the amylo-maize mutant has no effect on the normal basipetal transport of auxin, but lateral transport is reduced to as little as 20% of the control value.

Indole-3yl acetic acid is present in Zea coleoptiles at physiological concentrations (Greenwood et al., 1972) and there is little doubt that it is involved in the transmission of the geotropic stimulus to the growing zone. This inference may justifiably be extended to other coleoptiles and certain hypocotyls and epicotyls, but probably not beyond. The situation in roots has not been clarified with the advent of radio-isotope techniques. Growth regulators appear to be involved and there is evidence to implicate the lateral redistribution of a basipetally transported inhibitor, but this inhibitor is not thought to be IAA (Shaw and Wilkins 1972). Gravi-perception occurs in the root cap (Juniper et al., 1966; Gibbons and Wilkins, 1970; Pilet, 1971) and the information is basipetally transported into the root (Shaw and Wilkins 1972) but IAA appears to be transported acropetally, at least in root segments (Wilkins and Scott 1968a, 1968b; Scott and Wilkins, 1968; Wilkins and Cane, 1970; Wilkins, Cane and McCorquodale, 1972), and barrier experiments suggest that substances moving acropetally are not involved in the geotropic response in roots (Shaw and Wilkins 1972).

The observations that N-1-naphthylphthalamic acid (Netien and Conillot 1951) and 2-3-6 Trichlorobenzoic acid (Jones et al., 1954) could inhibit the tropistic responses without affecting growth provided evidence to suggest that the reaction sequences in roots and shoots were similar. Since both phototropism and geotropism were affected, the reaction chain was thought by most workers to be the site of inhibition, but this assumption was questioned by Schrank (1960,1961) who dismissed the effect on phototropism on the grounds of reduced sensitivity to inhibition, and suggested that the inhibitors affected gravi-perception.

A further group of compounds with antitropistic properties are included in a group of biologically active derivatives of fluorene-9-carboxylic acid

known as the morphactins. Jones et al. (1954) considered the reaction sequence to offer the most likely site of action for these substances, and by eliminating some of the other possibilities, proposed that inhibition was mediated through competition with IAA in the lateral transport system. Evidence in support of this hypothesis was provided by Krelle and Libbert (1969a) and Bopp (1969), but similarities between responses to TIBA and the morphactins led Krelle and Libbert (1968b) to suggest that the morphactins, like TIBA, might act primarily as inhibitors of polar auxin transport. This view has been substantiated by Pilet (1970) and Parups (1970), as well as by work to be reported in this thesis. Parups (1970) maintained that, in addition to their inhibitory effects on basipetal transport, the morphactins were able to promote the acropetal movement of IAA in vertical segments and the upward lateral movement of IAA in geotropically stimulated segments. Thus, although there is reasonable evidence in the literature to support the hypothesis that the morphactins interfere with the auxin transport systems, their mode of action requires clarification before they can be used as evidence for a general auxin involvement in geotropism.

The simplest explanation to the problem of deriving a transverse polarity from information acquired from the symmetrical redistribution of statoliths is to assume that gravi-receptors are localised within the statocyst cell, but at a time when the existence of specific receptors remains in doubt it may be necessary to develop a theory which is capable of explaining a transverse polarity on the assumption that all regions of the statocyst wall are equally sensitive.

One such hypothesis has been advanced by Hertel and Leopold (1963) and is based on the assumption that basipetal auxin transport in coleoptiles is controlled by a process of differential secretion. Leopold and Hall (1966) have employed mathematical models to investigate the possibility of explaining basipetal auxin transport along these lines and have shown, for example, that a cell polar ratio, or ratio of secretions between apical and basal cell

walls, of 1.003 will result in basipetal transport with a polarity quotient ($= \frac{\text{basipetal transport}}{\text{acropetal transport}}$) of 3 on integration through a column of 200 cells. Their concept, which may be formulated $p = \frac{1 + \log Q}{0.872\ N}$, where p = cell polar ratio, Q = polarity quotient and N = number of cells in the column, requires that the polarity quotient shall increase exponentially with increasing tissue length (N) and a study of the kinetics of polar transport (de la Fuente and Leopold 1966) shows this to be the case. If the migration of statoliths to a tangential wall during geotropic stimulation could promote auxin secretion from the cytoplasm lining that side of the cell, then it would be possible to modify the polarity of auxin transport through the coleoptile tip (Hertel and Leopold 1963). Such a theory could certainly explain the development of a transverse polarity in organs in which a continuous plate of statenchyma exists, but its application to tissues where the statenchyma is localised is questionable.

In decapitated Zea coleoptile segments the starch statenchyma is restricted to the immediate vicinity of the vascular bundles, but lateral transport occurs between bundles as well as across them (Cane and Wilkins, 1971, 'side tissue experiments'). The vascular bundles are distributed bilaterally in coleoptiles and the curvatures developed in response to geotropic stimulation are affected by the alignment of these bundles with respect to the plane of curvature. The development of a transverse polarity, as measured by the development of the true geo-electrical effect, is not affected however by orientation with respect to the bundles (Grahm 1964, Johnsson 1971) and it must be concluded that the difference between curvatures results from a difference in the mechanical resistance to bending in the two planes, and not from a difference in the degree of polarization.

The ways in which hormone asymmetries give rise to differential growth remain uncertain, and they will not be clarified until the mechanisms of cell growth are better understood. Growth mechanisms may be considered under the headings of tip growth and multinet growth, and the problem of tip growth

and its control by orientation has already been considered in relation to the rhizoids of Chara. The problem of multinet growth is far more complicated and it is here that growth hormones are most implicated. The theory of multinet growth was first proposed by Roelofsen and Houwink (1953) and is based on the rearrangement of cellulose microfibrils during cell expansion. The primary wall has a skeleton of crystalline cellulose microfibrils which are always laid parallel to one another in a plane perpendicular to the main axis of the plant (Roelofsen 1951, Roelofsen and Houwink 1953). The fibril deposition shows no preferred orientation on the cross walls, but there is only one possible orientation in the side walls, and the fibrils build up in a series of loops. The loops restrict expansion at right angles to the main axis and the effect is strengthened by the random distribution of the fibrils in the end walls, but the restriction does not affect expansion along the long axis of the organ and polarised growth is therefore favoured. As the cell expands in the longitudinal direction the loops of microfibrils spread like the coils of an extending spring and their pitch changes from an orientation perpendicular to the direction of growth to one much more parallel with it. The diameter of the cell does not decrease because the fibrils are able to slip over one another as their pitch changes, but the thickness of the existing wall becomes somewhat reduced. This reduction in thickness is not important, however, because fibrils are deposited continuously on the inside of the wall, and it is this continuous deposition in the growing cell which results in the gradation of fibrils from nearly transverse on the inner surface of the wall to almost vertical at the middle lamella.

The microfibrils control the direction of expansion but they do not by themselves limit rate. They are embedded in an amorphous gel consisting mainly of non-cellulosic polysaccharides and proteinaceous materials, and it is the cross linkages in this amorphous material which are thought to exert the main constraint on growth.

Growth is dependent on cell wall extensibility and cell turgor, and factors affecting either of these parameters are likely to affect growth. Attention has centred on the control of cell wall extensibility and models may be divided into two categories depending on whether growth is assumed to be elastic or plastic. The earliest models assumed that extension was elastic and that biochemical changes were required to render it irreversible (Sachs 1887), but since extension is now known to be at least partially plastic, such biochemical changes may be superfluous. Biochemical changes do occur, however, in conjunction with growth. Masuda (1959) showed that auxin induced growth in Avena coleoptile segments could be inhibited by incubation with ribonuclease and his findings, which infer an effect of auxin at the level of gene transcription, have been substantiated by many workers using base analogues and actinomycin D to inhibit transcription. The requirement for transcription has been challenged on the grounds that the inhibitory effects of actinomycin D are often neither immediate nor absolute, but it appears from the data of Courtney, Marré and Key (1969) that the extent of growth inhibition may be correlated with the extent of the inhibition of RNA synthesis. The synthesised proteins may be concerned with the enhancement of cell turgor, the production of wall material or the promotion of wall softening, and each possibility has been utilised in the construction of models to explain cell growth. Plant growth hormones are known to stimulate the production of reducing sugars, and a theory involving the enhancement of turgor by reducing sugar production has been advanced by Arslan and Bennet-Clark (1960) to explain geotropically induced growth at the wheat node. There is however little definite evidence to suggest that increased turgor is involved in cell elongation, and evidence against such a requirement may be inferred from the demonstration that an equivalent extension rate for auxin induced growth in Avena coleoptile segments may be obtained when the turgor driving force is replaced by a constant applied force (Cleland 1971c).

If the function of the proteins is to produce more wall material, then this new material may be considered to have one of two fates. It may be required for the consolidation of the wall during elongation, or it may cause growth by a process of intussusception. There is a general increase in the gross synthesis of cell wall materials in response to auxin (Baker and Ray 1965a, 1965b) and both intussusception (deposition within the wall) and apposition (deposition at the membrane) are affected (Ray 1962). The effect appears to be induced by the auxin treatment and not by the subsequent elongation (Ray and Baker 1965), but time course studies have shown that increased synthesis is only detectable after a lag period of the order of one hour (Baker and Ray 1965; Abdul-Baki and Ray 1971). Auxin induced growth is known to occur within 15 minutes of auxin application (Evans and Ray 1969) and it seems unlikely therefore that the increased synthesis of wall materials is necessary for cell elongation.

The type of extension which a material will undergo when stressed is dependent on the number of cross linkages in the material. Materials which are extensively cross linked undergo a virtually instantaneous elastic extension whilst materials which are only slightly cross linked undergo a much slower extension which tends to be irreversible. This latter extension is known as viscous flow and its development is directly proportional to the stress time. Polymers of the type found in cell walls show an extension of an intermediate 'viscoelastic' form, and this viscoelastic extension may be divided into reversible elastic and irreversible plastic components. The plastic component in such physical systems is connected with the breakage of bonds by physical force, but these bonds may also be attacked by chemical agents, allowing the limited viscoelastic deformation to be converted into a more continuous extension, and this characteristic has obvious implications when considering the action of growth hormones in physiological systems. Cells are able to elongate at a reasonably constant rate for a considerable period of time whilst isolated cell walls show only a constantly diminishing

viscoelastic response (Cleland 1967; Hager, Menzel and Krauss 1971). Cleland (1967, 1971b, and Haughton 1971) has found that the extensibility of Avena cell walls can be increased if the coleoptiles are first pretreated with auxin, and Cleland and Haughton (1971) have shown that, if the curves of extension v. log time for auxin and control pretreated walls are extrapolated, they intersect at the time axis. These phenomena will only occur if the size of the viscoelastic flow units is changed, and it therefore seems probable that the prolonged response in the intact cell can be explained in terms of the operation of a series of viscoelastic extensions in response to chemical stimuli produced in the cell.

Physical extension is insensitive to temperature and treatment with metabolic inhibitors but cell elongation shows a marked temperature dependency and can be blocked completely by metabolic inhibitors (Cleland 1968). The biochemical requirement could involve one of several agencies and hypotheses implicating pectin methyl esterases which rupture the covalent calcium bonds in calcium pectate, and polysaccharide hydrolases which cleave various saccharide polymers, have been numerous. There is little doubt that such enzymes exist in plant cells and it is quite probable that their levels are affected by plant hormones (see Masuda and Nade 1967; Maclachlan, Davis and Fan 1968), but the magnitude of their effects and the kinetics of their appearance do not seem to correlate with observed growth responses. It is also difficult to reconcile their involvement with the observed responses to inhibitors because experiments involving the use of metabolic inhibitors (Cleland 1968) and inhibitors of protein synthesis (Courtney, Morré and Key 1967; Cleland 1970, 1971a) suggest that the growth regulating proteins are rather unstable whilst the polysaccharide hydrolases are renowned for their extreme stability.

Considerable advances in our understanding of cell expansion have resulted from recent investigations into the acid growth response. Bonner (1934) was first to note the growth promoting properties of low pH, but he

interpreted his results in terms of the activation of endogenous auxins and the subject received little further attention. Recently Rayle and Cleland (1970) and Evans, Ray and Reinhold (1971) have reported an immediate promotion of growth in response to low pH treatment. The response is optimal at pH3 and resembles the auxin induced response in several important aspects, namely maximal growth rate, relationship to inserton extensibility, Q_{10} and dependency on cell turgor. The responses differ firstly in that the acid response is not dependent on respiration or protein synthesis (Ganot and Reinhold 1970; Evans, Ray and Reinhold 1971; Hager, Menzel and Krauss 1971) and, secondly, in that it may also be induced in 'frozen-thawed' material if a force is applied to replace the missing cell -turgor (Rayle and Cleland 1972). The great significance of these findings is that the kinetics of the in vivo response to low pH are not affected by the inhibitor treatments or by the 'frozen-thawed' treatment. It seems unlikely therefore that low pH is involved in the activation of wall softening enzymes, as suggested by Hager, Menzel and Krauss (1971), and the failure to alter the acid response in 'frozen-thawed' segments by the application of treatments designed to inhibit, denature or extract wall proteins may be taken as further evidence against this possibility (Rayle and Cleland 1972). Any requirement for the synthesis of consolidatory cell wall materials during elongation may also be ruled out because both in in vivo and in vitro acid growth responses are irreversible.

It is now possible to envisage the control of growth by hydrogen ions, the production of which is controlled by the growth regulator and the function of which is to reversibly cleave some acid-labile bond in the cell wall. The rapid reversibility of the hydrolyses will mean that, if hydrolysis and extension fail to occur simultaneously, the bond will reform in its original position;thus explaining the lack of stored growth under water stress, whilst if the two do occur simultaneously the bond will reform in a different position and give rise to consolidated growth. The growth limiting proteins

(Cleland 1971a) may be required for the generation or transport of hydrogen ions, and the rapid effects of inhibitors may be explained by their action on this part of the system.

INTRODUCTION

The first and probably most detailed investigation into the geotropic response in the grass culm was carried out by Von Sachs (1887) who observed that, whilst the leaves were completely developed, the leaf sheath bases showed anatomical and physiological features which were consistent with a persistent condition of youth'. The parenchymatous cells of the leaf sheath base remained highly turgid and lignification in the vascular tissues was greatly suppressed. When geotropically stimulated, curvatures developed at two or three nodes on the stem and the curvatures could be attributed to cell expansion on the lower sides of the leaf sheath bases.

The ability to respond geotropically at the node is not restricted to the Gramineae but is also found in several other plant orders, notably the Commelinaceae and the Caryophyllaceae. Miehe (1902) was interested in the response in the Commelinaceae and found, working with two node preparations from Tradescantia fluminensis, that a geotropic response at the lower node was only possible when the upper node was intact. The mere presence of the upper node was not sufficient to initiate the response; the axillary bud had to be present, and it was the development of this bud which governed the magnitude of the response at the lower node. The individual responses in multinode preparations appeared to be well co-ordinated because, when the combined responses at the nodes returned the apex to vertical, the reaction at the lower nodes ceased despite the fact that they had only realised a fraction of their potential curvature.

Essentially similar 'geotonic' phenomena have been reported for the responses at the nodes of stems of Triticum aestivum and Bromus sterilis. Arslan and Bennet-Clark (1960) have found that the magnitude of the response at the node is dependent upon whether the stem segment is tip held or base held, and they have suggested that the response is conditioned by the amount of leaf sheath tissue excised with the segment. Since both tip held Bromus

stem segments (Arslan and Bennet-Clark 1960) and single node Tradescantia segments (Miehe 1902) are reported to have the capacity to respond through 90°, it seems reasonable to assume that the integrated responses are under the control of some form of geotonic agency, but the analogy cannot be complete if, as suggested by Arslan and Bennet-Clark (1960), it is the length of the leaf sheath which governs the response in the grass culm, because the leaf sheath offers no means of communication between nodes.

Rawitscher (1932) thought that the tonic influence of gravity could be attributed to differences in auxin production in various parts of the plant and evidence to implicate auxin in the geotropic response in the grass culm was quickly forthcoming. Schmitz (1933) reported an increased auxin production in the lower halves of the leaf sheath bases of several seedling grasses, and Van Overbeek (1938,1945) reported substantial increases in the auxin content of the nodal zones of maize and sugar cane stems (the geotropic responses in maize, sugar cane and many other tropical grasses occur in the basal regions of the stem as well as in the leaf sheath base). Additional evidence to implicate auxin in the geotropic response in Zea came from the work of Van Overbeek (1936,1938) and Shafer (1939) who connected the ageotropic behaviour of 'Lazy Maize' with its inability to redistribute the abnormally high levels of 'auxin' found in the nodal regions of the stem. Ageotropism in Lazy Maize is a character associated with the mutation of a single recessive gene (Jenkins and Gerhardt 1931), and Van Overbeek believed that this gene locus was directly responsible for auxin redistribution.

It must be remembered that the above mentioned demonstrations of an auxin involvement were all inferred from the biological activity associated with diffusates and crude extracts. While IAA has been shown to be active in promoting the growth of the wheat leaf sheath base (Nagda 1956; Arslan and Bennet-Clark 1960), it has never been shown to be as effective as the geotropic stimulus, and attempts to extract IAA from maize nodes have been singularly unsuccessful (Arslan and Bennet-Clark 1960). Although preliminary

chromatographic separations have indicated the presence of growth promoters in the nodal regions of *Zea* stems, the distribution of these substances has suggested that they are not connected with the geotropic response (Arslan and Bennet-Clark, 1960). The involvement of growth substances in geotropic responses of this type is clearly in need of clarification.

The research reported in this thesis has been undertaken to elucidate the physiology of the geotropic response in the wheat culm, and experiments have been designed with a view to investigating two basic problems, namely the nature of the growth mechanism and the means of its control. The project is thought to be of special academic interest, firstly because it extends research in the field of geotropism to a system which is operative in green tissues and, secondly, because it provides an opportunity to test theories propounded largely for seedling geotropism on a system which is functional throughout the life of the plant. Commercial significance may also be attached to the project because the geotropic response under investigation provides the only means of rectification in grasses following storm damage during the reproductive phase of growth. Information concerning the physiology of this response may be of value in breeding programmes designed to increase crop yield.

MATERIALS AND METHODS

1. Plant Materials

A. Triticum aestivum L. var. Kolibri

Fruits ('seeds') obtained from Charles Sharp and Co. Ltd., Lincs., U.K. were sown under glass in drills 150 mm apart. Drills were arranged in beds measuring 3.3 m x 1.3 m and plants were staked as required. Successional sowings were made at 14-day intervals to provide for continuous sampling. Supplementary lighting was supplied by 1000 w MBFR/U high pressure mercury vapour reflector lamps (Thorn Lighting Ltd., Glasgow, U.K.) which were arranged at 2 m spacings in two rows 1.6 m apart and 2.75 m above soil level. The units measured 0.75 m in length so that the total clearance was 2.0 m and the lamps provided a 16-hour photoperiod. Some additional material was grown outside under normal daylight conditions during the summer months.

Plants were harvested just prior to anthesis (but see Results section Fig. 33 for the effect of sampling date on geosensitivity at individual nodes) and the response at the apical node was considered unless otherwise stated in the Results section. Where two node preparations were involved segments containing the two uppermost nodes were excised just prior to anthesis.

Experiments involved the use of one of two types of segment:-

type 1 - leaf sheath bases excised with 50 mm of culm to either side,

type 2 - 2.4 mm portions excised from the leaf sheath base.

Segments of type 1 were used in all curvature experiments and segments of type 2 were used in 24-h bioassays and quick growth studies.

Experiments were carried out in diffuse white light and all experimental

chambers except those used in rotation experiments were kept in diffuse white light during the experimental period. The material did not show a phototropic response, and equivalent geotropic responses were obtained in diffuse white light and total darkness (Table 1A).

Table 1A. The response to 24-h stimulation.

Unilateral white light		Total Darkness	
Vertical material photo-stimulus	Horizontal material geo- & photo-stimuli	Vertical material No stimulus	Horizontal material geo- stimulus
2.071 ± 0.286°	31.0 ± 2.04°	1.300 ± 0.324°	29.2 ± 1.34°

t for horizontal material = 0.738^{NS}

t for vertical material = 0.523^{NS}

B. Avena sativa L. var. Svalof Victory I

Zea mays L. var. Burpee Snowcross

Avena coleoptiles were used in auxin assays, and both Avena coleoptiles and Zea coleoptiles were used in the morphactin study (Results Section 10). Similar growing conditions were used for both species and the two will be considered together.

Avena seeds were obtained from the Svälof General Seed Company, Sweden, and Zea seeds were obtained from W. Atlee Burpee Co., Philadelphia, U.S.A. They were soaked in running tap water for up to five hours and then sown thickly in plastic sandwich boxes of dimensions 250 mm x 200 mm x 80 mm in a medium of moist vermiculite (Alexander Products Ltd., Somerset, U.K.).

The seeds were covered to a depth of 20 mm with moist vermiculite to give support to the emergent coleoptiles and boxes were placed in a ventilated cabinet in total darkness at 25 ± 1°C. Avena coleoptiles were ready for use after 4 days, and Zea coleoptiles after 5 days in the growing cabinet.

All subsequent handling was carried out under a dim green light of 10×10^{-12} einsteins $cm^{-2} sec^{-1}$ in the spectral band 510 - 535 nm and experimental chambers were kept in a dark box during the experimental period.

2. Microscopy

Material was prepared for microscopy in one of three ways.

A. Temporary preparations using a freezing microtome

Material was mounted eccentrically in a blob of 'Tissuetek' O.C.T. compound (Ames Company, Indiana, U.S.A.) on an aluminium chuck. Chucks were partially immersed in liquid nitrogen until the Tissuetek was frozen and they were then transferred to a cryostat cabinet (Bright's, Huntingdon, U.K.) at -18°C. After equilibrating chucks were positioned on the freezing microtome and sections cut. The knife angle was 12° from vertical. Sections were lifted from the knife surface on to glass slides where they were mounted in water and covered with 20 mm x 20 mm coverslips. They were stained using the irrigation technique, and coverslips were then sealed by ringing with DPX mountant (B.D.H. Chemicals Ltd., Poole, U.K.). Specimens were photographed within 24 hours of ringing.

B. Permanent preparations using wax embedded material sectioned on a Cambridge rocking microtome

Material was fixed and cleared in F.A.A. (50% absolute ethanol : 35% water : 10% formaldehyde [37-40%] : 5% glacial acetic acid) before

dehydrating in solvents of paraffin. The dehydrating and embedding procedures may be summarised:-

Dehydration

Change No.	n-Butyl alcohol (ml)	ethyl alcohol (ml)	water (ml)
1	10	20	70
2	15	25	60
3	25	30	45
4	40	30	30
5	55	25	20
6	70	20	10
7	85	15	0
8	100	0	0

Infiltration & embedding in wax

9	Add wax chips to butanol, stopper tubes and place in melting oven at 55°C. Remove stopper after a few hours.
10	Change wax and then cast blocks.

Blocks were sectioned at 20 μ on a Cambridge rocking microtome and ribbons were affixed to slides with saliva. Ribbons were then warmed to expand the wax (but not to melt it) and sections were dewaxed and stained according to the following procedure:-

Removal of wax. Slides were immersed in:-

xylene/wax
↓
xylene
↓
xylene/ethyl alcohol
↓
ethyl alcohol
↓
50% ethyl alcohol

Staining

1% aqueous basic fuchsin
↓
50% ethyl alcohol
↓
98% ethyl alcohol
↓
ethyl alcohol/xylene
↓
xylene
↓
Canada balsam

C. Permanent preparations using Araldite embedded material

Material was fixed in 3% phosphate-glutaraldehyde fixative which was composed of 88 mls phosphate buffer pH 7.3 ($NaH_2PO_4.2H_2O$ buffered with NaOH) and 12 mls 25% glutaraldehyde. All traces of gluteraldehyde were subsequently removed by repeated washings in phosphate buffer (pH 7.3) before post-fixation with 1% osmic acid (pH 7.3) and the material was again washed in phosphate buffer (pH 7.3). Material was then dehydrated through an alcohol series (25% → 50% → 75% → 90% → 100% → 100%). The dehydrated material was transferred via propylene oxide and propylene oxide : Araldite (1 : 1) to pure Araldite (46 ml resin + 54 ml hardener + 1.5 ml accelerator) and the Araldite was polymerised for 24 hours in an oven at 60°C. Sections (2μ) were cut on an L.K.B. ultratome and stained in a solution of 0.1% toluidine blue in 1% borax (1 : 1 mix). Sections were washed in 1% borax and distilled water and they were then mounted in Canada balsam.

3. Rotation Experiments

A belt driven apparatus was constructed using a Citenco electric motor type KQ/507 (Manorway, Borehamwood, Herts, U.K.) and pulley wheels of various diameters. The apparatus produced uniform rotation about an horizontal axis, and further axes at 15°, 30°, 45°, 60° and 75° displacement

from vertical were added for one set of experiments. The experimental chambers consisted of wax lined aluminium screw cap containers which measured 75 mm in diameter and 120 mm in length. Moist cotton wool was applied to the basal ends of 100 mm stem segments which were pinned to cylindrical corks of either 20 mm diameter (straight growth tests) or 40 mm diameter (acceleration tests) which were fixed in the centres of the screw caps (Fig. 1).

Calculation of acceleration for a given angular velocity

$$F = MW^2r$$

$$F = Ma$$

$$a = W^2r \quad \text{---} \quad (1)$$

$$W = \frac{R}{120\pi} \quad \text{---} \quad (2)$$

substituting (2) in (1)

$$a = \frac{R^2r}{(120\pi)^2}$$

where M = mass
a = acceleration
W = angular velocity rad/sec
R = angular velocity rev/min
r = radius of rotation

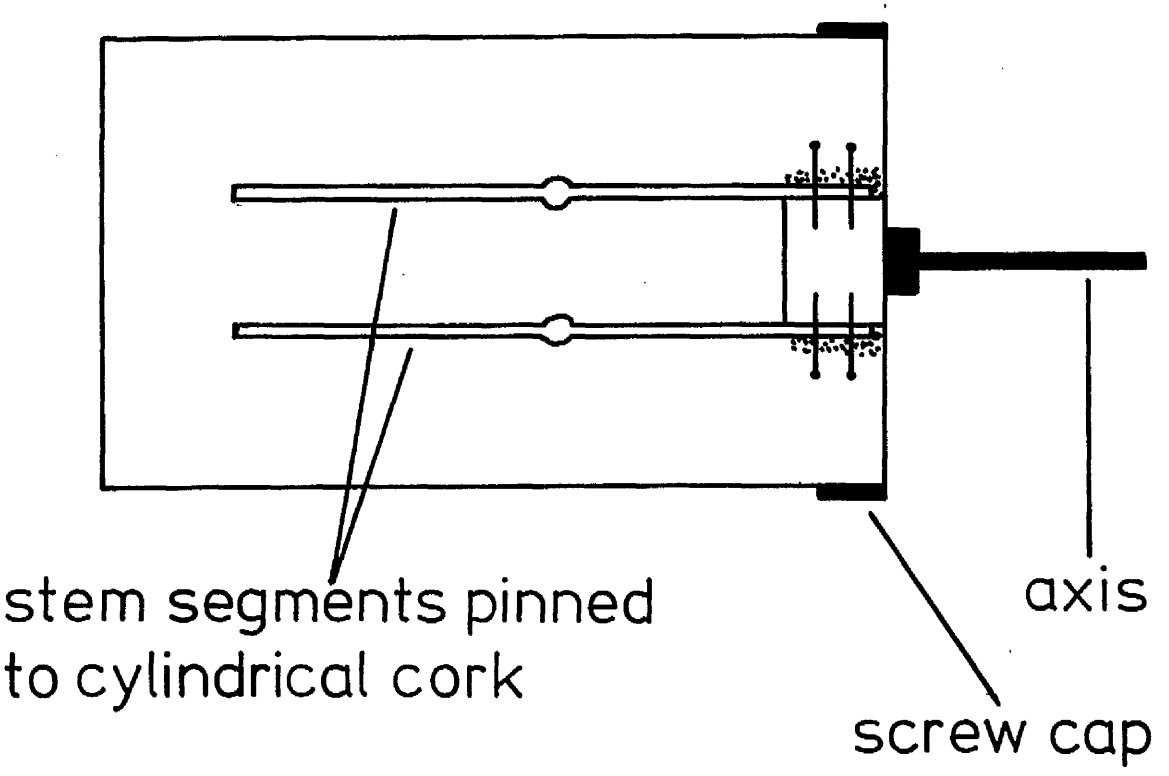
4. Anoxia Experiments

Anoxia experiments were conducted in an atmosphere of flowing nitrogen in parallel sided Pyrex desiccators. The desiccators, which were 200 mm in diameter, were fitted with intake and exhaust pipes and they each received six evacuations and six nitrogen flushes before final connection to a nitrogen stream. The nitrogen was supplied at low pressure from a cylinder, and was moistened by passage through two woolff bottles containing water. The nitrogen was replaced by air in the aerobic controls.

Fig. 1.

Design of the experimental chamber used in the rotation experiments.

See text for full description.


stem segments pinned
to cylindrical cork
axis
screw cap

5A. Bioassays involving the wheat leaf sheath base

(i) Curvature tests

a. Preparations

Single node preparations:- Leaf sheath bases were excised with 50 mm of culm to either side.

Two node preparations:- Segments were excised with 50 mm of culm below the lower node and 50 mm above the upper node.

The preparations, which included the apical node unless otherwise stated, were pinned either apically (tip held) or basally (base held) to polystyrene blocks and moistened cotton wool was applied to their pinned ends (Fig. 2B). During the experimental period the assembled units were held in plastic containers in which a high humidity was maintained. Curvature at the node was measured with a protractor at the end of the experimental period.

b. Quick growth responses

One node preparations were supported basally in test tubes and balanced kymograph levers were placed horizontally above their apical ends (Fig. 3B). Each kymograph lever measured 250 mm in length and it traversed the segment at a point 25 mm from the pivot. Permanent records of the magnified responses were traced on smoked charts which were affixed to 6-inch kymograph drums. Completed charts were removed from the drums and varnished by dipping in an ethanolic shellac solution.

c. Destarching techniques

Starch was removed using the destarching procedure of Pickard-Gillespie and Thimann (1966). One node preparations were placed in boiling tubes containing 5×10^{-5}M GA_3 + 5×10^{-5}M kinetin + 100 ppm streptomycin and the tubes were maintained in darkness at 30°C. Control incubations in 2% sucrose + 100 ppm streptomycin and distilled water + 100 ppm streptomycin were also

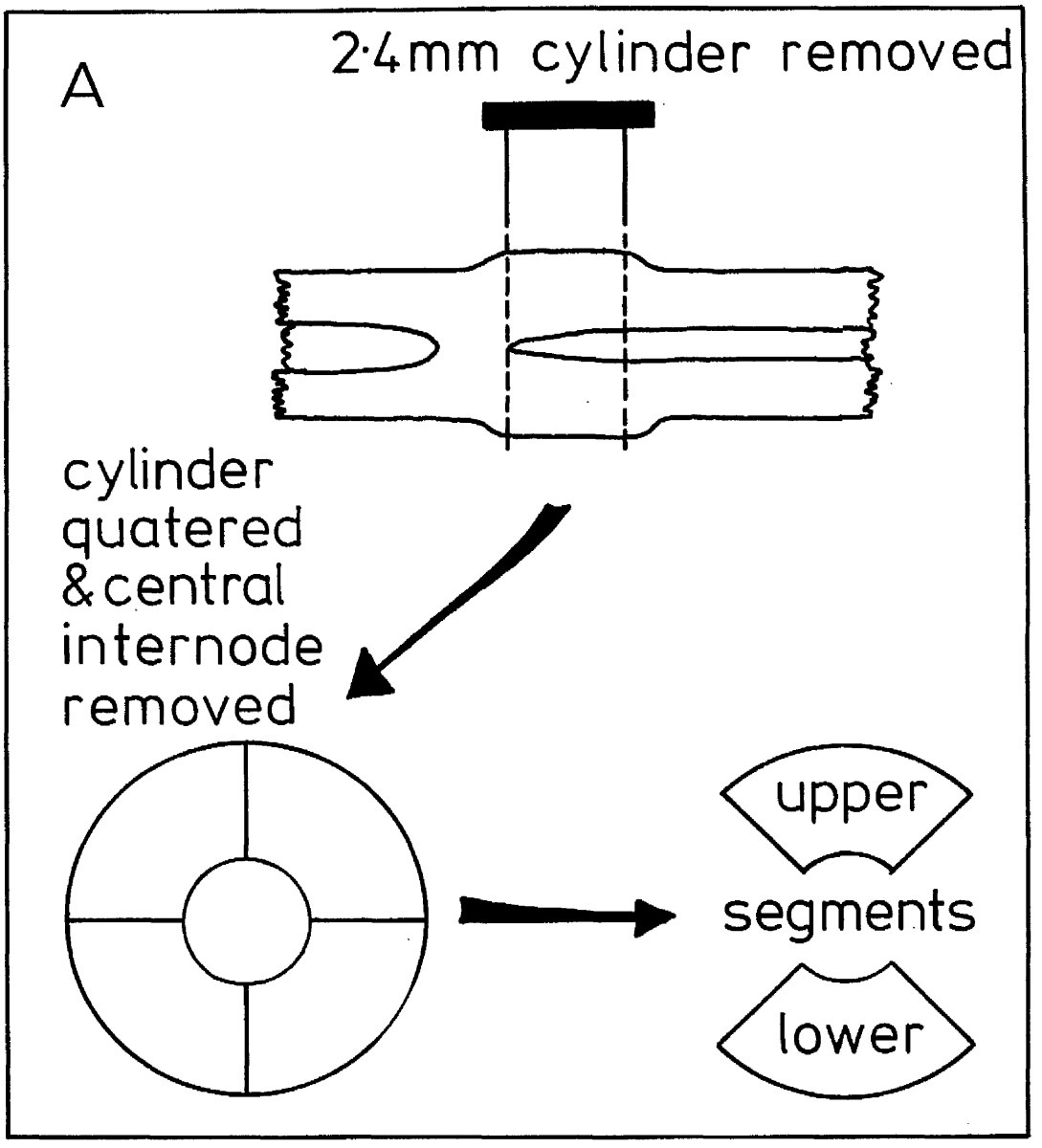
Fig. 2.

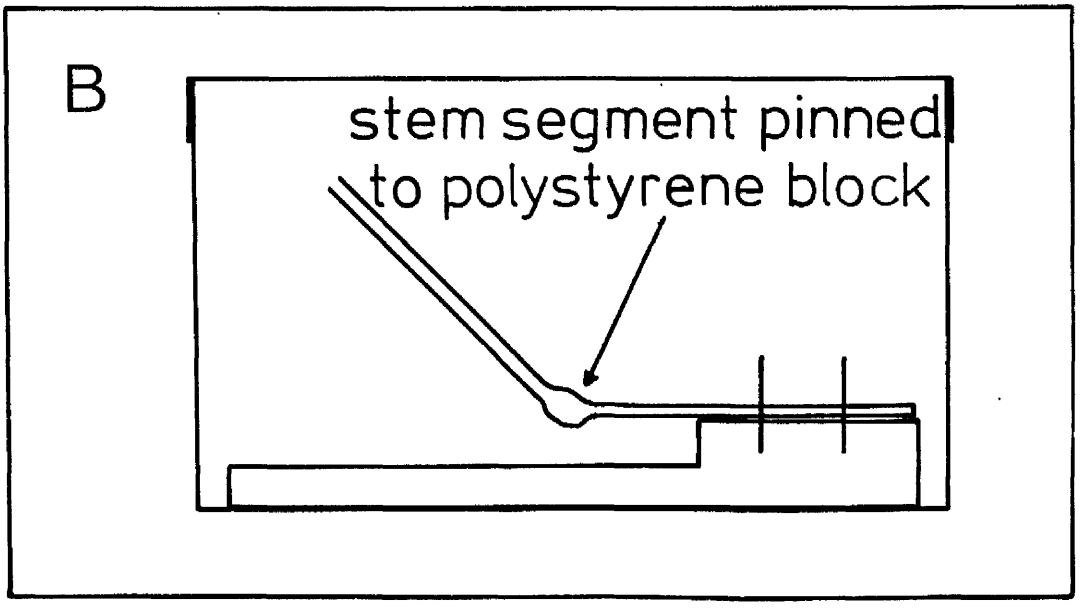
Preparation of material for use in bioassays involving the wheat leaf sheath base.

Fig. A : Excision of segments from the leaf sheath base.

Fig. B : Experimental design for curvature tests involving one or two node preparations.

See text for full descriptions.


A
2·4mm cylinder removed
cylinder quatered & central internode removed
upper
segments
lower


B
stem segment pinned to polystyrene block

carried out. All solutions were changed at 24-hour intervals and the treatments were concluded after a 72-hour incubation period. Half the material was then removed for assay and the remainder was transferred to tubes containing 2% sucrose + 100 ppm streptomycin. These tubes were kept in bright light at 25°C for 24 hours and the material was then removed for assay. The ability to respond to geotropic stimulation after the various treatments was assessed at the end of a 24-hour period of horizontal exposure in diffuse white light at 25°C.

(ii) Straight growth tests

a. 24-h Bioassays:- Portions of leaf sheath base 2.4 mm in length were excised and quartered, and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 mls of solution (Fig. 2A). Segments were shadowgraphed after 24 hours treatment.

The significance of segment orientation

It is possible to induce a growth response in isolated segments excised from the leaf sheath base (Fig. 14). Growth is only induced if segments are orientated with the outer epidermis facing downwards and segments so orientated will be called 'lowers'. The reverse treatment, where segments are orientated as 'uppers', does not result in the induction of growth. Segments orientated as 'uppers' may be used in experiments designed to investigate the effects of growth promotors on non-geotropically induced material whilst segments orientated as 'lowers' may be used in experiments designed to investigate the effects of promotors and inhibitors on geotropically induced growth.

b. Quick growth studies

Portions of leaf sheath base 2.4 mm in length were excised and, after removal of the pieces of stem contained therein, the leaf sheath bases were

threaded, in batches of five, on glass rods which were submerged horizontally in 250 mls of solution. The growth response was magnified through a vertical lever 350 mm in length which was pivoted 50 mm above the experimental material and the response was further magnified by a kymograph lever 250 mm in length which was pivoted 25 mm from the vertical lever (see Fig. 3A). The horizontal force component exerted on the plant material by the lever system was eliminated by balancing the kymograph lever. A permanent record of the development of the response was obtained using the kymograph technique described previously.

5B. Bioassays involving Avena and Zea Coleoptiles

(i) Curvature Tests

Curvature experiments were conducted with the detached apical 15 mm of the coleoptile from which the leaves had been withdrawn. Segments were held basally between pieces of moist filter paper in perspex frames and assembled frames were placed in plastic containers in which a high humidity was maintained. Segments were shadowgraphed after a 4-hour period of geotropic stimulation.

(ii) Straight growth experiments

Zea coleoptile segments 10 mm in length, and Avena coleoptile segments 5 mm in length were taken 1 mm behind the apex. Segments were incubated in 50 mm petri dishes containing 10 mls of solution and they were shadowgraphed after 24 hours treatment.

5C. Solutions used in bioassays

(i) Growth regulators

Growth regulators, with the exceptions of kinetin and gibberellic acid, were dissolved in minimal quantities of methanol. Kinetin was dissolved

Fig. 3.

Experimental designs for the apparatus used in the measurement of quick growth responses.

Fig. A : Measurement of straight growth in segments excised from the leaf sheath base.

Fig. B : Measurement of curvature in 100 mm stem segments.

See text for full descriptions.

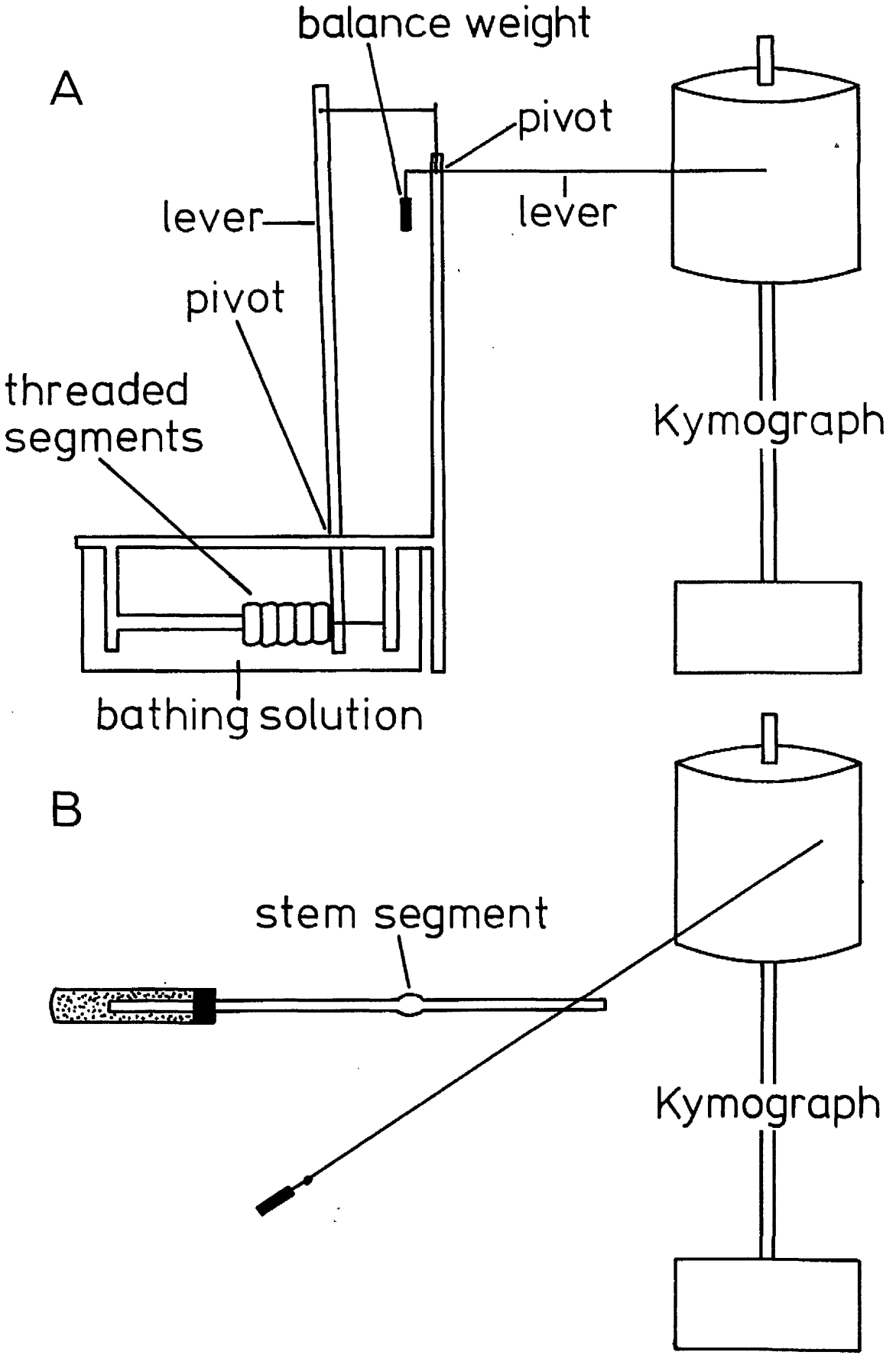
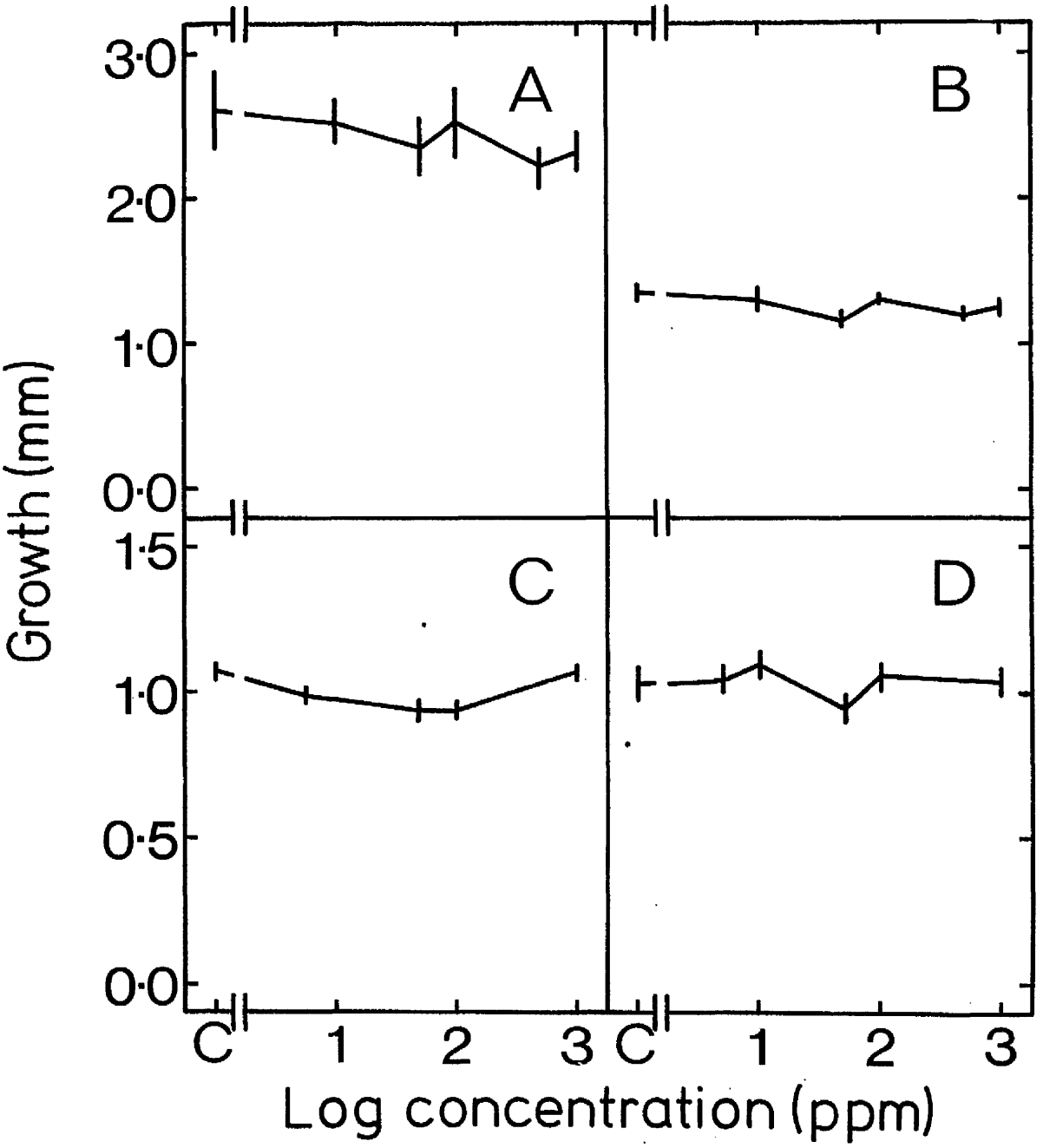

A
balance weight
pivot
lever
lever
pivot
threaded
segments
Kymograph
bathing solution
B
stem segment
Kymograph

Fig. 4.

The effects on growth of the solvents used to dissolve the growth regulators.

The figure shows the effects of methanol on the growth of 10 mm *Zea* coleoptile segments (A), 5 mm *Avena* coleoptile segments (B).

Also shown are the effects of methanol (C) and dimethylformamide (D) on the growth of geotropically induced segments ('lowers') excised from the wheat leaf sheath base.


3·0
2·0
1·0
0·0
1·5
1·0
0·5
0·0
A
B
C
D
Growth (mm)
C
1
2
3
C
1
2
3
Log concentration (ppm)

in warm dimethyl formamide and gibberellic acid was dissolved directly in water. Once dissolved the solutions were added to distilled water to give stock solutions which contained the growth regulator at a working concentration of 2×10^{-4}M and the solvent at about 500 ppm and solutions for use in bioassays were prepared by serial dilution. The stock solutions were stored in a refrigerator at 2°C.

The effects of methanol and dimethyl formamide in the bioassays are shown in Fig. 4. Since the solvents had no significant effect up to 1000 ppm it was not thought necessary to include a dilution series in all experiments and a fixed concentration of 100 ppm methanol or dimethyl formamide was added to the solvent controls. The following plant growth regulators have been used:

Indole acetic acid	[IAA]	Sigma Chemical Co. St. Louis, U.S.A.
Gibberellic acid	[GA_3]	Koch Light Labs., Bucks., U.K.
Kinetin	[K]	" " " " "
Coumarin	[C]	" " " " "
Abscisic acid	[ABA]	Hoffman-La Roche, Basle, Switzerland.
Morphactin	[CFM]	E. Merck AG., Darmstadt, W. Germany.

CFM = 2-chloro-9-hydroxyfluorene-carboxylic acid-9-methyl ester.

(ii) Buffers

a. Citrate : phosphate buffer

The following chart was used to make up a range of citrate : phosphate buffers. Buffer pH was checked, using a Pye pH meter (Model 79), and corrected if necessary.

pH	0.2M Disodium hydrogen orthophosphate (Analar) ml	0.1M Citric acid (Analar) ml
2.5	1.71	18.29
3.0	4.11	15.89
3.5	6.05	13.95
4.0	7.71	12.29
4.5	9.08	10.92
5.0	10.30	9.70
6.0	12.63	7.37
7.0	16.47	3.53
8.0	19.45	0.55

b. Glycine : HCl buffer

0.1M glycine (Analar) solution was buffered with 2N HCl to a final pH of pH3, pH3.5 or pH4.

(iii) Metabolic inhibitors

Inhibitors were dissolved in distilled water and solutions were stored in a refrigerator.

a. Respiratory inhibitors and uncouplers

Sodium azide

Potassium cyanide

Di-nitro phenol.

b. Inhibitors of protein synthesis

Cycloheximide (Calbiochem., San Diego, U.S.A.)

Chloramphenicol (Sigma Chemical Co., St. Louis, U.S.A.)

c. Inhibitors of nucleic acid synthesis

Actinomycin D (Sigma Chemical Co., St. Louis, U.S.A.)

5D. The Shadowgraph technique

Segments were removed from the incubation media and blotted dry. They were then arranged in rows on glass plates and the assembled plates were placed in a photographic enlarger (Universal Alpha II). Permanent records of the images, which were focussed at a final magnification of x5 (x1 for coleoptile curvature tests), were obtained by shadowgraphing on photographic paper (Ilfobrom 4 IB4 IP). Papers were developed using Ilford contrast developer (1 : 4 dilution) and fixed in Kodafix solution (1 : 4 dilution). They were then dried and glazed on a Kodak glazing machine (Model 15TC).

6. Radioactive Tracer techniques

A. Solutions and experimental procedures

(i) IAA-5-3H. IAA-5-3H of specific activity 1.7 $CimM^{-1}$ (C.E.A. Gif-sur-Yvette, France) was incorporated in blocks of 1.5% Oxoid Ionagar No.2 (B.P. Oxoid Ltd., London, U.K.) at a concentration of 10^{-6}M. The incorporation procedure, which involved adding the IAA to a molten agar solution (?60°C), had no effect on the radiochemical constitution of the tracer (Fig. 6).

The transport of IAA was studied in oat and corn coleoptile segments and in segments excised from the wheat leaf sheath base.

a. Polar auxin transport

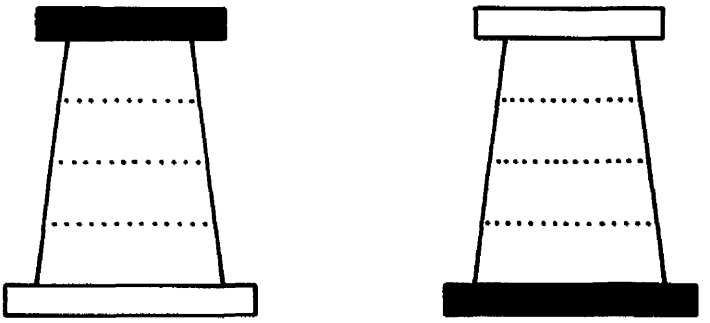
Acropetal and basipetal transport was studied in 10 mm coleoptile segments and 2.4 mm segments excised from the wheat leaf sheath base. Segments were held vertically between blocks of 1.5% agar and the morphologically apical ends of the segments were always uppermost (Fig. 5). Radioactive IAA was supplied in either the apical or the basal agar block.

Fig. 5.

Tissue arrangements in transport experiments.

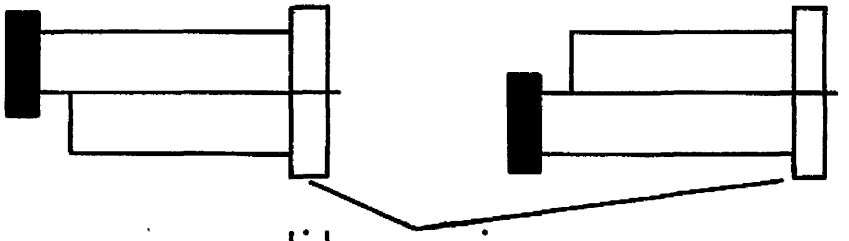
The tissue arrangements used in transport experiments designed to study longitudinal transport, lateral transport in coleoptiles and lateral transport in leaf sheath bases are shown in Figs. A, B and C respectively. At the end of the transport period the tissues were divided into subsections for extraction. Segments utilised in longitudinal transport experiments were cut transversely into four equal subsections in the case of the 10 mm coleoptile segments, and two equal subsections in the case of 2.4 mm leaf sheath base segments, and segments used in lateral transport experiments were bisected longitudinally into upper and lower halves.

Longitudinal movements



Basipetal Acropetal

Lateral movements in coleoptiles



split receivers

Lateral movements in leaf sheath bases

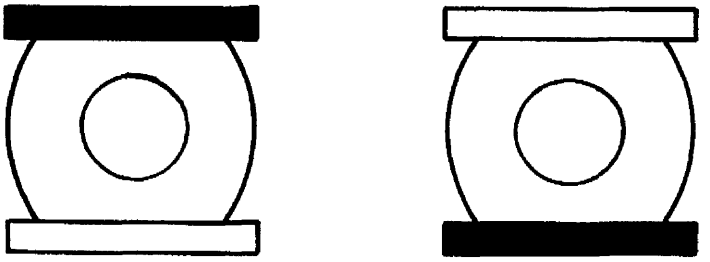
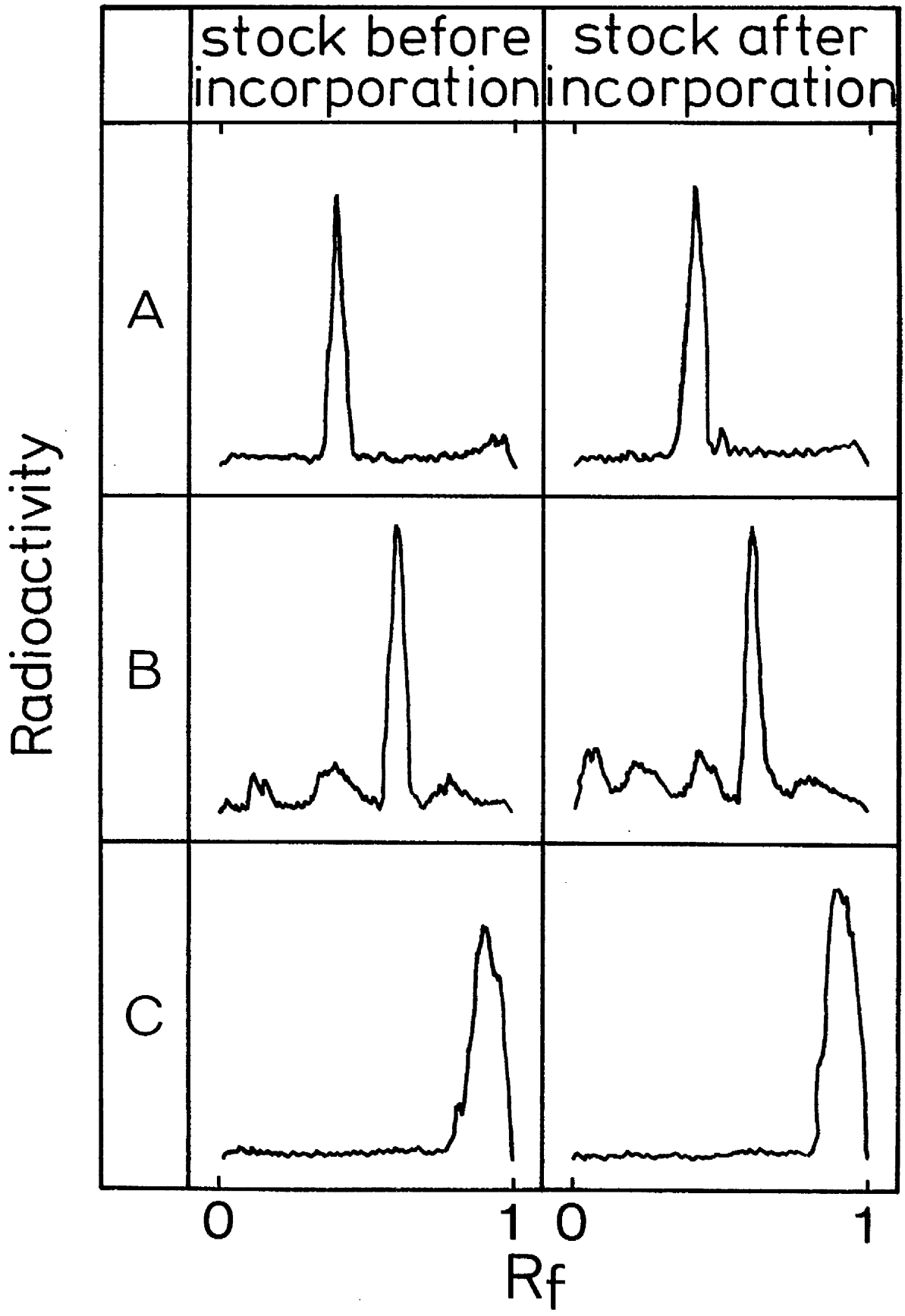


Fig. 6.

Analyses of IAA-5-3H stock solutions before and after incorporation into agar donor blocks.

Thin layer chromatography was employed to test the radio-chemical purity of the IAA-5-^{3}H stock solution before and after incorporation into hot agar (>80°C). Chromatograms were scanned using a Panax radio-chromatogram scanner after chromatography, through 100 mm, using the basic solvent system 45 : 35 : 20 methyl acetate : isopropanol : ammonia (A), the acid solvent system 95 : 5 chloroform : acetic acid (B), or the acid solvent system 5 : 1 : 2.2 butanol : acetic acid : water (C).


stock before incorporation
stock after incorporation
A
B
C
Radioactivity
0
1
0
1
Rf

At the end of the transport period the segments were cut transversely into four equal subsections in the case of 10 mm coleoptile segments, and two equal subsections in the case of 2.4 mm leaf sheath bases. Subsections and receiver blocks were extracted in 95% ethanol and extracts were analysed for radioactivity.

b. Lateral transport in corn coleoptiles

The lateral transport of IAA in horizontally held segments was determined by the method of Goldsmith and Wilkins (1962, 1964). Zea coleoptile segments were supplied at their apical ends with asymmetric sources of IAA-5-3H, and agar blocks, separated by fine glass slivers which slightly penetrated the segments, were placed in contact with the upper and lower halves of the basal ends of the segments (Fig. 5). At the end of a 2-hour transport period the segments were divided into upper and lower halves and tissues and receiver blocks were extracted with 95% ethanol. The extracts were analysed for radioactivity.

c. Lateral transport in wheat leaf sheath bases

Thin radial sections were removed from two opposite faces of excised leaf sheath bases which measured 2.4 mm in length. The segments were placed horizontally and agar blocks were applied to the upper and lower cut surfaces (Fig. 5). At the end of the transport period the segments were divided into upper and lower halves and the tissues and receivers were extracted with 95% ethanol. The extracts were analysed for radioactivity.

d. Segment pretreatment procedures

Segments for use in the morphactin studies were pretreated by submersion for one hour in a solution containing CPM at one of a range of concentrations. Control segments were pretreated in distilled water.

B. Assessment of Radioactivity

A Packard Tricarb Triple A scintillation spectrometer was employed to detect radioactivity in extracts from tissues and receiver blocks. Material for scintillation counting was extracted for 24 hours in 1 ml 95% ethanol at 1°C. After evaporation of the ethanol under vacuum, 10 mls of a scintillation fluid containing 4 gms PPO (2,5-diphenyloxazole; B.D.H., Poole, U.K.) and 200 mgms POPOP ((1,4-D:[2-(5-phenyloxazolyl)]benzene); Koch Light Labs., Bucks, U.K.) per litre of toluene were added to each vial.

The efficiency of scintillation counting is governed by the quench level and this is measured automatically by the Tricarb spectrometer. The quench level is computed by counting a compound external standard (americum 241 and radium 226) of known activity through the sample vial and the value is electronically adjusted to a whole number integer (A.E.S. ratio) between 0 and 10 (0 = complete quench, 10 = no quench). The adjustment is brought about by changing the electron optics between the photo-cathode and the first dynode of the photomultiplier tubes by means of an induced magnetic field. The automatic activity analyser is programmed to convert cpm to dpm simply by providing it with the counting efficiencies at the fixed A.E.S. ratios and these efficiencies may be calculated from the following formula provided the absolute activity (dpm) of the standards is known.

$$\text{Efficiency at a given A.E.S. ratio} = \frac{\text{net cpm for standard}}{\text{known dpm for standard.}}$$

Acetone quenched hexadecane-1,2-^{3}H standards of known specific activity were prepared from stock supplied at sp. activity 2.0 μ Ci/gm by the Radiochemical Centre, Amersham, U.K. and these were used to calculate the counting efficiencies for A.E.S. ratios 2 to 8. The absolute activity analyser was programmed with these efficiencies (see Table 1B) and it was then able to compute absolute activity using the simple equation

$$\text{dpm} = \frac{\text{cpm}}{\text{efficiency.}}$$

Table 1B.

Tritium standardisation for single isotope counting using a Packard Tricarb Triple A scintillation spectrometer.

The table presents counting efficiencies for acetone quenched hexadecane-1,2-3H samples of known specific activity. These efficiencies were used in conjunction with the combined 'Quickset' factory optimised discriminator and gain controls.

Table 1B

A.E.S. Ratio	Efficiency %
0.2	11.3
0.3	17.3
0.4	23.9
0.5	29.3
0.6	36.2
0.7	43.8
0.8	50.6

C. Chromatographic analysis of radioactivity

C. Chromatographic analysis of radioactivity

Material was extracted for 24 hours in absolute methanol in an atmosphere of nitrogen at 1°C and the methanol was then evaporated to low volume under reduced pressure in a vacuum desiccator. The extracts were adjusted, where possible, to contain approximately equal amounts of radioactivity per unit volume (50,000 dpm per 10 µl), and aliquots (10 µl) were applied under nitrogen to the starting lines of Polygram thin layer chromatography plates (Macherey-Nagel & Co., Düren, Germany). Polygram silica G plates (0.25 mm silica G layer) were used with the basic solvent system 45 : 35 : 20 methyl acetate : isopropanol : ammonia, and the acid solvent 95 : 5 chloroform : acetic acid, whilst Polygram cel300 plates (0.1 mm cellulose layer) were used with the acid solvent system 5 : 1 : 2.2 butanol : acetic acid : water. A Panax radiochromatogram scanner was used to detect and record the distribution of radioactivity on the plates.

7. Extraction and determination of growth substances in wheat leaf sheath bases

A. Diffusion experiments. Leaf sheath bases were excised and bisected. Segments were placed with their longitudinal cut surfaces adjacent to agar receiver blocks which were held on glass slides, and the slides were orientated so that the segments constituted either upper or lower halves. After a 24-hour diffusion period the blocks were assayed for auxin- and gibberellin-like activities.

(i) Auxin assay. Blocks were applied apically to vertical Avena coleoptile segments, 10 mm in length, which were excised 1 mm below the apex. The assembled holders were held in damp chambers and the segments were shadowgraphed after 24 hours treatment.

(ii) Gibberellin assay. Lettuce seeds (var. Arctic King) were germinated on damp filter paper. Germinated seedlings were placed on the blocks and hypocotyl lengths were determined after 72 hours.

B. Extraction experiments.

i. Tests for the occurrence of IAA in leaf sheath bases

Stem segments were geotropically stimulated for 12 hours and the leaf sheath bases were then excised and bisected into upper and lower halves for extraction. The extraction procedure is summarised below:-

1. Homogenise in 80% methanol in Waring blender for 1 min.
2. Extract for 24 h in 80% methanol under nitrogen at 1°C.
3. Filter through cheesecloth and then through Whatman No.1 filter paper.
4. Reduce to aqueous phase on rotary evaporator at 35°C.
5. Adjust pH to 3 using 2N hydrochloric acid and partition three times with diethyl ether (3 x 50 ml partitions).
6. Partition pooled ether extract three times with 5% sodium bicarbonate (3 x 50 ml partitions).
7. Backwash pooled sodium bicarbonate fraction with diethyl ether.
8. Acidify pooled sodium bicarbonate fraction with concentrated hydrochloric acid and partition three times with diethyl ether (3 x 50 ml partitions).
9. Concentrate ether fraction to low volume and streak on Whatman No. 1 Chromatography papers.

Chromatograms were developed through 250 mm using either the acid solvent system 5 : 1 : 2.2, butanol : acetic acid : water or the basic solvent system 10 : 1 : 1, isopropanol : ammonia : water. Chromatograms were then dried and divided into 10 equal units for bioassay. Bioassays were conducted with 5 mm Avena coleoptile segments in 1 dram vials

(23.5 mm x 24 mm) which contained 1 ml of solution. Segments were shadowgraphed after 24 hours treatment.

ii. Tests for the occurrence of indoles in leaf sheath bases

A preliminary search for indoles in the leaf sheath base was carried out. Leaf sheath bases were excised and homogenised in 80% methanol in a Waring blender and the homogenates were extracted for 24 hours under nitrogen at 1°C. The extracts were then filtered and reduced to low volume on a 'Buchi' rotary evaporator. The crude aqueous extracts were streaked on to Whatman 3MM Chromatography papers and the papers were chromatographed, through 30 cm, using either the acid solvent system 5 : 1 : 2.2, butanol : acetic acid : water or the basic solvent system 10 : 1 : 1, isopropanol : ammonia : water. Papers were dried and sprayed with Ehrlich's reagent (1 gm p-dimethylaminobenzaldehyde + 50 mls absolute ethanol + 50 mls concentrated hydrochloric acid).

Two Ehrlich positive substances were found in reasonably high yield and these were further investigated by chromatography with known standards. The Ehrlich positive regions were eluted from the chromatogram with methanol and the eluates were rechromatographed with different solvent systems, the purification procedure usually consisted of three chromatographic separations using the acid system followed by the basic system followed by the original acid system. Eluates from the third chromatographic system were co-chromatographed with known standards. One of the eluates was evaporated to dryness and subjected to mass spectroscopy using an A.E.I. MS12 mass spectrometer with direct probe insertion, but this experiment has yet to be repeated.

8. Sugar Extraction and Determinations

A. Extraction

Material was extracted for 24 hours in 80% ethanol at 1°C and the

ethanol was removed under reduced pressure using a 'Buchi' rotary evaporator.

B. Determinations

(i) Reducing sugar determination by the Nelson-Somogyi method (1951)

Extracts were evaporated to dryness and sugars were taken up in warm distilled water. This treatment effectively removed plant pigments without affecting sugar yield. Three 2 ml samples were taken from each extract, and 2 mls of Somogyi's reagent were added to each sample. The mixtures were contained in 6 in. x ½ in. test tubes and glass marbles were placed in the necks of the tubes to prevent losses during the ten-minute boiling period. After cooling 2 mls of Nelson's chromogenic reagent were added and the mixtures were shaken until all copper oxide was dissolved. Each solution was diluted to a final volume of 25 mls and its optical density at 660 nm was determined with a Unicam SP500 series 2 UV and visible spectrometer using the null transmission method. A calibration curve was prepared for an equimolar mixture of glucose and fructose (Fig. 7).

Somogyi's Reagent

Solution I contains 24 gms of anhydrous sodium carbonate (Analar) + 12 gms of potassium sodium tartrate (Analar) + 16 gms of sodium bicarbonate (Analar) + 144 gms of anhydrous sodium sulphate (Analar) dissolved in 800 mls of distilled water and solution II contains 4 gms of copper sulphate ($CuSO_4.5H_2O$ Analar) and 36 gms of anhydrous sodium sulphate (Analar) in 200 mls of distilled water. Four volumes of solution I are mixed with one volume of solution II at the time of usage.

Fig. 7.

Calibration data relating reducing sugar concentration to optical density for the Nelson-Somogyi method.

An equimolar mixture of glucose and fructose was dissolved in distilled water, and a series of dilutions was prepared. These solutions were assayed by the Nelson-Somogyi method, and a calibration graph relating reducing sugar content to OD at 660 nm was prepared.

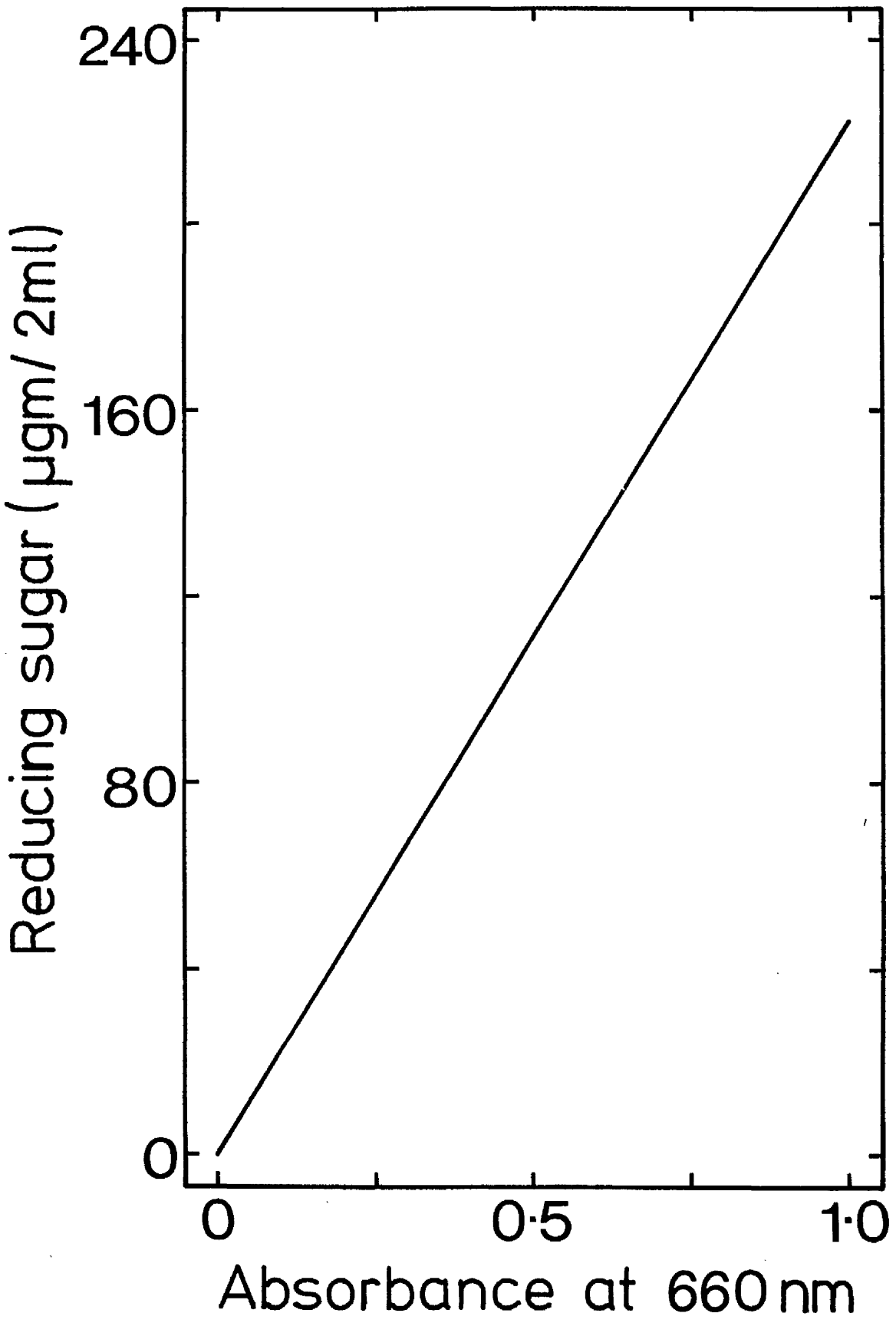
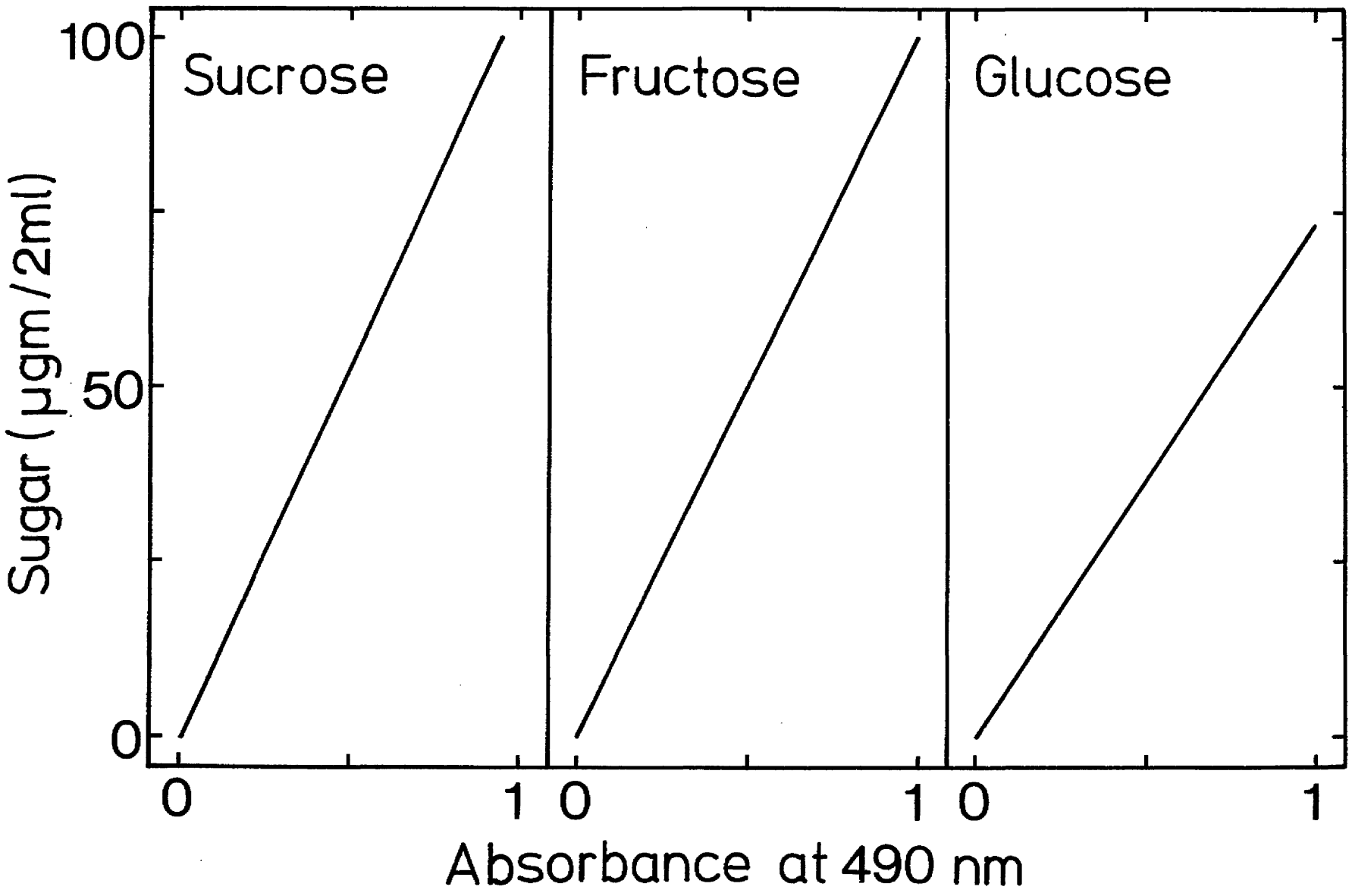

240
160
80
0
Reducing sugar (µgm/2ml)
0
0·5
1·0
Absorbance at 660 nm

Fig. 8.

Calibration data relating sugar concentration to optical density for the phenol : sulphuric acid determination

Glucose, fructose and sucrose solutions were prepared at concentrations of 5 and 10 gm l^{-1}. Aliquots (in units of 10 µl) were applied to the starting lines of Whatman 3MM chromatography papers and dilution series were built up for the three sugars. After chromatography for 36 h using the solvent system 140 : 20 : 40 isopropanol : n-butanol : water, the chromatograms were dried and the sugars were eluted in 10 ml of distilled water. Aliquots (2 ml) were assayed by the phenol : H_2SO_4 method, and calibration graphs relating sugar content to O.D. at 490 nm were prepared.


Sucrose
Fructose
Glucose
100
50
0
Sugar (µgm/2ml)
0
1
0
1
0
1
Absorbance at 490 nm

Nelson's Reagent

The following are united to give 500 mls of reagent.

(1) 25 gms of ammonium molybdate tetrahydrate (Analar) in 450 mls of distilled water.

(2) 21 mls concentrated sulphuric acid (Analar).

(3) 3 gms sodium arsenate (Analar) dissolved in 25 mls of distilled water.

ii. Qualitative Paper Chromatography of mono- and di-saccharides

Extracts were reduced to small volume and streaked on the starting lines of Whatman 3MM Chromatography papers. Papers were developed by downward chromatography with the solvent system 140 : 20 : 40, isopropanol : n-butanol : water, and about 36 hours was allowed for a good separation. The solvent fronts ran off the ends of the chromatograms during this period. Chromatograms were dried, dipped in aniline-diphenylamine reagent, and placed in an oven at 95°C. Colour development was complete in 4-5 minutes.

Aniline-Diphenylamine reagent

Solution I contained 1 ml aniline, 1 gm diphenylamine and 100 mls acetone and solution II consisted of 85% orthophosphoric acid. Ten volumes of solution I were mixed with one volume of solution II immediately prior to usage.

III. Quantitative determination of mono- and di-saccharides

Extracts were chromatographed as in the last subsection but known sugar standards were spotted in lanes to either side of the extract streaks. The lanes were cut from the chromatograms after chromatography, and the marker spots were developed using the aniline-diphenylamine reagent. The sugar containing areas of the extract chromatograms were then identified

from the positions of the marker spots and sucrose, glucose and fructose containing regions were cut from the extract chromatograms. The paper strips were placed in boiling tubes containing 10 mls of distilled water and the tubes were shaken on a Gallenkamp orbital incubator for 15 minutes. The extracts were filtered through sintered glass to remove any paper fibres and 2 ml samples were analysed for sugar content using the method of Dubois et al. (1951). An 80% phenol solution was prepared and 0.14 ml of the lower layer (phenol saturated with water) was added to each 2 ml sample. This was followed by the rapid addition of 5 mls of concentrated H_2SO_4. The samples were mixed thoroughly and left to cool for 30 minutes and their optical densities at 490 nm were determined with a Unicam SP500 series 2 U.V. and visible spectrometer using the null transmission method. The Nelson-Somoygi method was also used as a check for the glucose and fructose determinations.

Calibration graphs were prepared for sucrose, glucose and fructose (Fig. 8).

Standard sugars. Sugars were dissolved in 10% aqueous isopropanol to yield 0.5% solutions for monosaccharides and 0.7% solutions for disaccharides.

9. Invertase Determinations

A. 100 mm stem segments. Leaf sheath bases were bisected into upper and lower halves after a measured period of geotropic stimulation.

B. 2.4 mm leaf sheath base segments. Growth was stimulated either geotropically (orientated as 'lowers') or chemically ('uppers' and IAA or buffer treatments). Segments orientated as 'uppers' in distilled water served as controls.

At the end of the stimulation period the tissues were crushed in a 0.05 M sucrose solution using a 60 mm mortar and pestle. The homogenates

were transferred to boiling tubes together with the washings (the mortar and pestle were washed with 0.05 M sucrose) and the final volume was made up to 10 mls. Tubes were incubated at 25°C on a Gallenkamp orbital incubator and the reaction was terminated after three hours by plunging the tubes into boiling water for 5 minutes. The incubation media were filtered through sintered glass filter funnels and their reducing sugar contents were determined by the Nelson-Somogyi method. Controls omitting either the tissue or the sucrose were also run.

10. Statistical Analyses

A. Replication. The high specific activity of the IAA-5-^{3}H used in the transport experiments permitted transport in individual segments to be investigated and this facility enabled treatments to be replicated ten times in each experiment. Bioassays were conducted with populations of at least 20 segments in each replicate and both bioassays and transport experiments were repeated on at least three separate occasions.

Quick growth studies were carried out on either individual stem segments (curvature) or batches of 5 excised leaf sheath bases (straight growth) and experiments were replicated at least ten times.

B. Standard error. The standard error of the mean value for each series of observations was calculated from the relationship

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{\text{number of observations}}}$$

It was calculated using an Olivetti programma 101 desk top computer from the formula

$$\text{Standard error} = \sqrt{\frac{\Sigma x^2 - \frac{[\Sigma x]^2}{n}}{n(n-1)}}$$

Where n = the number of observations and x = the individual value of each observation. The term $\Sigma x^2 - \frac{[\Sigma x]^2}{n}$ represents the corrected sum of

squares which will be referred to as CS.

Standard errors are shown on the graphs as vertical bars. The bars are usually symmetrical about the points and equal to 2 x SE but where space is limited the bars are applied to one side of the points and represent only 1 x SE.

C. The Student's t test. The t test was used to test the difference between two means.

$$t = \frac{\text{mean difference}}{\text{SE of the difference}}$$

where SE of the difference $= \sqrt{SE_{\bar{x}_1}^{\ 2} + SE_{\bar{x}_2}^{\ 2}}$

$\bar{x}$ = mean value $= \frac{\Sigma x}{n}$

It was calculated using an Olivetti programma 101 desk top computer from the formulae:-

(1) SE of the difference $= \sqrt{\frac{CS_1}{n_1(n_1-1)} + \frac{CS_2}{n_2(n_2-1)}}$

(2) Mean difference $= \bar{x}_1 - \bar{x}_2$

(3) $t = \frac{\text{Mean difference}}{\text{SE of the difference}} = \frac{(2)}{(1)}$

It was not necessary for the number of observations in each population to be equal.

Percentage data. The t test may only be applied to populations which are normally distributed. Percentage values have fixed limits (0% and 100%) and are not therefore normally distributed. To overcome this difficulty statistical analyses were carried out after angular transformation of the percentage data using tables quoted by Fisher and Yates (in 'Statistical Tables' 6th edition. Oliver & Boyd, London pp. 74. 1963). This transformation has the effect of restoring the 'tails' to the distribution.

Significance levels for t values were obtained from tables (Fisher and Yates Statistical Tables pp. 46) and the indices *, ** and *** are used to

denote significant differences at probabilities better than 95%, 99% and 99.9% respectively. Values which are not significantly different even at the 95% probability level show the suffix NS (= not significant).

D. The analysis of variance. The analysis of variance enables the effects of different factors to be compared at the same time. Replicated 4^n and 5^n factorial designs were employed to investigate the effects of pairs of chemical growth regulators on growth. Data were analysed using an Olivetti programma 101 desk top computer and the significance levels for F values were obtained from tables (Fisher and Yates 'Statistical Tables' pp. 49-57). Significance levels are indexed as recorded above for t values.

The computer programme may be tabulated:-

Stage 1. Calculate : Mean of replicates ij $= \frac{1}{r} Sij$

Mean of rows i $= \frac{1}{mr} SRi$

Mean of columns j $= \frac{1}{nr} SCj$

Grand mean $= \frac{1}{nmr} \Sigma x$

Correction factor for sum of squares $= \frac{1}{nmr} (\Sigma x)^2$

where r = number of replicates, m = number of columns (factor 1) and n = number of rows (factor 2). The correction factor for the sum of squares will be denoted CF.

Stage 2.

(1) Calculate total sum of squares, TSS $= \Sigma x^2 - CF$.

(2) Calculate Error sum of squares, ESS $= \Sigma x^2 - \frac{1}{r} \Sigma s_{ij}^2$ and error mean square, EMS $= \frac{ESS}{nm(r-1)}$

(3) Calculate Row sum of squares, RSS $= \frac{1}{mr} \Sigma s_R^2 - CF$ and row mean square RMS $= \frac{RSS}{n-1}$

(4) Calculate Column sum of squares, CSS $= \frac{1}{nr} \Sigma s_C^2 - CF$ and column mean square CMS $= \frac{CSS}{m-1}$

(5) Calculate Interaction sum of squares, $ISS = \frac{1}{n} \Sigma S_{ij}^2 - RSS - CSS - CF$ and interaction mean square $\underline{IMS} = \frac{ISS}{(n-1)(m-1)}$

The divisors used in the mean square calculations in Stages (2), (3), (4) and (5) represent the degrees of freedom for error, rows, columns and interaction respectively.

(6) Calculate F values for rows, columns and interaction by dividing respective mean square values by the error mean square.

$$F \text{ rows} = \frac{RMS}{EMS}$$

$$F \text{ columns} = \frac{CMS}{EMS}$$

$$F \text{ interaction} = \frac{IMS}{EMS}$$

RESULTS

1. The nature of the response

The first experiments were designed to establish the nature of the geotropic response in the wheat culm. The 100 mm stem segments used in the curvature experiments are shown before and after a 72-h period of geotropic stimulation in Plates 1A and B respectively, and a comparison between the plates reveals two distinct regions of growth. Growth in the internode is apparent in both vertical and horizontal preparations whereas growth in the leaf sheath base is only observed during geotropic stimulation. The vertical leaf sheath bases are not growing organs (Fig. 9 curve b) but growth is induced on the lower sides when the organs are displaced and the geotropic response proceeds for 4 to 5 days (Fig. 9 curve a). Growth is not induced in the upper half of the organ where a certain amount of shrinkage may occur as the tissue becomes compressed by growth in the lower regions (Fig. 9 curve c). These findings indicate that the geotropic response in the wheat culm is connected with the differential induction of growth in the leaf sheath base.

The growth of the lower half of the leaf sheath bases is accompanied by a rapid increase in fresh weight and, whilst the increase is due mainly to water uptake (Fig. 10 curve b), there is also a slow increase in dry weight (curve a). The changes in dry weight are considered in terms of % fresh weight in Fig. 11. The percentage dry matter in the lower half of the leaf sheath base declines with the onset of curvature and, although the dry matter content shows a subsequent increase, the percentage dry matter fails to reach the control value for the upper half of the organ during the 6-day experimental period. These data suggest that growth results initially from cell expansion with consolidation following as a secondary process.

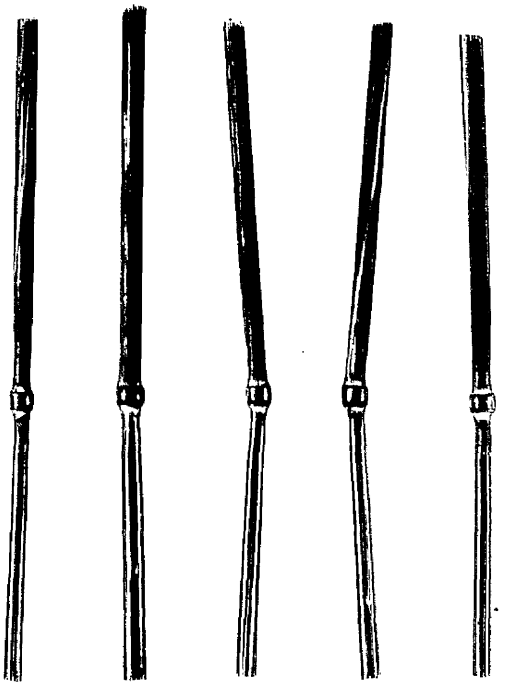
Plate 1.

Triticum aestivum L. var. Kolibri.

The geotropic response in stem segments.

Stem segments of the type used in the curvature experiments are shown before (A) and after (B) a 72-h period of geotropic stimulation.

A



B

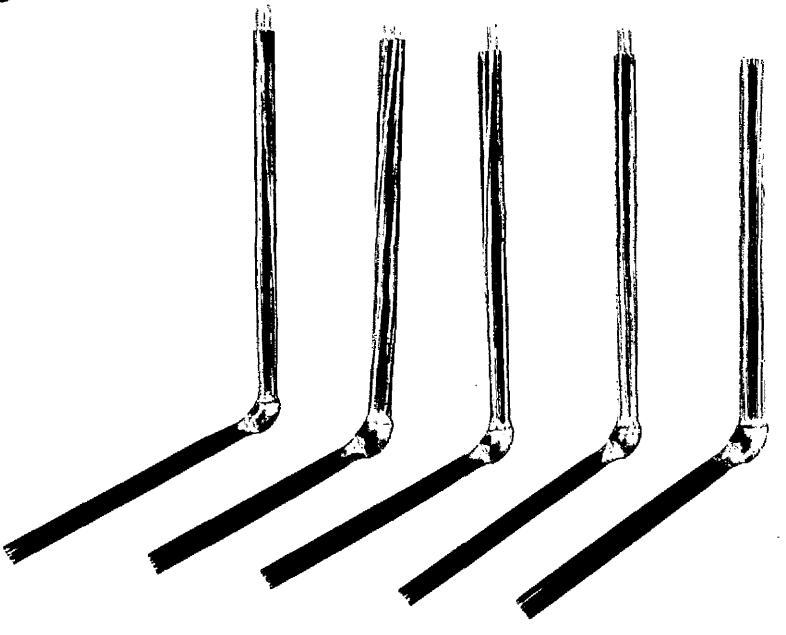


Fig. 9.

Triticum aestivum var. Kolibri.

The development of curvature with time.

Treatment: Batches of 25 stem segments 100 mm in length were pinned either horizontally or vertically. The increases in the lengths of the upper (——^{c}) and lower (– – ^{a}) faces of the horizontal leaf sheath bases and the vertical faces of the vertical leaf sheath bases (·····^{b}) were measured at 24-h intervals. The curvatures developed in the horizontal segments were also measured at 24-h intervals (————).

White light 25°C.

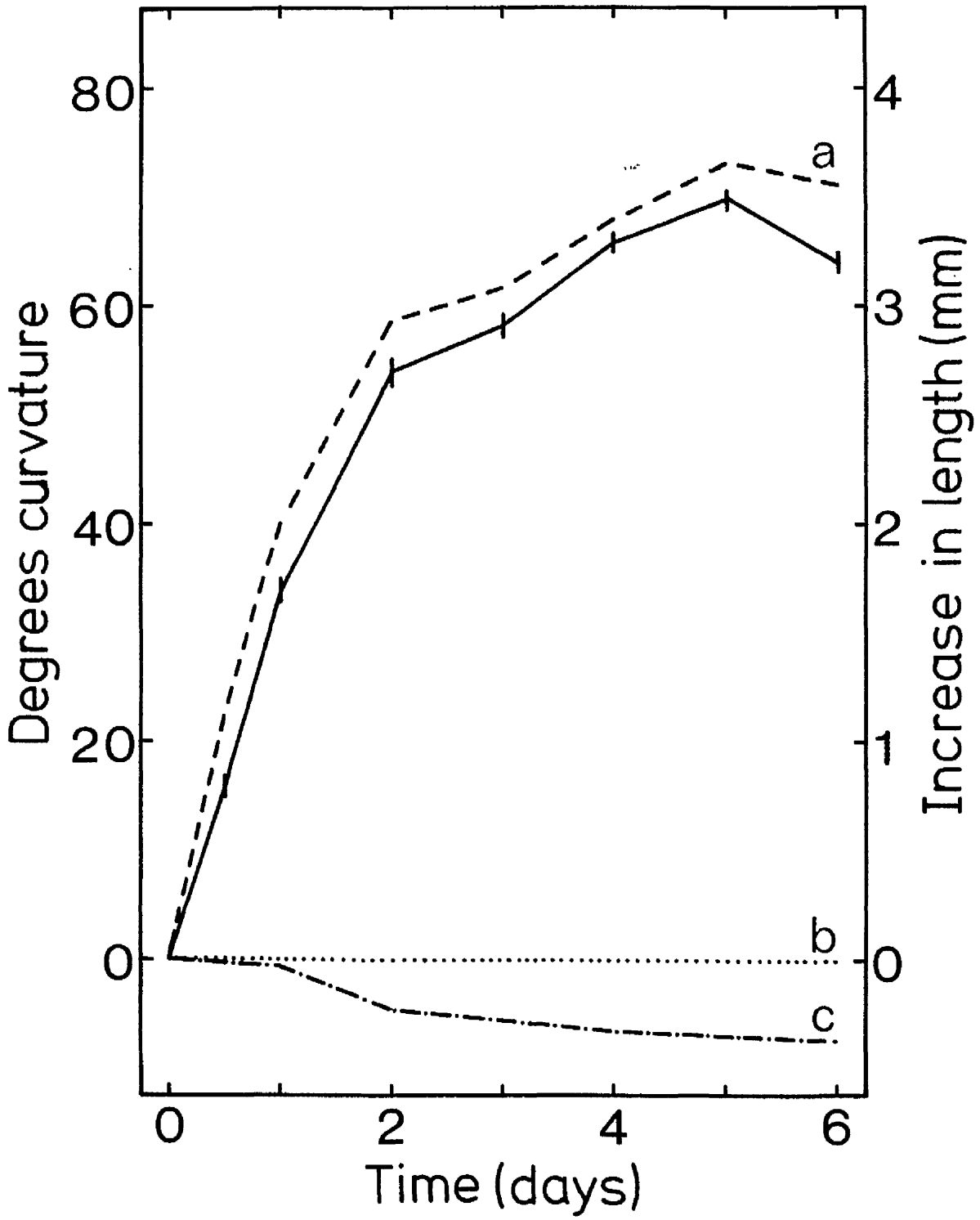

80
60
40
20
0
4
3
2
1
0
a
b
c
0
2
4
6
Time (days)
Degrees curvature
Increase in length (mm)

Fig. 10.

Triticum aestivum var. Kolibri.

Changes in the weights of the upper and lower halves of the leaf sheath base with time during geotropic stimulation.

Treatment: Stem segments 100 mm in length were pinned horizontally and the fresh and dry weights of the upper (U) and lower (L) halves of batches of 25 leaf sheath bases were determined at 24-h intervals. The differences (L - U) in fresh weight (———[a]), dry weight (– – [c]), and water content (·····[b]) were calculated.

White light 25°C.

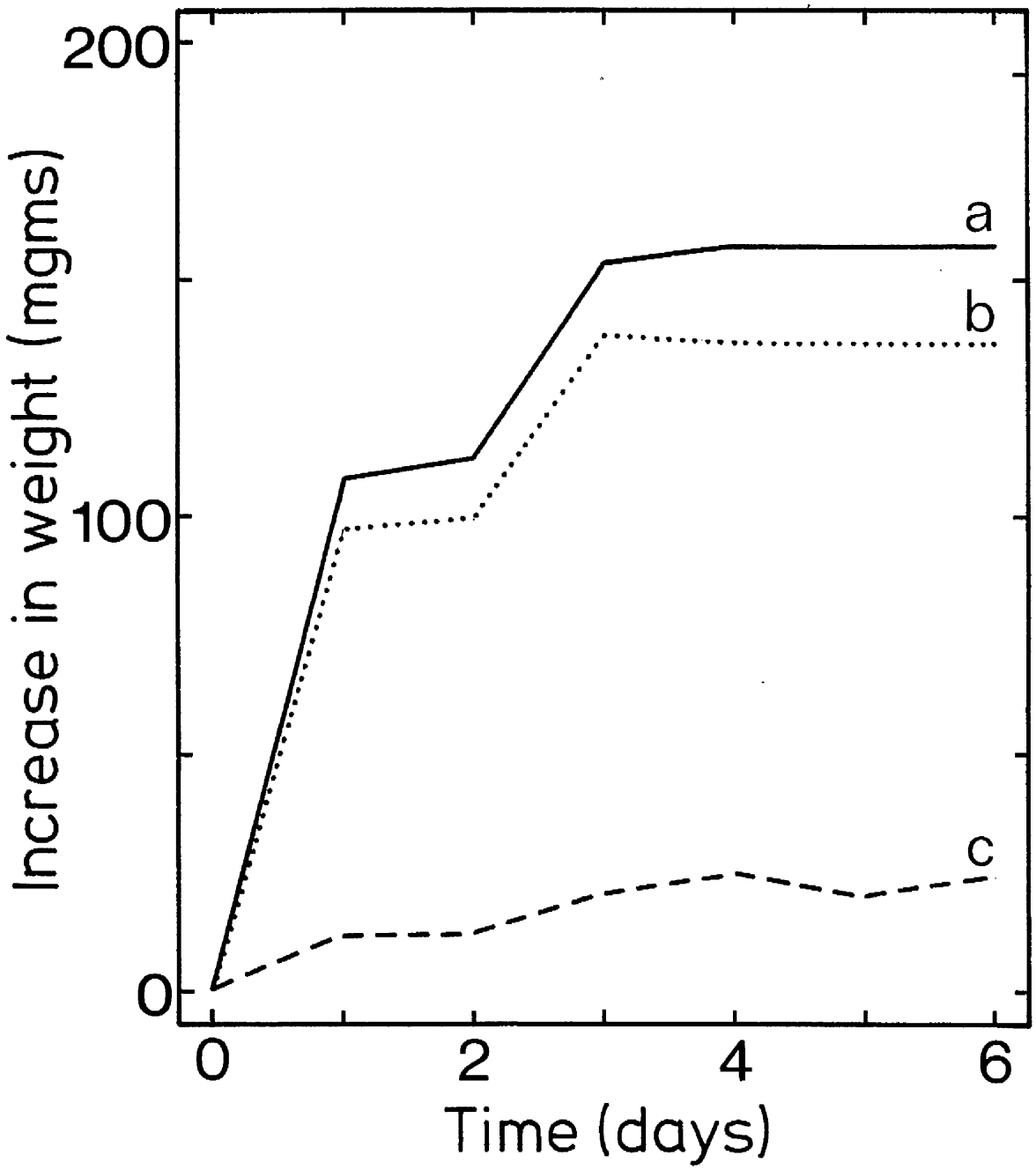

200
100
0
Increase in weight (mgms)
a
b
c
0
2
4
6
Time (days)

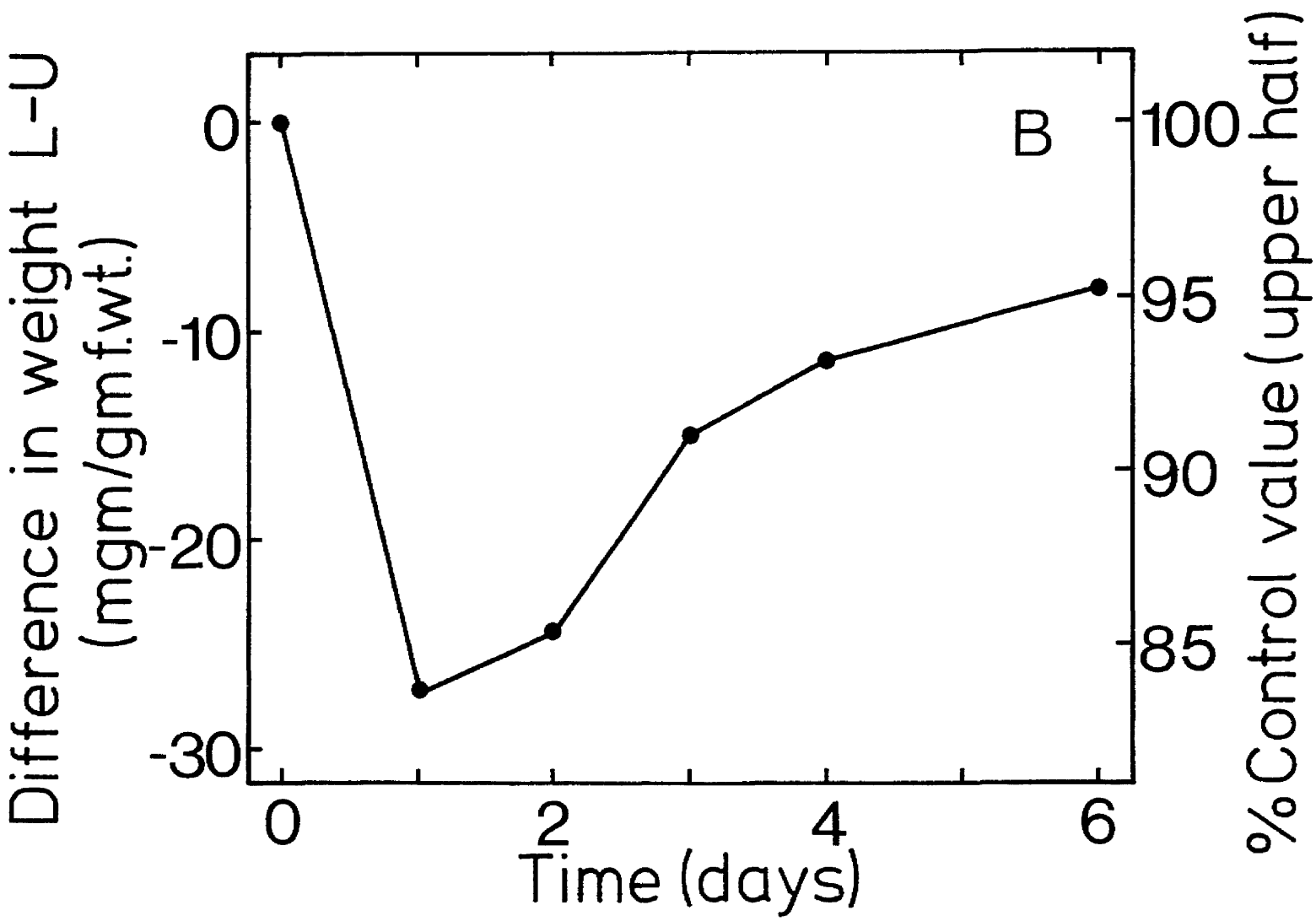
Fig. 11.

Triticum aestivum var. Kolibri.

Changes in dry weight during periods of geotropic stimulation.

Treatment: Stem segments were pinned horizontally and batches of 25 were sampled at 24-h intervals. Leaf sheath bases were excised and bisected, and the fresh and dry weights of upper and lower halves determined. The differences in dry weight per gram fresh weight between lower and upper halves were calculated and plotted against time.

White light 25°C.


Difference in weight L-U
(mgm/gm f.wt.)
0
-10
-20
-30
B
100
95
90
85
% Control value (upper half)
0
2
4
6
Time (days)

2. Co-ordination of the response

The typical stem contains four leaf sheath bases, and the question arises as to whether a correlation in growth activity exists between these growth centres. The curvatures developed at the morphologically apical and basal nodes of two-node preparations which are held above the apical node, between apical and basal nodes or below the basal node, are shown in Figs. 12A and B respectively. When the curvatures developed at the two nodes during the four-day stimulation period are summed, the combined curvatures are found to be 90.3 $\pm$ 1.09° for base held preparations, 106 $\pm$ 2.7° for tip held preparations and 117 $\pm$ 2.8° for centrally held preparations. The value obtained for the tip held preparation is greater than that obtained for the base held preparation ($t = 5.29^{***}$) and the value obtained for the centrally held preparation is greater than those obtained for both base held and tip held preparations ($t = 8.48^{***}$ and 2.88^{***} respectively). These experiments provide unequivocal evidence for an interaction between nodes, but the concept of chemical co-ordination is dispelled by the demonstration that living continuity may be broken, by cutting through the internode and inserting a wooden splint into the hollow stem, without affecting the curvatures developed at the two nodes (compare lines ——— and —·—·— in both Fig. 12A and Fig. 12B). Scrutiny of the data presented in Fig. 12 shows that it is curvature at the physically upper node, rather than the morphologically apical or basal node, which is most reduced by the treatment. Curvature at the morphologically apical node is greatest in the tip held preparation and least in the base held preparation, whilst the reverse is true for the morphologically basal node. As the physically upper node is lifted away from the horizontal, the proportion of the net response which it carries out is progressively reduced. Although this behaviour cannot be explained in terms of chemical co-ordination it can be correlated with the angles at which the nodes are exposed to gravity, and it may therefore be possible to explain the observed behaviour

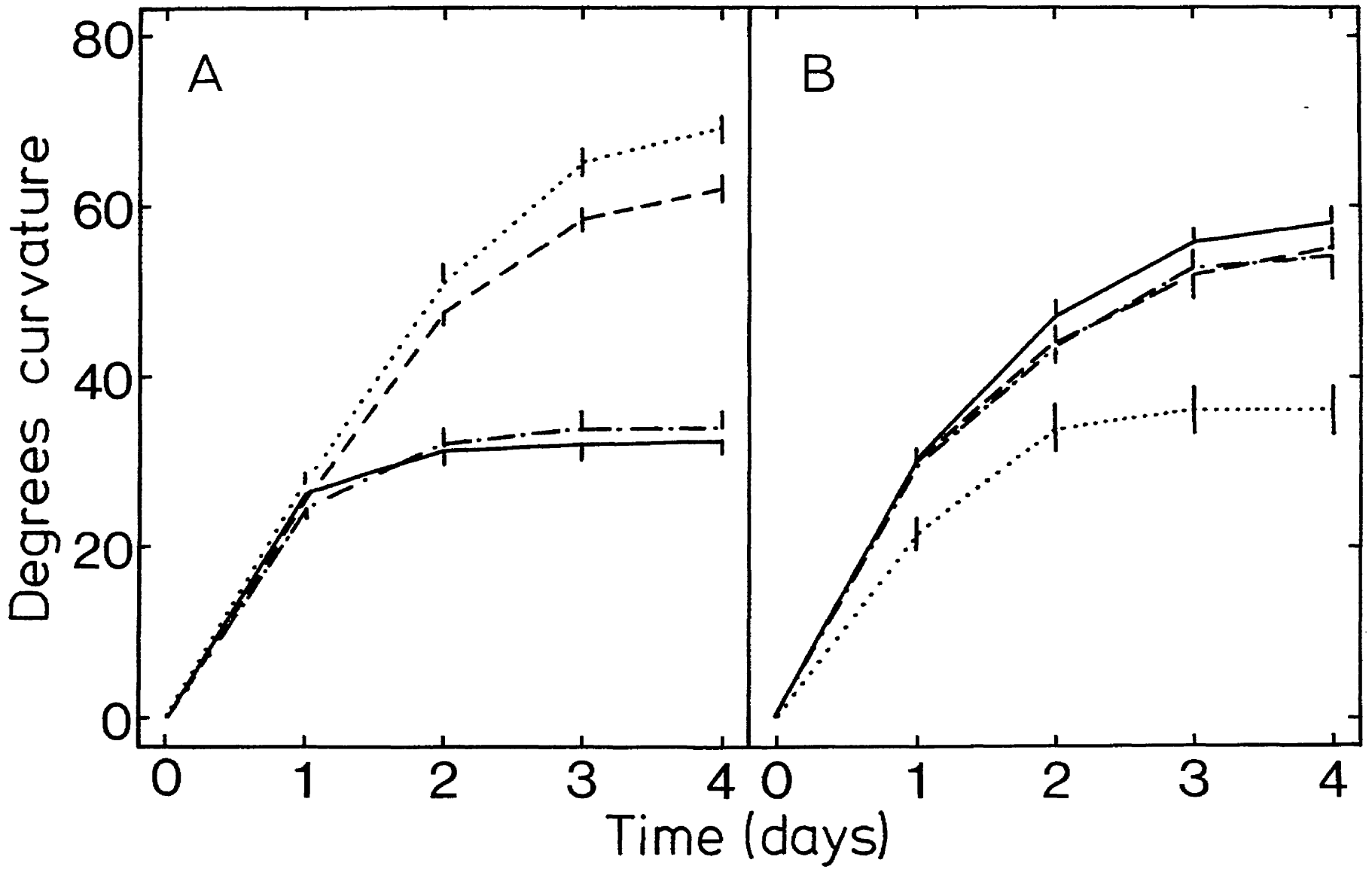
Fig. 12.

Triticum aestivum L. var. Kolibri.

The independence of the responses at each node.

Treatment: Segments containing the apical two nodes with 50 mm lengths of internode below the basal node and above the apical node, were pinned above the morphologically apical node (tip held preparation ·····), between the apical and basal nodes (– – – –) or below the morphologically basal node (base held preparation (————). In some base held preparations living continuity between nodes was broken by cutting through the central internodal region and inserting a wooden splint (–·–·–). Curvatures at the morphologically apical (A) and basal (B) nodes were measured at 24-h intervals.

White light 25°C.


A
B
Degrees curvature
80
60
40
20
0
0
1
2
3
4
Time (days)

in terms of gravi-perception if perception, and hence response, can be shown to bear some proportionality to the angle at which the organ is displaced from vertical.

Geotropic responses in the growing apices of higher plants can be divided into three major steps: the perception of the stimulus, the transmission of the information to the site of the response, and the response. The transmission step involves the movement of chemical 'messengers' and the involvement of these messengers can be studied by means of barrier experiments. The effects of the insertion of barriers into the leaf sheath base are shown in Fig. 13. Barriers which are inserted to bisect the leaf sheath base horizontally are no more effective in reducing curvature than are barriers which bisect vertically, but both treatments reduce curvature to about 85% of the value for the intact control. The stem is physically bent by the growth of the lower side of the leaf sheath base, and the pressure exerted by the leaf sheath base is reduced following bisection, when some of the effort is lost in parting the cut surfaces around the barrier. Measurements of the lengths of the upper and lower surfaces of the leaf sheath bases are not subjected to this limitation and measurements of these parameters remain unaffected by the barrier treatments.

If geotropic curvature is indeed independent of the transport of chemical growth regulators it ought to be possible to induce the response in segments excised from the leaf sheath base. This possibility is examined in Fig. 14. Growth is induced when the segment is laid with its outer epidermis facing downwards (lowers) but not when the outer epidermis is orientated facing upwards (uppers). Thus the induction of the response at the cellular level appears to be determined solely by the orientation of the outer tangential wall with respect to the gravity vector. A comparison between histograms 1 and 3 in Fig. 14 shows that the magnitude of the response is reduced to 50% of the control value when segments are excised and orientated as 'lowers' in distilled water, but this reduction cannot

Fig. 13.

Triticum aestivum var. Kolibri.

The requirement for tissue integrity at the leaf sheath base.

Treatment: Stem segments 100 mm in length were pinned horizontally after one of the following pretreatments.

a. Insertion of a polythene barrier 4 mm in length to bisect the leaf sheath base into upper and lower halves.

b. Insertion of a polythene barrier 4 mm in length to bisect the leaf sheath base into left and right halves.

c. No barrier insertion.

The lengths of the upper (U) and lower (L) faces of the leaf sheath bases (Fig. B) and the curvatures developed in the segments (Fig. A) were determined after a 24-h treatment period.

White light 25°C.

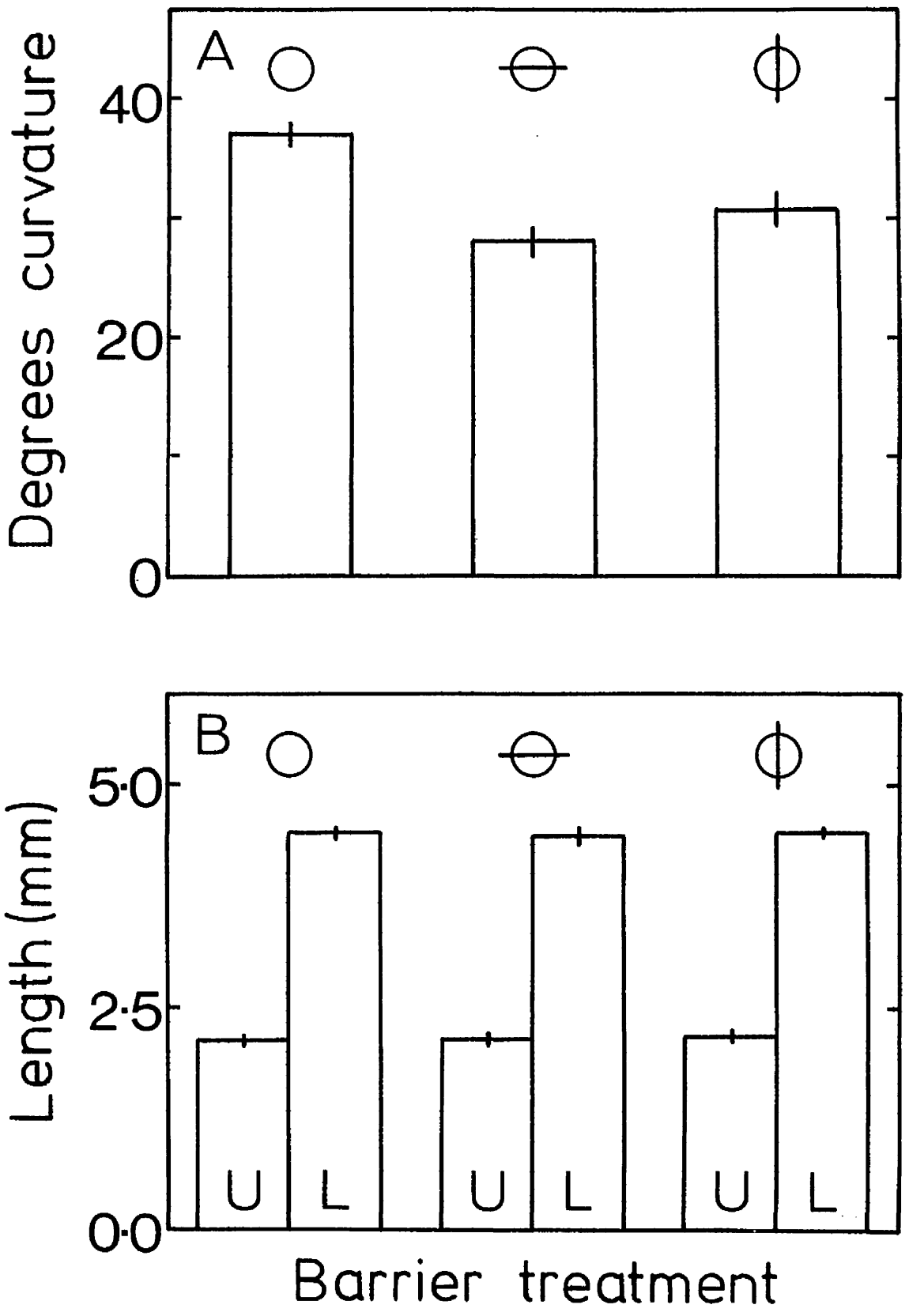

A
Degrees curvature
40
20
0
B
Length (mm)
5·0
2·5
0·0
U
L
U
L
U
L
Barrier treatment

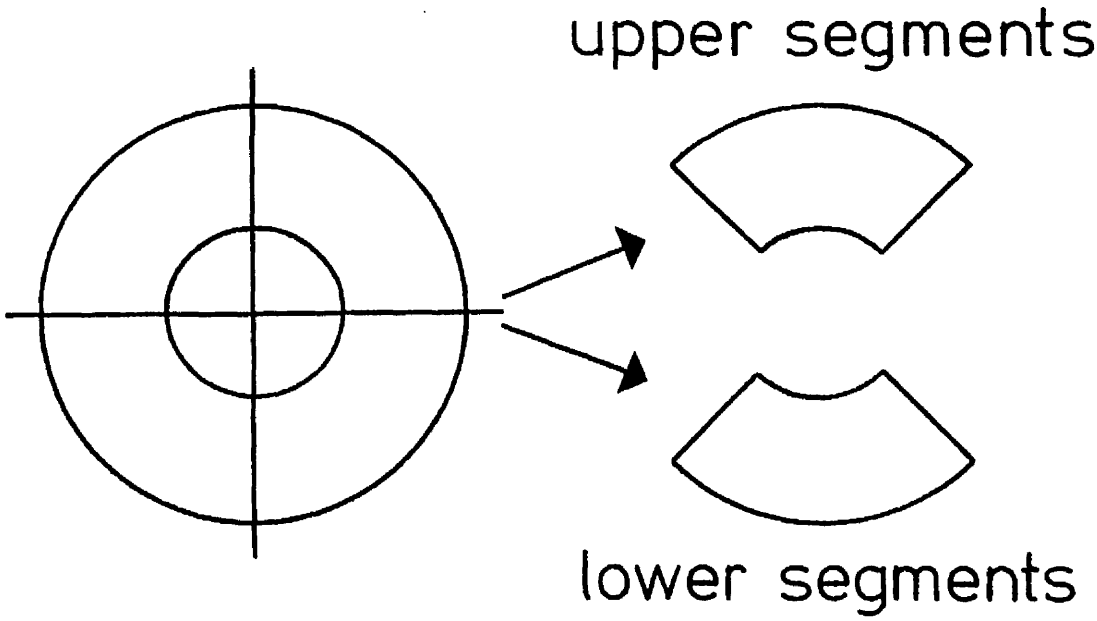
Fig. 14.

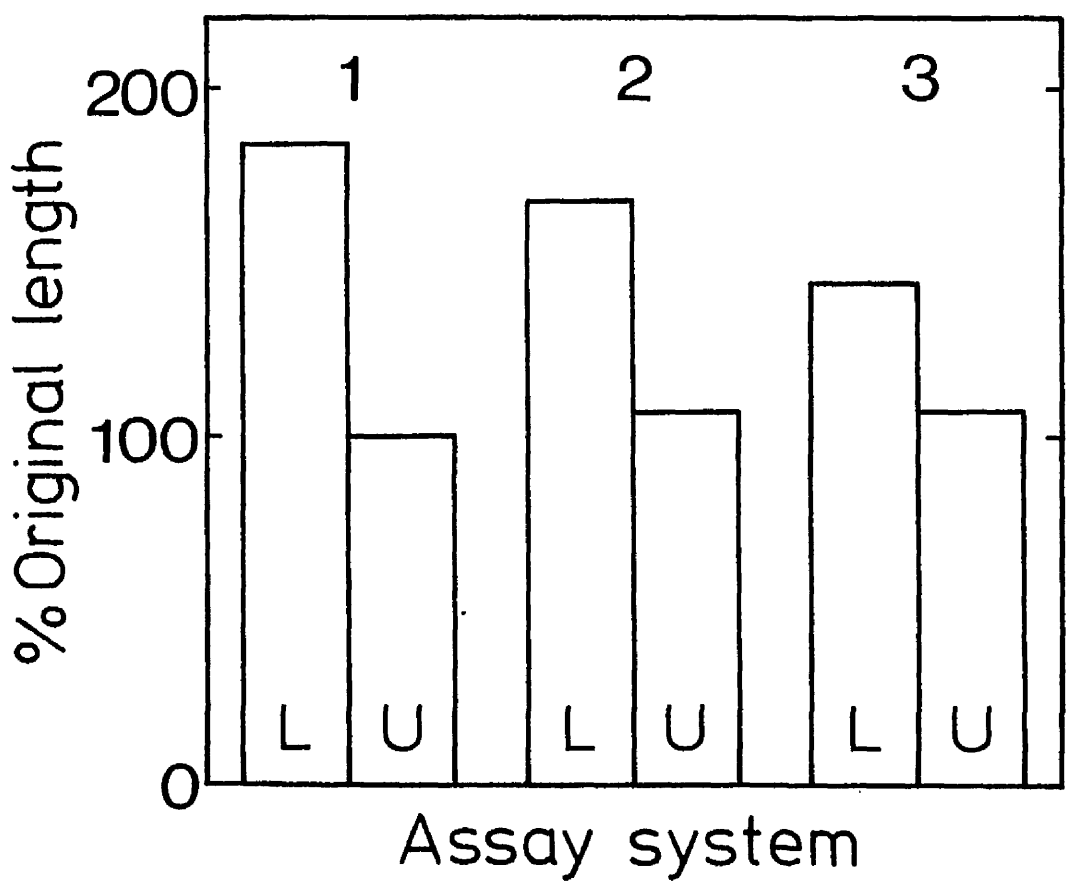
Triticum aestivum var. Kolibri.

The growth of segments excised from the leaf sheath base.

Treatment: Portions of the leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (U) or 'lowers' (L) in 50 mm petri dishes containing 2.5 ml of either 2% sucrose (2) or distilled water (3). Segments were shadowgraphed after 24-h treatment and their growth was compared with that measured on the upper and lower faces of the intact organ following a 24-h period of geotropic stimulation (1).

White light 25°C.


upper segments
lower segments


200
100
0
1
2
3
L
U
L
U
L
U
% Original length
Assay system

represent a decline in the capacity for gravi-perception because the magnitude of the response is increased to approach control values when 2% sucrose is added to the incubation medium. A very small increase in length is often observed in segments orientated as uppers, and the magnitude of this response is usually of the order of 0.1 mm or about 4% of the initial length of the segment. The response is not affected by the 2% sucrose treatment and probably represents water uptake to satisfy diffusion pressure deficits in the material at the time of sampling.

3. The effect of orientation on the development of the response

The data presented in Fig. 12 provide strong evidence for growth correlation between nodes. The base held two node preparations produce a net curvature of 90°, but the capacity for curvature is clearly greater because centrally held two node preparations, in which one end of each leaf sheath base remains horizontal, are able to produce a net curvature of 117° during the four-day stimulation period. The restriction of curvature in the base held two node preparations is brought about by the premature termination of the response at the physically upper node, and this can be seen clearly from a comparison between the responses at equivalent nodes shown in Figs. 12A and B. The restriction cannot be explained in terms of a chemical co-ordination system, but physical co-ordination determined solely by the quantity of stimulus perceived at the individual leaf sheath bases could provide a theoretical explanation. Before considering the effect of orientation on the development of the response it is necessary to obtain some idea of the distribution of responsive tissues in the leaf sheath base, because any regionalisation of these tissues may also be expected to exert a controlling influence on the response.

Growth in the apical, central and basal regions of the lower sides of tip held and base held leaf sheath bases is shown in Fig. 15. Growth occurs throughout the leaf sheath base, but the response at a point is again determined by the orientation at that point. Essentially similar amounts of growth occur in the apical, central and basal regions of the base held leaf sheath base, but the data presented in Fig. 15B show that growth in the apical and central regions increases whilst that in the basal region decreases when the organ is tip held, and the organ must therefore be considered to have a greater overall capacity for growth in its apical regions.

The observation that all regions of the leaf sheath base are capable of response to geotropic stimulation cannot be taken as evidence for the distribution of the gravi-perception mechanism throughout the organ, because

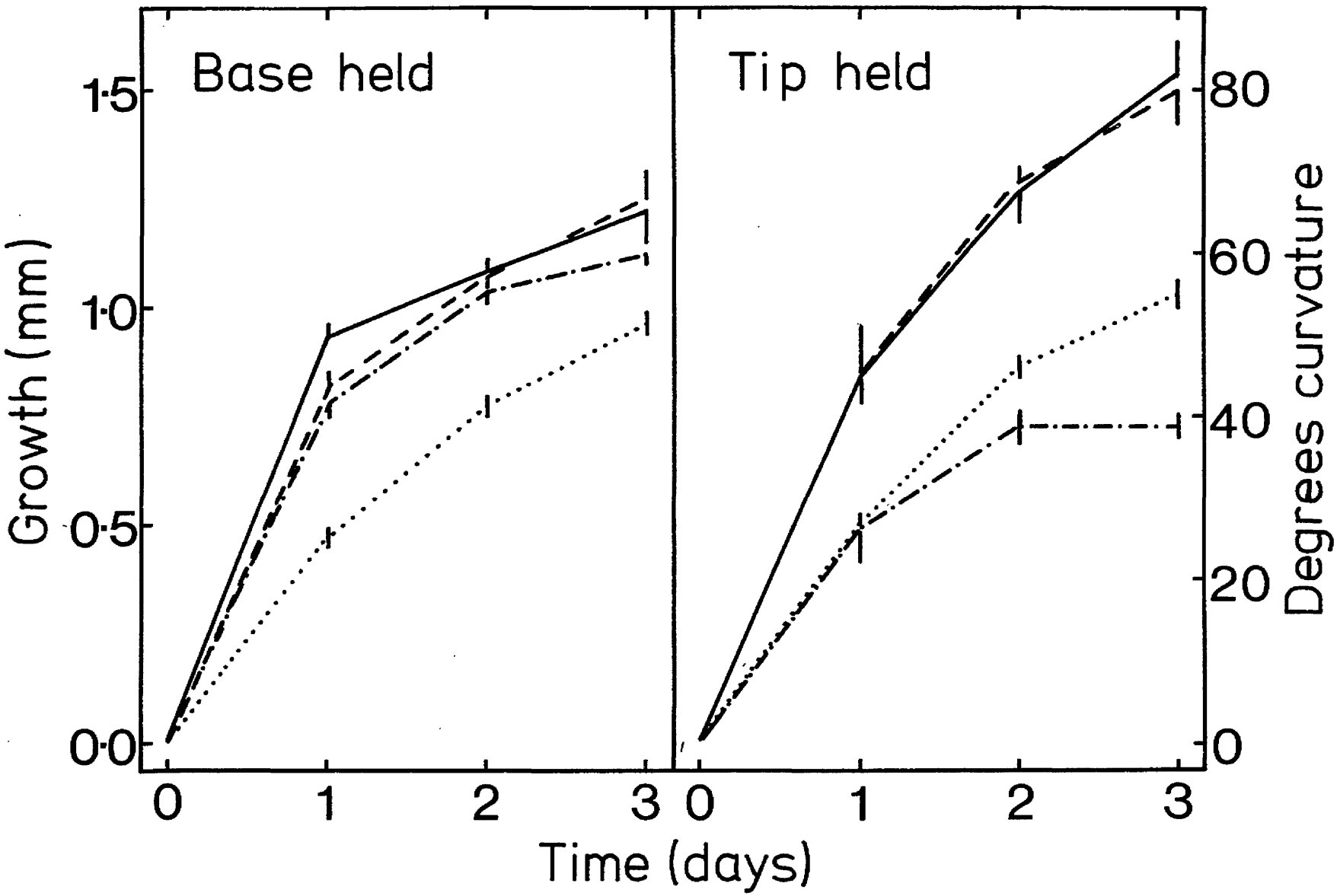
Fig. 15.

Triticum aestivum L. var. Kolibri.

The region of growth in the lower half of the leaf sheath base.

Treatment: One node preparations 100 mm in length were prepared from stems which contained an apical leaf sheath base measuring 3 mm in length. Indian ink dots were arranged at 1 mm intervals up one side of the leaf sheath bases and the stem segments were pinned horizontally either basally (base held) or apically (tip held) with the ink dots lowermost. The distances between the ink dots were measured at 24-h intervals and the growth of the apical (———), central (— — —) and basal (—·—·—) 1 mm regions were calculated. The curvatures developed in the segments were also recorded (·····).

White light 25°C.


Base held
Tip held
Growth (mm)
Degrees curvature
Time (days)
0·0
0·5
1·0
1·5
0
20
40
60
80
0
1
2
3

information may be longitudinally transported to various regions, but the observation that segments excised from different regions of the leaf sheath base retain the ability to grow when stimulated may be taken as evidence in this respect. A further impression of the distribution of the gravi-perception mechanism can be obtained from a consideration of the effect of stem orientation on the development of curvature in the leaf sheath base, and such a study can also be used to provide a critical assessment of the hypothesis that the responses developed at neighbouring nodes are controlled individually by a process dependent solely on the orientation of each growth centre.

The effects of stem orientation on the curvatures developed in base held and tip held stem segments are shown, following periods of 24-h and 48-h continuous geotropic stimulation in Figs. 16A and B respectively. The magnitude of the response in base held segments shows a steady increase with increasing displacement from vertical, and this finding may be taken as evidence in favour of a co-ordination system based on the quantity of stimulus received at each growth centre. The observation that curvature is maximal at 120° displacement from vertical, and not 90° displacement as might be anticipated if the response was related to the sine of the angle of displacement, may be explained in terms of the quantity of stimulus received during the prolonged stimulation period. The apex of a base held segment fixed at a deviation of 120° [redacted] from vertical will bend up past the 90° displacement mark towards vertical, and the apical regions of the leaf sheath base will be subjected to maximum stimulation for a longer period than would be the case for an initial displacement of 90°. If perception is confined to the apical regions of the leaf sheath base, then tip held segments subjected to continuous stimulation ought to receive stimulation at a constant level determined only by the initial deviation of the stem segments from vertical. As seen in Fig. 17 this reasoning appears to hold for the Avena coleoptile in which the perception mechanism is known to be mainly confined to the extreme apex. The curvatures developed in base held Avena coleoptile

Fig. 16.

Triticum aestivum L. var. Kolibri.

The effect of orientation on the development of the response.

Treatment: Stem segments 100 mm in length were pinned either apically (tip held) or basally (base held) at an angle ranging from 0° to 180° displacement from vertical. The curvatures developed in base held (—·—·—) and tip held (- - - -) segments were measured after 24 h (Fig. A) and 48 h (Fig. B) continuous geotropic stimulation. The values are compared with theoretical values calculated assuming a direct proportionality between response and the sine of the initial angle of displacement (═══).

White light 25°C.

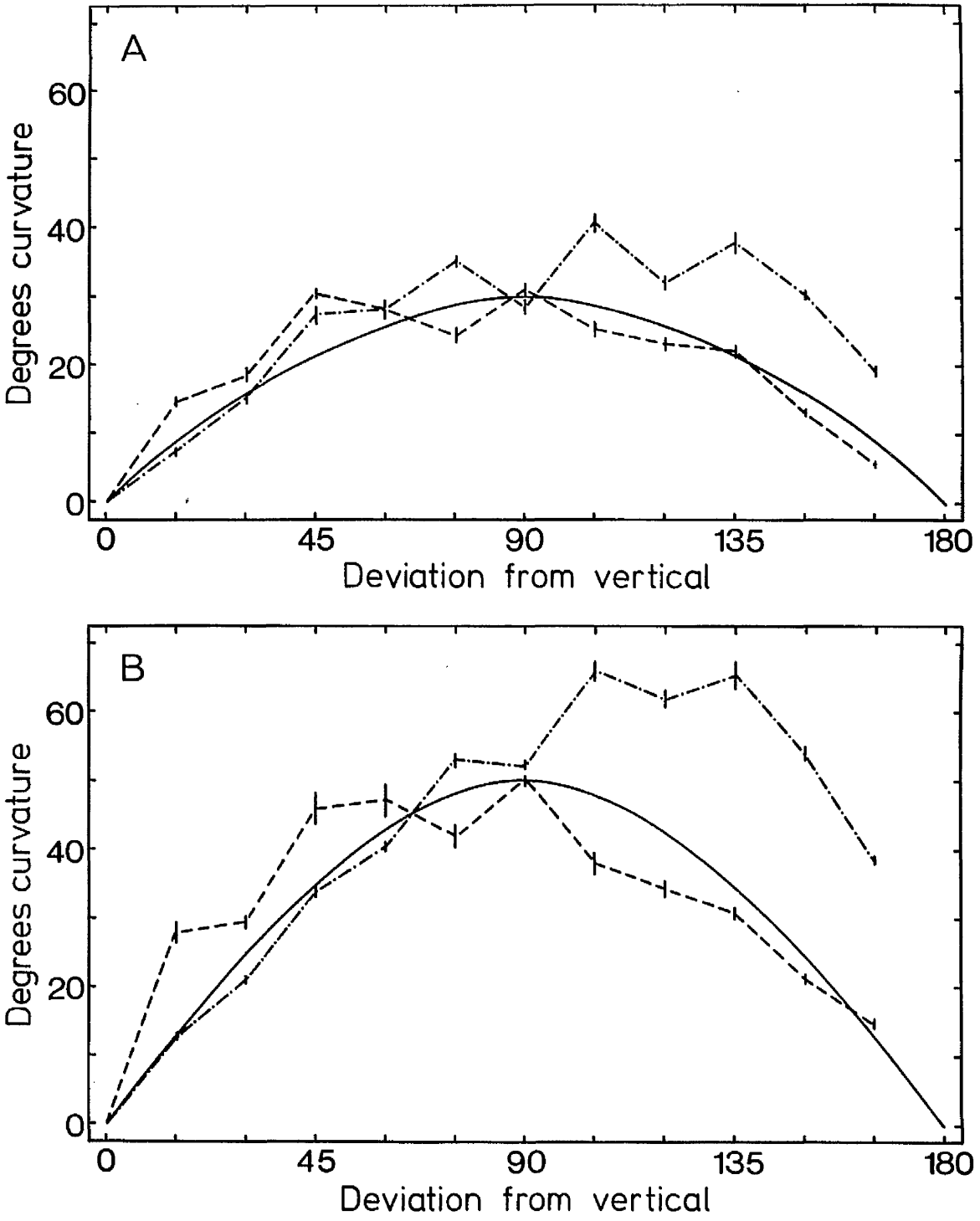

A
Degrees curvature
60
40
20
0
0
45
90
135
180
Deviation from vertical
B
Degrees curvature
60
40
20
0
0
45
90
135
180
Deviation from vertical

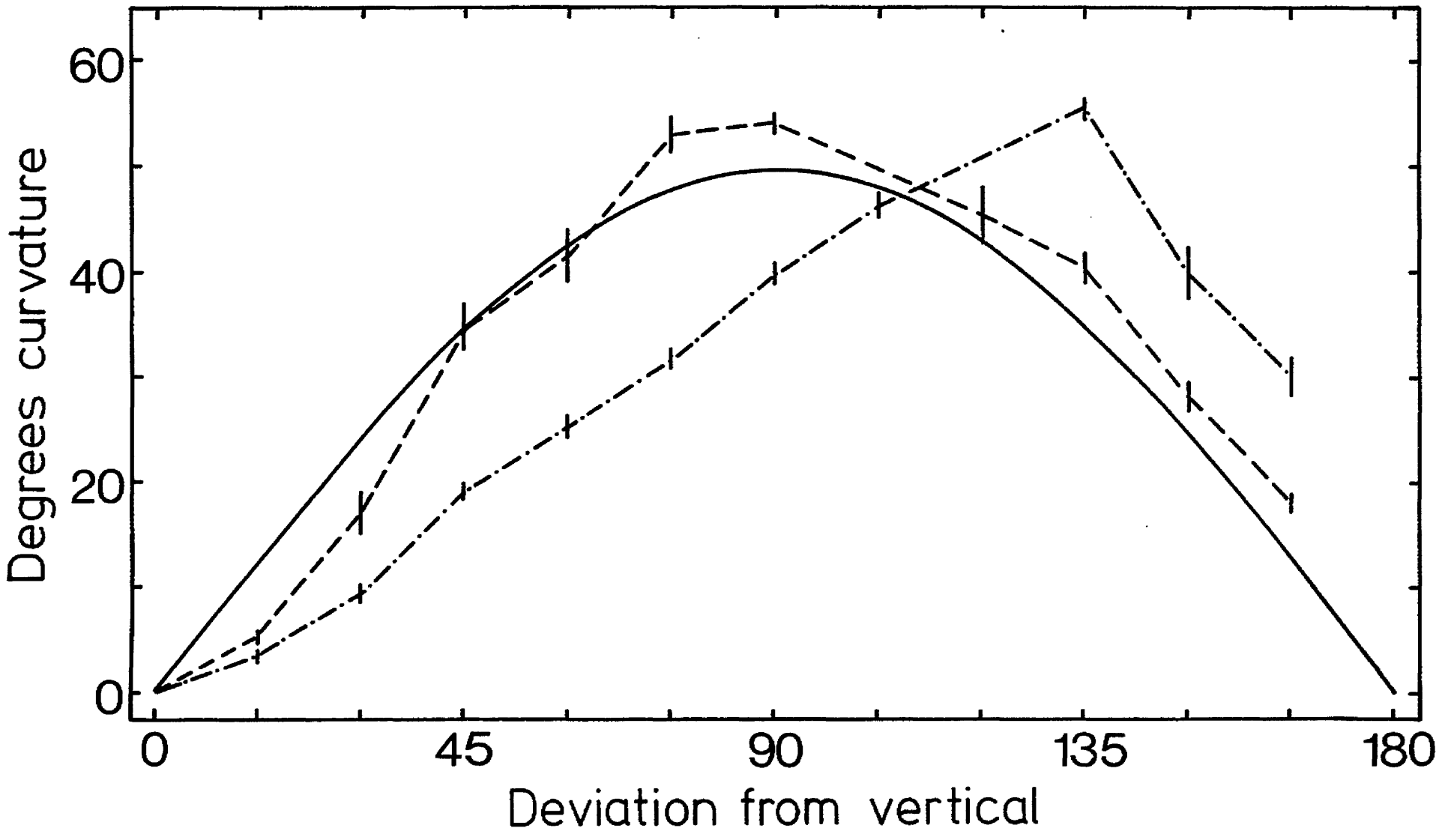
Fig. 17.

Avena sativa L. var. Svalov Victory.

The effect of orientation on the development of the response in Avena coleoptiles.

Treatment: Coleoptile segments 15 mm in length were held either apically (tip held) or basally (base held) in perspex frames fixed at angles ranging from 0 to 180° displacement from vertical. The curvatures developed in base held (—·—·—) and tip held (— — —) segments were measured after a 4-h period of continuous geotropic stimulation. The values are compared with theoretical values calculated assuming a direct proportionality between response and the sine of the angle of displacement (———).

Darkness 25°C.


60
40
20
0
Degrees curvature
0
45
90
135
180
Deviation from vertical

segments and base held wheat stem segments are similar and show response maxima in the region 120-135° displacement from vertical, but the response developed in tip held Avena coleoptile segments is maximal at 90° displacement from vertical and shows a pattern of development proportional to the sine of the angle of displacement. The response in the tip held wheat stem segments is not proportional to the sine of the angle of displacement, but curvatures are greatest at angles of less than 90° displacement from vertical. The response in the tip held segment is an endeavour to bend the apex up to vertical, but because this region is fixed the effect is to bring the basal region of the stem up past the 90° displacement mark towards vertical. The arc to be described during the response in a tip held segment fixed at 45° displacement from vertical is therefore the same as the one to be described by a base held segment fixed at 135° displacement from vertical. If all regions of the leaf sheath base are equally sensitive to gravity, the deviation from the sine distribution observed in the base held segments at angles greater than 90° displacement ought to be replaced in tip held segments by a similar deviation at angles less than 90° displacement, and the observation that the optimum displacement for curvature shifts from angles greater than 90° for base held segments to angles less than 90° for tip held segments may be taken as evidence in this respect. The fact that the asymmetry is greatest for base held segments cannot be taken as evidence for a regionalised concentration of the perception apparatus because an apical concentration of either perceptive or reactive capacities in the manner shown in the diagrammatic representation in Fig. 18A will explain the experimental results. The difference in magnitude between the two asymmetries may of course be explained in terms of a genuine deviation from the sine rule, but it is not possible to test for such a discrepancy using experiments which involve continuous stimulation because the angle of stimulation is changing constantly with the development of curvature. The observation that similar curvatures develop in tip held and base held

preparations when these are laid horizontally (see dotted lines in Figs. 15A and B) may be taken as evidence in favour of the operation of the sine rule because, although the arcs to be described during curvature are equivalent, the regions of the statocytes to be stimulated are opposite (see diagrammatic representation in Fig. 16B).

The relationship between the response and the sine of the angle of displacement will receive further attention in the section dealing with the gravi-perception process.

Fig. 18A.

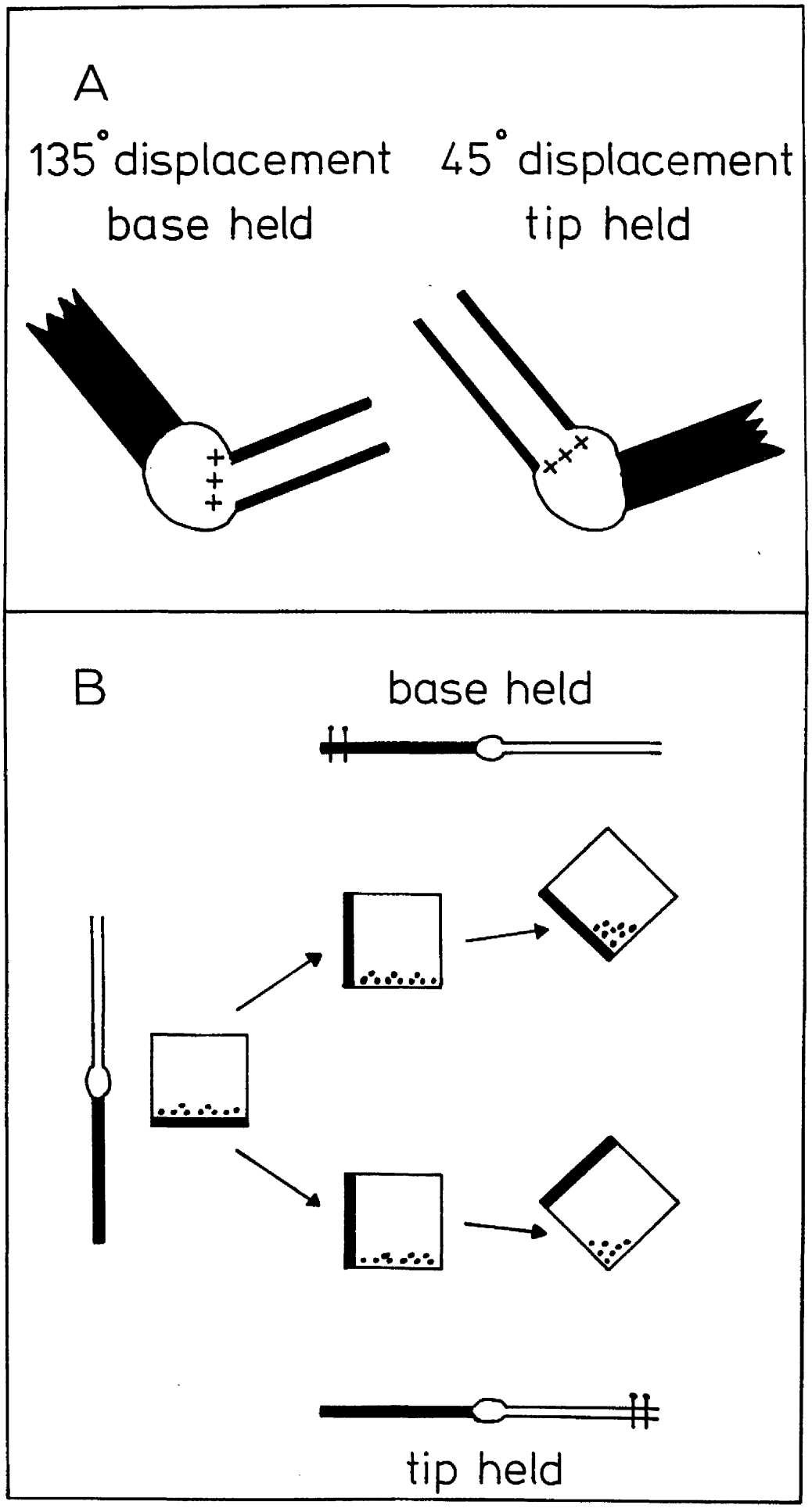
The stimulus received by the apical regions of leaf sheath bases during base held stimulation at 135° displacement, and tip held stimulation at 45° displacement from vertical.

If either receptive or reactive capacities are concentrated at the apical end of the leaf sheath base the stimuli received during the two treatments will differ (see text).

Fig. 18B.

Displacement of statoliths in idealised statocytes for base held and tip held preparations.

The arcs described during curvature are equivalent, but the regions of the statocyte which are stimulated are opposite (see text).


A
135° displacement
base held
45° displacement
tip held
B
base held
tip held

4. Anatomy of the wheat culm

A transverse section through the leaf sheath base is presented in Plate 2. The tissues are continuous in the leaf sheath base, but they give rise to the laminate leaf sheath which is rolled round the stem, and the vascular bundles are arranged in an open ring to allow for this transition. The vascular bundles are distributed amongst tightly packed parenchymatous tissues which are composed, in the transverse plane, of roughly circular cells in a band some 25 cells thick.

The vascular bundles of the leaf sheath base are shown at higher magnification in Plate 3A. The vascular elements are entirely primary with functional metaphloem and metaxylem elements. The protoxylem is mostly collapsed to form the protoxylem lacunae, but in some instances non-functional protoxylem is still in evidence. The vascular bundles of the leaf sheath proper and the internodal regions of the stem are seen from Plates 3B and 3C to be of similar construction. The bundles of the internode are arranged in two circles. The outer bundles are embedded in a continuous layer of sclerenchyma whilst the inner bundles are individually enclosed in sclerenchymatous sheaths which protrude into the central pith. The internodal regions of the stem are hollow except in the meristematic regions directly above the nodes. The vascular bundles of the leaf sheath proper (Fig. 3C) are arranged in a single row immediately within the lignified outer epidermis, and are individually enclosed in sheaths of sclerenchyma which extend through the leaf to the outer epidermis. The sheaths surrounding the larger bundles also extend to the inner epidermis. The bundle sheaths of the leaf sheath are replaced by large bundle caps in the leaf sheath base and the two structures are seen in Plates 3B and 3A respectively. The bundle caps of the leaf sheath base are unlignified and the transition from lignified to unlignified material is most dramatic when viewed in longitudinal section. Plate 4 presents a longitudinal section of the entire leaf sheath base and

Plate 2.

Triticum aestivum L. var. Kolibri.

A transverse section through the stem and leaf sheath tissues in the region of the leaf sheath base.

Treatment: Sections 25μ thick were cut on a freezing microtome, stained with 1% thionine blue and mounted in water.

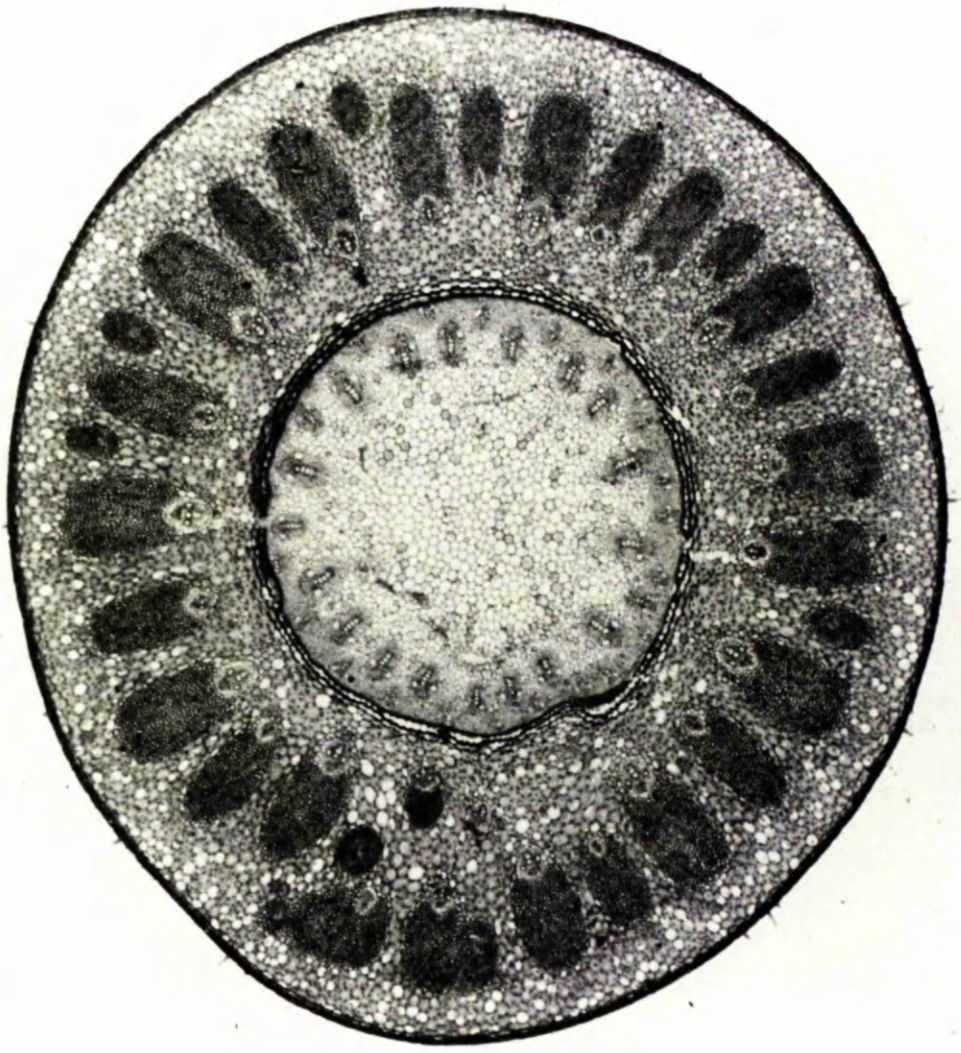
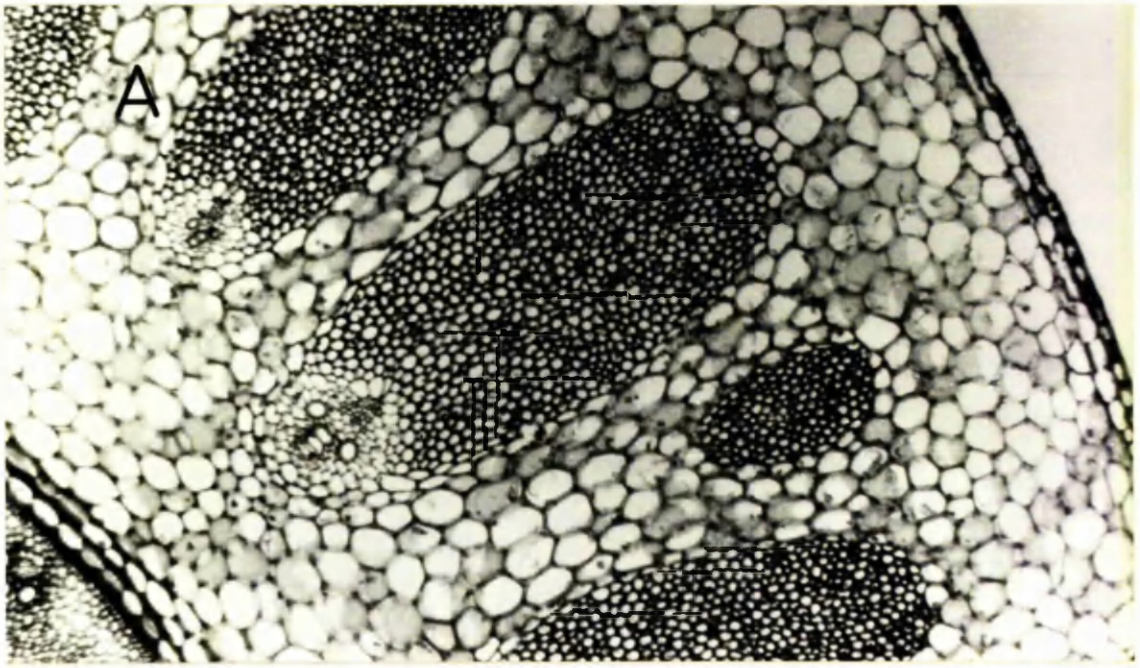


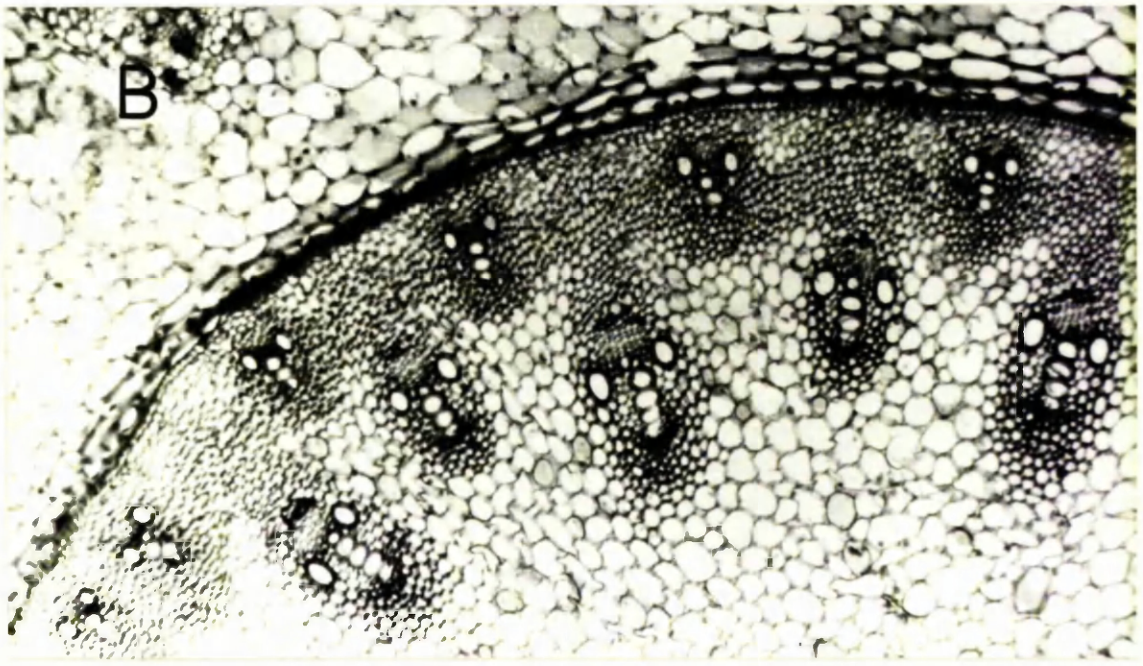
Plate 3.

Triticum aestivum L. var. Kolibri.

The arrangement of vascular tissues in the region of the leaf sheath base.

Treatment: Transverse sections through the leaf sheath base (A), internode (B) and leaf sheath (C) were cut on a freezing microtome, stained in 1% thionine blue and mounted in water.


A


B

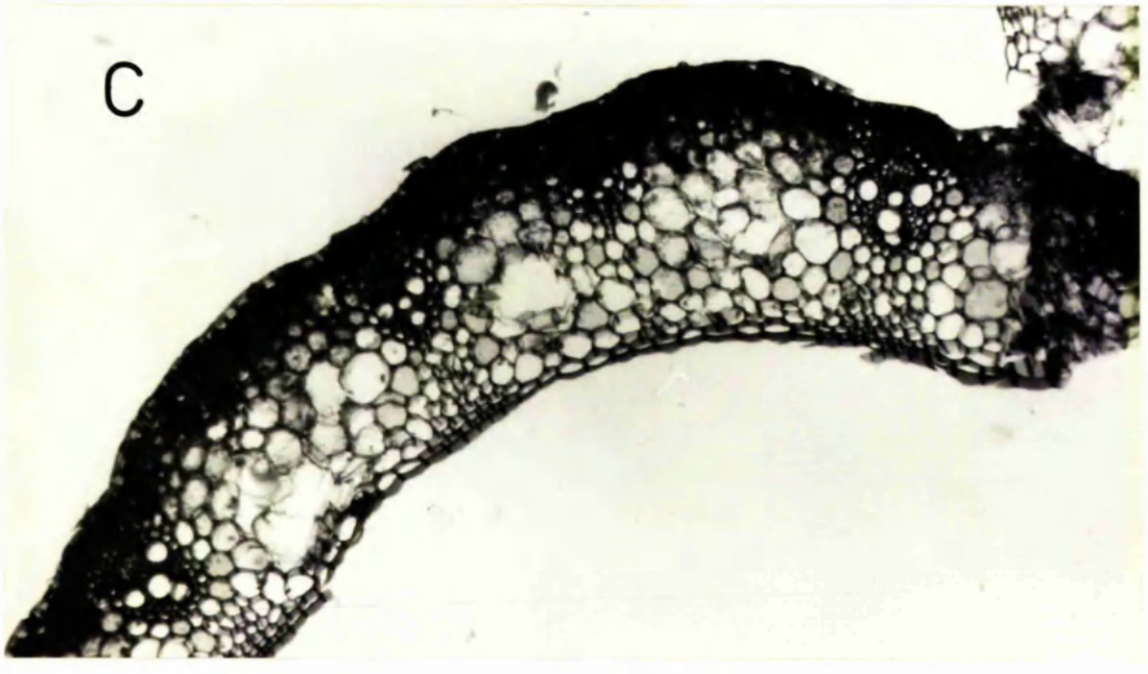
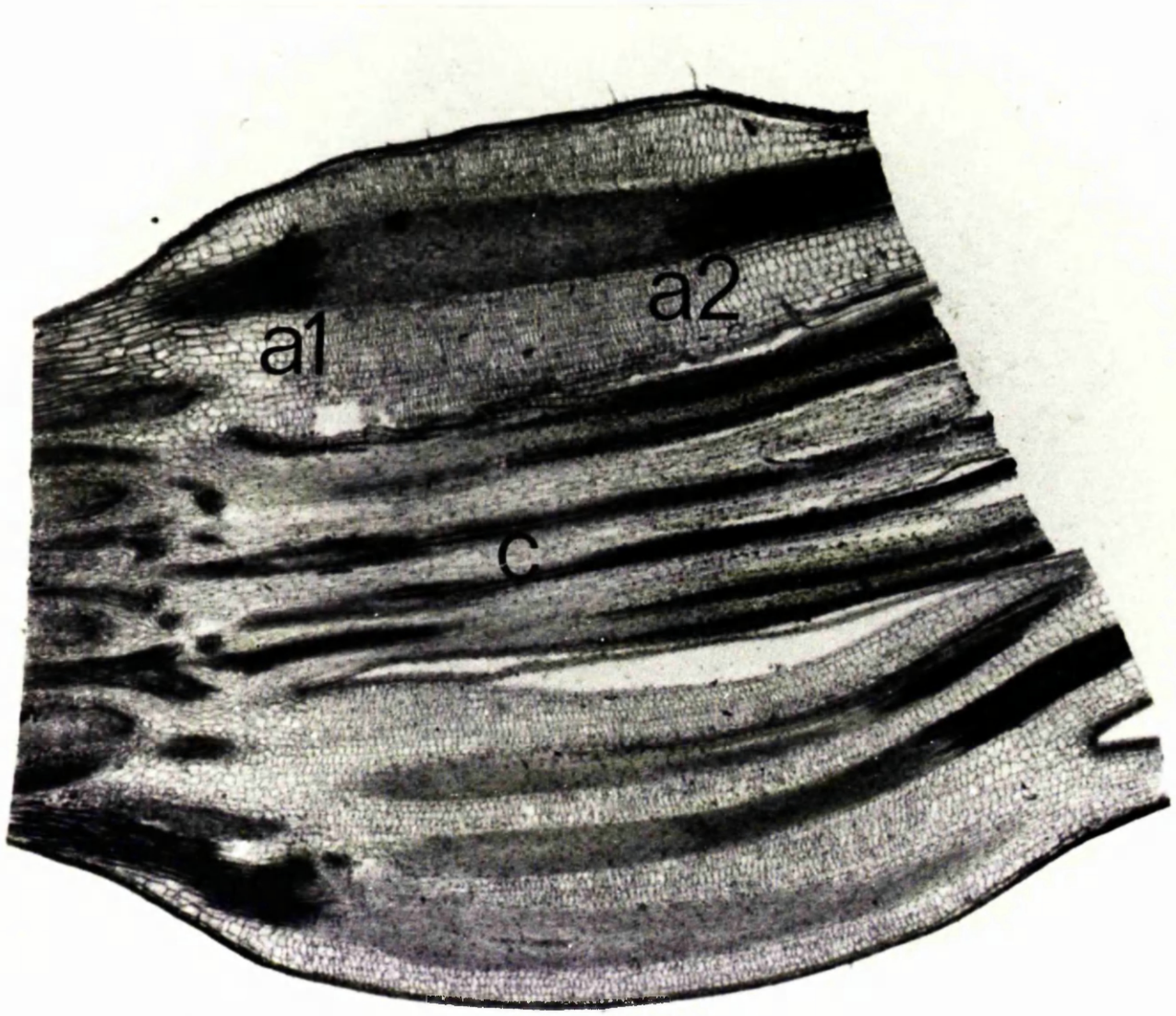

C

Plate 4.

Triticum aestivum L. var. Kolibri.

A longitudinal section through the stem and leaf tissues in the region of the leaf sheath base.

Treatment: Section 25μ thick were cut on a freezing microtome, stained in 1% thionine blue and mounted in water. The extent to which the vascular bundles had become lignified was examined. The transition from the lignified bundle cap near the node (a1) to the unlignified cap in the leaf sheath base and back to the lignified cap at the base of the leaf sheath proper (a2) is immediately apparent, as is the reduction in lignification in the basal regions of the internode (c).


a1
a2
c

shows the region of vascular anastomosis in the node on the left hand side, the development of the true leaf sheath and internode on the right hand side, and the specialised region of geotropically sensitive tissues in the centre. The material has been stimulated geotropically for 48-h before sectioning and the elongation of the lower half of the leaf sheath base is clearly visible. The abrupt transition from the lignified bundle cap near the node (a1) to the unlignified cap in the leaf sheath base and back to the lignified bundle cap in the base of the leaf sheath proper (a2), is immediately apparent, and a similar reduction in the degree of lignification in the basal regions of the internode is also observed (a). As the plant matures and the elongation of the internode becomes complete, the sheaths surrounding the vascular elements in the basal regions of the internode become lignified as do the sclerenchymatous tissues immediately beneath the epidermis. The bundle caps of the leaf sheath base eventually become lignified, but this process is considerably retarded.

The constituent cells of the bundle caps may be observed in TS in Plate 3A. The tissue is composed of angular cells which pack tightly so that intercellular spaces are not apparent. The tissue is seen in LS in Plate 5. In this plane the cells are elongated and their end walls are tapered. At the extremities of the leaf sheath base the cells are heavily lignified and devoid of protoplasts, but in the central regions they retain their protoplasmic lining and contain nuclei which are clearly visible.

The parenchymatous cells of the leaf sheath base are hexagonal in longitudinal section, but they do not appear to exhibit the thick longitudinal walls reported for the wheat var. Atlee by Arslan and Bennet-Clark (1960). In thin 2 μ sections the longitudinal walls appear no thicker than the transverse walls (see Plate 6). Growth in response to geotropic stimulation is brought about by an increase in the longitudinal diameters of the parenchymatous cells, but the increase is not associated with any corresponding decrease in radial or tangential diameters. The mean radial and tangential

Plate 5.

Triticum aestivum L. var. Kolibri.

A longitudinal section through the bundle cap.

Treatment: Material was embedded in wax and sectioned longitudinally at a thickness of 20μ. Sections were stained in 1% basic fuchsin. The cells in the unlignified regions of the bundle caps were examined for evidence of protoplasm and nuclei.

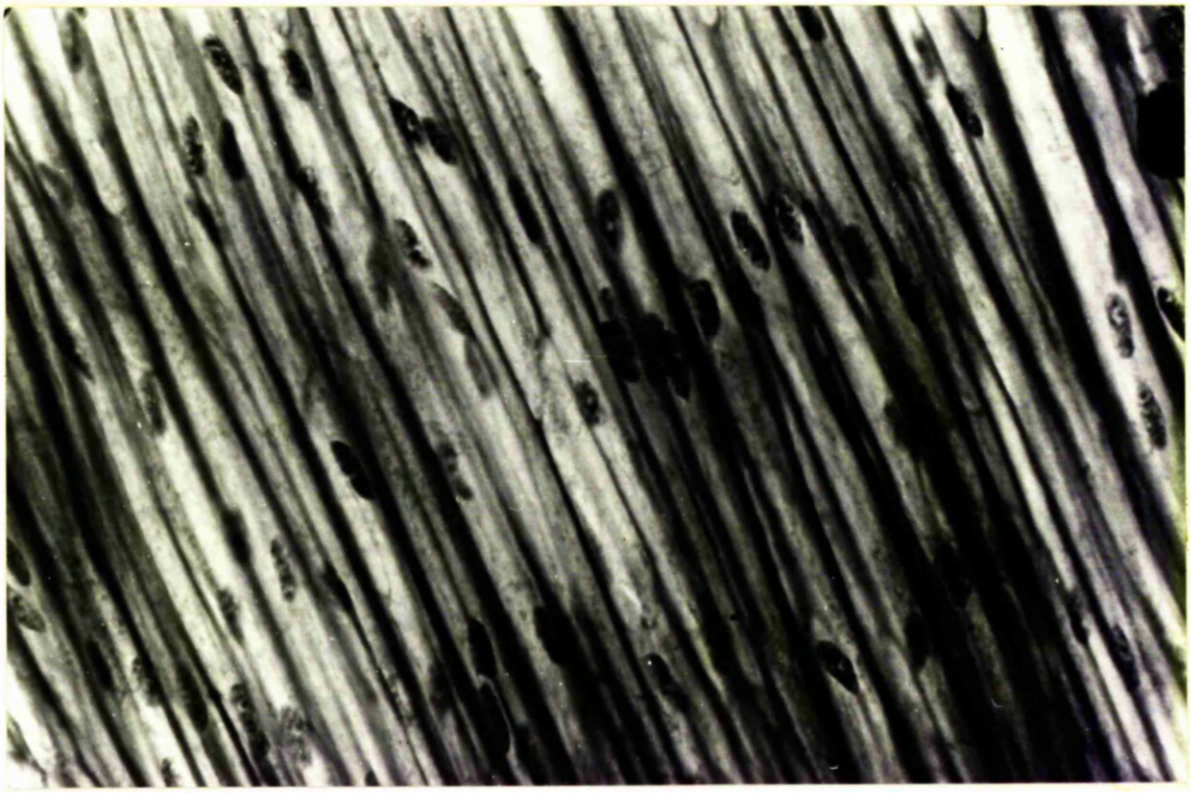
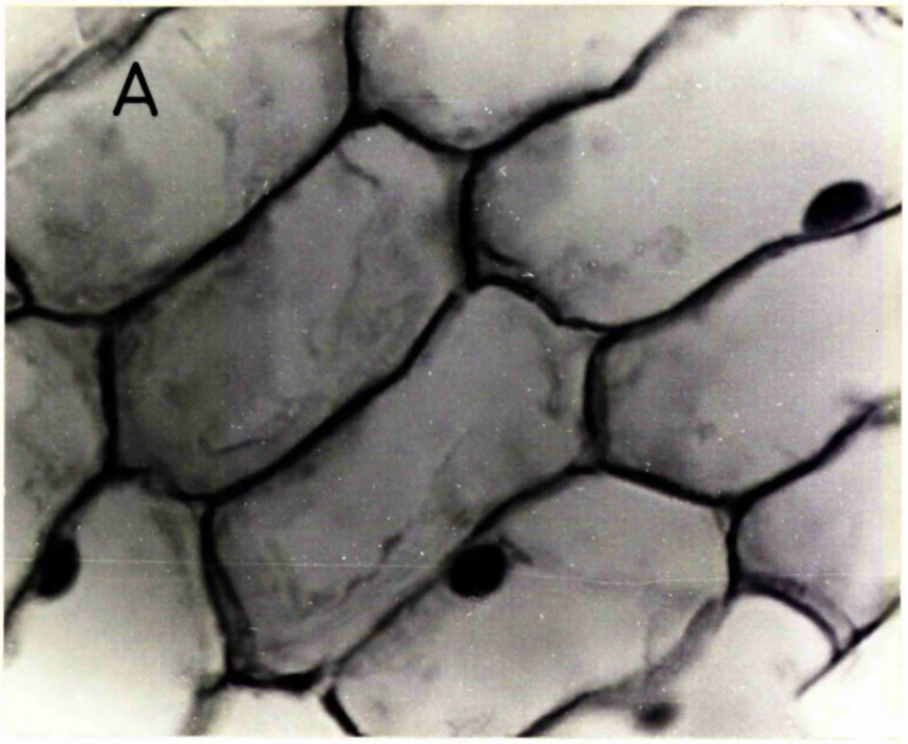


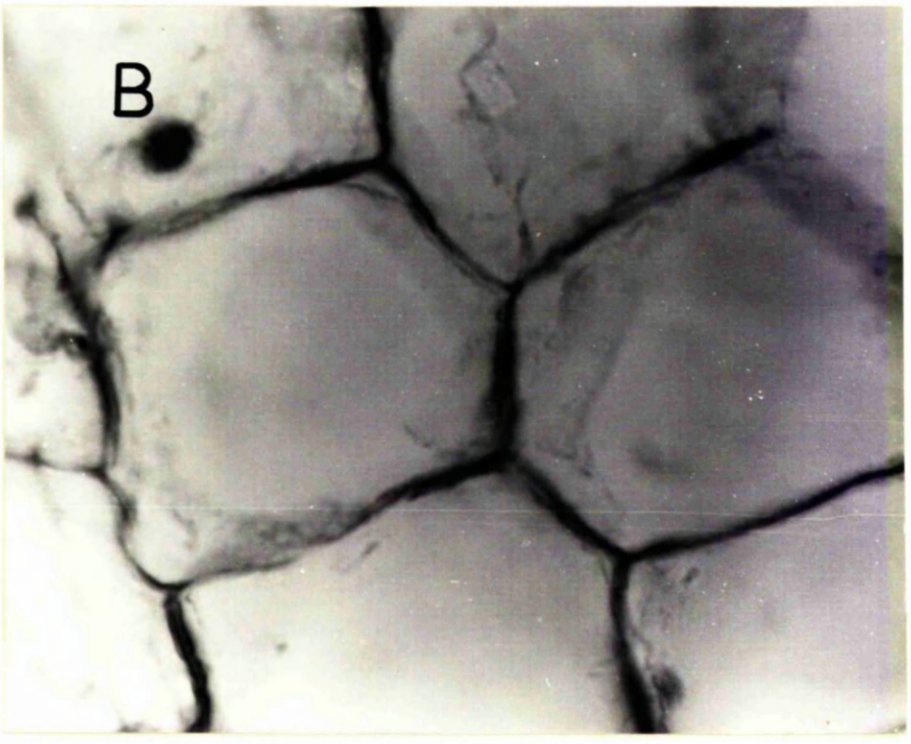
Plate 6.

Triticum aestivum L. var. Kolibri.

Longitudinal sections through the cortical cells of the leaf sheath base.

Treatment: Stem segments 100 mm in length were stimulated geotropically for 48 h. Portions of tissue from the upper (A) and lower (B) regions of the stimulated leaf sheath bases were then mounted in araldite. Longitudinal sections 2μ thick were cut on an ultrazone, stained in 0.1% toluidine blue and mounted in Canada balsam.


A


B

diameters of the cells in a transect taken through the upper and lower sides of the leaf sheath base after a 48-h period of geotropic stimulation are given in Table 2. The longitudinal diameters of the cells on the lower side of the organ double during this period, but no such decreases in tangential and radial diameters are apparent. The absence of dramatic changes in radial and tangential diameters may be taken as conclusive evidence for the involvement of cell expansion rather than cell realignment in the geotropic response mechanism.

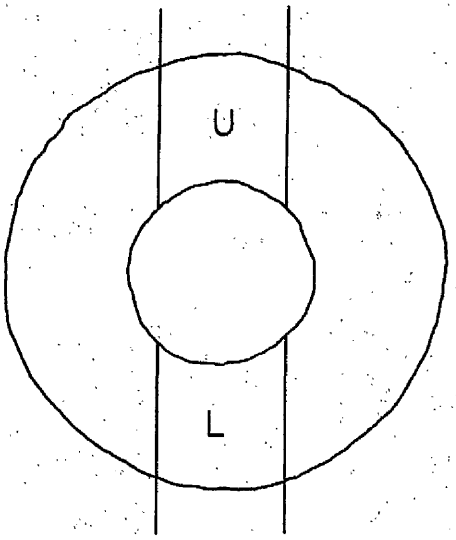
Table 2.

Triticum aestivum L. var. Kolibri.

The radial and tangential diameters of the parenchymatous cells of the upper and lower halves of the leaf sheath base 48 h after the onset of the geotropic response.

Treatment: Stem segments 100 mm in length were laid horizontally for 48 h. The leaf sheath bases were then excised, mounted in tissue tek and frozen in liquid nitrogen. Transverse sections 25 μ thick were cut on a freezing microtome at -18°C and aqueous mounts were stained with thionine blue. Sections were photographed, and cell diameters were measured in millimetres from the prints. The two sampling regions are shown in the diagram opposite.

Table 2



	Diameter (mm from photograph)	
	Radial	Tangential
Upper Sector (U)	12.634 ± 0.345	13.863 ± 0.352
Lower Sector (L)	13.259 ± 0.353	15.176 ± 0.068
t values between lower & upper sectors	1.280	2.600*

5. The gravi-perception mechanism

Anatomical studies have revealed the presence of two potential statoliths in the leaf sheath base and these are shown in Plate 7. Crystals are to be found in most of the parenchymatous cells and these are usually seen as rods or rhomboids in thick sections (Plate 7A). Starch grains are also plentiful, but their distribution is restricted to the parenchyma in the immediate vicinity of the vascular bundles (Plates 7B and C).

A time course for the sedimentation of these potential statoliths is given in Fig. 19 and shows that both types of particle are capable of complete sedimentation within 7-10 min. The role of these potential statoliths has been further assessed by experiments designed to remove the starch grains preferentially from the statenchyma. Material has been destarched using the gibberellin/kinetin treatment first developed by Gillespie-Pickard and Thimann (1966) and, although a time course for destarching has not yet been completed, a 3-day incubation in darkness at 30°C has been shown to remove all traces of starch from the leaf sheath bases. The effect of the destarching treatment on the distribution of starch grains is shown in Plate 8C whilst the effects of the control treatments involving incubation in 2% sucrose or distilled water are shown in Plates 8A and B. The control treatments do not result in destarching, but the size of the individual grains is somewhat reduced by incubation in distilled water. None of the treatments removes the crystal inclusions from the parenchyma.

The curvatures developed during a 24-h period of geotropic stimulation following incubation in 2% sucrose, distilled water or gibberellin plus kinetin are shown in Fig. 20A. The destarching treatment results in the complete abolition of the response, but curvatures develop normally following the control incubation with 2% sucrose. Curvature is slightly reduced following the control incubation in distilled water. Transference from the original incubation media to a medium of 2% sucrose in bright light is

Plate 7.

Triticum aestivum L. var. Kolibri.

Potential statoliths in the leaf sheath base.

Treatment: Transverse sections were cut on a freezing microtome, mounted in water and examined for potential statoliths.

Crystals occur in most of the parenchymatous cells and these are clearly visible in thick (35μ) sections (A). Sedimentable starch grains are also apparent in material stained with iodine solution, but these are restricted to the cells lying adaxial to the vascular bundles (B & C).

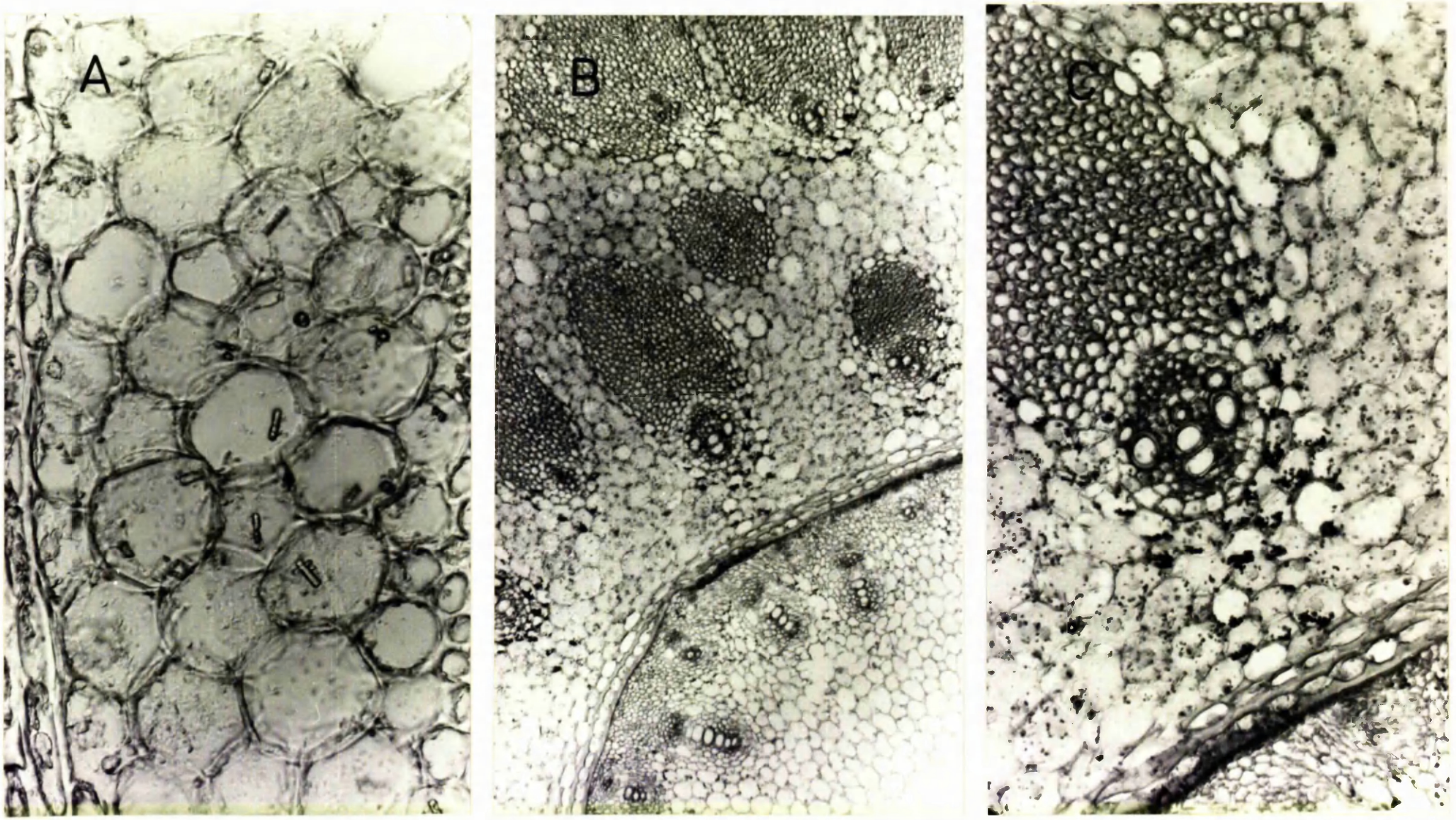

A
B
C

Fig. 19.

Triticum aestivum L. var. Kolibri.

Sedimentation rates for potential statoliths.

Treatment: Stem segments 100 mm in length were laid horizontally for 30 min and then rotated through 180°. Leaf sheath bases were excised at 2 min intervals starting 1 min after the rotation treatment, and the segments were mounted in tissue tek and frozen in liquid nitrogen. Transverse sections 25 μ thick were cut on a freezing microtome at -18°C and aqueous mounts were stained with iodine. Sections were observed under a Vickers 'Patholux' microscope and the extent to which starch grains (—●—)= centre of band) and crystal inclusions (—▲—) had sedimented was estimated with the aid of a micrometer eyepiece.

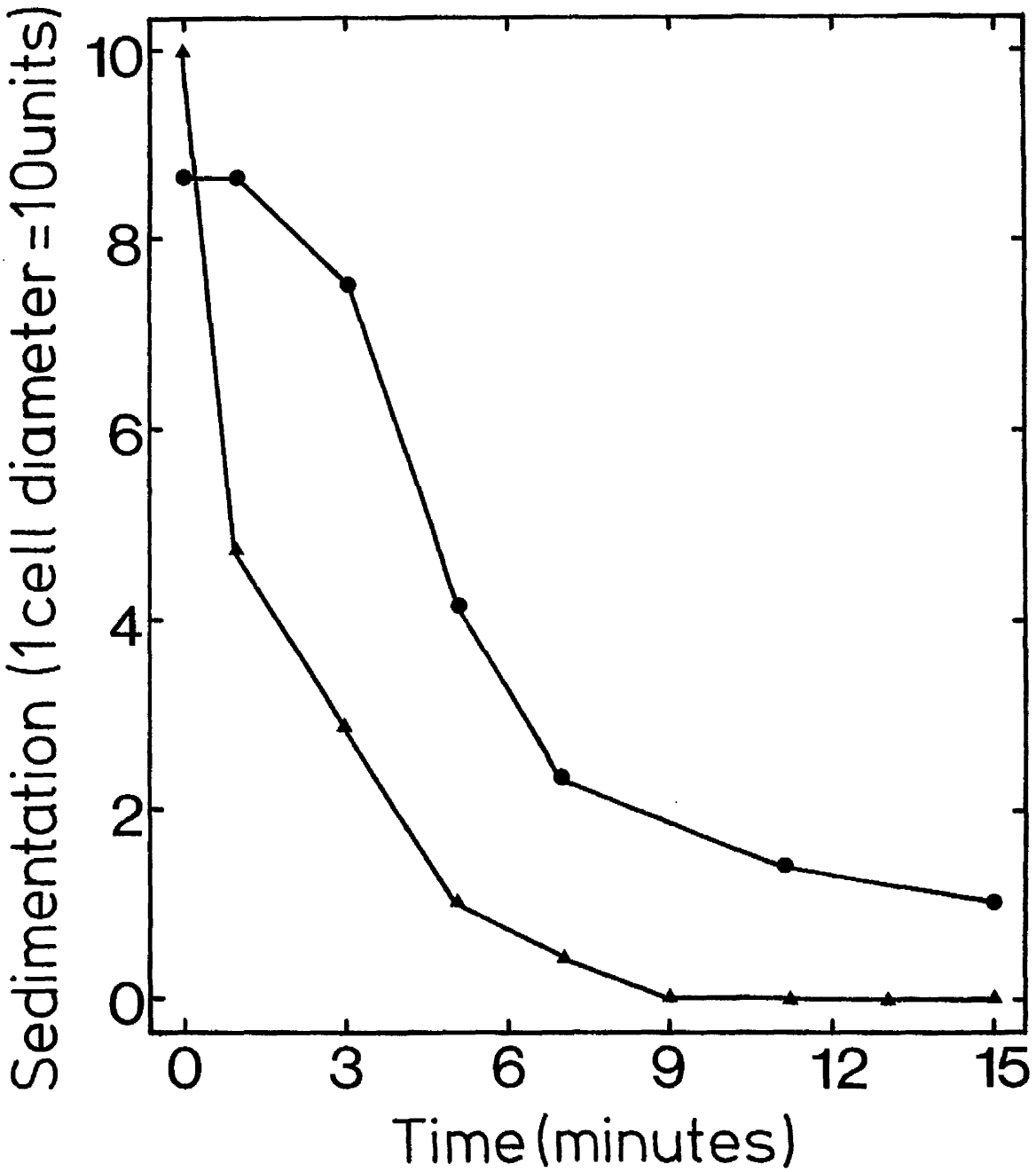

Sedimentation (1cell diameter=10units)
10
8
6
4
2
0
0
3
6
9
12
15
Time (minutes)

Plate 8.

Triticum aestivum L. var. Kolibri.

The extent of destarching during incubation in a solution of gibberellin and kinetin.

Treatment: Stem segments 100 mm in length were placed vertically in boiling tubes containing 2% sucrose + 100 ppm streptomycin; distilled water + 100 ppm streptomycin; or 5×10^{-5}M GA_3 + 5×10^{-5}M kinetin + 100 ppm streptomycin. The effect of a 72-h destarching treatment on the distribution of starch grains (C) is compared with the effects of the control incubations in 2% sucrose (A) or distilled water (B). Resynthesis of starch following transference to 2% sucrose and maintenance in bright light for 24 h is seen in Fig. D.

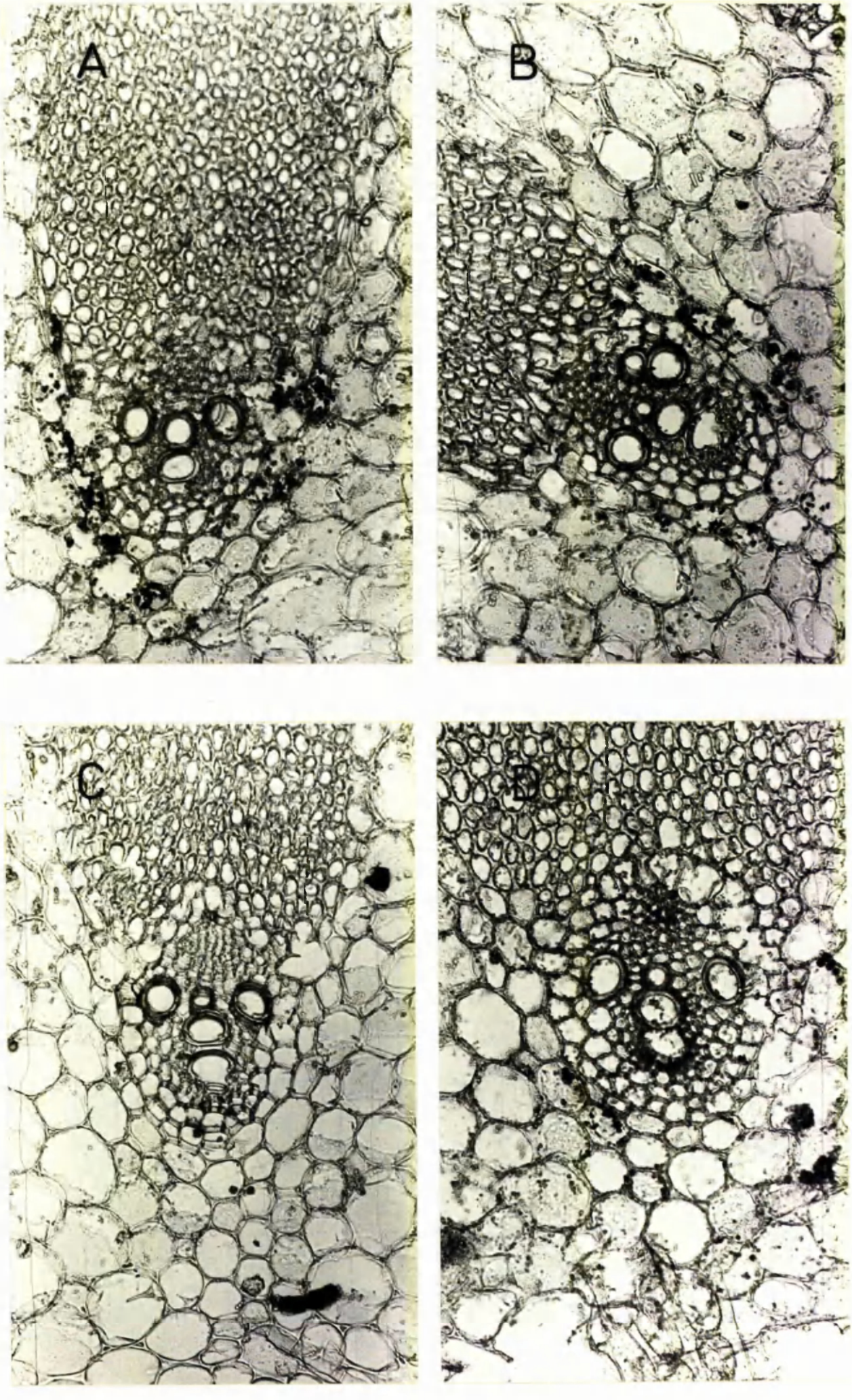
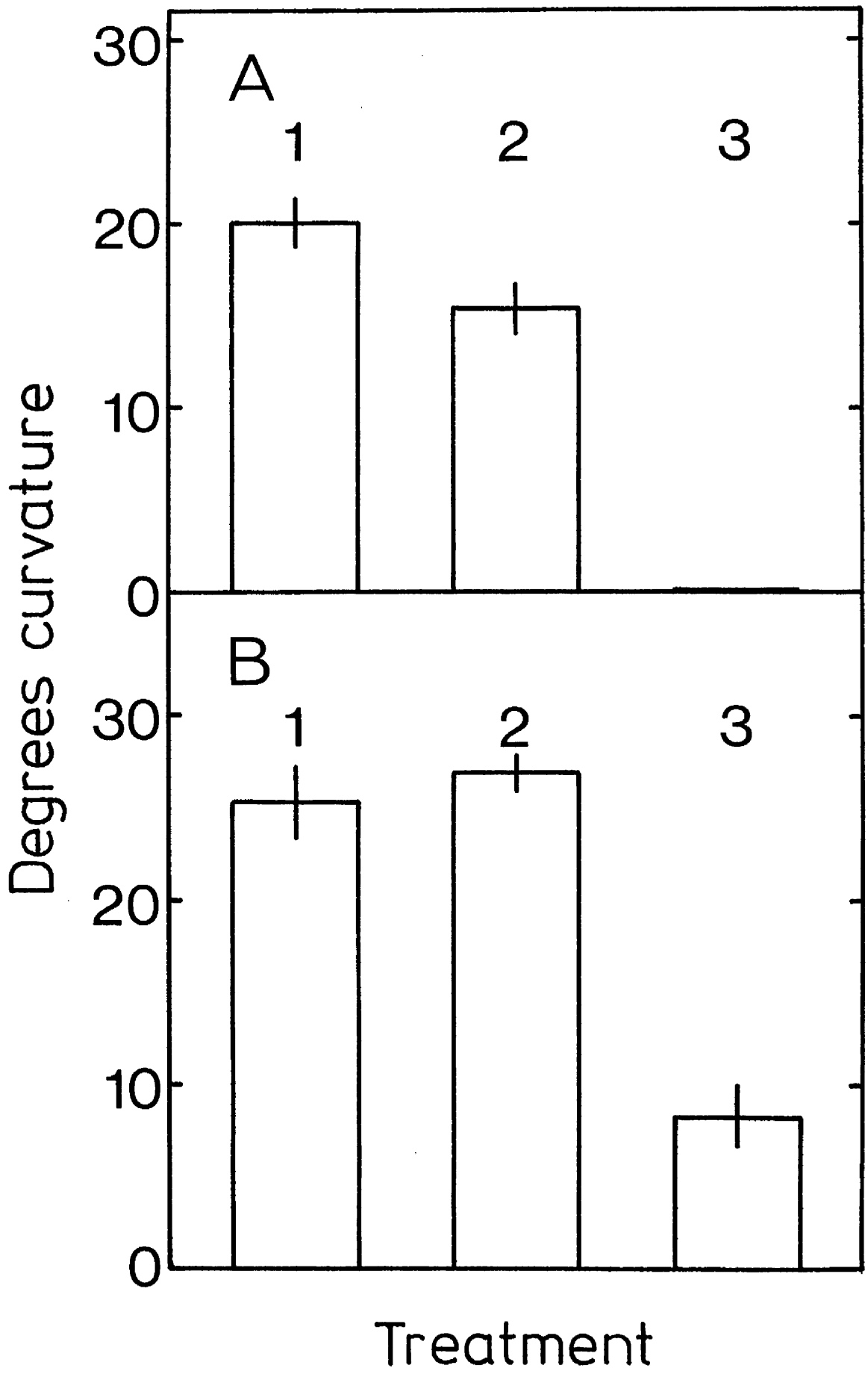

A
B
C
D

Fig. 20.

Triticum aestivum L. var. Kolibri.

The effect of the removal of starch grains from the statonchyma.

Treatment: Stem segments 100 mm in length were placed vertically in boiling tubes containing: (1) 2% sucrose + 100 ppm streptomycin; (2) distilled water + 100 ppm streptomycin; and (3) 5×10^{-5}M GA_3 + 5×10^{-5}M Kinetin + 100 ppm streptomycin. Tubes were held in darkness at 20°C and solutions were changed at 24-h intervals. Half of the stem segments were removed and pinned horizontally after a 72-h incubation period, and the remainder were transferred to tubes containing 2% sucrose which were maintained in bright light at 25°C. These segments were removed and pinned horizontally after a 24-h incubation period. Geotropic stimulation took place in the normal diffuse white light at 25°C. The curvatures developed during a 24-h stimulation period are shown for the dark treatment and combined dark and light treatments in Figs. A & B respectively.


A
B
1
2
3
30
20
10
0
Degrees curvature
Treatment

sufficient to initiate the synthesis of starch grains in the destarched statocnhyma, and the resynthesised starch grains are seen in Plate 6D. Resynthesis is far from complete after a 24-h treatment period. The curvatures developed during a 24-h period of geotropic stimulation following the 24-h restarching treatment are shown in Fig. 20B. Geotropic curvatures develop in the restarched material (histogram B3) and the restarching treatment also has the effect of increasing the curvature developed in the original water controls. The curvatures developed in the sucrose and water controls (histograms B1 and B2) are comparable with the curvature developed in untreated material.

Since the crystal inclusions are unaffected by the destarching treatment their role as statoliths must be questioned. The evidence against them is however only circumstantial, as it may always be argued that the destarching treatment affects other aspects of the response sequence in addition to the starch grains.

The study of the gravi-perception mechanism is complicated by the fact that gravity can never be eliminated in an earth bound laboratory, but the problem has been alleviated for many plant systems by the use of the clinostat. The effect of rotating 100 mm stem segments about a horizontal axis is shown in Fig. 21. Rotation at speeds between 1/6 RPH and 5 RPM about a 20 mm radius of rotation has no visible effect on unstimulated material, but rotation at slower speeds promotes straight growth, and rotation at faster speeds promotes centripetal curvature. Rotation at speeds between 1/6 RPH and 5 RPH ought therefore to be suitable for gravity nullification in this material, but rotation at these speeds has been found to cause straightening in material which is already responding to geotropic stimulation (Fig. 22). This phenomenon may be connected with the long periods of rotation required to study the relatively slow development of the geotropic response but, since its effect is to prevent the development of curvature in material which is stimulated prior to rotation, it has prevented the use of the horizontal clinostat as a means of gravity nullification.

Fig. 21.

Triticum aestivum L. var. Kolibri.

The effect of rotation about a horizontal axis.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Assembled containers were rotated about horizontal axes at one of a range of speeds between $^{1}/_{50}$ RPM and 500 RPM and the effect on the leaf sheath base was observed. Low rotation speeds induced straight growth which was measured after 72 h rotation (—●—) whilst faster rotation resulted in centripetal curvature which was measured after 24 h rotation (—■—).

Darkness 25°C.

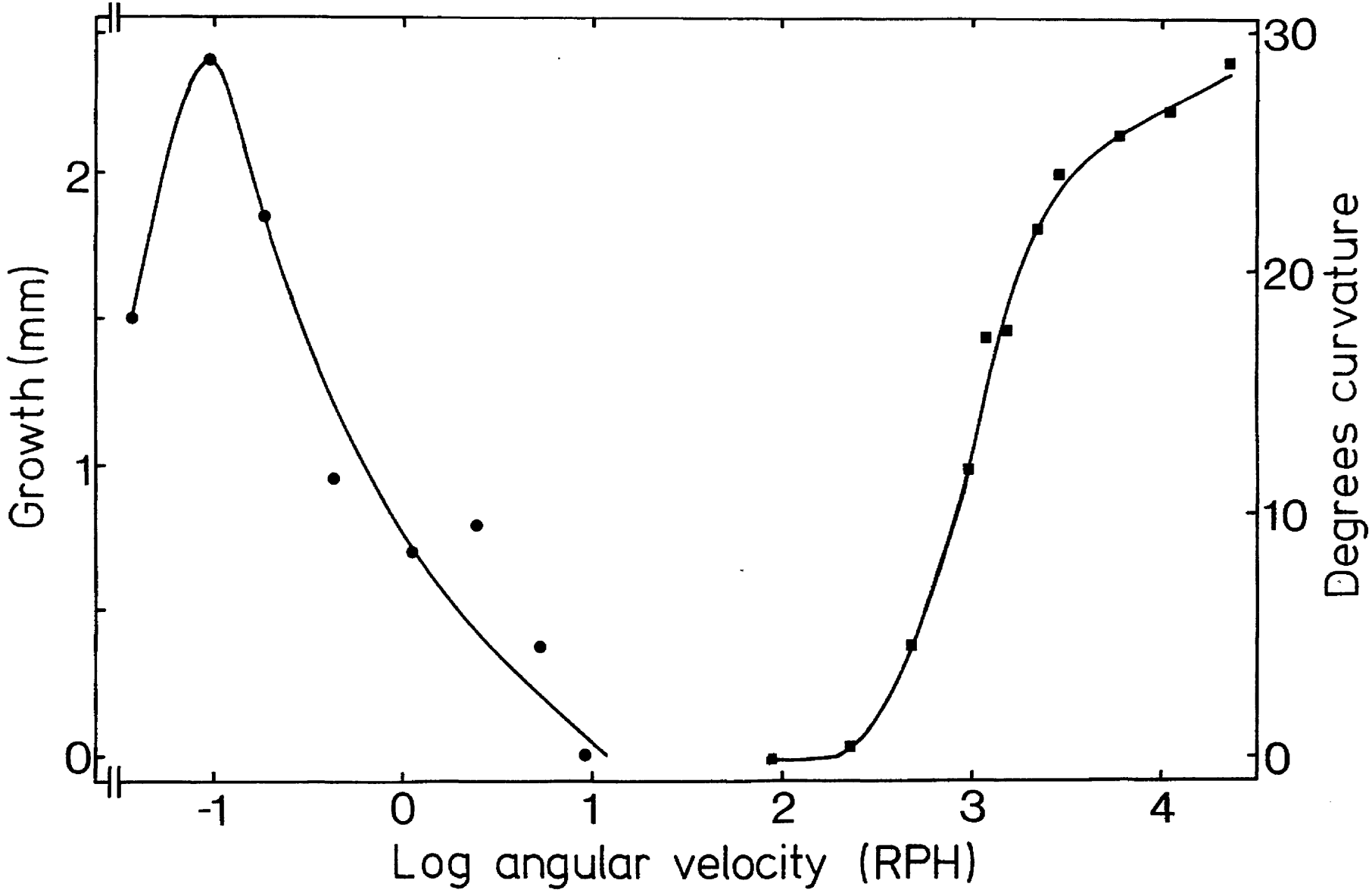

Growth (mm)
2
1
0
-1
0
1
2
3
4
Log angular velocity (RPH)
30
20
10
0
Degrees curvature

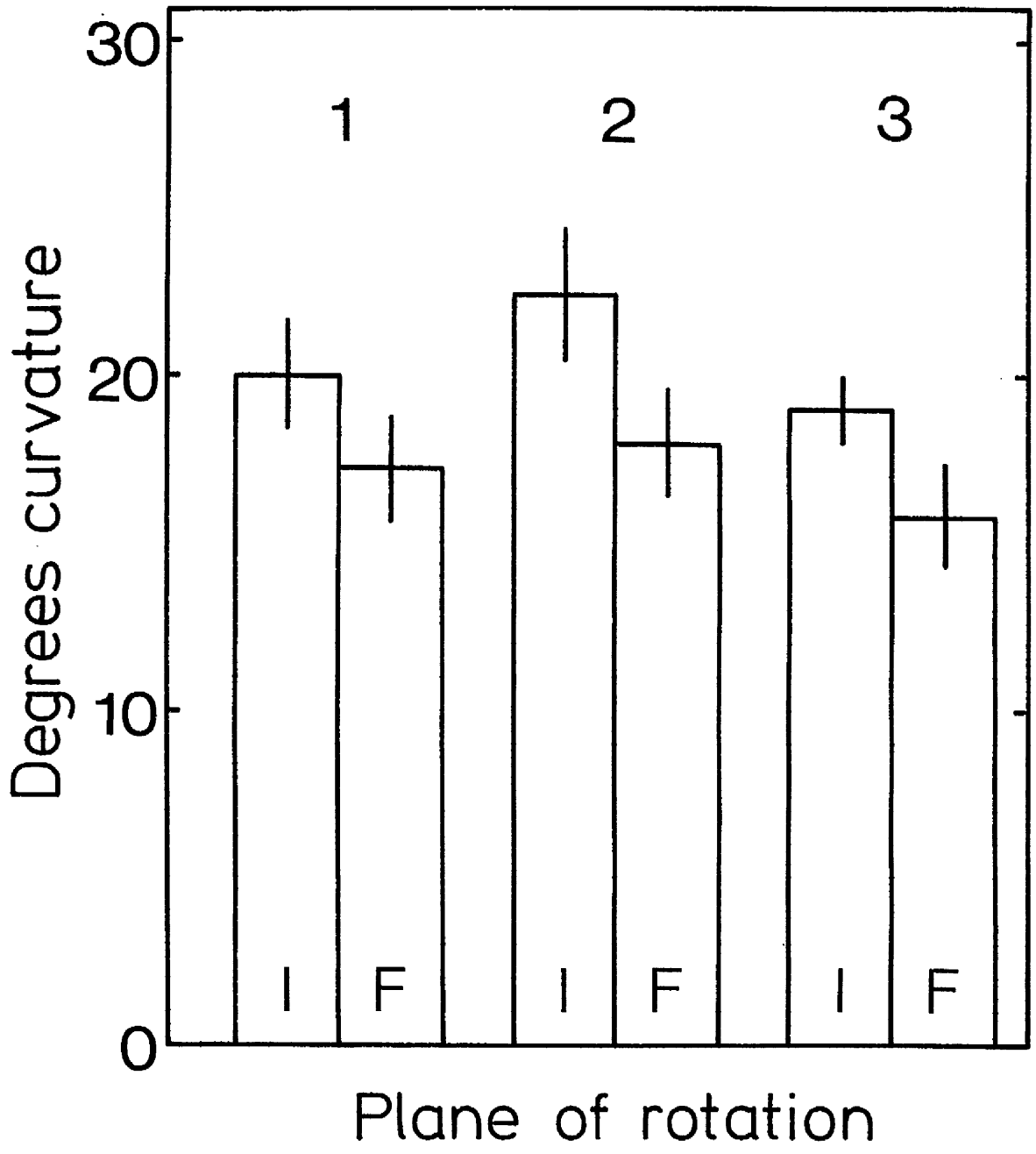
Fig. 22.

Triticum aestivum L. var. Kolibri.

Gravity nullification on the horizontal clinostat.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Containers were held horizontally in one of three positions such that segments bent: (1) towards the clinostat axis; (2) away from the clinostat axis; or (3) in the plane of the subsequent rotation. When the segments had bent through between 15° and 25° the initial curvature (I) was measured and rotation at 2 RPM was commenced. Final curvature (F) was measured after a 6-h rotation period.

Darkness 25°C.


30
20
10
0
1
2
3
I
F
I
F
I
F
Degrees curvature
Plane of rotation

Rotation about a horizontal axis has been used to investigate other aspects of the perception mechanism. Rotation at speeds between 2 RPH and 500 RPH has been used to calculate the threshold acceleration required to induce visible curvature. Data presented in Fig. 23 show that accelerations between 1/10,000 xg and 1/1,000 xg are required to yield a measurable response after 24 h rotation, and that the centripetal curvature obtained from an applied acceleration of 1 xg is comparable with the curvature obtained during normal exposure to gravity.

Straight growth may be induced by horizontal rotation at slow speeds and the relationship between rotation speed and the induced growth is shown in Fig. 24. Straight growth is apparent at a rotation speed of 1 RPH and is maximal at 1/10 RPH. The response at this latter rotation speed is seen in Plate 9 which shows the 100 mm stem segments before (A) and after (B) the inducement of straight growth during a 72-h rotation period. The distribution of reactive tissues in the leaf sheath base is shown in Fig. 25. The capacity for straight growth is greatest in the apical regions and least in the basal regions of the organ, and the distribution of reactive tissues is similar to that found for the normal geotropic response (see Fig. 15). The inference from these experiments is that growth results from the stimulation of all sides of the organ as the statoliths tumble slowly over the statocyte walls, and this inference is substantiated by the demonstration that curvature develops immediately on terminating rotation (Fig. 26). The normal geotropic response develops only after a latent period of 2h-20min at 25°C, but the rotation treatment has the effect of priming the tissues so that curvature develops immediately in the plane of the gravitational vector when the treatment is terminated.

The statoliths are held at the lowest point in the cell under the influence of gravity, and because the segments remain straight during rotation, the orbitals described about the statoliths by the revolving cell walls remain fixed and the cells are subjected to continous stimulation at a constant

Fig. 23.

Triticum aestivum L. var. Kolibri.

Threshold 'g' for the geotropic response at the node.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Assembled containers were rotated about horizontal axes at speeds which yielded accelerations between $^{1}/_{10,000}$ x g and 5 x g. Curvature towards the axis of rotation was measured after 24 h rotation (—●—) and curvature resulting from normal horizontal exposure to gravity (1 x g) was also determined after 24 h (✪).

Darkness 25°C.

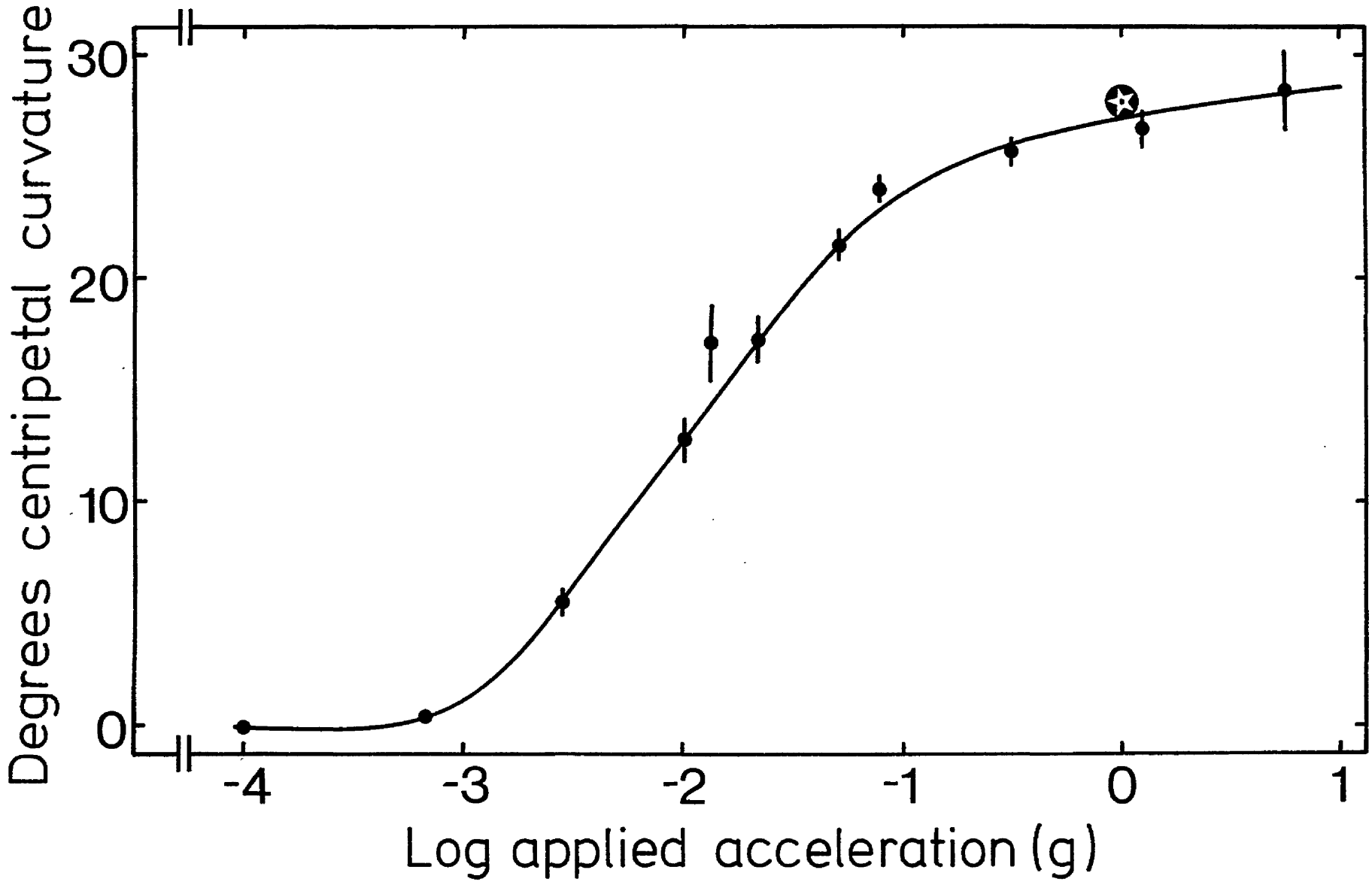

Degrees centripetal curvature
30
20
10
0
-4
-3
-2
-1
0
1
Log applied acceleration (g)

Fig. 24.

Triticum aestivum L. var. Kolibri.

The straight growth response to slow rotation about a horizontal axis.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Assembled containers were rotated about horizontal axes at one of a range of speeds between $^{1}/_{50}$ RPH and 72 RPH. The growth of the leaf sheath base was measured after a 72 h rotation period.

Darkness 25°C.

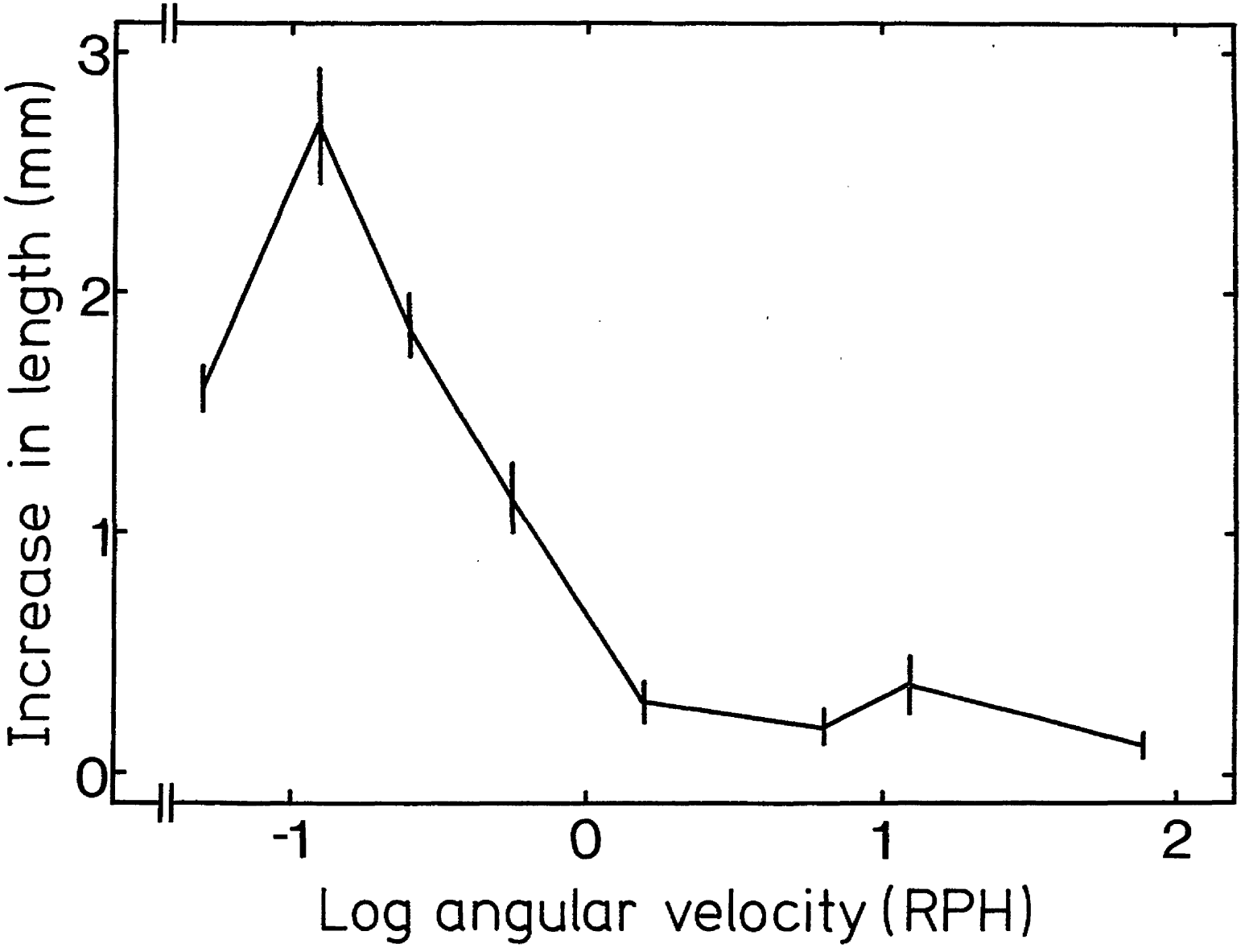

Increase in length (mm)
3
2
1
0
-1
0
1
2
Log angular velocity (RPH)

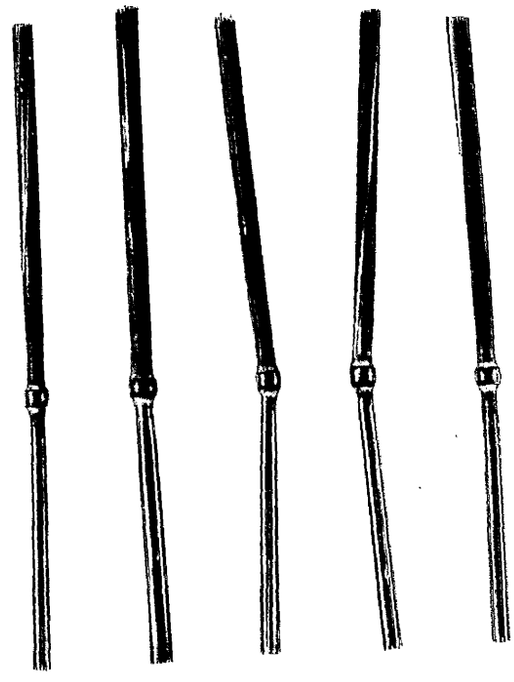
Plate 9.

Triticum aestivum L. var. Kolibri.

Straight growth in stem segments subjected to slow rotation about a horizontal axis.

Treatment: Stem segments of the type used in experiments involving slow rotation about horizontal axes are shown before (A) and after (B) in a 72-h period of horizontal rotation at $^{1}/_{10}$ RPH.

A



B

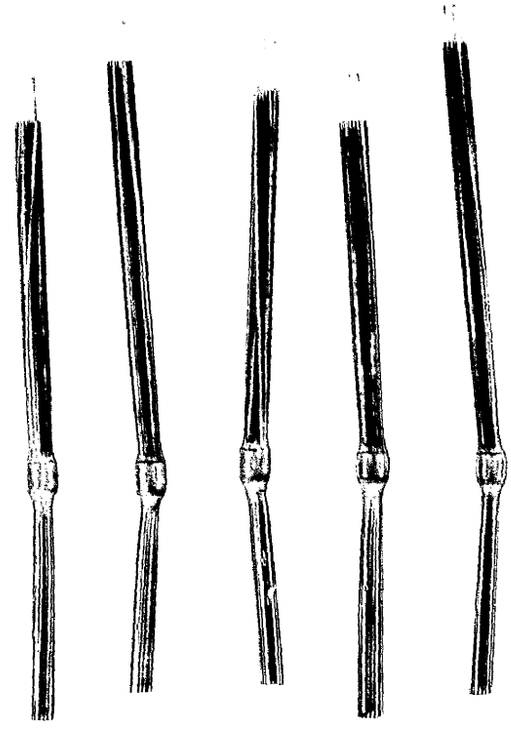


Fig. 25.

Triticum aestivum L. var. Kolibri.

Straight growth induced by rotation at $^{1}/_{10}$ RPH.

Treatment: One node preparations 100 mm in length were prepared from stems which contained an apical leaf sheath base measuring 3 mm in length. Indian ink dots were arranged 1 mm apart up one side of the leaf bases, and the preparations were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Assembled containers were rotated about horizontal axes at $^{1}/_{10}$ RPH and the growth responses in basal (a), central (b) and apical (c) 1 mm regions of the leaf sheath bases were determined after a 72-h rotation period.

Darkness 25°C.

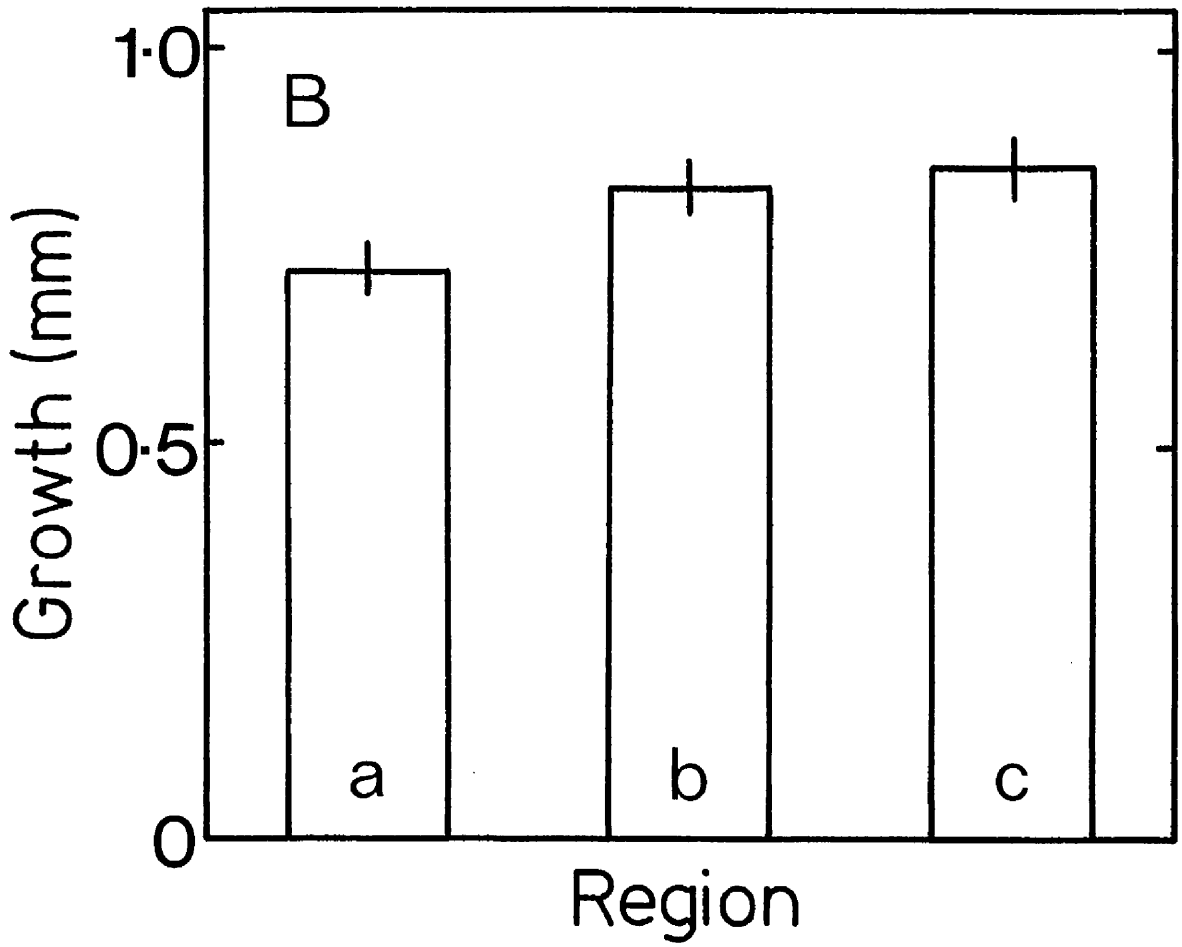

1·0
0·5
0
B
Growth (mm)
a
b
c
Region

Fig. 26.

Triticum aestivum L. var. Kolibri.

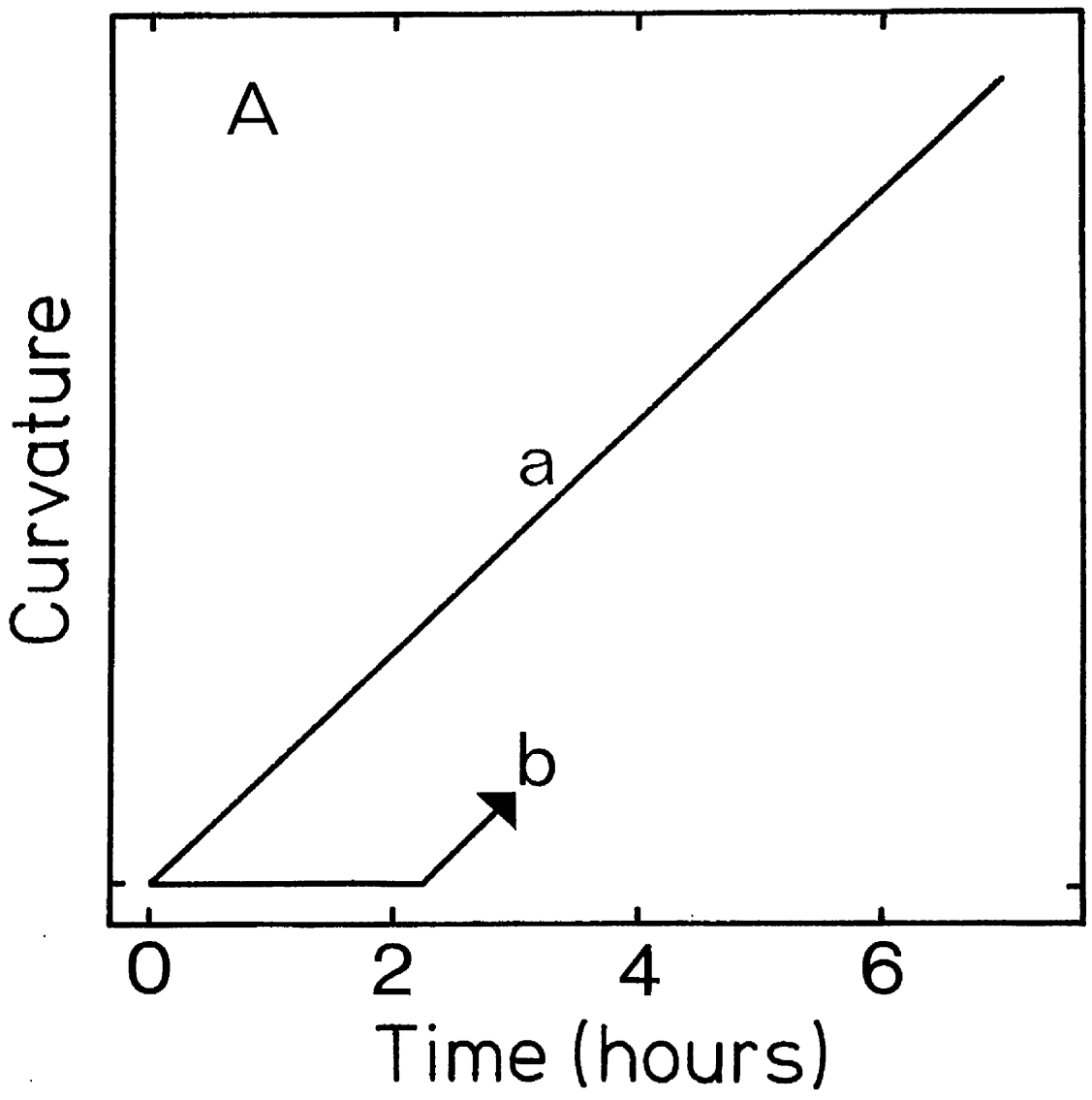
The response on terminating rotation at $^{1}/_{10}$ RPH.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers, and assembled containers were rotated about horizontal axes at $^{1}/_{10}$ RPH. Rotation was terminated after 6 h and the development of curvature was recorded on a smoke drum rotated at 50 mm h^{-1} (Fig. A). The development of the response, shown by line (a), is compared with the development of the response in material which did not receive the rotation treatment (line b). The arrows in Fig. B indicate the direction of curvature on terminating rotation.

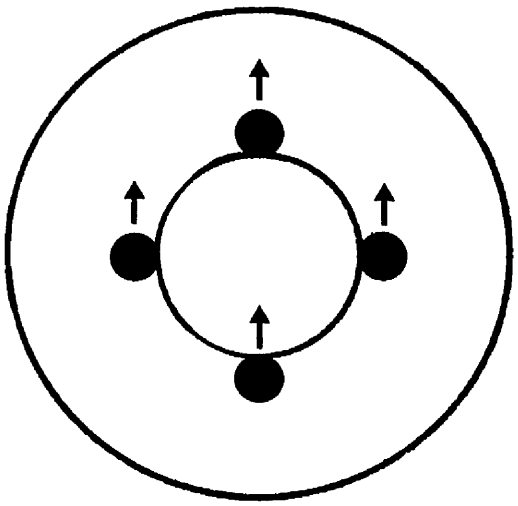
Darkness 25°C.

Lag periods. For response on terminating rotation at $^{1}/_{10}$ RPH = 0 min.

For normal response = 2.27 $\pm$ 0.057 h.



B



level. The stimulus can be applied to different regions of the statocyte wall by rotation about axes fixed at different inclinations to vertical, and the technique can therefore be used to test the application of the sine rule to geotropism in the leaf sheath base. The technique eliminates the problems of curvature and straightening encountered on the clinostat, and the continuous nature of the stimulus may also be expected to minimise any stimulatory effects which might ensue whilst the statoliths are sliding to their equilibrium orbitals.

Straight growth induced by rotation at 1/10 RPM about axes fixed at angles ranging from 0° to 180° displacement from vertical is shown in Fig.27. Growth is maximal at 90° displacement from vertical, and the response shows a symmetrical development about this point. The response shows a broader plateau than would be anticipated from a rule requiring direct proportionality with the sine of the angle, but the sine rule as first propounded by Sachs required only that the response should be proportional to a function of the sine of the angle of displacement. Because the angle of inclination remains constant during rotation the level of stimulation must also remain constant, and the differences in magnitude between responses developed at various displacements from vertical must therefore be attributed to differences in response rate. This reasoning is substantiated by data presented in Fig. 28 for the initial development of curvature at displacements of 90° and 15° from vertical. The reaction time is not affected by the orientation with respect to vertical, but the response rate is reduced markedly at the latter displacement.

Fig. 27.

Triticum aestivum L. var. Kolibri.

The validity of the sine rule for the geotropic response at the wheat node.

Treatment: Stem segments 100 mm in length were pinned to cylindrical corks which were fixed in the centres of screw cap containers. Assembled containers were rotated at 1/10 RPH about axes fixed at angles ranging from 0° to 180° displacement from vertical. Growth was measured after a 72-h rotation period (– – – –). The values are compared with theoretical values calculated assuming a direct proportionality between response and the sine of the angle of displacement (———).

Darkness 25°C.

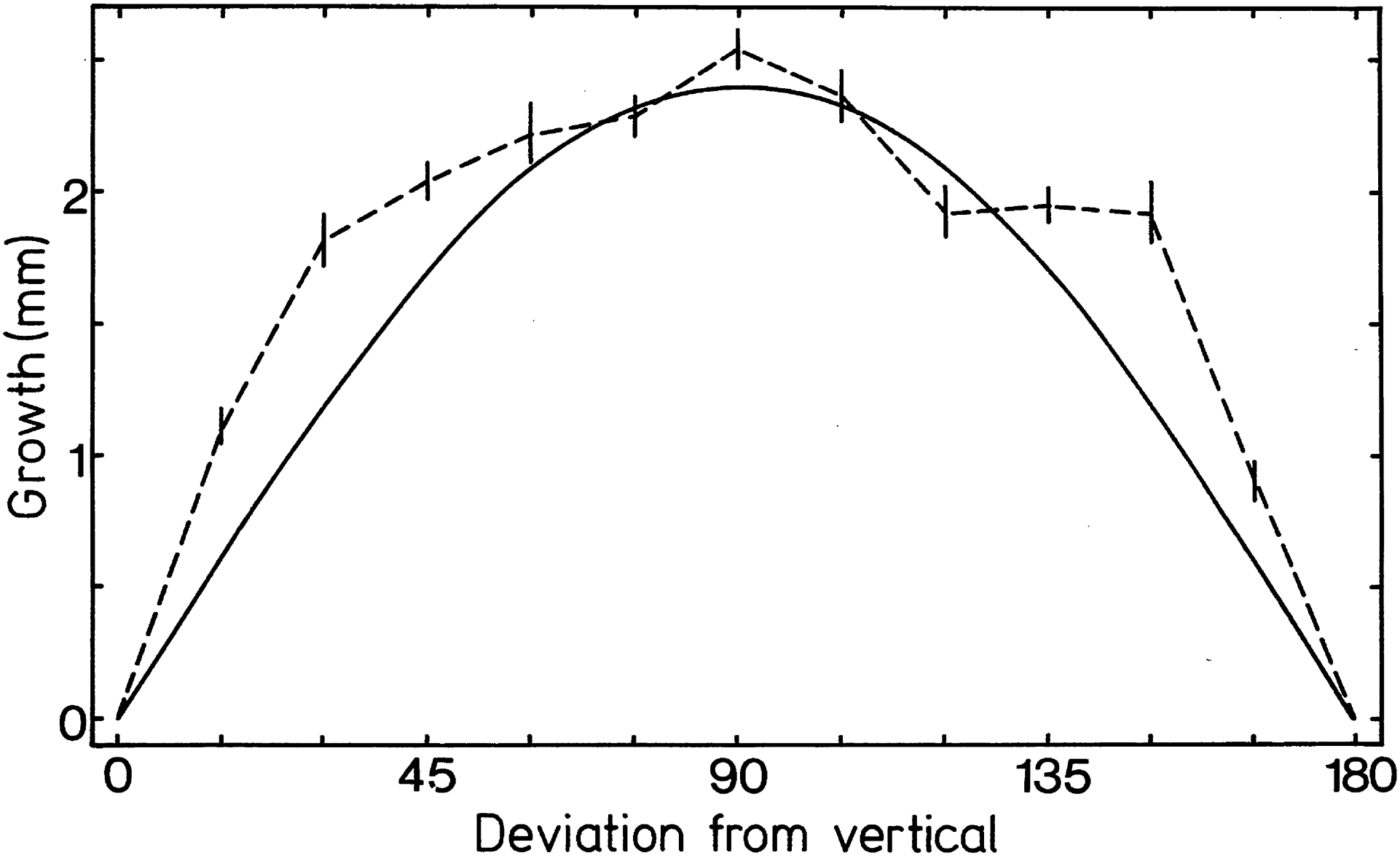

Growth (mm)
0
1
2
0
45
90
135
180
Deviation from vertical

Fig. 20.

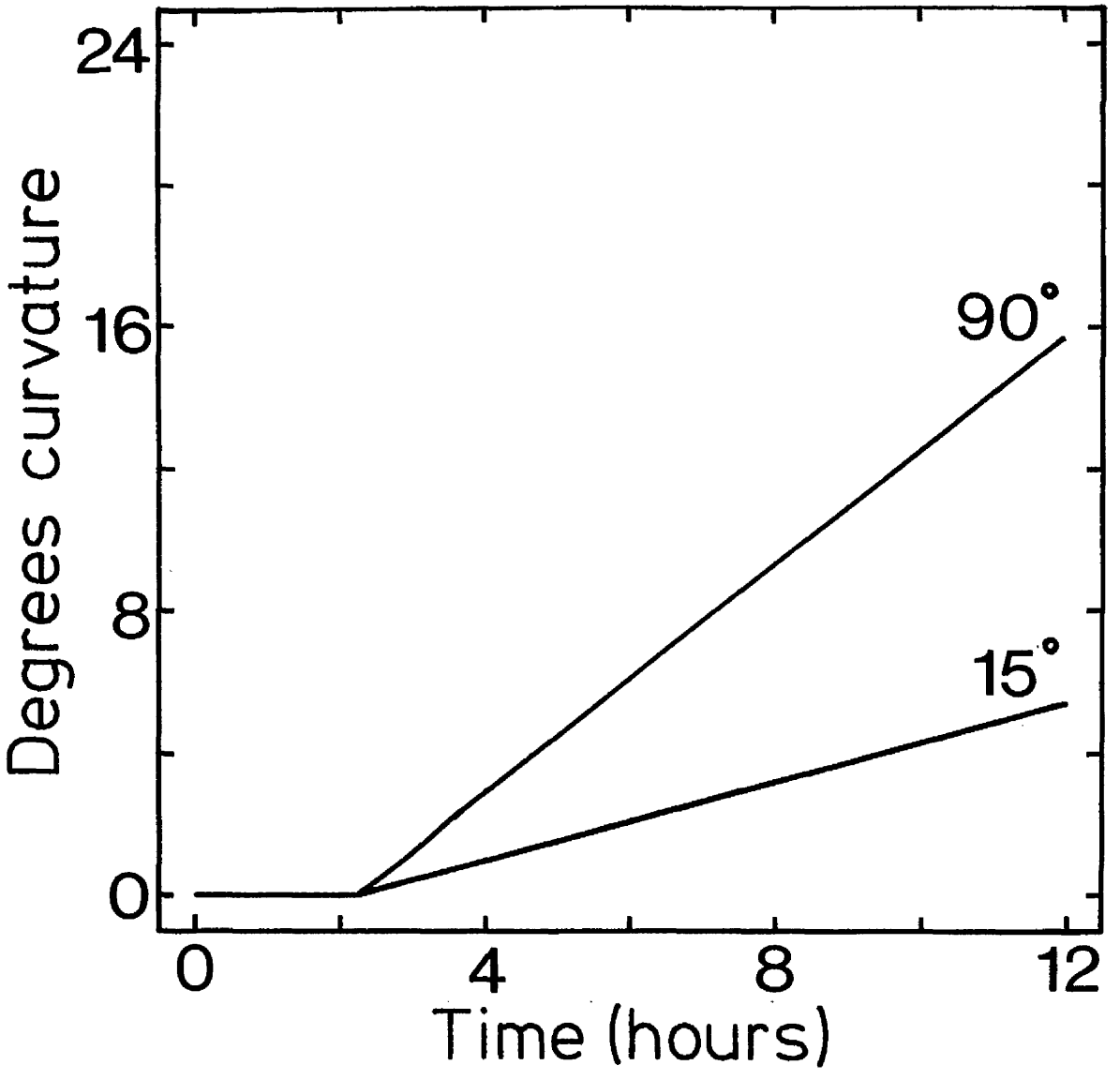
Triticum aestivum var. Kolibri.

The kinetics of the responses at 15° and 90° displacement from vertical.

Treatment: Stem segments 100 mm in length were held at either 15° or 90° displacement from vertical. Curvature was magnified through a kymograph lever and recorded on a smoke drum rotated at 50 mm h^{-1}.

White light 25°C.

Reaction time = 2.325 h.


24
16
8
0
Degrees curvature
90°
15°
0
4
8
12
Time (hours)

6. The reaction sequences of the latent and response periods

Geotropically induced curvature proceeds at a steady rate for many hours (see Fig. 9), but an initial lag period must elapse before any curvature occurs. The existence of a lag period is a characteristic of all geotropic systems, and the time involved is usually referred to as the reaction time or latent period. The reaction time constitutes a period of metabolic activity and, in order to establish the lag period observed in Fig.30 as a true reaction time, it is necessary to investigate the metabolic requirements of the organ during this period.

The effect of anoxia on the development of the geotropic response in the leaf sheath base is shown in Fig. 29. The response does not develop under nitrogen, but if the nitrogen is replaced by air the response develops normally. The reverse treatment, in which the material is transferred from air to nitrogen, results in the immediate termination of the response, and the response can therefore be considered dependent on aerobic metabolism at all stages in its development.

The development of the response in segments maintained in air is compared in Fig. 30 with the development of the response in segments which were maintained horizontally in an atmosphere of nitrogen for 2 h prior to transference to air. The requirement for oxygen during the latent period is shown by the extension of the lag period from the normal 2h-20min at 25°C in air to 4 h following the initial 2-h nitrogen pretreatment.

The temperature dependencies of the latent period and response are shown in Figs. 31A and B respectively. Both sequences are temperature dependent and the Q_{10} values of 1.81 for the latent period, and 1.80 for the response, are comparable and indicative of the operation of a chemical response sequence.

Fig. 29.

Triticum aestivum L. var. Kolibri.

The effect of anoxia on the geotropic response at the wheat node.

Treatment: Stem segments 100 mm in length were held horizontally in an atmosphere of flowing air (A) or nitrogen (N). Curvatures were measured after 24 h treatment (left hand histograms in each pair), and the treatments were changed. Curvatures were measured again after a further 24-h treatment period (right hand histograms in each pair).

White light 25°C.

Statistical Analysis. The t test was used to test the difference between mean curvatures for 24-h and 48-h responses.

t(Air/Air) = -7.895***

t(Air/Nitrogen) = -0.405^{NS}

t(Nitrogen/Nitrogen) = 0

t(Nitrogen/Air) = -14.892***

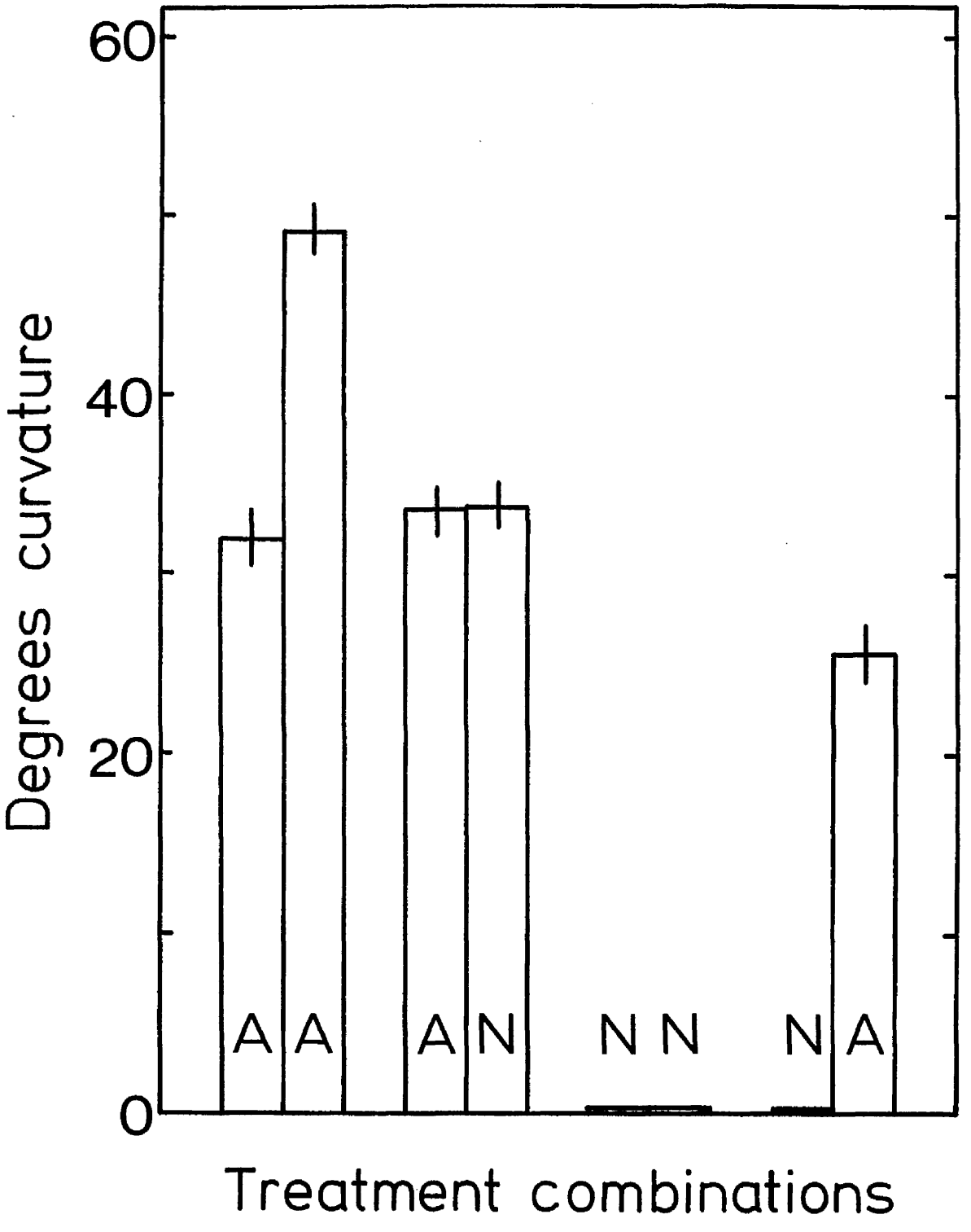

60
40
20
0
Degrees curvature
A A
A N
N N
N A
Treatment combinations

Fig. 30.

Triticum aestivum var. Kolibri.

The effect of anoxia on the latent period for the geotropic response at the wheat node.

Treatment: Stem segments 100 mm in length were held horizontally in an atmosphere of flowing air (A) or nitrogen (N) for 2 h. They were then removed to an atmosphere of air, and the development of curvature was recorded on a smoke drum rotated at 50 mm h^{-1}.

White light 25°C.

Lag periods

Latent time in air = 2.27 ± 0.057 h.

Latent time when first 2 hrs spent under nitrogen = 3.778 ± 0.111 h

Difference = 1.508 h < 2 h.

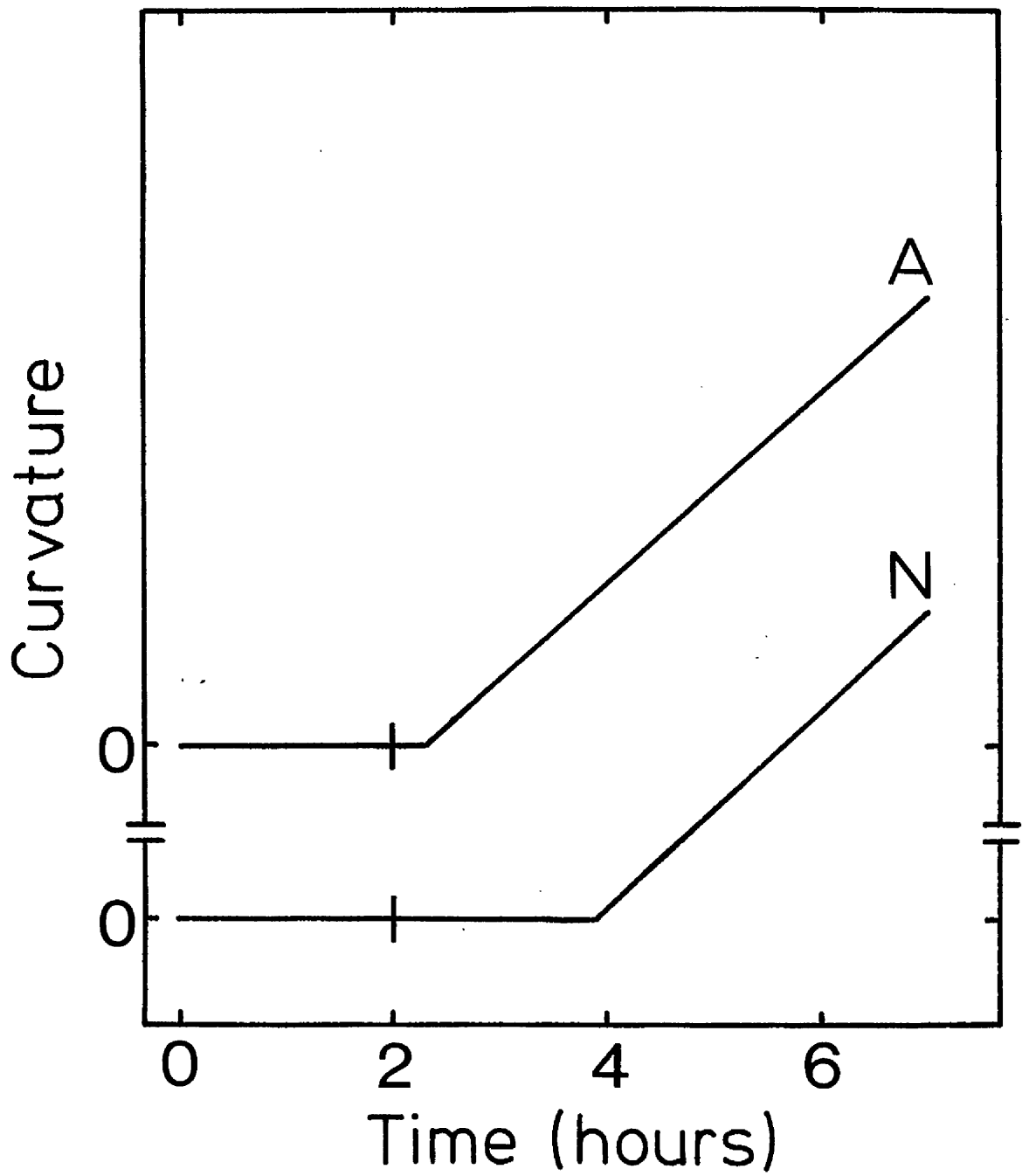

Curvature
A
N
0
0
0
2
4
6
Time (hours)

Fig. 31.

Triticum aestivum L. var. Kolibri.

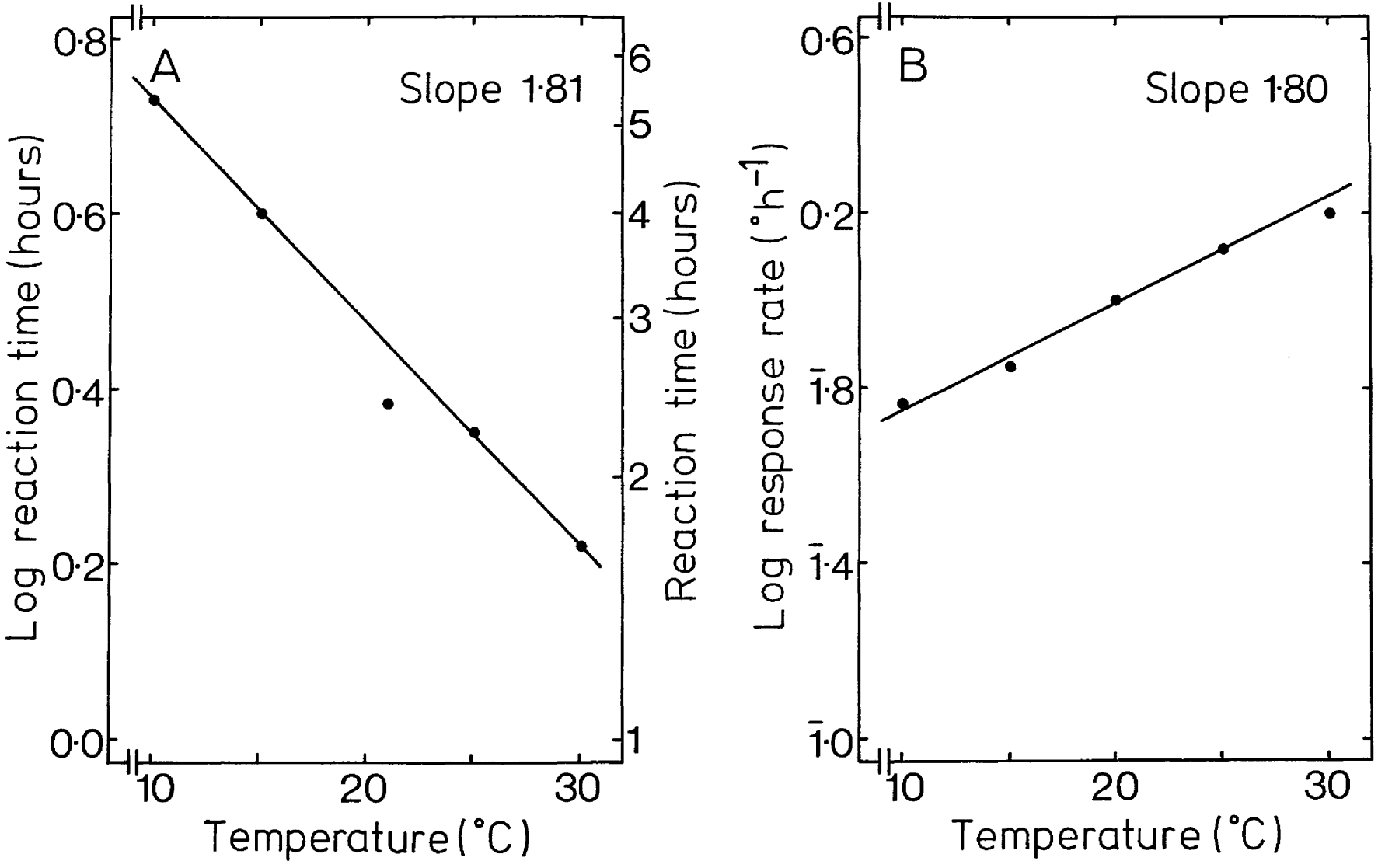
Q_{10} values for the latent period and geotropic response.

Treatment: Stem segments 100 mm in length were held horizontally and the responses at different temperatures were recorded on kymograph drums rotated at 50 mm h^{-1}. The latent periods (fig. A) and subsequent response rates (fig. B) were determined and plotted against temperature. The Q_{10} values were determined from the slopes of the lines which were fitted through the points by eye.

White light 10 to 30°C.

Q_{10} values. Latent period = 1.81.

Response = 1.80.


A
Slope 1·81
Log reaction time (hours)
Reaction time (hours)
0·8
0·6
0·4
0·2
0·0
6
5
4
3
2
1
10
20
30
Temperature (°C)
B
Slope 1·80
Log response rate (°h⁻¹)
0·6
0·2
1̄·8
1̄·4
1̄·0
10
20
30
Temperature (°C)

f. The persistence of the stimulus

All stages in the development of the response appear to be dependent on metabolism, and a knowledge of the extent to which the organ can regulate this metabolism is vital when considering the integration of the overall response. The persistence of the response on returning a stimulated segment to vertical is shown in Fig. 32. A lag period of 46.3 $\pm$ 2.5 min exists between the termination of horizontal stimulation and the termination of the response, but the curvature developed during this period is less than 0.25° compared with an initial response rate of $1.5°h^{-1}$. The recovery response proceeds maximally at a uniform rate of $1.5°h^{-1}$, but only after a lag of some 2 h, and it is attractive to speculate on the operation of identical mechanisms involving induction on opposite sides of the organ when attempting to explain the response and recovery reactions. The rapid termination of the initial response on righting the organ cannot be explained in terms of a counter reaction induced in such a manner, and it is necessary therefore to consider the question of the persistence of the stimulus. If the response is dependent on continual stimulation, then termination of the stimulus will result in the termination of the response, and the length of the lag period between the two events will reflect the number of steps in the reaction chain which are beyond the control of the receptor mechanism.

The short lag period observed in this response may indicate a short reaction chain which is closely controlled by the receptor mechanism, and the logic behind this argument will be further developed in the Discussion section.

Fig. 32.

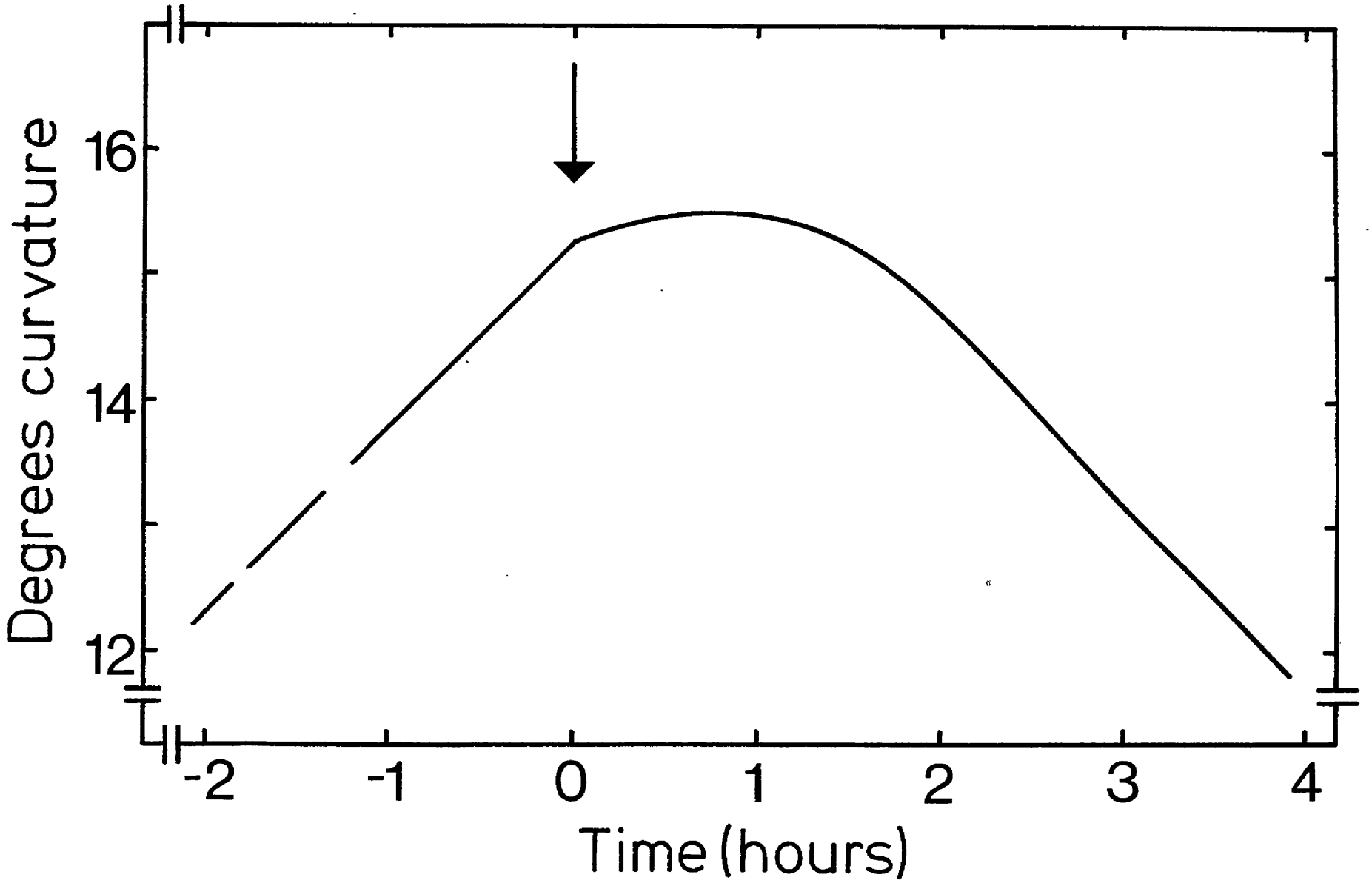
Triticum aestivum L. var. Kolibri.

The persistence of the geotropically induced response.

Treatment: Stem segments 100 mm in length were held horizontally for about 12 h and then returned to vertical (zero time at arrow). Subsequent curvature was magnified through a kymograph lever and recorded on a smoke drum which was rotated at 100 mm h^{-1}.

Measurements taken:

1. Additional curvature after righting = $0.224 \pm 0.075°$.
2. Lag between cessation of horizontal stimulation and cessation of response = 46.3 ± 2.5 min.
3. Initial steady response rate = $1.462 \pm 0.03° \ h^{-1}$.
4. Final steady recovery rate = $1.483 \pm 0.10° \ h^{-1}$.


Degrees curvature
16
14
12
-2
-1
0
1
2
3
4
Time (hours)

8. The relationship between the physiological age of the leaf sheath base and its ability to respond to geotropic stimulation

The ability to respond at the leaf sheath base is greatest in the more apical organs, and the effect of physiological age on the geotropic response sequence is therefore of interest. Data presented in Fig. 33 show that the leaf sheath base has the greatest capacity for curvature when the internode immediately above is growing rapidly. When elongation is complete the ability to bend is gradually lost and the response passes up the stem to the next leaf sheath base. The decline in responsiveness may represent the loss of either the capacity for gravi-perception or the ability to respond once stimulated, but evidence to be presented in Section 14 will show that changes in invertase activity occur in response to geotropic stimulation in organs at all stages in their development, and an effect on the capacity for gravi-perception is therefore unlikely.

The effect of the weight of tissue above the node is shown in Fig. 34. Actively bending segments are able to lift relatively considerable weights without any significant reduction in curvature, and the weight of tissue above the node must therefore be considered of marginal importance. When the internode is growing rapidly the internodal tissues immediately above the node are unthickened and the stem is supported by the leaf sheath. Wall thickening occurs as extension becomes complete, and the development of secondary walls in the leaf sheath base and surrounding tissues may be expected to exert a constraint on the development of the geotropic response. The dry weights of the leaf sheath base, leaf sheath and stem are considered in relation to both physiological age and geotropic responsiveness in Fig.35. The percentage dry matter in the lower regions of the internode rises sharply as the growth of the internode becomes complete and, because the new material remains insoluble after refluxing in a mixture of one part ethyl alcohol and two parts benzene for 30 h (Table 3), the increase in dry weight may be taken

to represent the production of materials required for the development and thickening of cell walls. The percentage dry matter present in the leaf sheaths remains stable with increasing age, but a moderate increase is observed in the leaf sheath bases, and this increase is again associated with the production of cell wall materials.

Fig. 33.

Triticum aestivum L. var. Kolibri.

The relationship between physiological age and the ability to respond.

Treatment: Stem segments 100 mm in length containing the first (N1), second (N2) or third (N3) node from the apex were laid horizontally and the ability to respond at each node was determined. The experiment was repeated at weekly intervals when the plants were at the following stages of development:-

Week 1. Elongation of the internode between nodes 3 and 2 from the apex.

Week 2. Elongation of the internode between nodes 2 and 1 just commencing. Tissues above node 1 not yet self-supporting.

Week 3. Elongation of the internode between node 1 and the inflorescence commencing. Tissues above node 1 now self-supporting.

Week 4. Elongation of internode between node 1 and the inflorescence continuing.

Week 5. Anthesis.

Week 6. Anthesis more or less complete.

Week 7. Ear developing.

White light 25°C.

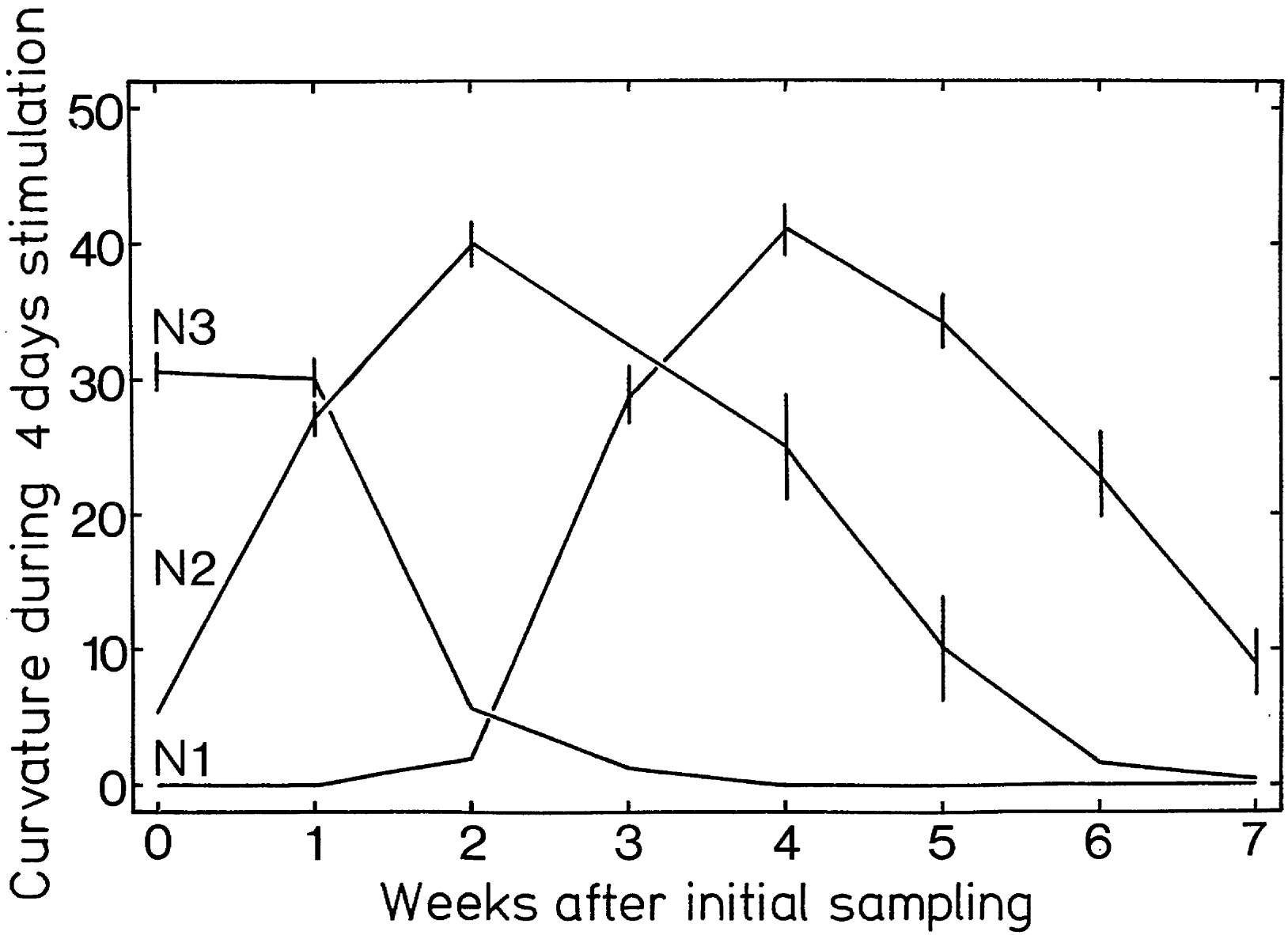

Curvature during 4 days stimulation
50
40
30
20
10
0
N3
N2
N1
0
1
2
3
4
5
6
7
Weeks after initial sampling

Fig. 34.

Triticum aestivum L. var. Kolibri.

The effect on curvature of the weight of tissue above the node.

Treatment: Stem segments 100 mm in length were laid horizontally and small leadweights were suspended from their apices. Curvature was measured after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control segments (C) and segments from which weights were suspended (W).

Weight (W)	1 gm	2 gm	3.5 gm	5 gm
t (C/W)	0.494 NS	0.230 NS	1.208 NS	2.411*

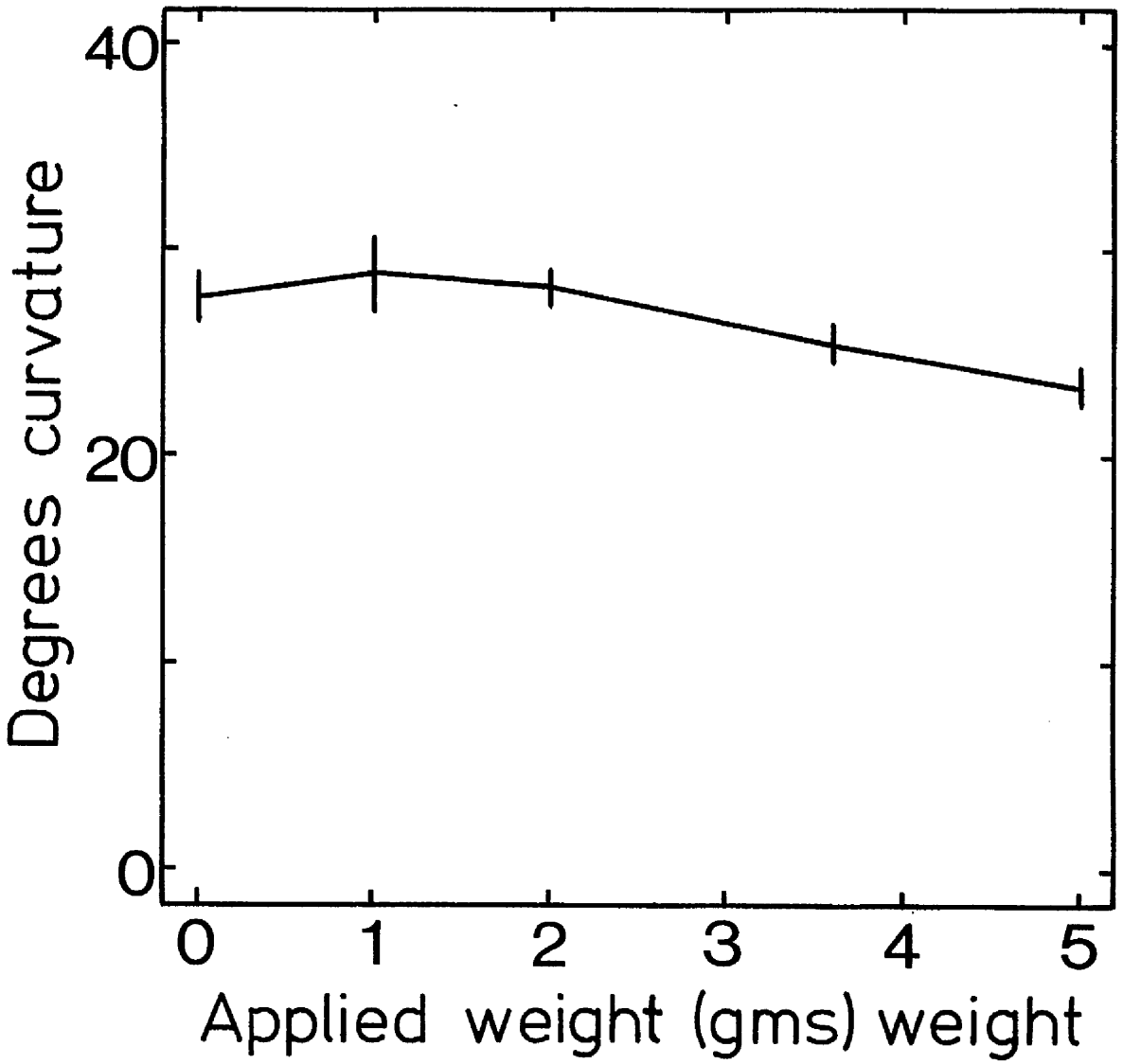

40
20
0
Degrees curvature
0
1
2
3
4
5
Applied weight (gms) weight

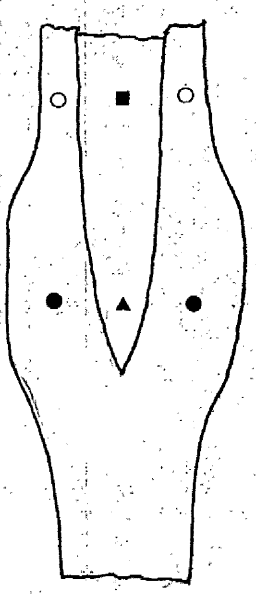
Fig. 35.

Triticum aestivum L. var. Kolibri.

The relationship between dry matter and curvature.

Treatments: 1. Stem segments 100 mm in length, containing the first, second or third node from the apex were laid horizontally. Curvatures (— — — —) were measured after a 24-h stimulation period.

2. Fresh and dry weights were recorded for four tissue regions in the vicinities of the first, second and third nodes from the apex. The percentage dry matter in each tissue region was calculated.



—●— The leaf sheath base.

—▲— The internode immediately within the leaf sheath base.

—○— The first 10 mm of leaf sheath immediately above the leaf sheath base.

—■— The internode within the first 10 mm leaf sheath above the lenf sheath base.

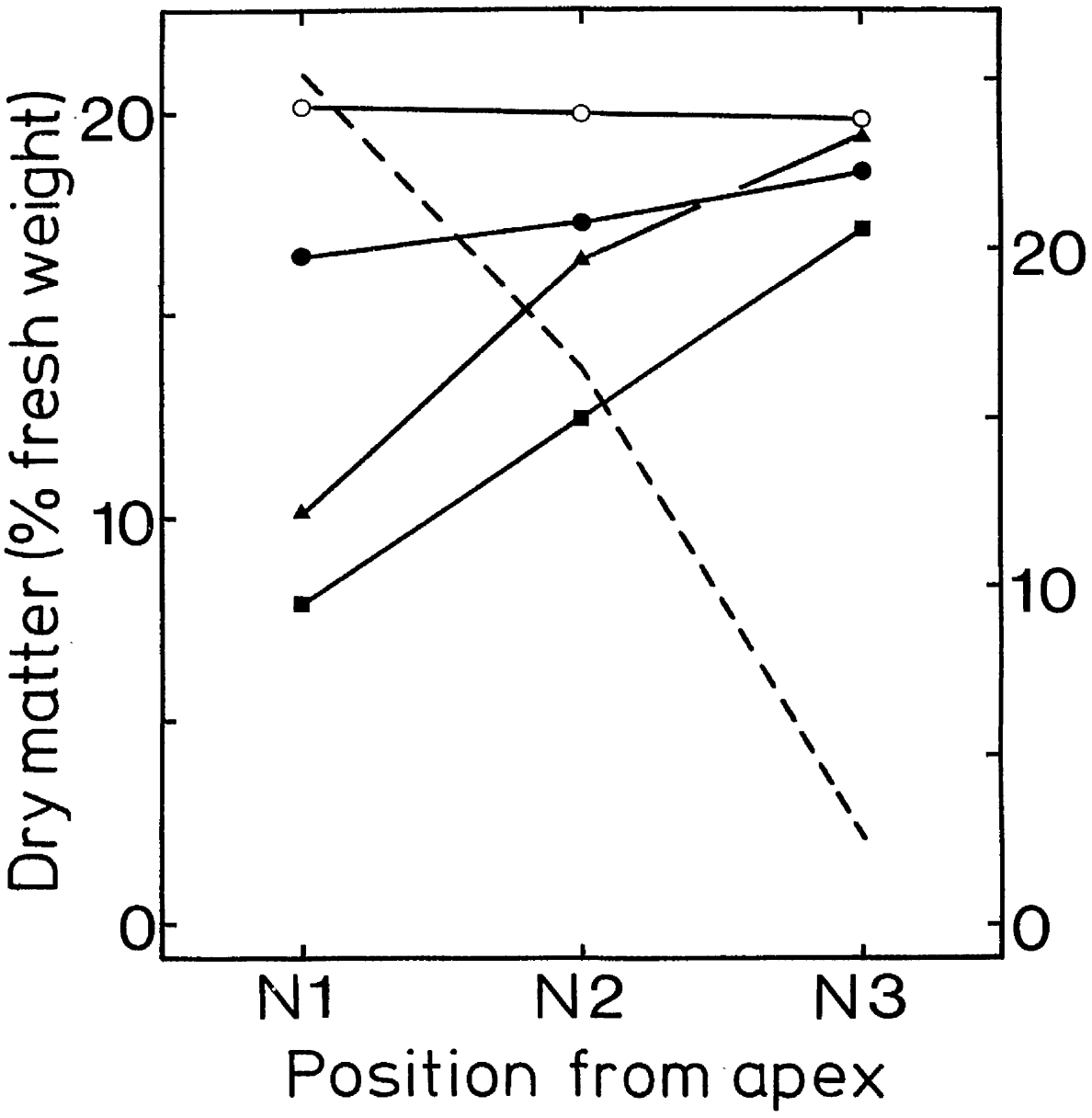
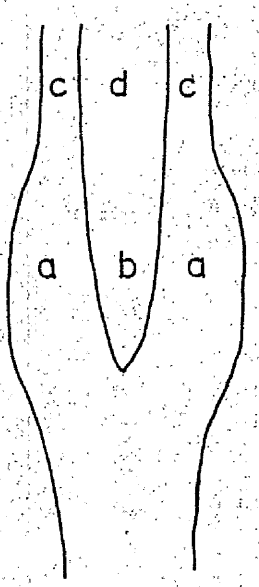

Dry matter (% fresh weight)
20
10
0
20
10
0
N1
N2
N3
Position from apex

Table 3.

Triticum aestivum L. var. Kolibri.

Composition of the tissues in the region of the node.

Treatment: Fresh weights, dry weights and dry weights after refluxing with a mixture of 1 part ethanol : 2 parts benzene were recorded for four tissue regions in the vicinities of the first, second and third nodes from the apex.



a. The leaf sheath base.

b. The internode immediately within the leaf sheath base.

c. The first 10 mm of leaf sheath immediately above the leaf sheath base.

d. The internode within the first 10 mm leaf sheath above the leaf sheath base.

Table 3

Position	Tissue Region	Fresh Weight (gm/unit)	% Fresh Weight		
			Water	Dry Matter	Dry Matter Insoluble in EtOH:C_6H_6
Node 1	a	0.06156	83.5	16.5	14.78
	b	0.04063	89.8	10.2	8.39
	c	0.03040	79.7	20.3	17.54
	d	0.00719	91.9	8.1	6.35
Node 2	a	0.03805	82.7	17.4	15.54
	b	0.05266	83.5	16.5	14.59
	c	0.02375	79.9	20.1	16.80
	d	0.01000	87.4	12.6	10.74
Node 3	a	0.02273	81.2	18.8	17.05
	b	0.04086	80.5	19.5	17.02
	c	0.01283	80.2	19.8	16.97
	d	0.00923	82.8	17.2	15.48

9. The involvement of growth regulators in the geotropic response

Several models involving the participation of growth regulators may be designed to explain the geotropic response in the leaf sheath base. These may be grouped under the following headings.

(a) Control by Inhibitor levels

> The presence of the inhibitor(s) prevents growth in the vertical organ. Inhibitor levels on the lower side of the organ decrease in response to geotropic stimulation, permitting growth in this region.

The decrease in inhibitor levels may result from differential rates of destruction or from lateral transport of the inhibitor away from the lower side of the organ.

(b) Control by Inhibitor-Promoter interaction

> The presence of an inhibitor prevents growth in the vertical organ. The effect is offset on the lower side of the geotropically stimulated organ by an increase in relative concentration of a growth promoter.

The change in concentration may result from differential rates of destruction of the inhibitor(s) or synthesis of the promoter(s). It may also result from the lateral transport of inhibitors, promoters, or both.

(c) Control by Promoter levels

> Promoters, if present in control material, are not sufficiently concentrated to induce growth. Promoter levels on the lower side of the organ increase in response to geotropic stimulation and induce growth in this region.

The increase in promoter levels may result from differential rates of synthesis, differential rates of longitudinal transport into the leaf sheath base, or lateral transport into the lower half of the geotropically stimulated organ.

Evidence from barrier experiments (see Fig. 13) and from the demonstration

that segments excised from the leaf sheath base will grow if correctly orientated (Fig. 14), argues against the involvement of lateral transport systems, but lateral redistribution within the segments may be sufficient to allow growth, especially if coupled with differential sensitivity in the tissues.

The fact that segments excised from the leaf sheath base will grow only if correctly orientated (Fig. 14) may be used in the development of bioassays to examine the role of chemical growth promoters and inhibitors. Segments orientated as 'uppers' are not geotropically induced, and serve as controls when assessing the effects of promoters, whilst segments orientated as 'lowers', which are geotropically induced, serve as controls when considering the effect of inhibitors on the geotropic response. The effect of promoters on this geotropically induced material may also be tested.

Experiments involving the use of non-induced (uppers) and geotropically induced (lowers) segments in 24-h straight growth assays have been conducted to test the effects of the plant growth regulators indole-3-acetic acid (Fig. 36), gibberellic acid (Fig. 37), kinetin (Fig. 38), abscisic acid (Fig. 39) and coumarin (Fig. 40). Both gibberellic acid and kinetin are without effect even when 2% sucrose is provided as a carbohydrate source, but IAA is highly effective in inducing growth in unstimulated material. A comparison between the broken and solid lines in Fig. 36 shows that the size of the IAA induced response in 'upper' segments approaches that of the geotropically induced response in 'lower' segments, but at no concentration is IAA able to promote the growth of geotropically induced segments above the control value.

No evidence for the involvement of inhibitors has been forthcoming from this investigation. Abscisic acid (Fig. 39) and coumarin (Fig. 40) are both without effect when applied to geotropically induced material.

The relationship between IAA induced growth and the geotropic response has been investigated with the aid of the synthetic growth regulator CFM.

Fig. 36.

Triticum aestivum L. var. Kolibri.

The effect of IAA on the growth of excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (-- — --) or 'lowers' (————) in 50 mm petri dishes containing 2.5 ml of a solution of IAA in the absence or presence of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-4} M IAA treatments.

	t values	
	+ 2% sucrose	No sucrose
Lowers	$t(0/10^{-4}M) = 1.608^{NS}$	$t(0/10^{-4}M) = 3.011^{**}$
Uppers	$t(0/10^{-4}M) = -20.630^{***}$	$t(0/10^{-4}M) = -8.245^{***}$

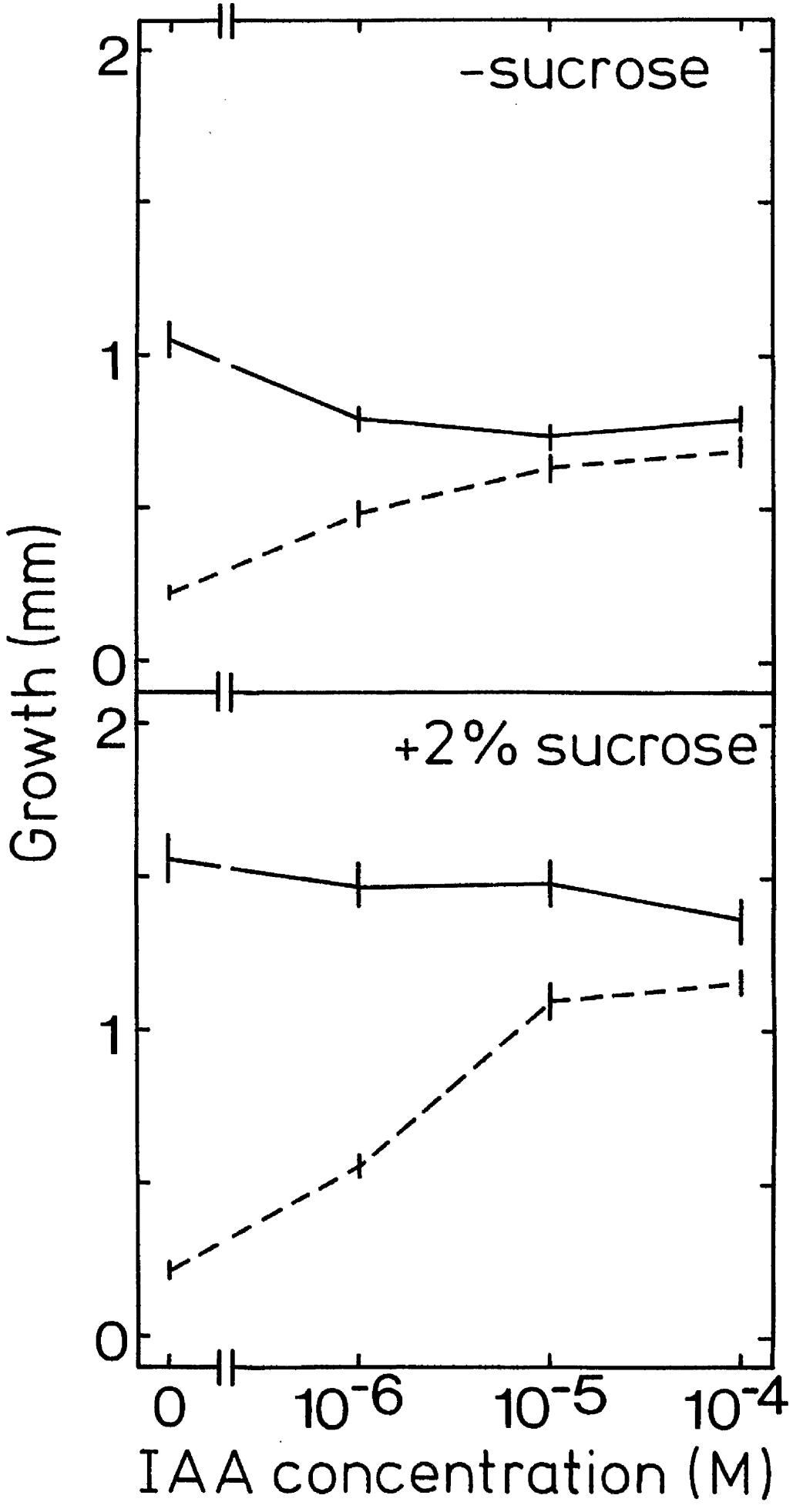

-sucrose
+2% sucrose
Growth (mm)
2
1
0
2
1
0
0
10^{-6}
10^{-5}
10^{-4}
IAA concentration (M)

Fig. 37.

Triticum aestivum L. var. Kolibri.

The effect of GA_3 on the growth of excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (– – – –) or 'lowers' (————) in 50 mm petri dishes containing 2.5 ml of a solution of GA_3 in the absence or presence of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-3}M GA_3 treatments.

	t values	
	+ 2% sucrose	No sucrose
Lowers	$t(0/10^{-3}M) = 0.970^{NS}$	$t(0/10^{-3}M) = 1.106^{NS}$
Uppers	$t(0/10^{-3}M) = -1.800^{NS}$	$t(0/10^{-4}M) = 2.036^{NS}$

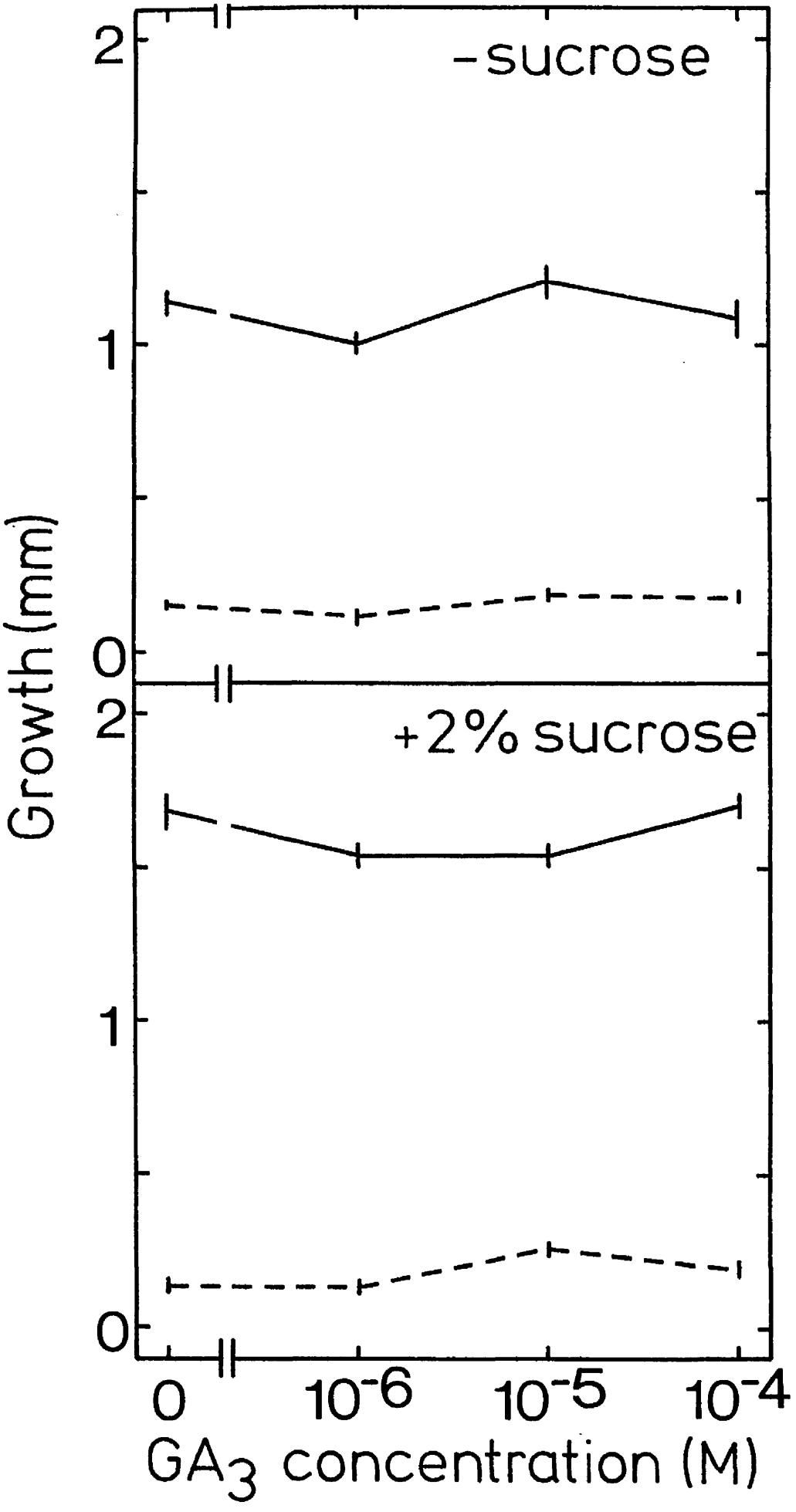

-sucrose
+2% sucrose
Growth (mm)
2
1
0
2
1
0
0
10^{-6}
10^{-5}
10^{-4}
GA_3 concentration (M)

Fig. 38.

Triticum aestivum L. var. Kolibri.

The effect of kinetin on the growth of excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (— — —) or 'lowers' (——————) in 50 mm petri dishes containing 2.5 ml of a kinetin solution. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Growth (mm)
1·5
1·0
0·5
0·0
0
10^{-8}
10^{-6}
K concentration (M)

Fig. 39.

Triticum aestivum L. var. Kolibri.

The effect of ABA on the growth of excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (– – –) or 'lowers' (———) in 50 mm petri dishes containing 2.5 ml of a solution of ABA in the absence or presence of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period.

White light. 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-4}M ABA treatments.

	t values	
	+ 2% sucrose	No sucrose
Lowers	$t(0/10^{-4}M) = 0.967^{NS}$	$t(0/10^{-4}M) = 1.051^{NS}$

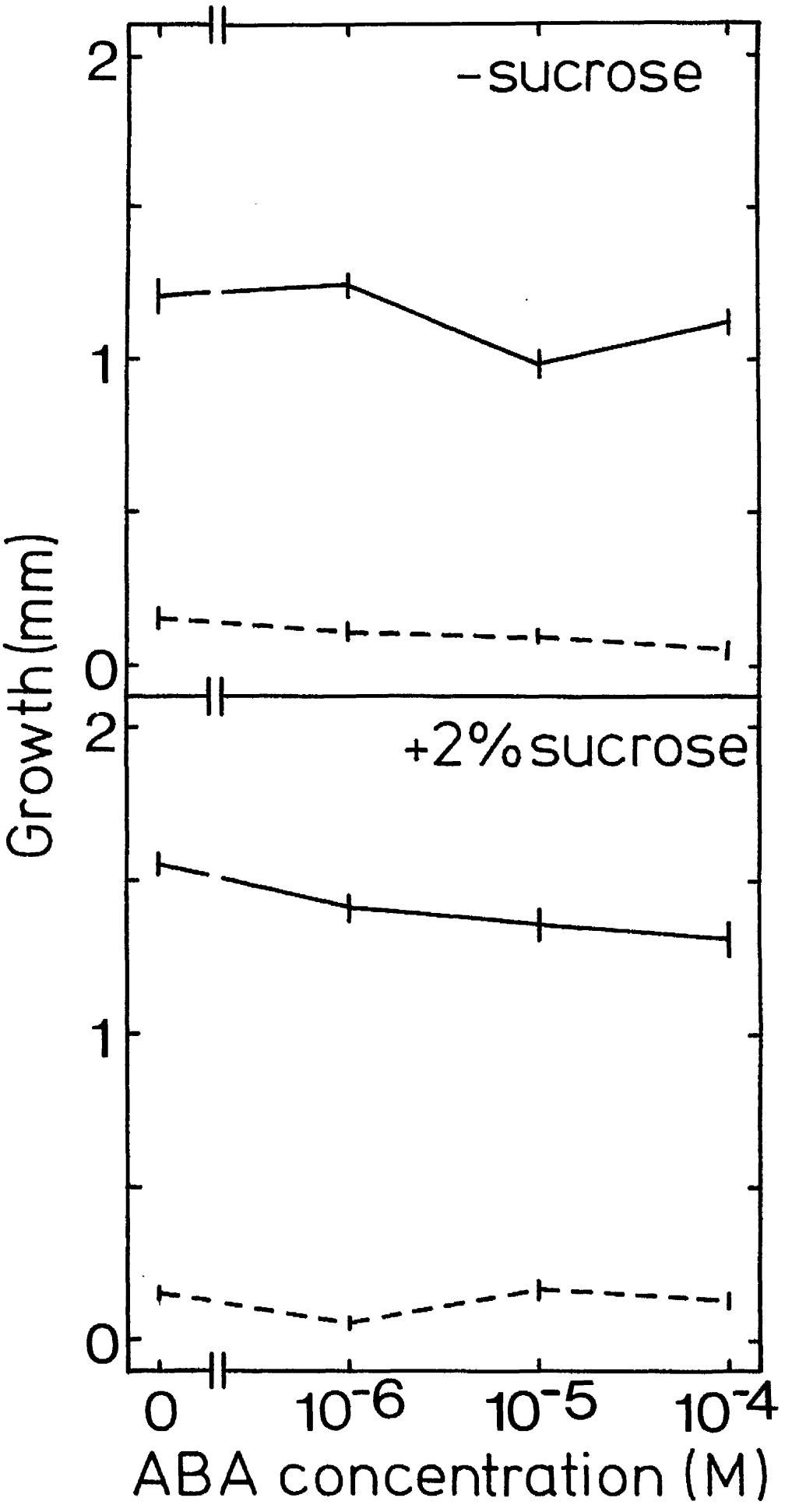

-sucrose
+2% sucrose
Growth (mm)
2
1
0
2
1
0
0
10^{-6}
10^{-5}
10^{-4}
ABA concentration (M)

Fig. 40.

Triticum aestivum L. var. Kolibri.

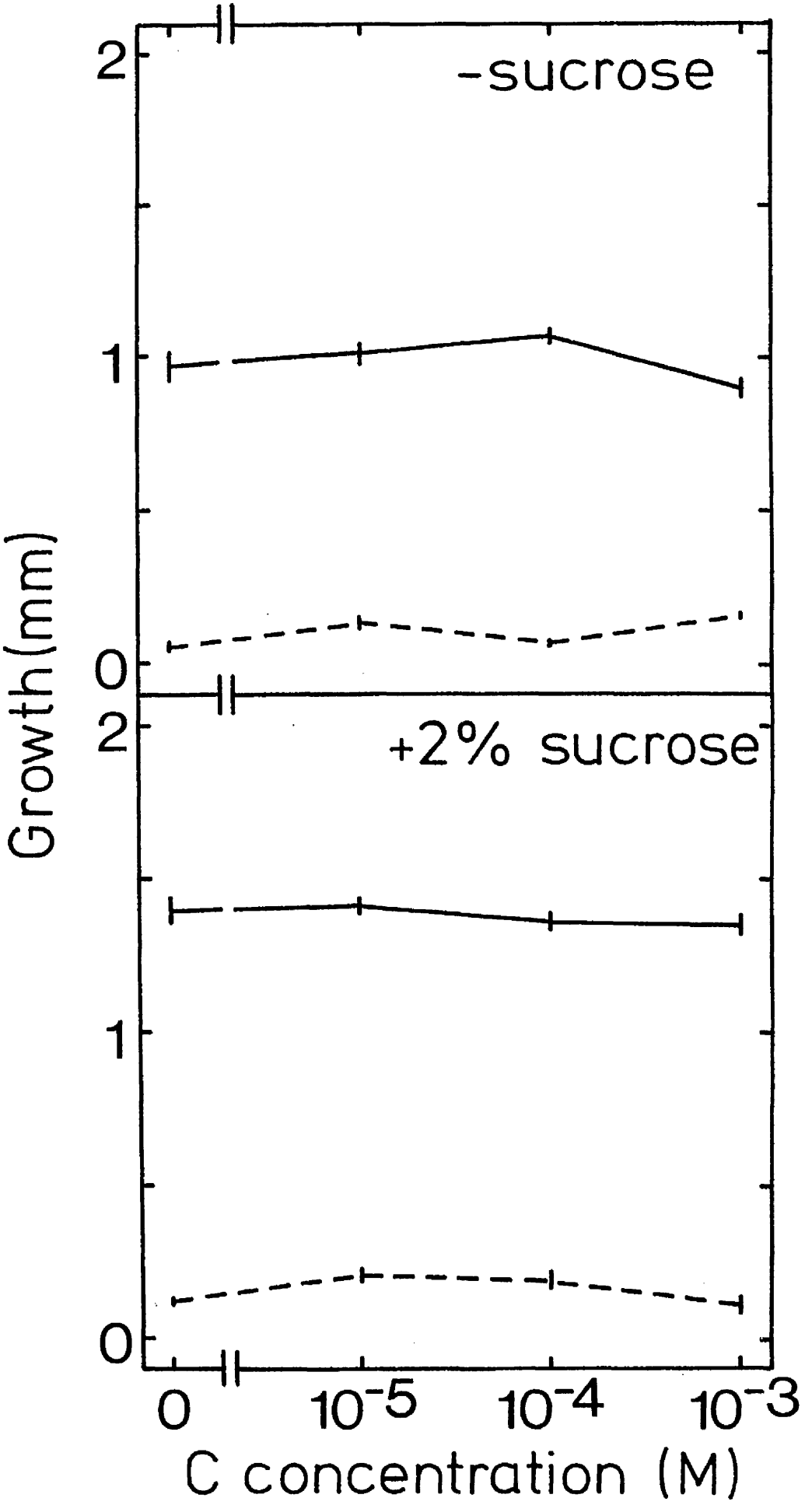
The effect of coumarin (C) on the growth of excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (– – –) or 'lowers' (———) in 50 mm petri dishes containing 2.5 ml of a solution of coumarin in the absence or presence of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-3}M coumarin treatments.

	t values	
	+ 2% sucrose	No sucrose
Lowers	$t(0/10^{-3}M) = 0.722^{NS}$	$t(0/10^{-3}M) = 1.425^{NS}$


-sucrose
+2% sucrose
Growth(mm)
2
1
0
2
1
0
0
10^{-5}
10^{-4}
10^{-3}
C concentration (M)

This substance is a member of a group of growth regulators known as the morphactins, and one of the characteristic properties of this group is the ability to abolish the tropic responses. The effects of CFM on geotropically induced growth in excised segments and curvature in 100 mm stem segments are shown in Figs. 41 and 42 respectively. The morphactin inhibits the growth of geotropically induced segments at a concentration of 10^{-7}M and it abolishes the response completely at a concentration of 10^{-5}M. A similar effect is observed when curvature in 100 mm stem segments is determined following pretreatment by submersion in CFM.

Fig. 41.

Triticum aestivum L. var. Kolibri.

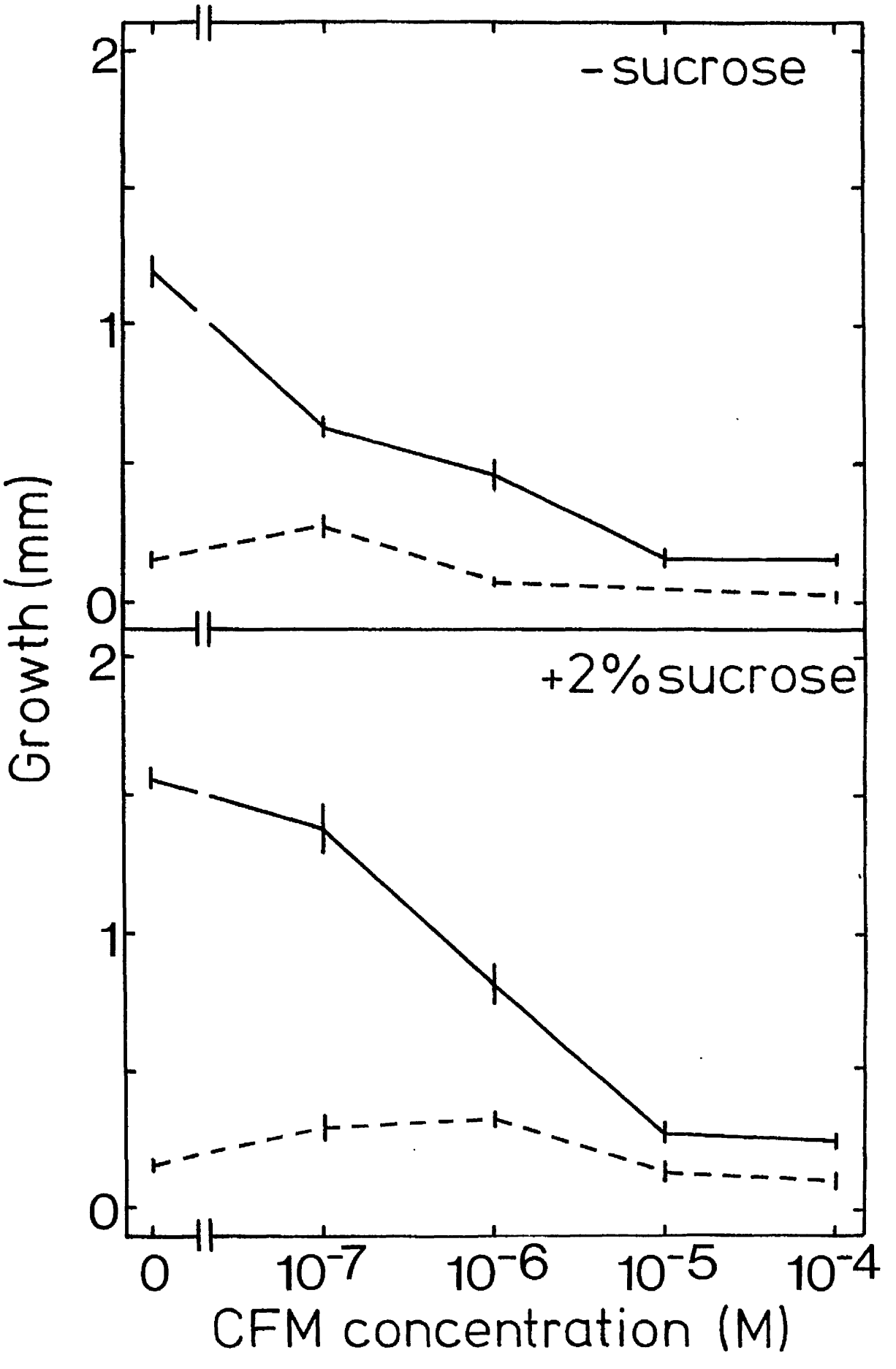
The effect of CFM on the growth of excised leaf sheath bases.

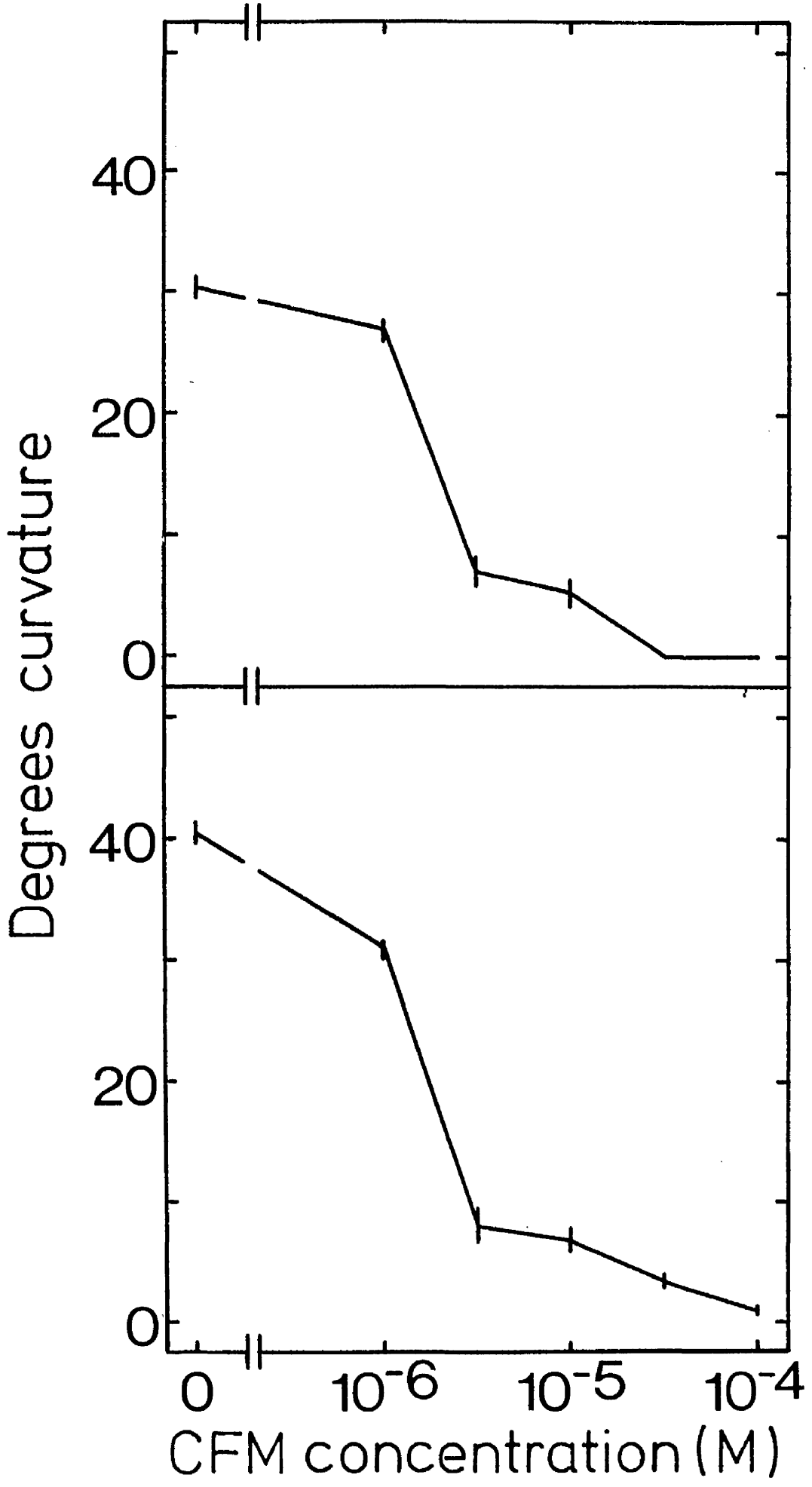
Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (– – – –) or 'lowers' (————) in 50 mm petri dishes containing 2.5 ml of a solution of CFM in the absence or presence of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-4}M CFM treatments.

	t values	
	+ 2% sucrose	No sucrose
Lowers	$t(0/10^{-4}M) = 29.863^{***}$	$t(0/10^{-4}M) = 14.375^{***}$


-sucrose
+2% sucrose
Growth (mm)
2
1
0
2
1
0
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
CFM concentration (M)


40
20
0
40
20
0
Degrees curvature
0
10-6
10-5
10-4
CFM concentration (M)

10. The mode of action of the morphactins

A. The auxin transport system in coleoptiles

Whilst there is reasonable evidence in the literature to support the hypothesis that the morphactins interfere with the auxin transport system, their exact mode of action is by no means clear. The geotropic response may be inhibited by a termination of the auxin supply to the growing zone, or by a more specific abolition of the lateral transport system. A randomization in the direction of transport may also account for the inhibition, and this latter suggestion could explain the promotion of growth in certain plant systems following morphactin treatment. The response to the morphactins could also be explained in terms of an effect on auxin metabolism or synthesis.

In an attempt to clarify the mechanism of action the effects of CFM on the geotropic responses in cereal coleoptiles have been examined. Zea seedlings grown in the presence and absence of 10^{-5}M CFM are shown in Plates 10A and B respectively. The seedlings grown in the presence of the morphactin are unable to reorientate themselves with respect to gravity. Plates 11A and B show the sedimentation of starch grains in morphactin treated Zea coleoptile apices. Sedimentation of starch grains remains unaffected but, as seen from Fig. 43, the capacity for polar auxin transport is markedly reduced by the treatment. Coleoptile segments excised from Zea seedlings grown in the presence of 10^{-5}M CFM are incapable of polar auxin transport (Fig. 43A), but acropetal movement is not affected by the treatment (Fig. 43B). Identical results are obtained when coleoptiles taken from seedlings grown normally in vermiculite are submerged vertically in 10^{-5}M CFM for 1 h prior to the transport period (compare Figs. 43C and A; D and B), and this latter procedure has been used for the subsequent morphactin experiments.

Dosage response curves for the effect of CFM on polar auxin transport are shown for Zea coleoptile segments in Fig. 44, and for Avena coleoptile segments in Fig. 45. Basipetal transport is abolished when CFM is supplied

Plate 10.

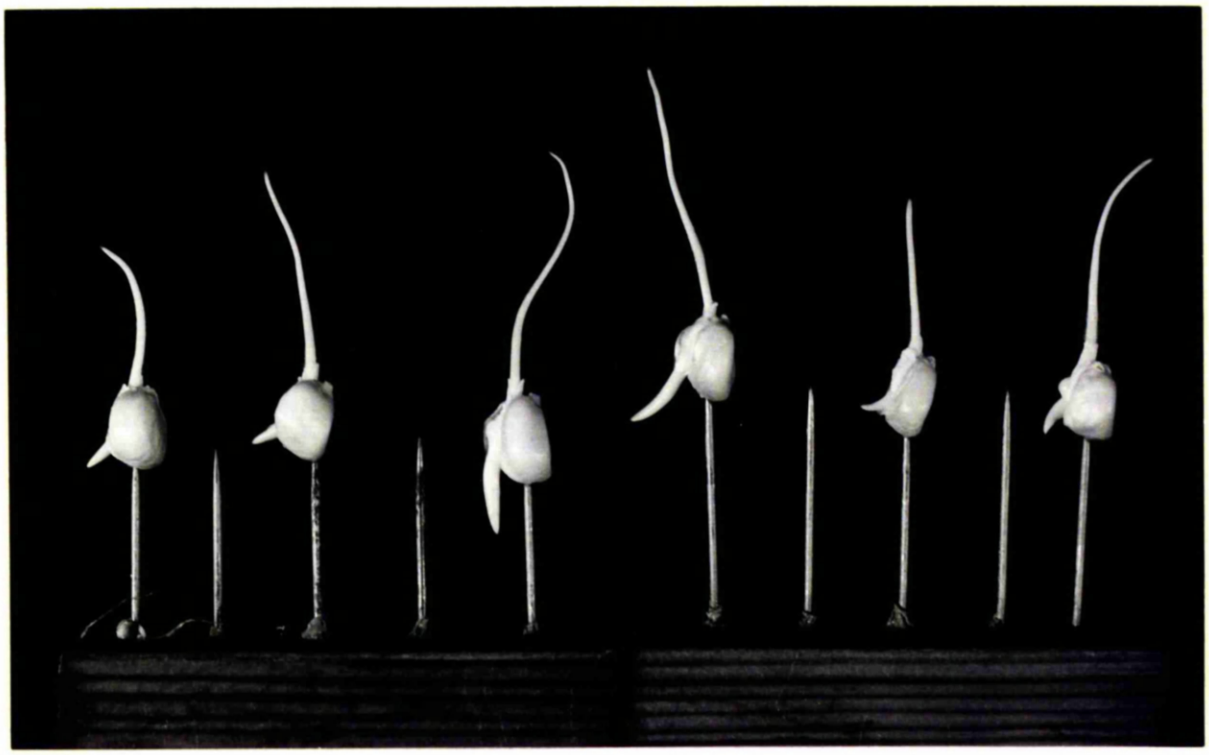
Zea mays L. var. Burpee Snowcross.

The effect of morphactin on the development of Zea seedlings.

Treatment: Zea seeds were imbibed in either 10^{-5}M CFM or distilled water. They were then impaled on steel pins and surrounded in cotton wool moistened with either 10^{-5}M CFM (A) or distilled water (B). The seeds were orientated so that the coleorhiza would emerge vertically upwards. Seedlings were photographed after 72 h treatment.

Darkness 25°C.

A



B

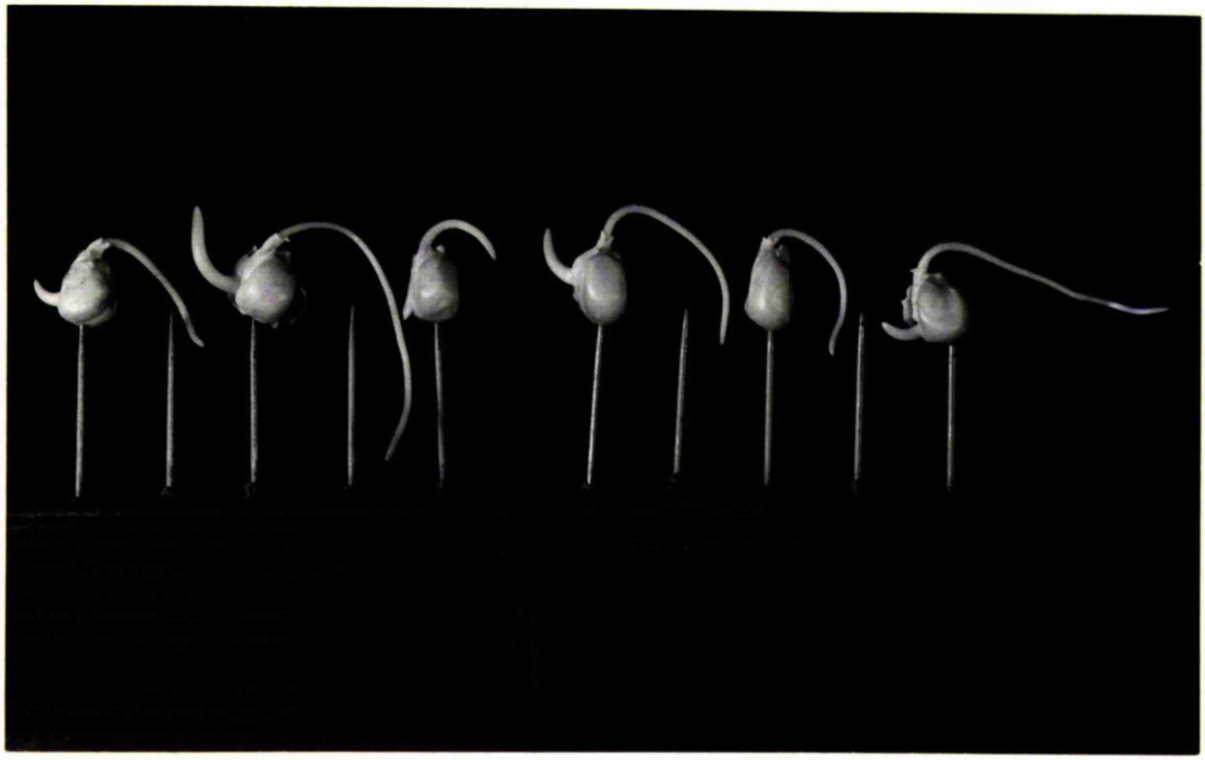
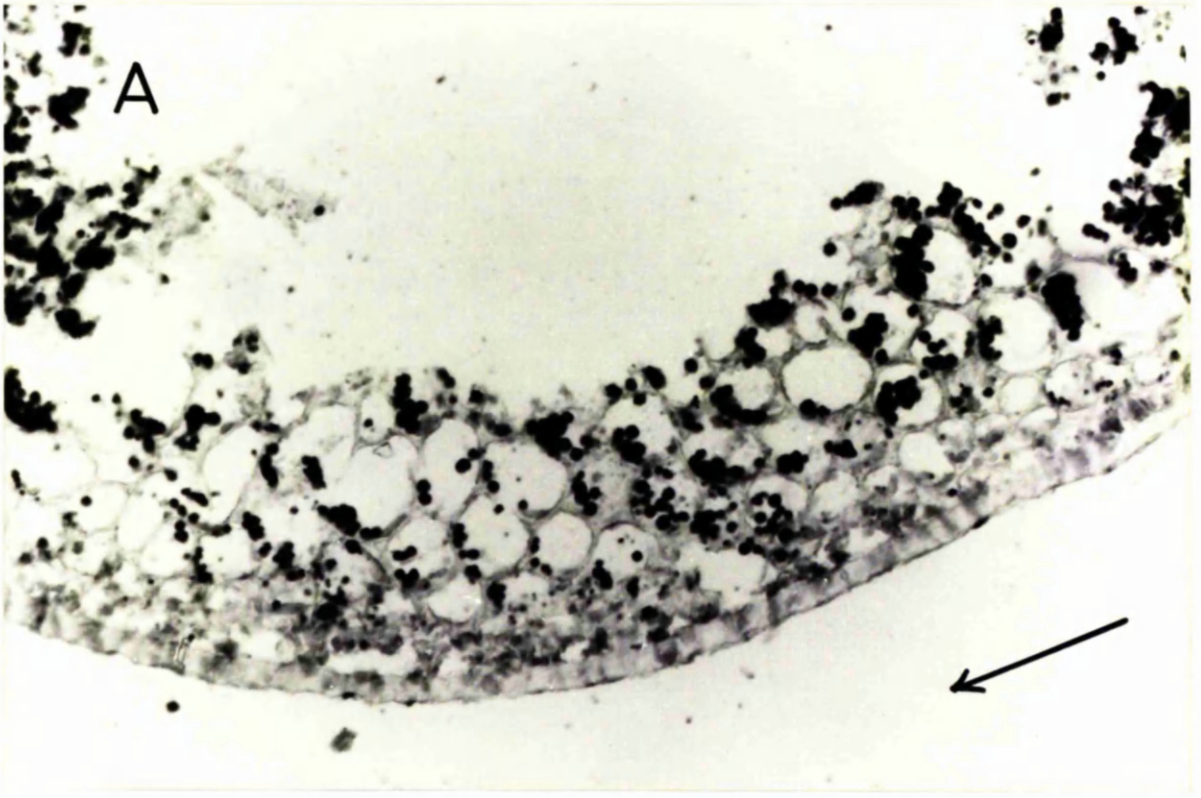


Plate 11.

Zea mays L. var. Burpee Snowcross.

The effect of morphactin on the sedimentation of starch grains in coleoptile apices.

Treatment: Excised coleoptiles 10 mm in length were pretreated by vertical submersion in a solution of 10^{-5}M CFM for 1 h. They were then removed and placed horizontally for 15 mins. after which time apices were excised and sectioned on a freezing microtome. Sections were stained in iodine solution. The sedimentation of starch grains is shown in Fig. A, and again at higher magnification in Fig. B. The arrows indicate the direction of the gravity force vector.


A

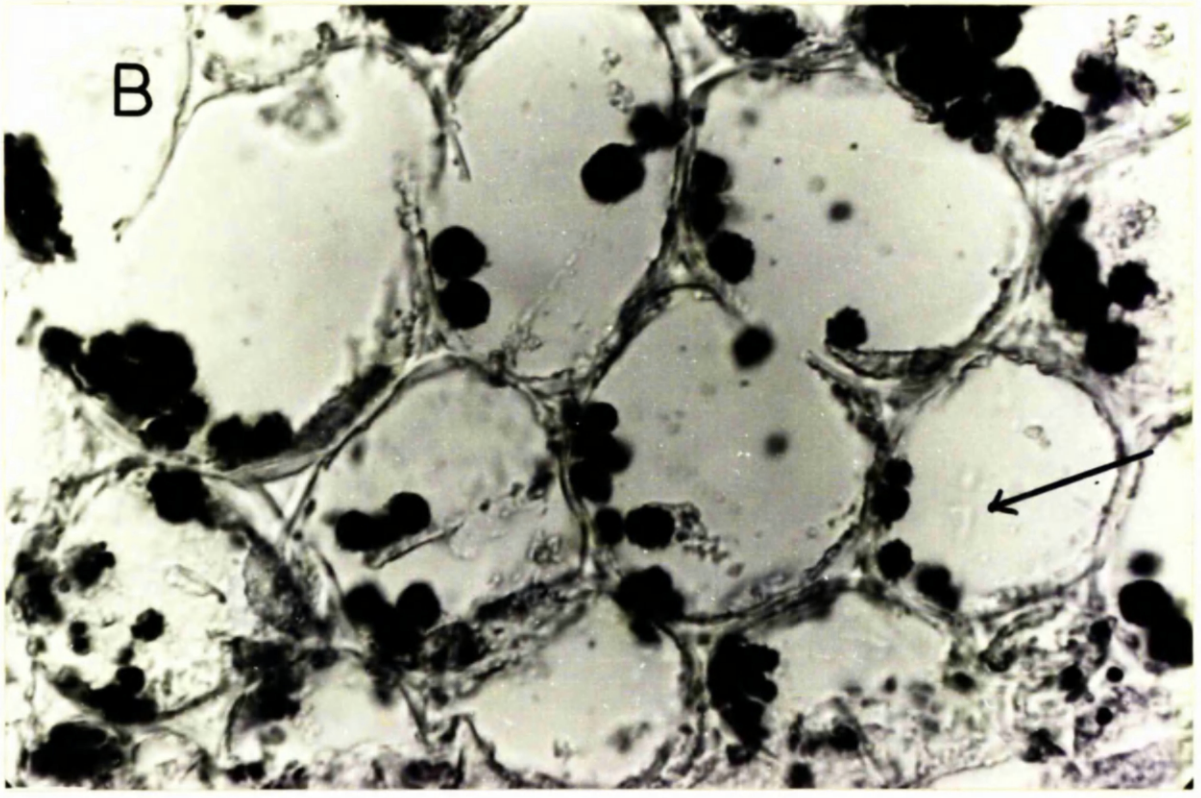

B

Fig. 43.

Zea mays L. var. Burpee Snowcross.

The effect of CFM pretreatment on polar auxin transport.

Treatments. Two pretreatments were examined. In the first (Figs. A & B) Zea seeds were planted on cotton wool moistened with 10^{-5}M CFM (·····) or distilled water (————) following imbibition in 10^{-5}M CFM and distilled water respectively. In the second (Figs. C & D) the seedlings were grown normally in vermiculite and the excised coleoptiles were pretreated by submersion in a solution of 10^{-5}M CFM (·····) or distilled water (————) for 1 h. The acropetal (Figs. B & D) and basipetal (Figs. A & C) movements of IAA-5-^{3}H in 10 mm coleoptile segments excised 1 mm beneath the apex were determined after a 2-h transport period.

Darkness 25°C.

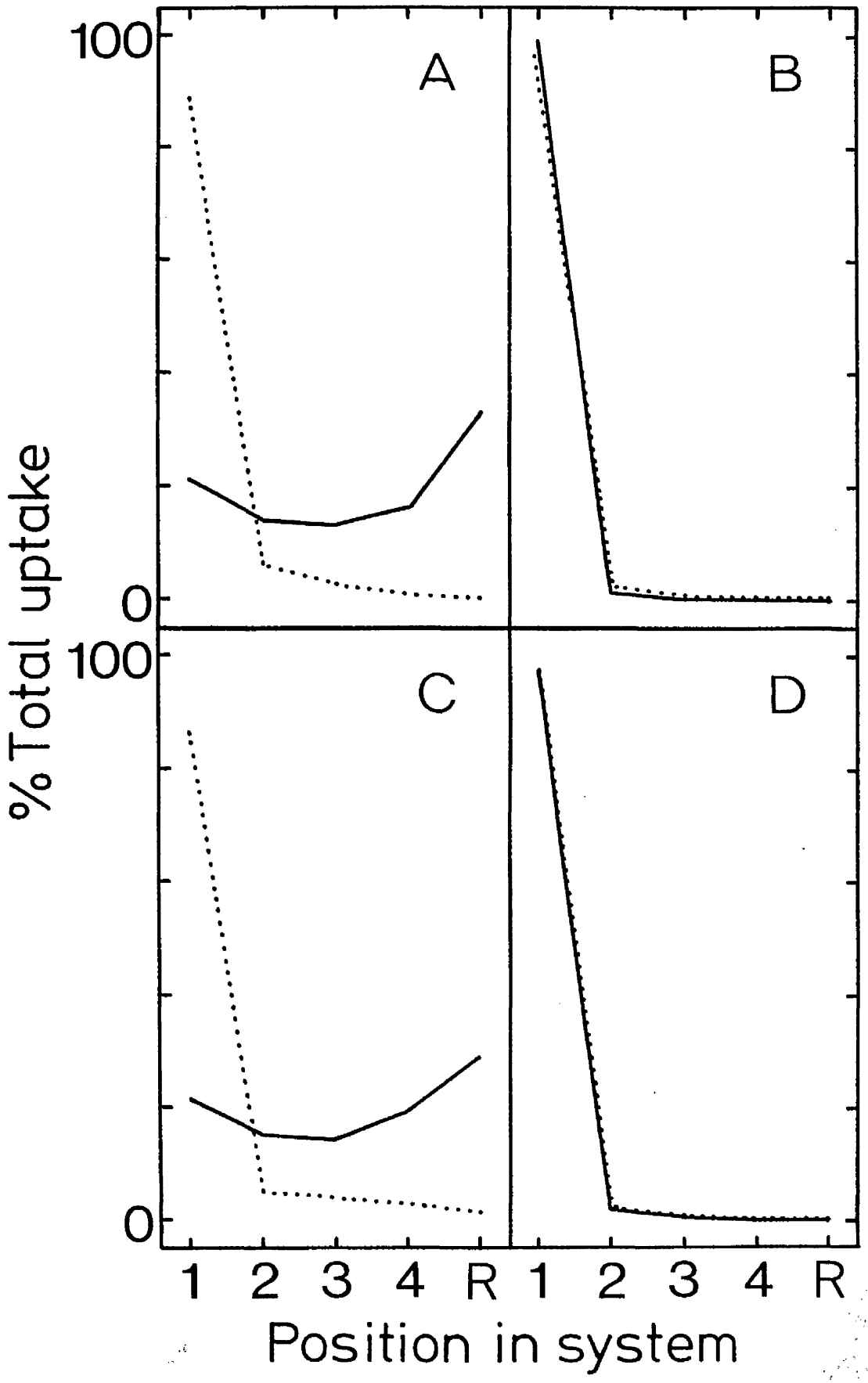

100
A
B
0
% Total uptake
100
C
D
0
1 2 3 4 R 1 2 3 4 R
Position in system

Fig. 44.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on the polarity of IAA movement.

Treatment: *Zea* coleoptile segments were pretreated by submersion in one of a range of concentrations of CFM for 1 h. The basipetal (A) and acropetal (B) movements of IAA-5-^{3}H were determined after a 2-h transport period. Radioactivity is expressed as percentage distribution through the system.

Darkness 25°C.

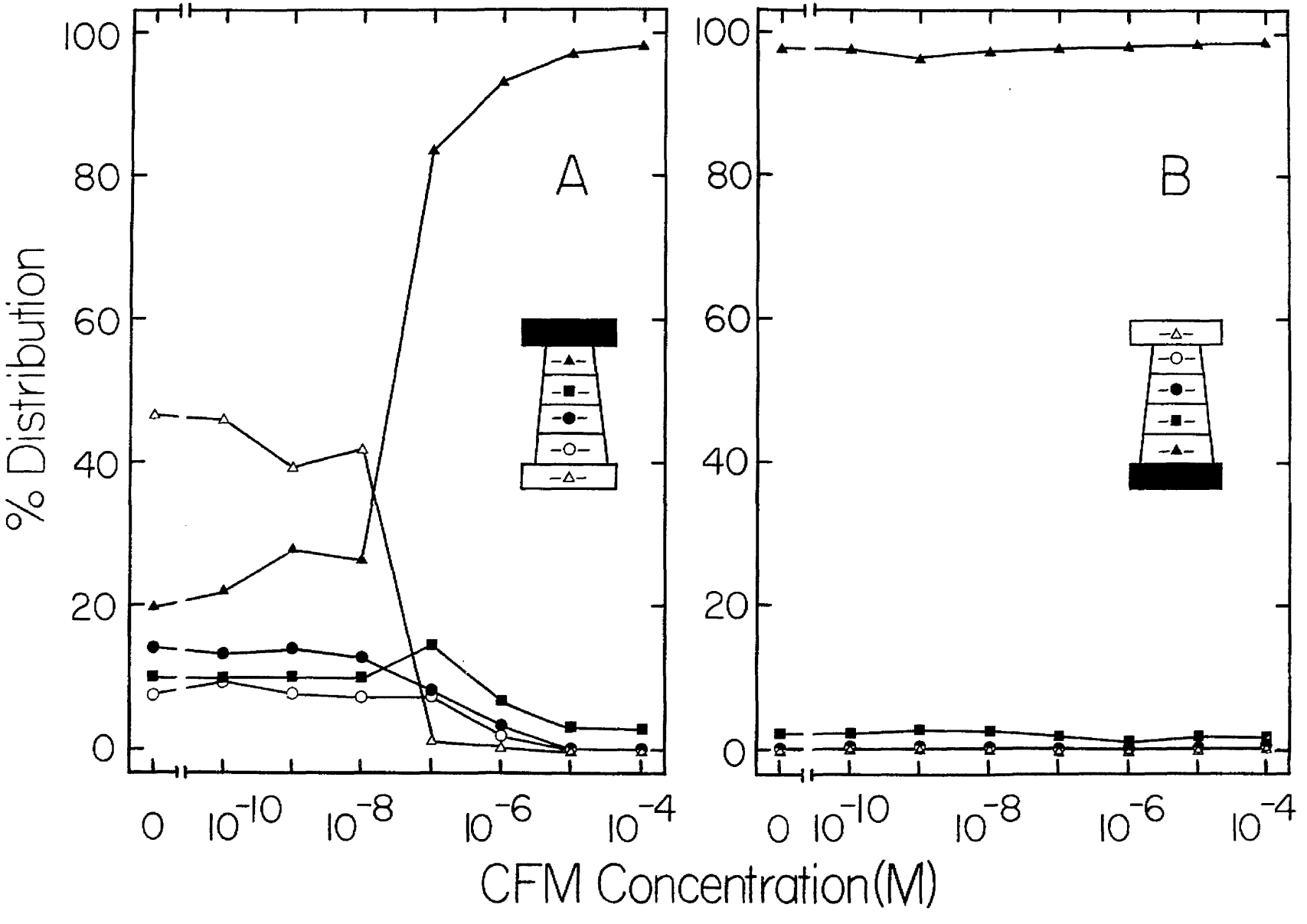

100
80
60
40
20
0
% Distribution
A
B
0
10^{-10}
10^{-8}
10^{-6}
10^{-4}
CFM Concentration(M)

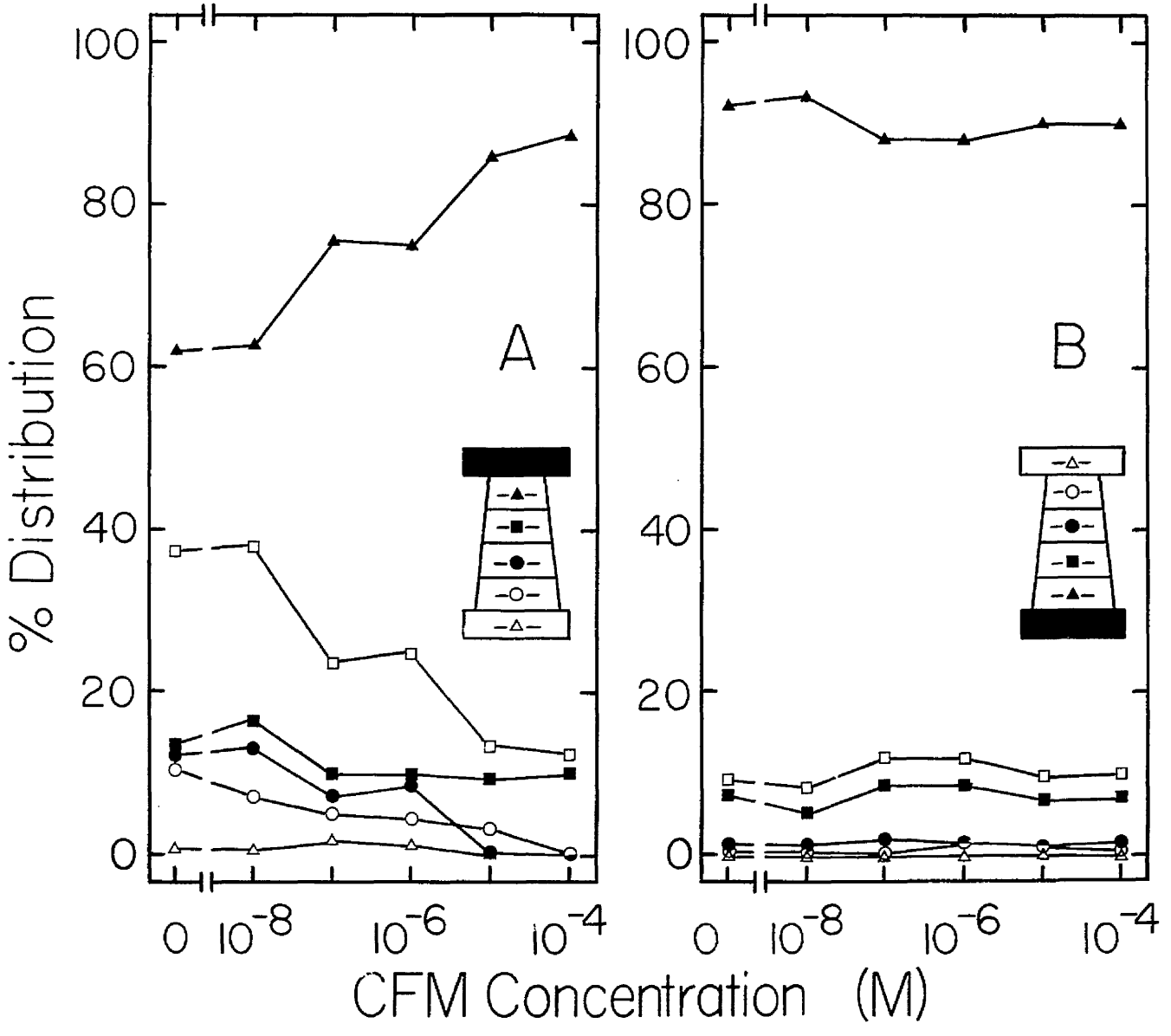
Fig. 45.

Avena sativa L. var. Svalov Victory.

The effect of CFM on the polarity of IAA movement.

Treatment: *Avena* coleoptile segments were pretreated by submersion in one of a range of concentrations of CFM for 1 h. The basipetal (A) and acropetal (B) movements of IAA-5-^{3}H were determined after a 2-h transport period. Radioactivity is expressed as percentage distribution through the system, and the combined uptake into subsections 2, 3, 4 and the receiver block is shown by the symbol (—— □ ——).

Darkness 25°C.


100
80
60
40
20
0
% Distribution
A
B
0
10^{-8}
10^{-6}
10^{-4}
CFM Concentration (M)

to either Zea or Avena coleoptile segments at concentrations in excess of 10^{-7}M, but concentrations of 10^{-8}M and below have no significant effect on the distribution patterns. Acropetal movement of IAA is slight and negligible radioactivity is detected in receiver blocks after a 2-h transport period. Pretreatment with CFM has no significant effect on the distribution patterns in either Zea or Avena coleoptile segments.

The effect of 10^{-5}M CFM on the acropetal and basipetal movements of IAA in Zea coleoptile segments are shown as a function of time in Figs. 46 and 47. Data are expressed in terms of percentage uptake into the system in Fig. 46 and absolute uptake into the system in Fig. 47, and data are further analysed in Tables 4 and 5. The acropetal movement of IAA in CFM pretreated segments rises slightly above control values with time, and the differences become significant after an 8-h transport period (compare Figs. 46B and D; 47 B and D; and see also Table 4B). Basipetal transport is abolished by the morphactin pretreatment, and the abolition remains complete for at least 24 h. During this period the distribution of radioactivity in CFM pretreated segments supplied with either apical or basal donor blocks remains identical (compare Figs. 46C and D and see also Table 4A). Data for the uptake of radioactivity into CFM pretreated segments are presented in Table 5. Uptake following basal donation exceeds uptake following apical donation (Table 5A), but the surface area available for basal donation also exceeds that available for apical donation because of the tapered construction of the segment. The majority of the radioactivity (>80%) is to be found in the first subsection from the donor block when IAA-5-^{3}H is supplied after CFM pretreatment, and the differences in uptake into these first subsections arising after apical or basal donation are no longer significant when the data are considered on a fresh weight basis (Table 5B).

The effect of CFM on the lateral redistribution of radioactivity in horizontally held Zea coleoptiles during a 2-h transport is shown in Fig. 48. The CFM pretreatment has no significant effect on the upward lateral movement

Fig. 46.

Zea mays L. var. Burpee Snowcross.

The effect of CFH on the polarity of IAA movement with time.

Treatment: Zea coleoptile segments were pretreated by submersion in either 10^{-5} M CFH or distilled water (control) for 1 h. The basipetal movements of IAA-5-^{3}H in control and CFH pretreated segments are shown in Figs. A & C respectively, whilst the acropetal movements in control and CFH pretreated segments are shown in Figs. B & D respectively. Radioactivity is expressed as percentage distribution through the system.

Darkness 25°C.

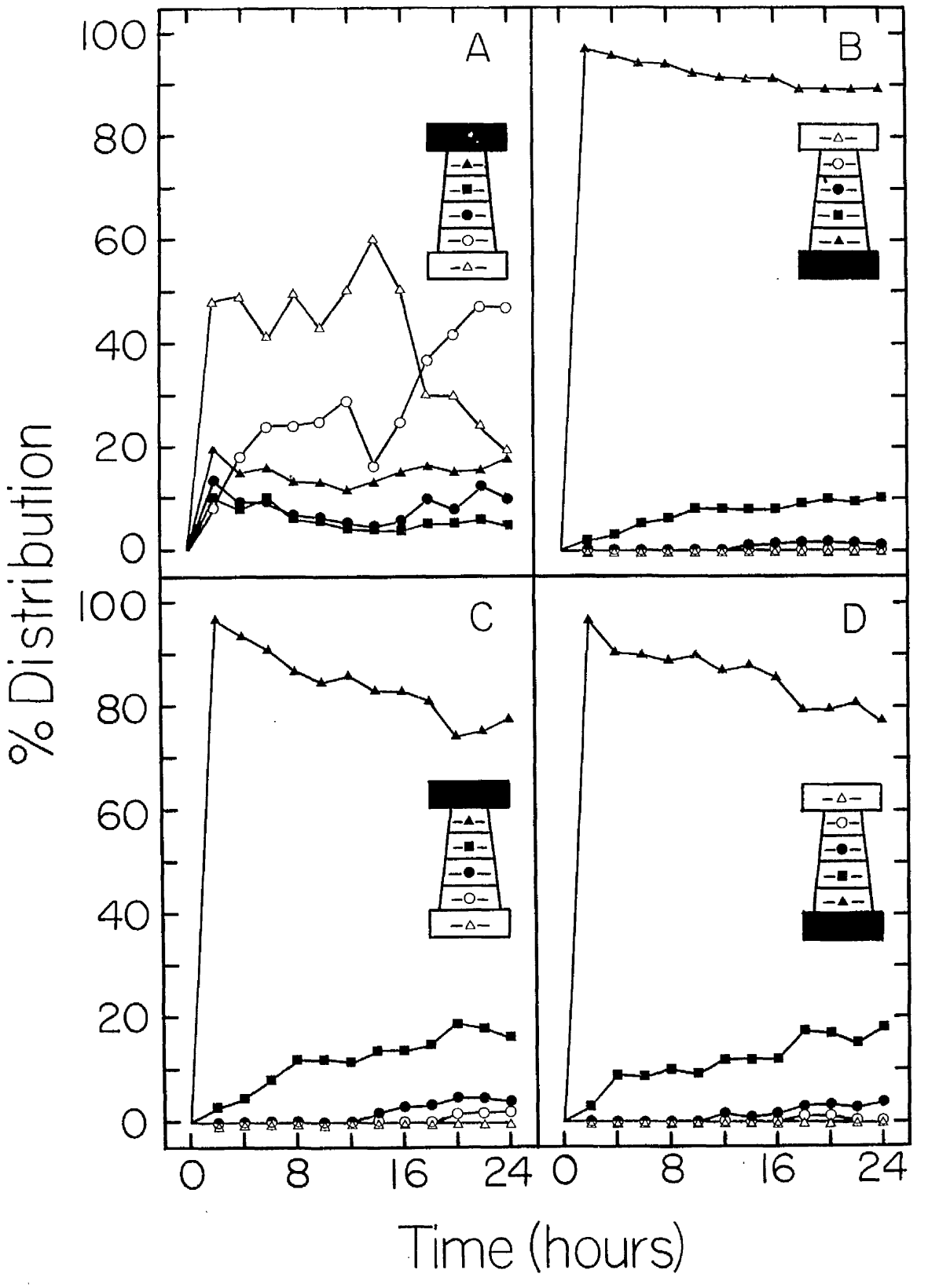

A
B
C
D
100
80
60
40
20
0
0
8
16
24
% Distribution
Time (hours)

Fig. 47.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on the polarity of IAA movement with time.

Treatment: (as Fig. 46) Zea coleoptile segments were pretreated by submersion in either 10^{-5} M CFM or distilled water (control) for 1 h. The basipetal movements of IAA-5-^{3}H in control and CFM pretreated segments are shown in Figs. A & C respectively, whilst the acropetal movements in control and CFM pretreated segments are shown in Figs. B & D respectively. The distribution of radioactivity is expressed in terms of the absolute activity (dpm) in subsections 2 (———), 3 (– – –), and 4 (••••••) and in receiver blocks (–·–·–).

Darkness 25°C.

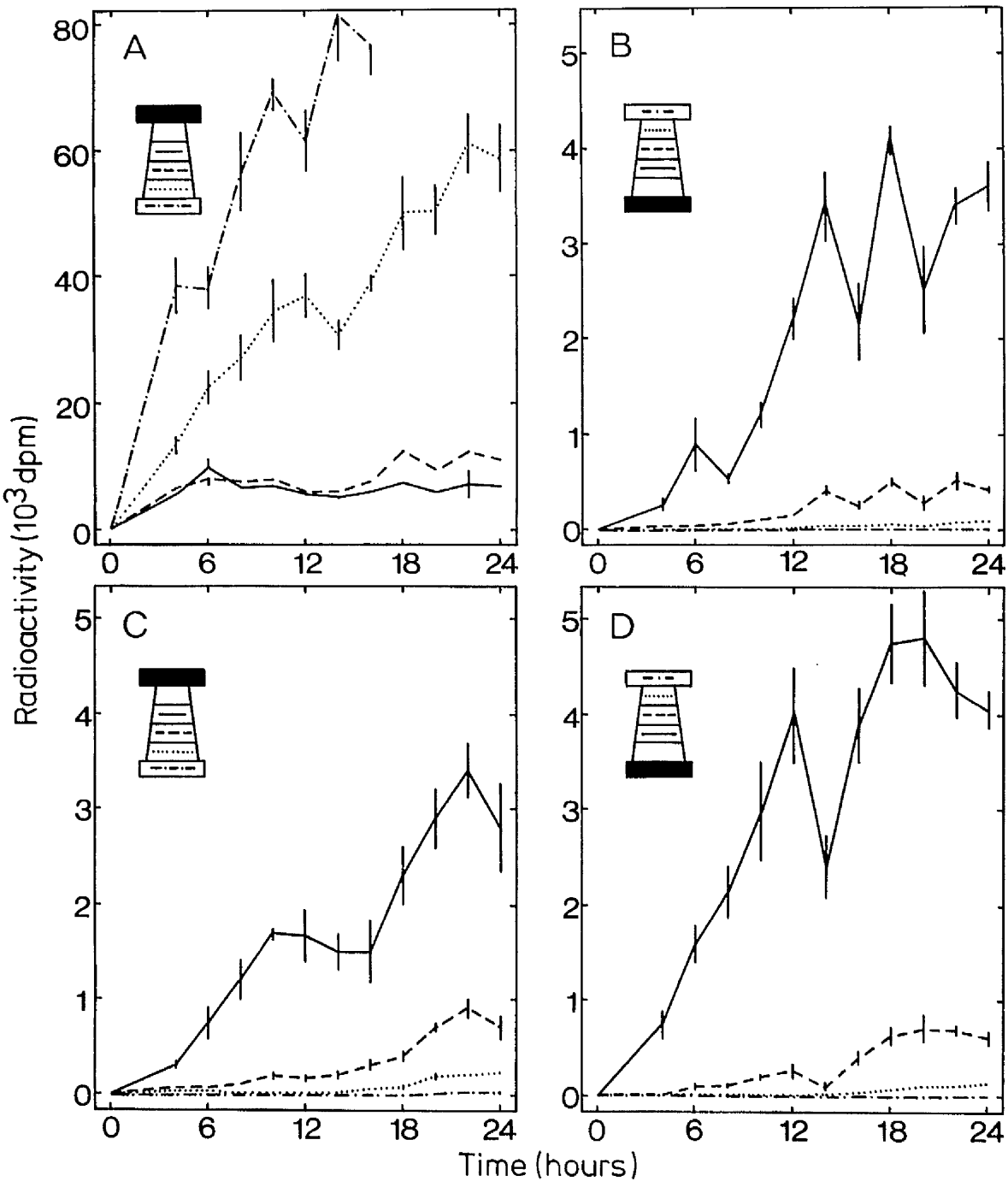

A
B
C
D
Radioactivity (10^3 dpm)
Time (hours)
0
6
12
18
24
20
40
60
80
1
2
3
4
5

Table 4.

Zea mays L. var. Burpee Snowcross.

Statistical analysis of the data presented in Figs. 46 & 47.

The combined uptake into subsections 2, 3, 4, and the receiver following the apical or basal donation of IAA-5-^{3}H to CFH pretreated segments, is shown in Table A. Uptake is presented in terms of absolute activity (dpm) and percentage total uptake into the system (subsections 1 + 2 + 3 + 4 + R) and the differences in uptake following apical or basal donation are analysed for both presentations.

Combined uptake into subsections 2, 3, 4, and the receiver following basal donation to control and CFH pretreated coleoptile segments, is shown in Table B. The data are presented in terms of absolute activity (dpm) and percentage total uptake, and the differences in uptake following the two treatments are analysed. The percentage data are tabulated after angular transformation.$^{\mp}$

Table 4A

Time hours	Uptake dpm (1000s)		Uptake % Total †		t values	
	Apical donation	Basal donation	Apical donation	Basal donation	DPM	%
4	± 0.4644 ± 0.1373	± 0.8590 ± 0.2366	± 14.4120 ± 2.3166	± 17.3160 ± 3.5157	-1.4427 NS	-0.6898 NS
8	± 1.3396 ± 0.2717	± 2.3155 ± 0.2938	± 21.0020 ± 0.8750	± 19.0520 ± 1.4072	-2.4391 *	1.1769 NS
12	± 1.8989 ± 0.3247	± 4.3912 ± 0.6300	± 21.6320 ± 0.6360	± 21.4600 ± 1.1474	-3.5167 **	0.1311 NS
16	± 1.8884 ± 0.4482	± 4.3198 ± 0.3780	± 22.2320 ± 1.8152	± 21.6020 ± 1.6050	-4.1477 ***	0.0600 NS
20	± 3.8203 ± 0.3124	± 5.6751 ± 0.7154	± 30.4640 ± 0.9994	± 26.3320 ± 1.5305	-2.2186 *	2.0607 NS
24	± 4.5741 ± 0.4258	± 5.0394 ± 0.3541	± 29.2040 ± 1.2528	± 24.8600 ± 0.8096	-0.8403 NS	2.7920 *

Table 4B

Time hours	Uptake dpm (1000s)		Uptake % Total †		t values	
	Control	CFM	Control	CFM		
	Basal donation	Basal donation	Basal donation	Basal donation	DPM	%
4	± 0.2773 ± 0.0218	± 0.8590 ± 0.2366	± 9.6540 ± 1.1433	± 17.3160 ± 3.5157	2.4492 *	2.0727 NS
8	± 0.6129 ± 0.0501	± 2.3155 ± 0.2938	± 14.4760 ± 0.5544	± 19.0520 ± 1.4072	5.7134 ***	3.0258 **
12	± 2.3836 ± 0.2400	± 4.3912 ± 0.6300	± 17.2220 ± 0.6622	± 21.4600 ± 1.1474	2.9781 **	3.1994 **
16	± 2.3920 ± 0.4871	± 4.3198 ± 0.3780	± 17.2200 ± 1.4363	± 21.6020 ± 1.4050	3.1270 **	2.2346 *
20	± 2.8530 ± 0.5381	± 5.6751 ± 0.7754	± 17.3200 ± 1.3581	± 26.3320 ± 1.5305	2.9904 **	4.4046 ***
24	± 4.0197 ± 0.2862	± 5.0394 ± 0.3541	± 19.4460 ± 0.4534	± 24.8600 ± 0.8096	2.2396 *	5.8353 ***

Table 5.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on the uptake of IAA-5-^{3}H.

The total uptake of radioactivity into tissue and receiver blocks with time after apical or basal donation of IAA-5-^{3}H to segments pretreated with either 10^{-5}M CFM or distilled water is shown in Table A. The t test is used to test the differences in uptake for the treatment combinations:- (a) apical v. basal donation to control coleoptiles.

(b) apical v. basal donation to CFM pretreated coleoptiles.

(c) apical donation to control coleoptiles v. apical donation to CFM pretreated coleoptiles.

(d) basal donation to control coleoptiles v. basal donation to CFM pretreated coleoptiles.

Uptake into the first subsection from the donor following the apical or basal donation of IAA-5-^{3}H to CFM pretreated segments is shown in Table B. A correction is made for the difference in fresh weight between the apical and basal subsections, and t values are calculated for the differences in uptake following apical and basal donation before (a) and after (b) application of this fresh weight correction. The basal subsections averaged 2.46 times heavier than the apical subsections.

Table 5A.

Time h	Uptake dpm (1000s)				t Values			
	Control		CFM					
	Apical donation	Basal donation	Apical donation	Basal donation	a	b	c	d
4	75.8227	11.4478	7.1361	11.0193	+11.462 ***	-1.664	+13.430 ***	-0.130
8	112.7675	9.9662	10.3406	21.6028	+21.840 ***	-5.417 ***	+20.924 ***	-7.327 ***
12	123.8321	27.7468	13.8142	31.9605	+17.662 ***	-5.976 ***	+25.100 ***	-0.951
16	131.9621	26.2695	11.3616	32.5060	+10.233 ***	-6.074 ***	+11.435 ***	-2.066
20	121.9447	31.0564	14.9925	28.3931	+26.359 ***	-5.805 ***	+33.813 ***	+0.991
24	107.6811	36.1552	14.9924	28.3687	+ 8.259 ***	-9.243 ***	+11.777 ***	+2.000

Table 5B.

Time h	Uptake dpm (1000s)			t values	
	Apical donation	Basal donation	Apical donation after fresh weight correction	a	b
4	6.6712	10.2323	16.4570	-1.444	+2.006
8	9.0009	19.2873	22.3040	-5.374 ***	+0.739
12	11.9153	27.5693	29.394	-6.384 ***	+0.382
16	9.4732	28.1865	23.369	-5.484 ***	+0.850
20	11.1721	22.7181	27.561	-6.227 ***	+1.579
24	12.8693	23.3292	31.579	-1.808	+1.746

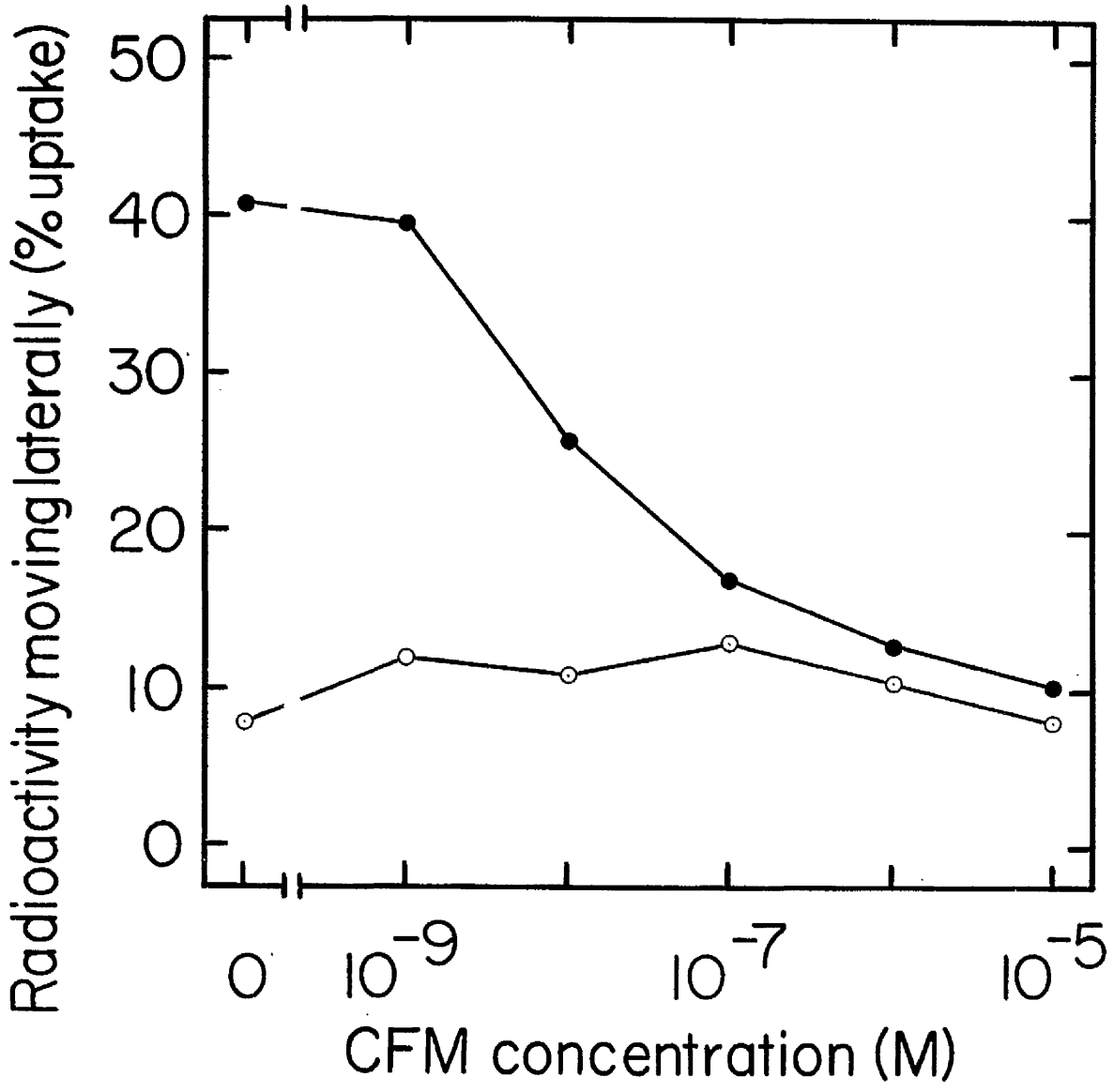
Fig. 48.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on the lateral movement of IAA-5-^{3}H.

Treatment: Zea coleoptile segments, 10 mm in length, were pretreated by submersion in one of a range of concentrations of CFM for 1 h. The segments were removed, laid horizontally, and supplied at their apical ends with asymmetric sources of IAA-5-^{3}H. Agar blocks, separated by fine glass slivers which slightly penetrated the segments, were placed in contact with the upper and lower halves of the basal ends of the segments. Radioactivity moving laterally into the opposite half of the system during the first 2 h following asymmetric donation to the upper (solid circles) or lower (open circles) halves of the segment is expressed in terms of percentage total uptake into the system.

Darkness 25°C.


Radioactivity moving laterally (% uptake)
50
40
30
20
10
0
0
10^{-9}
10^{-7}
10^{-5}
CFM concentration (M)

of radioactivity after donation to the lower half of the segment, but it is markedly effective in reducing the downward lateral movement after donation to the upper half of the segment. The downward lateral movement is reduced when CFM is applied at concentrations greater than 10^{-9}M, and it is abolished when concentrations exceed 10^{-7}M.

10b. Influence on IAA metabolism in coleoptiles

The radioactivity moving acropetally and basipetally through 10 mm Zea coleoptile segments has been analysed by thin layer chromatography using the basic solvent system 45:35:20 methyl acetate:isopropanol:ammonia (Fig. 49) and the non-polar acid solvent system 95:5 chloroform:acetic acid (Fig. 50). Chromatographic analyses of extracts from donor blocks used in the donation of IAA-5-^{3}H to Zea coleoptile segments show firstly that these donors do not become exhausted and, secondly, that they retain their radiochemical constitution. There is no evidence to indicate the export of metabolites or enzymes capable of metabolising IAA into the donor blocks during the transport period. Radioactivity is not exported into receiver blocks in detectable quantities following basal donation to control Zea coleoptile segments or apical or basal donation to CFM pretreated segments, but radioactivity is detectable in receiver blocks following basipetal transport in control segments and here the activity,as resolved on the two solvent systems, is entirely confined to the IAA molecule. The peaks detected at the IAA Rf on chromatograms spotted with aliquots from IAA-5-^{3}H stock solutions, or from extracts of donor or receiver blocks, are always narrow and symmetrical. There are no shoulders and the peaks never extend over more than 0.1 Rf at their bases.

Chromatographic analysis of tissue extracts using the basic solvent system yields one major peak in radioactivity which corresponds in Rf with stock IAA-5-^{3}H (Fig. 49 columns 1 and 2), but the peak is wide and its shape is never symmetrical. It extends over at least 0.2 Rf at its base and may be partially resolved into two zones. Complete resolution into two major

Fig. 49.

Zea mays L. var. Burpee Snoweross.

The effect of CFM on the metabolism of applied IAA-5-^{3}H.

Treatment: Zea coleoptile segments, 10 mm in length, were pretreated by submersion in either 10^{-5} M (MA & MB) CFM or distilled water (CA & CB) for 1 h. The radioactivity moving basipetally (B) or acropetally (A) during the first 2 h following the apical or basal donation of IAA-5-^{3}H was analysed by thin layer chromatography on silica coated plates using the basic solvent system 45 : 35 : 20 methyl acetate : isopropanol : ammonia. Represented are chromatograms of methanolic extracts from the proximal (1) and distal (2) halves of the segments (with respect to the donor), the used donors (Do) and the receiver blocks (R).

IAA ran to Rf 0.4 on this solvent system.

The divisors accompanying each chromatogram represent the relative dilution of the extract in the preparation of the aliquot for spotting.

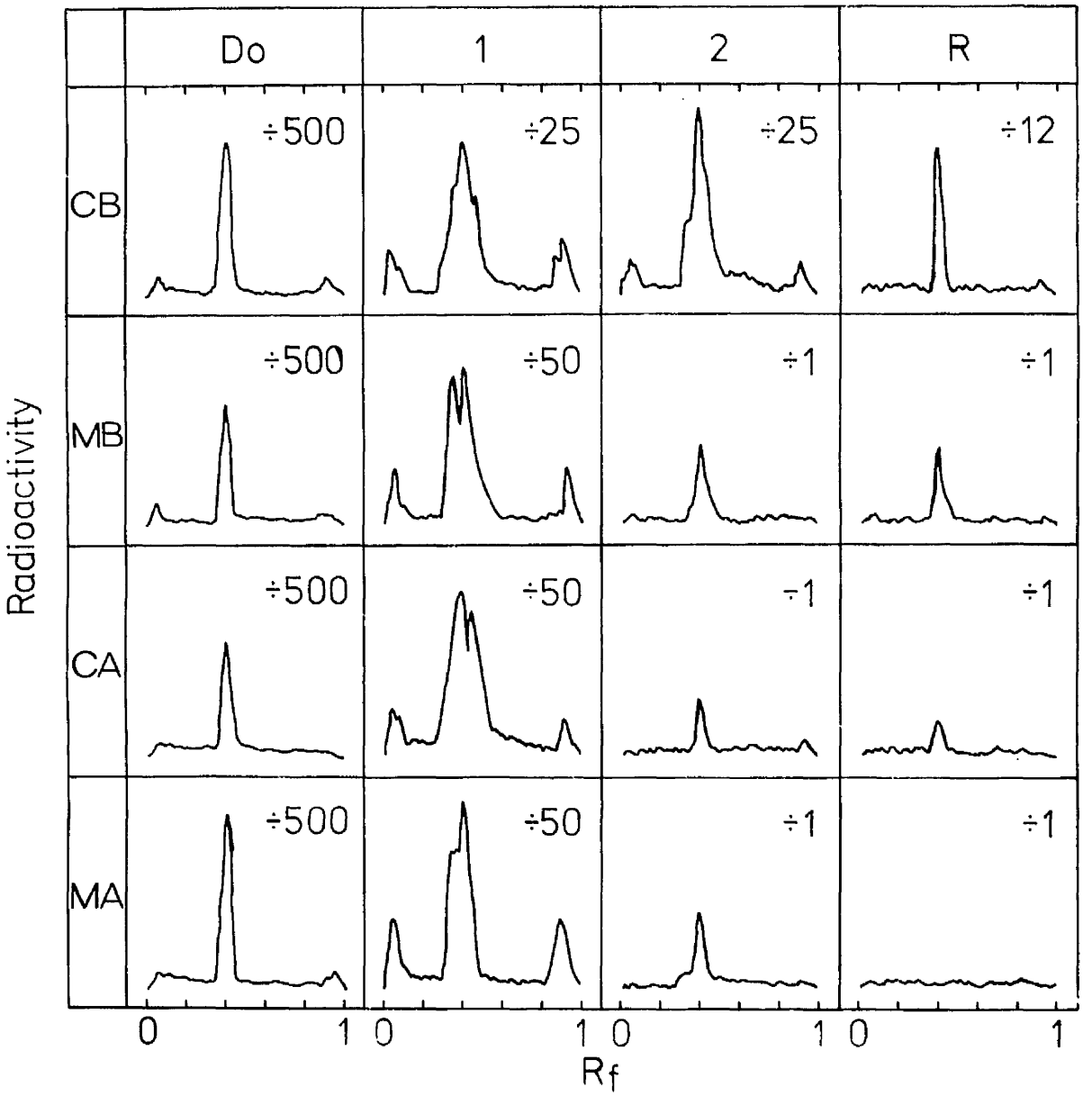

Do
1
2
R
CB
MB
CA
MA
÷500
÷25
÷25
÷12
÷500
÷50
÷1
÷1
÷500
÷50
÷1
÷1
÷500
÷50
÷1
÷1
0
1
0
1
0
1
0
1
Rf
Radioactivity

Fig. 50.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on the metabolism of applied IAA-5-^{3}H.

Treatment: Zea coleoptile segments, 10 mm in length, were pretreated by submersion in either 10^{-5} M CFM (PA & PB) or distilled water (CA & CB) for 1 h. The radioactivity moving basipetally (B) or acropetally (A) during the first 2 h following apical or basal donation of IAA-5-^{3}H was analysed by thin layer chromatography on silica coated plates using the acid solvent system 95 : 5 chloroform : acetic acid. Represented are chromatograms of methanolic extracts from the proximal (1) and distal (2) halves of the segments (with respect to the donors), the used donors (Do) and the receiver blocks (R).

IAA ran to Rf 0.6 on this solvent system.

The divisors accompanying each chromatogram represent the relative dilution of the extract in the preparation of the aliquot for spotting.

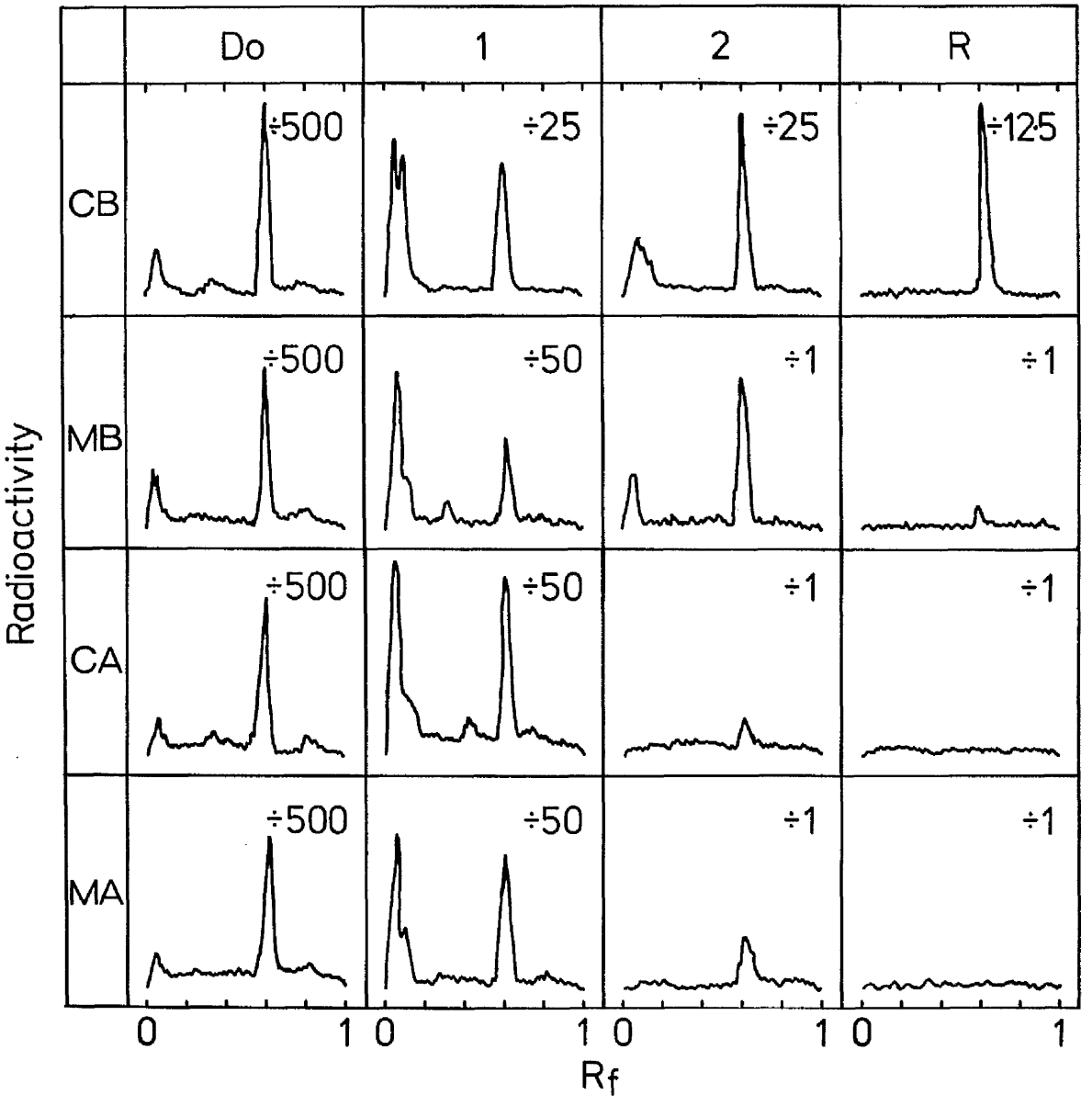

Do
1
2
R
CB
MB
CA
MA
÷500
÷25
÷25
÷125
÷500
÷50
÷1
÷1
÷500
÷50
÷1
÷1
÷500
÷50
÷1
÷1
Radioactivity
0
1
0
1
0
1
0
1
Rf

Fig. 51.

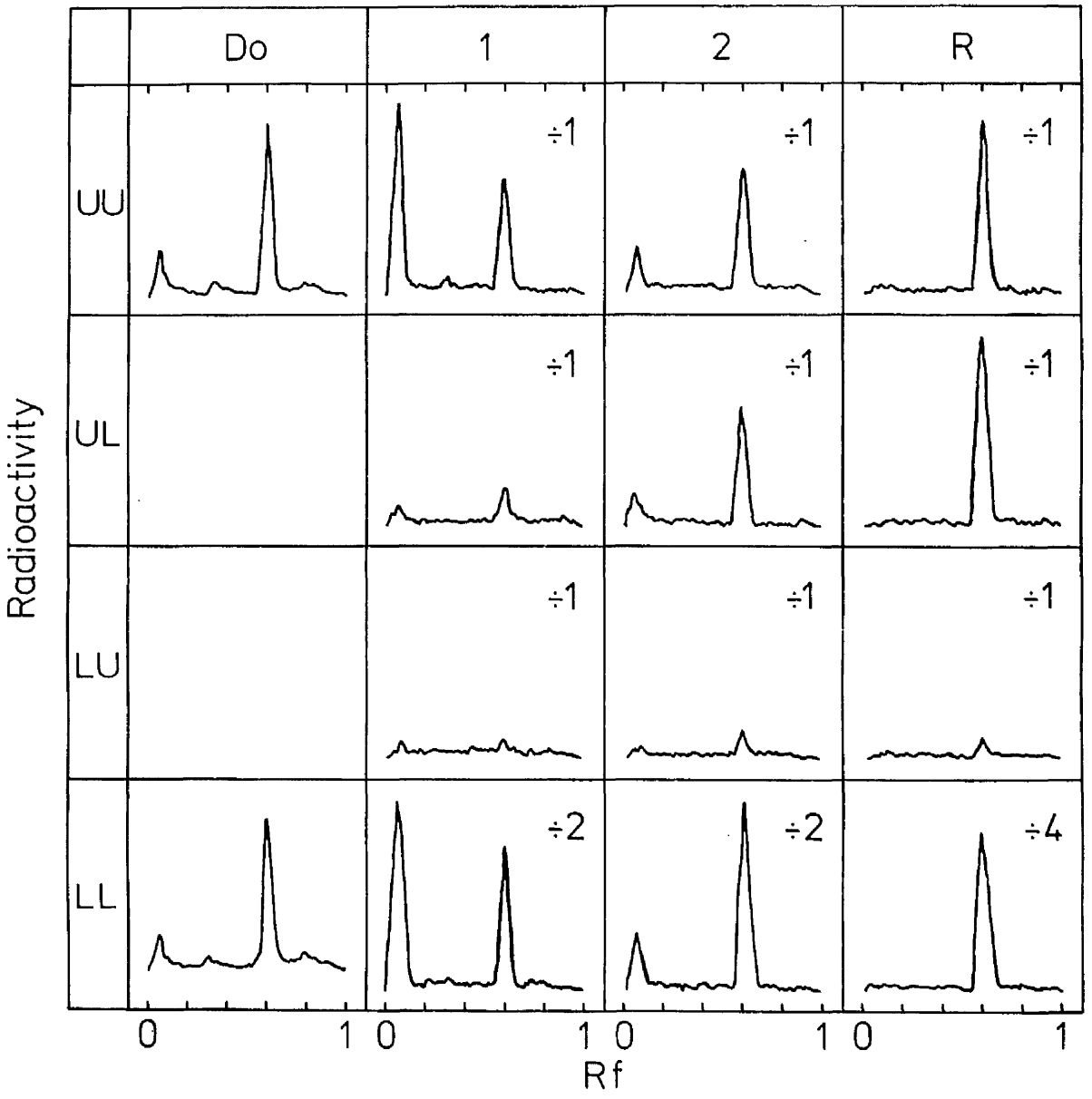
Zea mays L. var. Burpee Snowcross.

Chromatographic analysis of laterally transported radioactivity.

Treatment: Zea coleoptile segments, 10 mm in length, were placed horizontally with split receiver blocks in contact with the upper and lower faces of their basal cut ends. Asymmetric sources of IAA-5-^{3}H were applied to either the upper (UU) or lower (LL) halves of the segments and the radioactivity in the upper (UU & LU) and lower (UL & LL) halves of the segments was analysed after a 2-h transport period by thin layer chromatography on silica coated plates using the acid solvent system 95 : 5 chloroform : acetic acid. Represented are chromatograms of methanolic extracts from the proximal (1) and distal (2) halves of the segments (with respect to the donor), the used donors (Do) and the receiver blocks (R).

IAA ran to Rf 0.6 on this solvent system.

The divisors accompanying each chromatogram represent the relative dilution of the extract in the preparation of the aliquot for spotting.


Do
1
2
R
UU
UL
LU
LL
÷1
÷1
÷1
÷1
÷1
÷1
÷1
÷1
÷1
÷2
÷2
÷4
Radioactivity
0
1
0
1
0
1
0
1
Rf

zones of radioactivity is achieved with the non-polar acid solvent 95:5 chloroform:acetic acid (Fig. 50 columns 1 and 2). One of the resolved peaks corresponds in Rf with stock IAA and is narrow and symmetrical. The second peak is also narrow, but it widens to become asymmetric towards its base where a shoulder of slightly higher Rf may be partially resolved. This shoulder may represent one of the contaminants present in the stock solution, but the peak itself represents a major metabolic product. Production of this metabolic product is greatest where IAA is present at high concentrations and radioactivity in the product predominates in extracts from the tissue adjacent to the donor block after basal donation to control segments and both apical and basal donation to CFM pretreated Zea coleoptile segments. There is no evidence to suggest that CFM modifies IAA metabolism in Zea coleoptile segments.

The radioactivity moving laterally in horizontally held Zea coleoptile segments has also been chromatographed using the acid solvent system 95:5 chloroform:acetic acid and the chromatograms, which are shown in Fig. 51, reveal a similar pattern of metabolism to that found in the vertical controls. The metabolic product predominates in the tissues adjacent to the donor blocks, but the radioactivity transported laterally is almost entirely confined to the IAA molecule, and the radioactivity exported into receiver blocks is entirely confined to the IAA molecule after a 2-h transport period.

10c. Influence on coleoptile growth

The chromatographic analyses show firstly that the radioactivity transported through the coleoptile tissues is confined to the IAA molecule and, secondly, that IAA metabolism is not altered by CFM treatment. The morphactin does not cause a randomization in the direction of transport, but it exerts its effect through the inhibition of the polarised basipetal and lateral transport systems. The effect of CFM on the development of curvature in Zea and Avena coleoptile segments is shown in Fig. 52, and a

Fig. 52.

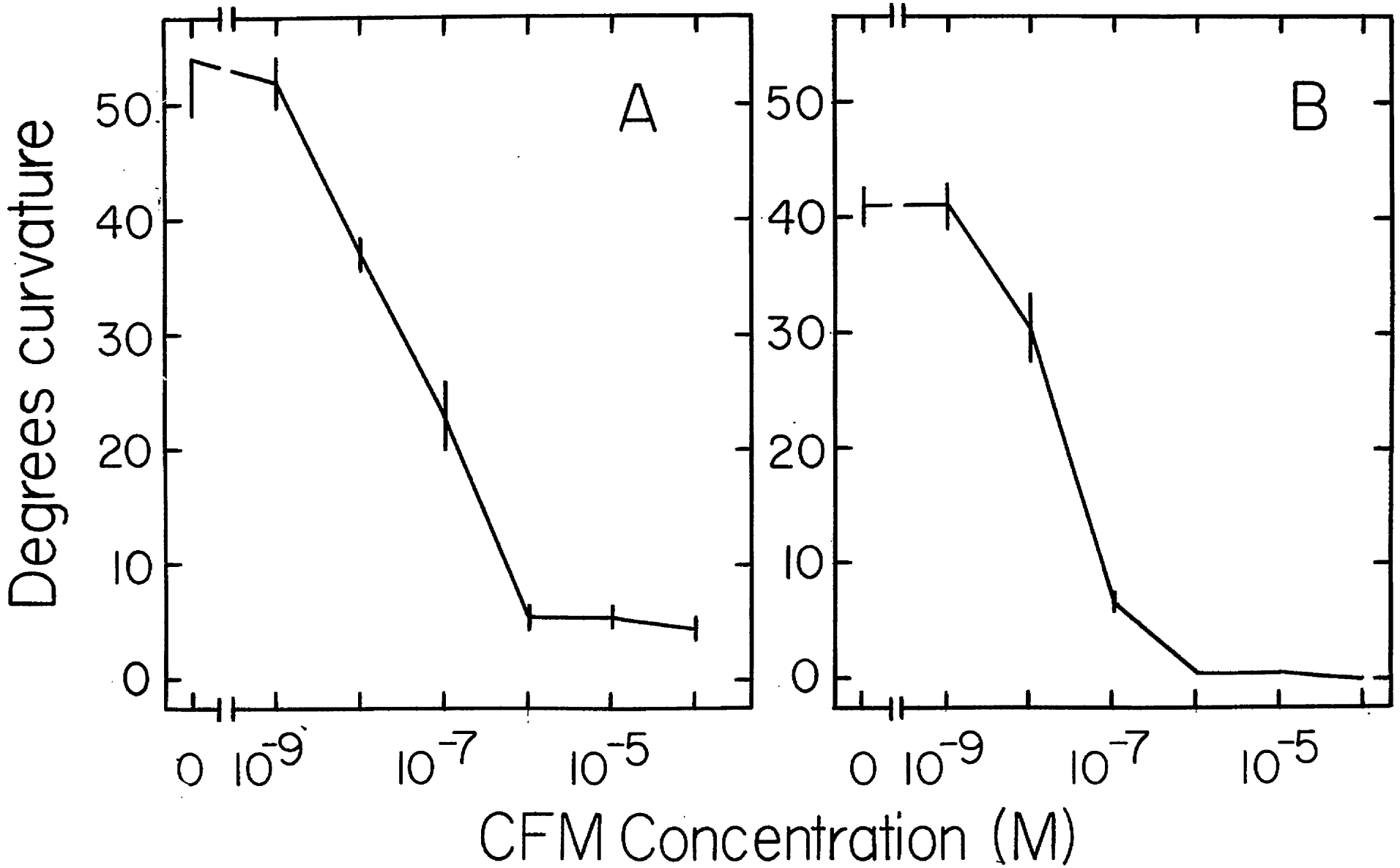
Zea mays L. var. Burpee Snoweross.

Avena sativa L. var. Svalov Victory.

The effect of CFH on the development of geotropic curvature.

Treatment: Zea coleoptile segments (A) and Avena coleoptile segments (B) were excised 15 mm below the apex and pretreated by submersion in one of a range of concentrations of CFH. They were removed after a 1-h pretreatment period and fixed horizontally with their basal ends sandwiched between pieces of filter paper which were moistened with the appropriate concentration of CFH. Segments were shadowgraphed after a 5-h treatment period.

Darkness 25°C.


Degrees curvature
50
40
30
20
10
0
A
B
0
10^{-9}
10^{-7}
10^{-5}
CFM Concentration (M)

comparison between these data and the auxin transport data presented in Figs. 44 and 45 suggests a close correlation between the inhibition of polarised transport and the geotropic response.

The effects of CFM on straight growth in Zea coleoptile segments are shown after a 24-h incubation period in Fig. 53. A significant promotion of growth occurs in response to CFM in the presence (curve 1a) and the absence (curve 2) of IAA and CFM only becomes inhibitory at 10^{-4}M when the two solutes are provided in aqueous solution. Maximum promotion is achieved when CFM is supplied at 10^{-7}M and similar results are obtained from the CFM pretreatment used throughout the transport experiments, except that in this assay inhibition is observed at a lower concentration (10^{-6}M to 10^{-5}M).

The effects of combinations of IAA and CFM on straight growth in Zea coleoptile segments are shown in Fig. 54, and the data concerning IAA application in conjunction with promotory concentrations of CFM are analysed using the analysis of variance. Highly significant values of F are obtained for IAA and CFM treatments, but the component for interaction is not significant. Similar experimentation has been employed to determine the effects of combinations of GA_3 and CFM on growth in Zea coleoptiles, and data for this assay are provided in Fig. 55. The analysis of variance again yields significant values of F for growth promotion by CFM, but the values obtained for GA_3 and interaction components are not significant. The additive nature of the CFM and IAA induced effects precludes the action of CFM as an auxin in the promotion of growth in Zea coleoptile segments, and the absence of a response to GA_3 may be taken to preclude its action as a gibberellin, at least of the GA_3 type. Neither IAA nor GA_3 is able to offset the inhibitory effects of CFM which are manifest at a concentration of 10^{-4}M.

Factorial experiments have also been used to determine the effects of CFM in conjunction with IAA or GA_3 on the growth of Avena coleoptile segments, and data are presented for the two treatments in Figs. 56 and 57. The

Fig. 53.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on straight growth.

Treatment: Coleoptile segments, 10 mm in length, were excised 1 mm below the apex. Some segments were pretreated by submersion in a solution of CFM prior to assembly between blocks of 1.5% agar which provided an apical source of 10^{-5}M IAA (1b) whilst others were floated directly in 50 mm petri dishes containing 10^{-5}M CFM in the presence (1a) or absence (2) of 10^{-5}M IAA. Segments were shadowgraphed after a 24-h treatment period.

Darkness 25°C.

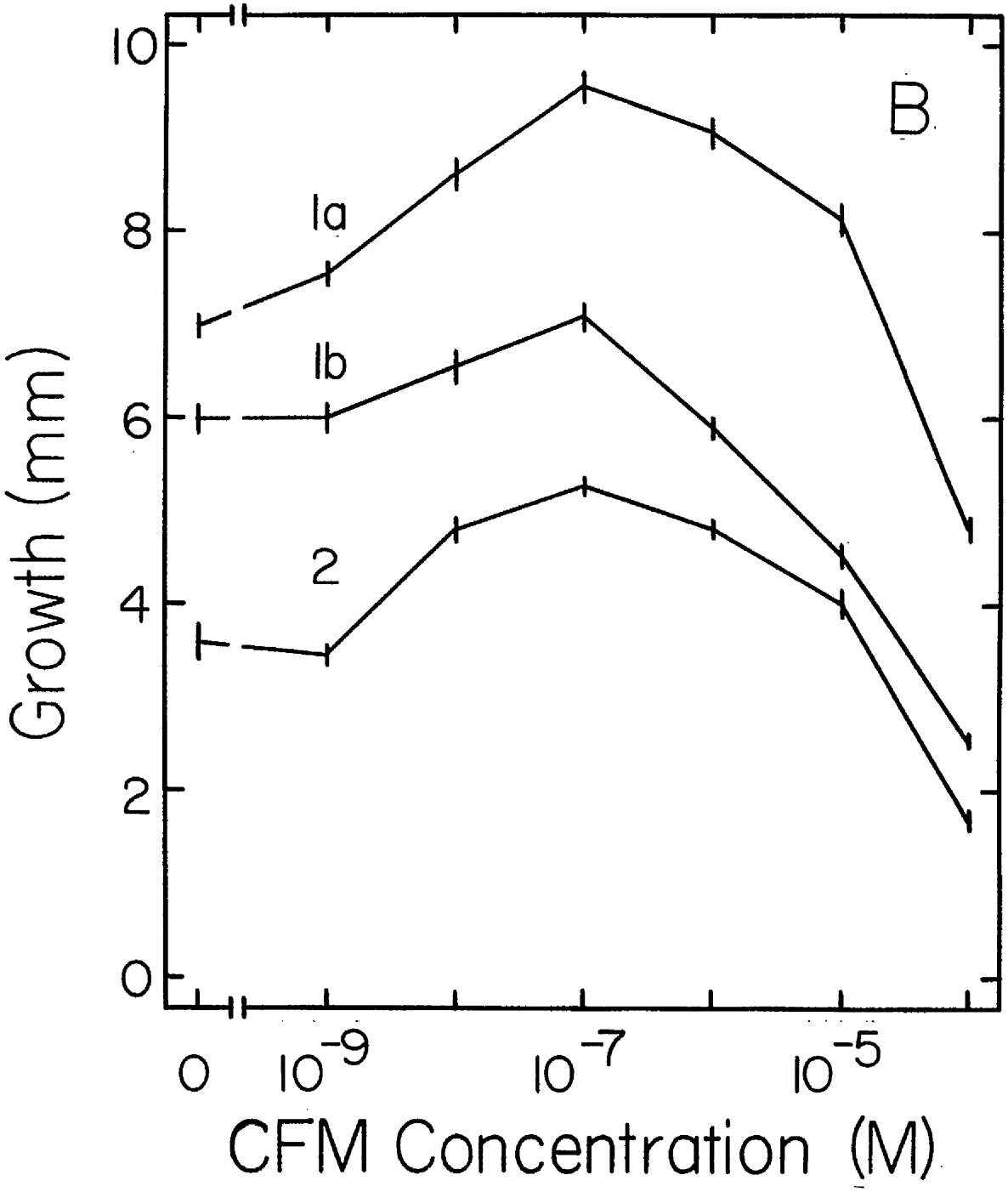

B
Ia
Ib
2
Growth (mm)
0
2
4
6
8
10
0
10^{-9}
10^{-7}
10^{-5}
CFM Concentration (M)

Fig. 54.

Zea mays L. var. Burpee Snowcross.

The effect of CFM on straight growth.

Treatment: Coleoptile segments 10 mm in length were excised 1 mm below the apex and floated in 50 mm petri dishes containing 10 ml of a solution of IAA and CFM in the following combinations. Segments were shadowgraphed after a 24-h treatment period.

IAA	CFM	a 0	b 10^{-8}M	c 10^{-7}M	d 10^{-6}M	e 10^{-5}M	f 10^{-4}M
g	0	3.969	5.546	5.784	5.260	4.531	2.504
h	10^{-6}M	4.655	6.301	6.821	6.315	5.391	3.319
i	10^{-5}M	7.082	8.363	8.594	8.330	7.488	3.786
j	10^{-4}M	8.234	8.242	9.903	9.317	9.038	4.733

The table presents growth in millimeters and the data are plotted with CFM (A) and IAA (B) as abscissae in the figures opposite.

Darkness 25°C.

Statistical analysis: The analysis of variance was used to test the effects of IAA (0 to 10^{-4}M) and promotory concentrations of CFM (0 to 10^{-7}M) on segment growth.

	Error	Rows (IAA)	Columns (CFM)	Interaction
df	12	3	2	6
ss	3.020	52.823	14.708	2.284
ms	0.251	17.607	7.354	0.380
f		70.147***	29.298***	1.513^{NS}

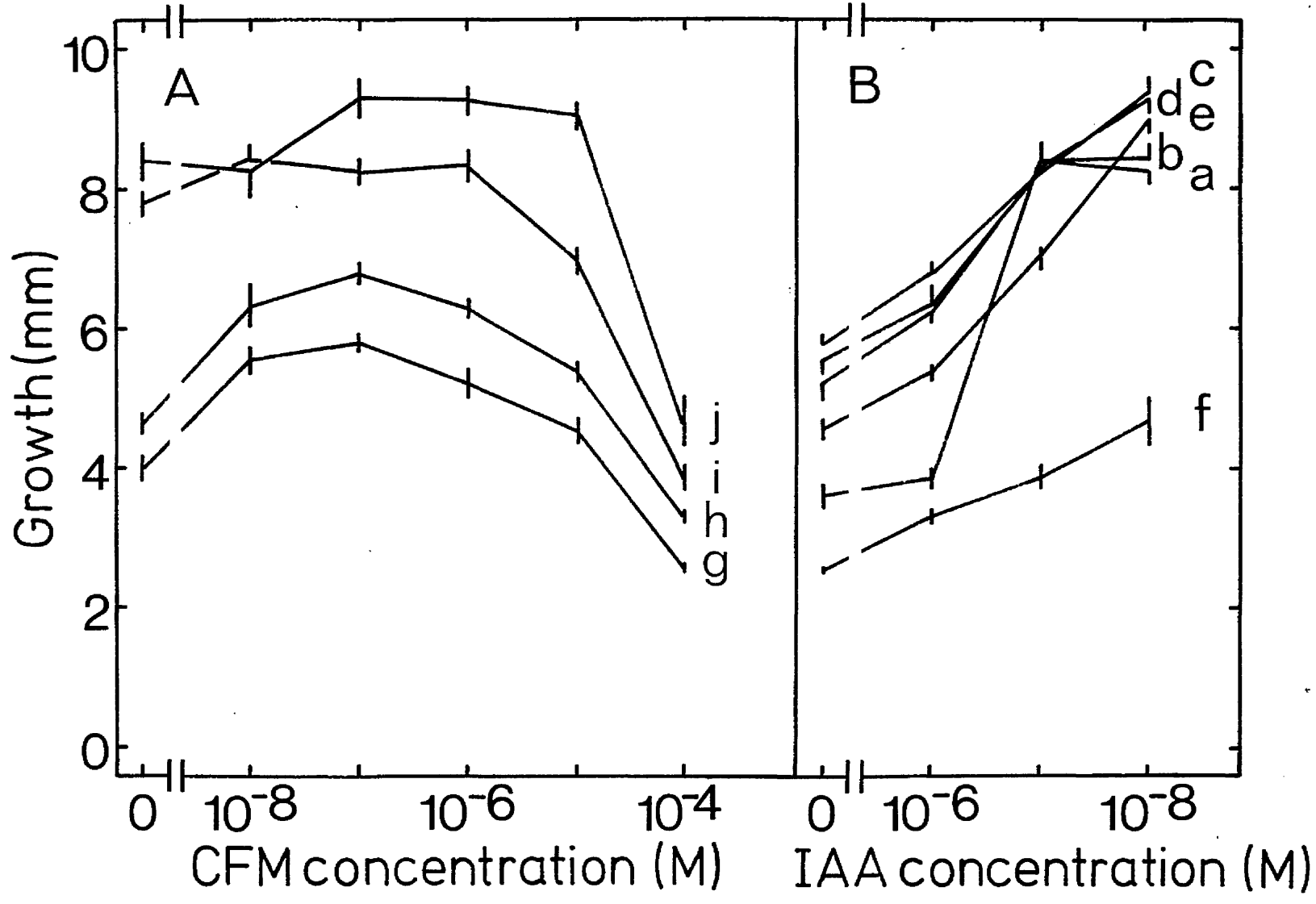

A
B
Growth (mm)
10
8
6
4
2
0
j
i
h
g
c
d
e
b
a
f
0
10^{-8}
10^{-6}
10^{-4}
CFM concentration (M)
0
10^{-6}
10^{-8}
IAA concentration (M)

Fig. 55.

Zea mays var. Burpee Snowcross.

The effect of CFM on straight growth.

Treatment: Coleoptile segments 10 mm in length were excised 1 mm below the apex and floated in 50 mm petri dishes containing 10 ml of a solution of GA_3 and CFM in the following combinations. Segments were shadowgraphed after a 24-h treatment period.

	CFM	a	b	c	d	e	f
GA_3		0	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
g	0	3.969	5.546	5.850	5.167	4.572	2.424
h	10^{-6}M	4.435	6.113	5.866	5.487	4.378	2.741
i	10^{-5}M	4.531	6.370	6.247	6.086	4.076	2.618
j	10^{-4}M	4.586	6.286	6.169	5.417	3.818	2.347

The table presents growth in millimetres and the data are plotted with CFM (A) and GA_3 (B) as abscissae in the figures opposite.

Darkness 25°C.

Statistical analysis. The analysis of variance was used to test the effects of GA_3 ($0 \rightarrow 10^{-4}$M) and promotory concentrations of CFM ($0 \rightarrow 10^{-7}$M) on segment growth.

	Error	rows GA_3	columns CFM	Interaction
df	12	3	2	6
ss	1.665	1.334	13.212	0.619
ms	0.138	0.444	6.606	0.103
f		3.217^{NS}	47.869***	0.746^{NS}

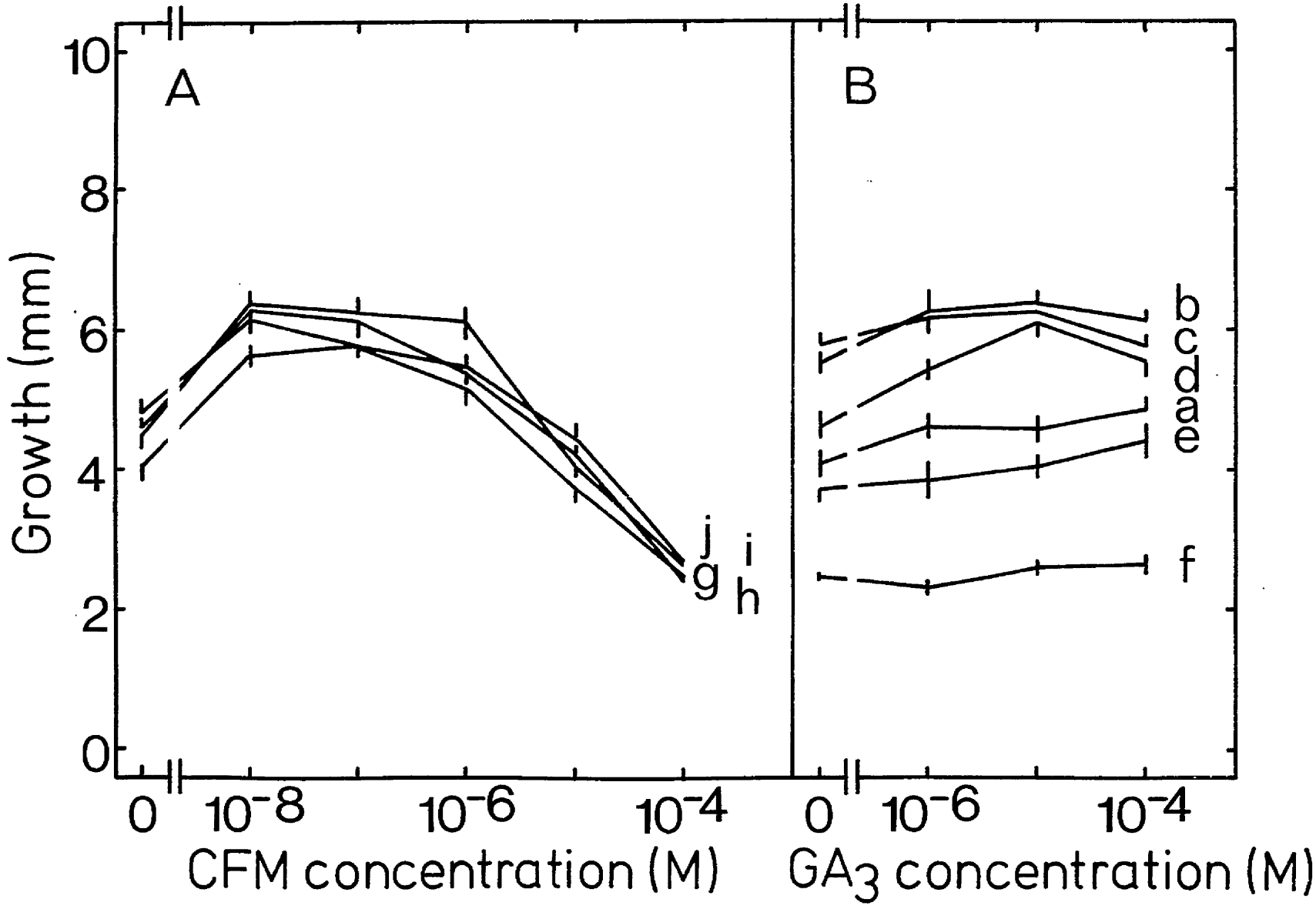

A
B
Growth (mm)
10
8
6
4
2
0
j
i
g
h
b
c
d
a
e
f
0
10^{-8}
10^{-6}
10^{-4}
0
10^{-6}
10^{-4}
CFM concentration (M)
GA_3 concentration (M)

Fig. 56.

Avena sativa L. var. Svalov Victory.

The effect of CFM on straight growth.

Treatment: Coleoptile segments 5 mm in length were excised 1 mm below the apex and floated in 50 mm petri dishes containing 10 ml of a solution of IAA and CFM in the following combinations. Segments were shadowgraphed after a 24-h treatment period.

IAA	CFM	a 0	b 10^{-8}M	c 10^{-7}M	d 10^{-6}M	e 10^{-5}M	f 10^{-4}M
g	0	3.850	2.985	2.946	2.157	1.628	1.354
h	10^{-6}M	4.539	4.827	4.754	4.446	3.444	1.719
i	10^{-5}M	6.024	5.706	6.141	5.240	3.656	1.862
j	10^{-4}M	6.171	7.083	6.747	6.592	6.234	2.024

The table presents growth in millimetres and the data are plotted with CFM (A) and IAA (B) as abscissae in the figures opposite.

Darkness 25°C.

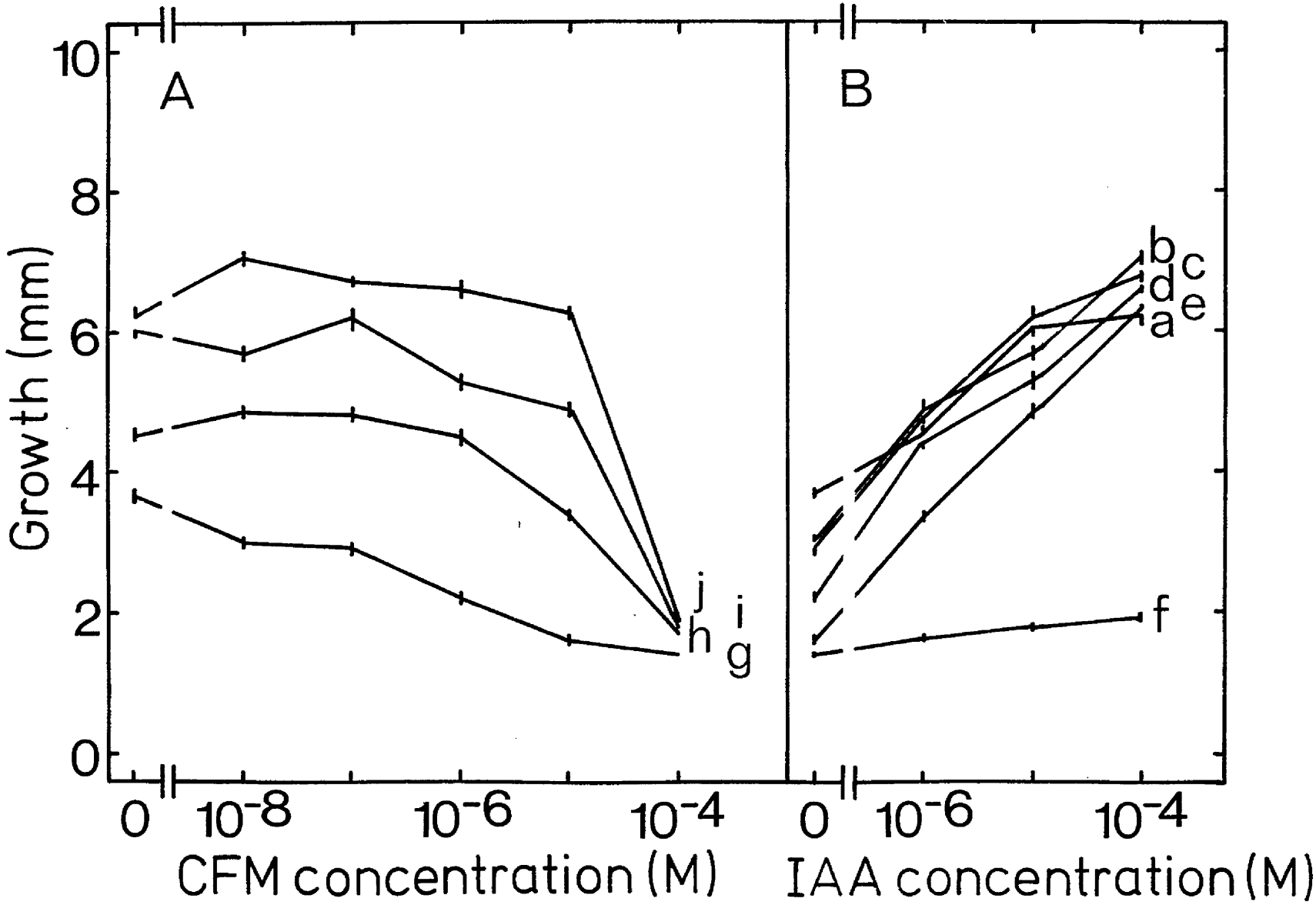

A
B
Growth (mm)
10
8
6
4
2
0
j
i
h
g
b
c
d
e
a
f
0
10-8
10-6
10-4
CFM concentration (M)
0
10-6
10-4
IAA concentration (M)

Fig. 57.

Avena sativa L. var. Svalov Victory.

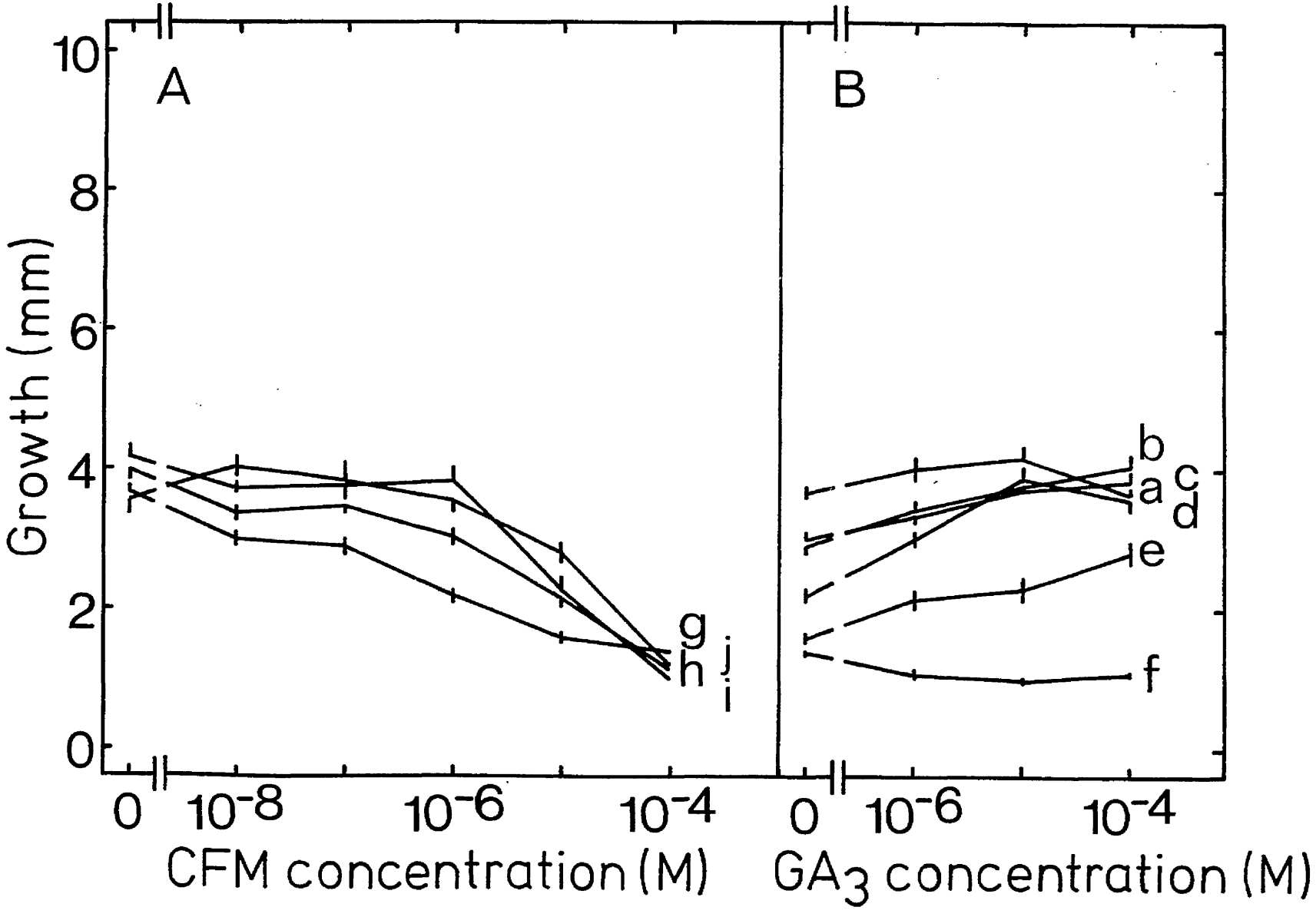
The effect of CFM on straight growth.

Treatment: Coleoptile segments 5 mm in length were excised 1 mm below the apex and floated in 50 mm petri dishes containing 10 ml of a solution of GA_3 and CFM in the following combinations. Segments were shadowgraphed after 24 hours treatment.

	CFM	a	b	c	d	e	f
GA_3		0	10^{-8}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
g	0	3.050	2.985	2.946	2.157	1.628	1.354
h	10^{-6}M	4.148	3.446	3.372	3.037	2.136	1.067
i	10^{-5}M	4.102	3.700	3.820	3.933	2.224	1.006
j	10^{-4}M	3.693	3.984	3.597	3.773	2.823	1.092

The table presents growth in millimetres and the data are plotted with CFM (A) and GA_3 (B) as abscissae in the figures opposite.

Darkness 25°C.


A
B
Growth (mm)
10
8
6
4
2
0
g
h
j
i
b
a
c
d
e
f
0
10-8
10-6
10-4
0
10-6
10-4
CFM concentration (M)
GA3 concentration (M)

morphactin does not promote growth at any of the concentrations tested, and the dosage response curves for the inhibition of growth are comparable with the dosage response curves for the inhibition of polar auxin transport shown in Fig. 45. Neither IAA nor GA_3 is able to offset the inhibitory effect of 10^{-4}M CFM, but the progressive inhibition of segment growth by increasing concentrations of CFM may be partially offset by the addition of high concentrations of IAA (Fig. 56A curve j). Such behaviour is to be expected when IAA is supplied in solution with CFM if the effect of the morphactin is indeed to prevent the polar transport of auxin, because the diffusion of IAA from the bathing solution may be expected to enhance the internal auxin concentration and thereby reduce the distance over which the auxin molecules must be transported to their site of action.

11. The role of IAA in the geotropic response in the wheat leaf sheath base

In order to investigate the auxin involvement more fully, a comparison has been made between the effects of CFM in the leaf sheath base and the coleoptile. The effects of combinations of IAA and CFM on growth in excised segments are shown for geotropically stimulated ('lowers') and unstimulated ('uppers') segments in Figs. 58 and 59 respectively. The morphactin is a powerful inhibitor of the geotropic response (Fig. 58 curve f), but the effect on the leaf sheath base may be overcome by the addition of IAA at relatively low concentrations (curves g and e). Auxin induced growth in the unstimulated segments is entirely insensitive to CFM at all concentrations tested. The analysis of variance yields values of F which are highly significant for IAA, but the values obtained for the CFM and interaction components are not significant at any significance level.

The relationship between auxin induced growth and geotropically induced growth is therefore different from that found in coleoptiles, and it seems unlikely that auxin can be involved in the type of co-ordinated reaction sequence which is found in the coleoptile. The action of IAA does, however, appear to be physiological. The effect of anoxia on the development of auxin and gravity-induced responses is shown in Fig. 60, and a comparison between the broken and solid lines in the treatments 'Lowers (w)' and 'Uppers (w)' reveals an equivalent dependency on aerobic metabolism for each of the two responses. The oxygen requirement for the IAA induced response may be overcome completely by the addition of 2% sucrose to the incubation medium, but this latter treatment is only partially able to replace the oxygen requirement for the geotropically induced response, and the treatment cannot be made more effective by the addition of IAA. Thus, although both processes appear to be physiological in nature, there do appear to be differences in their metabolic requirements.

The acropetal and basipetal movements of IAA-5-^{3}H in leaf sheath bases

Fig. 58.

Triticum aestivum L. var. Kolibri.

The effects of IAA and CFM on geotropically induced leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered, and quadrants were orientated as 'lowers' in 50 mm petri dishes containing 2.5 ml of a solution of IAA and CFM in the following combinations. Segments were shadowgraphed after a 24-h treatment period.

IAA	CFM	a 0	b 10^{-7} M	c 10^{-6} M	d 10^{-5} M	e 10^{-4} M
f	0					
g	10^{-6} M					
h	10^{-5} M					
i	10^{-4} M					
j	10^{-3} M					

Data are presented with CFM (fig. A) and IAA (fig. B) as abscissae.

White light 25°C.

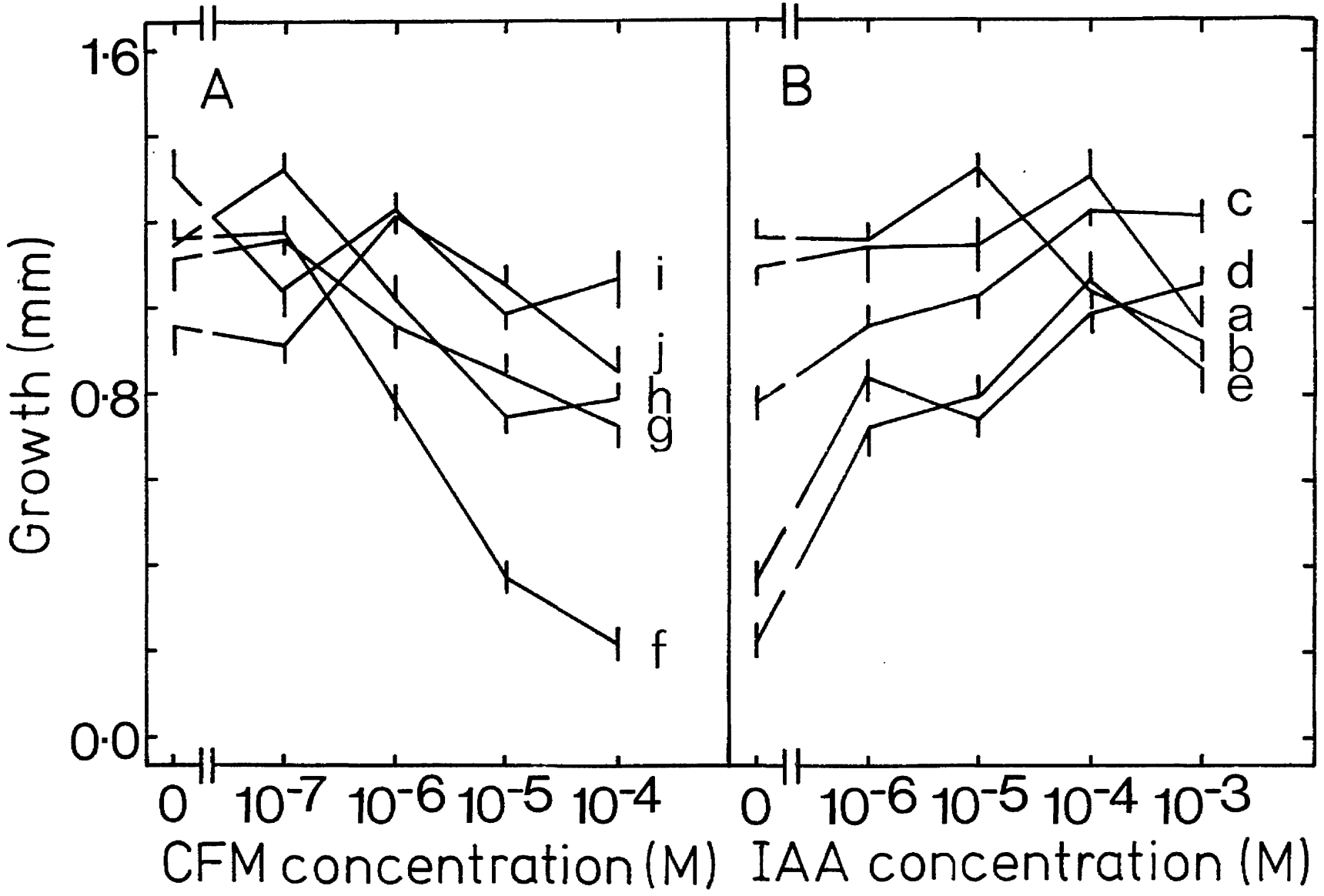

A
B
1·6
0·8
0·0
Growth (mm)
i
j
h
g
f
c
d
a
b
e
0
10-7
10-6
10-5
10-4
0
10-6
10-5
10-4
10-3
CFM concentration (M)
IAA concentration (M)

Fig. 59.

Triticum aestivum L. var. Kolibri.

The effects of IAA and CFM on auxin induced leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered, and quadrants were orientated as 'uppers' in 50 mm petri dishes containing 2.5 ml of one of the solutions tabulated for the previous figure (Fig. 58). Segments were shadowgraphed after 24 hours treatment. Data are presented with CFM (fig. A) and IAA (fig. B) as abscissae.

White light 25°C.

Statistical analysis. The analysis of variance was used to test the effects of CFM and IAA on segment growth.

	Error	rows (IAA)	columns (CFM)	Interaction
df	25	4	4	16
ss	0.1787	6.1456	0.0125	0.1734
ms	0.0071	1.5364	0.0031	0.0108
f		216.3943***	0.4366^{NS}	1.5211^{NS}

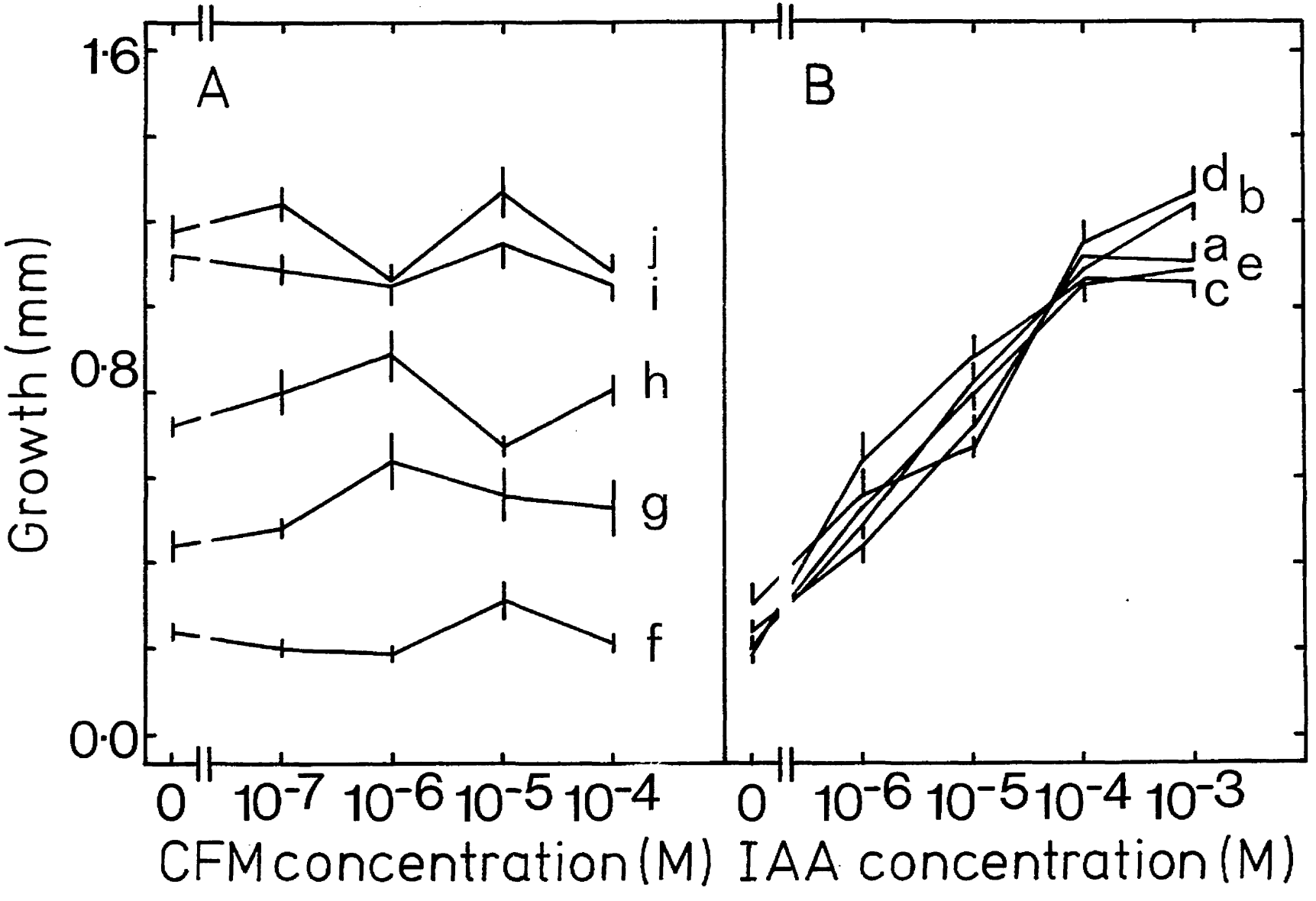

1·6
0·8
0·0
Growth (mm)
A
B
j
i
h
g
f
d
b
a
e
c
0
10-7
10-6
10-5
10-4
0
10-6
10-5
10-4
10-3
CFM concentration (M)
IAA concentration (M)

Fig. 60.

Triticum aestivum L. var. Kolibri.

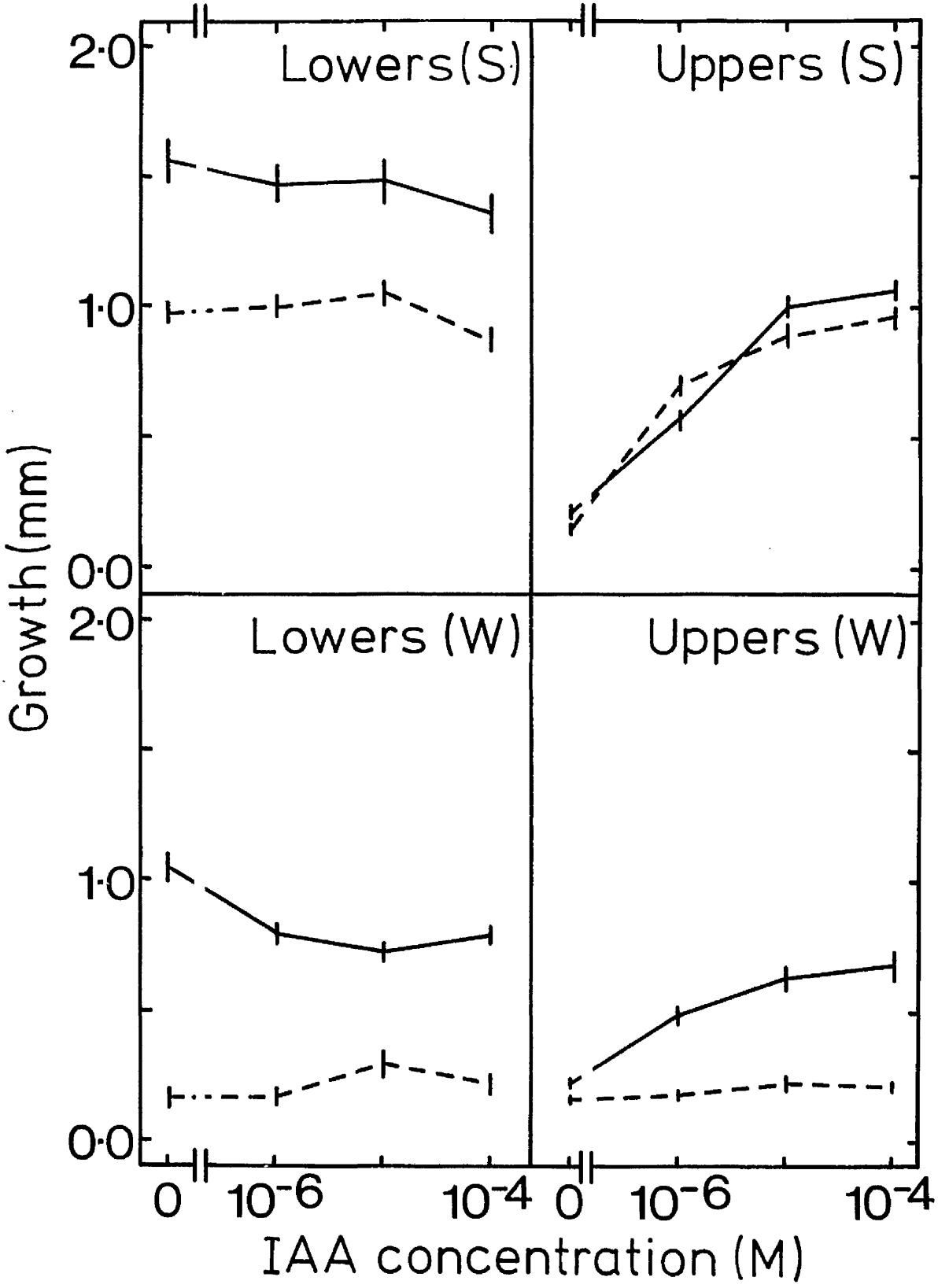
The effect of anoxia on IAA induced growth in the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of solution of IAA in the presence (S) or absence (W) of 2% sucrose. Dishes were maintained in a stream of air (———) or nitrogen (— — —) and segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and 10^{-4} M IAA treatments in air and nitrogen.

		t values	
		No sucrose	+ 2% sucrose
Air	Lowers	$t = 3.011^{**}$	$t = 1.600^{NS}$
	Uppers	$t = -8.245^{***}$	$t = -20.630^{***}$
N_2	Lowers	$t = -1.406^{NS}$	$t = 1.582^{NS}$
	Uppers	$t = -1.593^{NS}$	$t = -15.542^{***}$


Lowers (S)
Uppers (S)
Lowers (W)
Uppers (W)
2·0
1·0
0·0
Growth (mm)
0
10⁻⁶
10⁻⁴
IAA concentration (M)

are shown in Fig. 61, and the amounts of radioactivity passing into the distal halves of the segments are tabulated in the accompanying table. The leaf sheath bases have little capacity for auxin transport, but the data provide evidence to suggest a small basipetal polarity. More radioactivity is accumulated in the distal halves of the segments following apical donation, but the differential is only of the order of 5% total uptake. The effect of CFM on the distribution of apically and basally applied IAA-5-^{3}H is expressed as a function of time in Fig. 62, and an analysis of the data is also supplied in Table 6. The slight polarity of movement is abolished by the CFM pretreatment, but the shapes of the distribution curves remain virtually unaffected by the treatment.

Data presented in Figs. 63 and 64 show the lateral movement of labelled IAA in leaf sheath bases to be extremely sluggish. A significant polarity is developed after a transport period of 12 hours, but the differential is only of the order of 2 to 3% of total uptake. The polarity is again abolished by morphactin pretreatment (Table 7).

Fig. 61.

Triticum aestivum L. var. Kolibri.

The polarity of IAA movement through excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and held vertically between blocks of 1.5% agar. The radioactivity in extracts from the proximal (——) and distal (— — —) halves of the sections (with respect to the donor), and that in receiver blocks (—·—·—) was determined following apical (A) or basal (B) donation of IAA-5-^{3}H.

White light 25°C.

Statistical analysis. The t test was used to test the differences in combined uptake into the second subsection and receiver block following apical or basal donation of IAA-5-^{3}H.

Time h.	Uptake DPM (x100)		t value
	Apical donation	Basal donation	t A/B
4	8.057 ± 0.333	5.521 ± 0.707	2.321*
8	9.894 ± 0.855	5.259 ± 0.334	5.045***
12	11.040 ± 0.584	8.155 ± 0.986	2.699*
16	9.810 ± 0.650	6.765 ± 0.616	2.516*
20	11.877 ± 0.824	9.105 ± 1.311	3.397**
24	10.080 ± 0.674	6.913 ± 1.466	1.789^{NS}

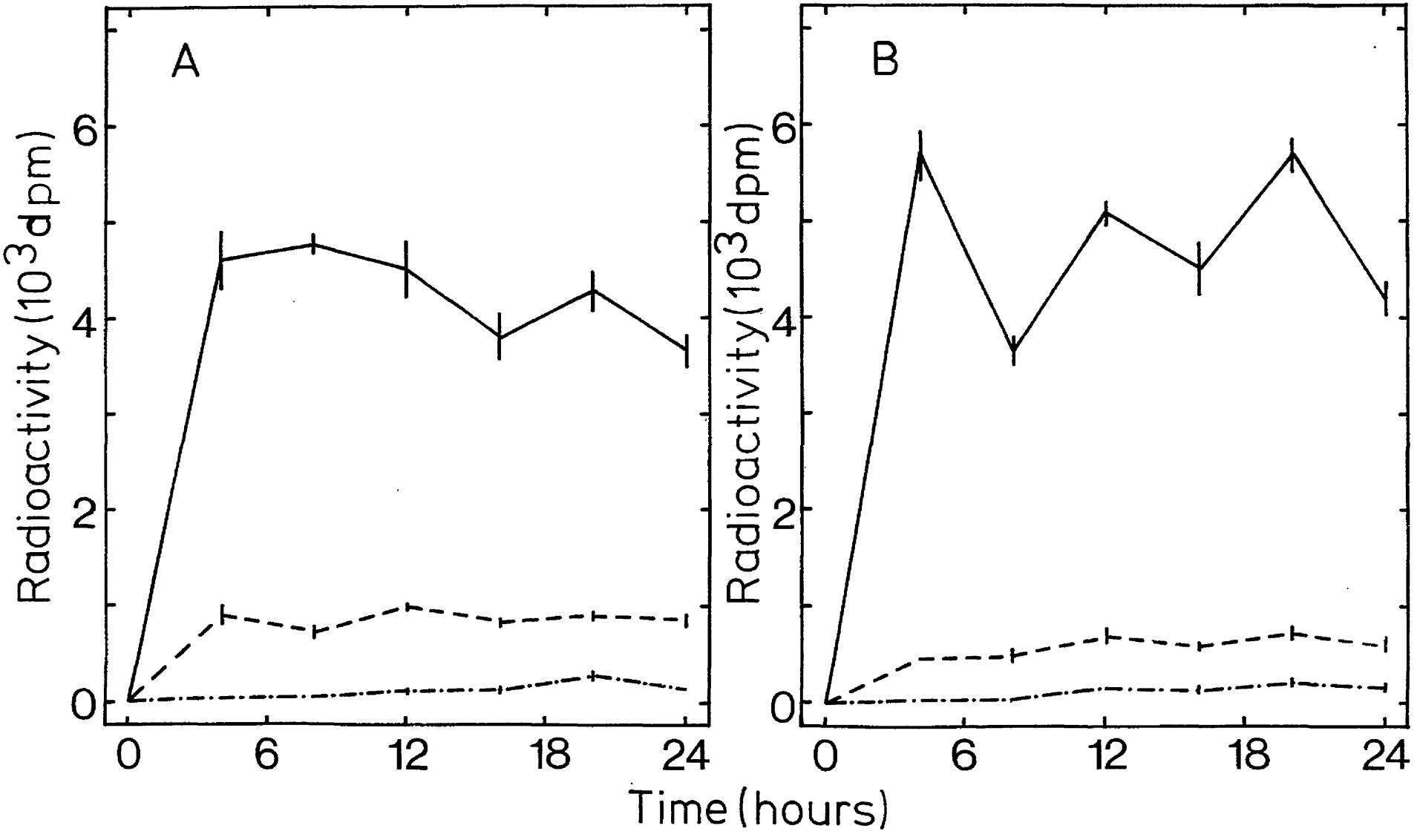

A
B
Radioactivity (10^3dpm)
Radioactivity (10^3dpm)
0
2
4
6
0
6
12
18
24
Time (hours)

Fig. 62.

Triticum aestivum L. var. Kolibri.

The effect of CFM on the polarity of IAA movement through excised leaf sheath bases.

Treatment: Portions of the leaf sheath base 2.4 mm in length were excised and pretreated by vertical submersion in a solution of 10^{-5} M CFM (broken lines) or distilled water (solid lines). Segments were removed after a 1-h pretreatment period and they were then placed vertically between blocks of 1.5% agar. The radioactivity in the proximal (circles) and distal (triangles) halves of the segments (with respect to the donor) and that in the receiver blocks (squares) was determined following apical (open symbols) or basal (solid symbols) donation of IAA-5-^{3}H.

White light 25°C.

Statistical Analysis (Table 6). The t test was used to test the differences in combined uptake into the second subsection and receiver block following apical or basal donation to control or CFM pretreated segments. The percentage data are tabulated after angular transformation.†

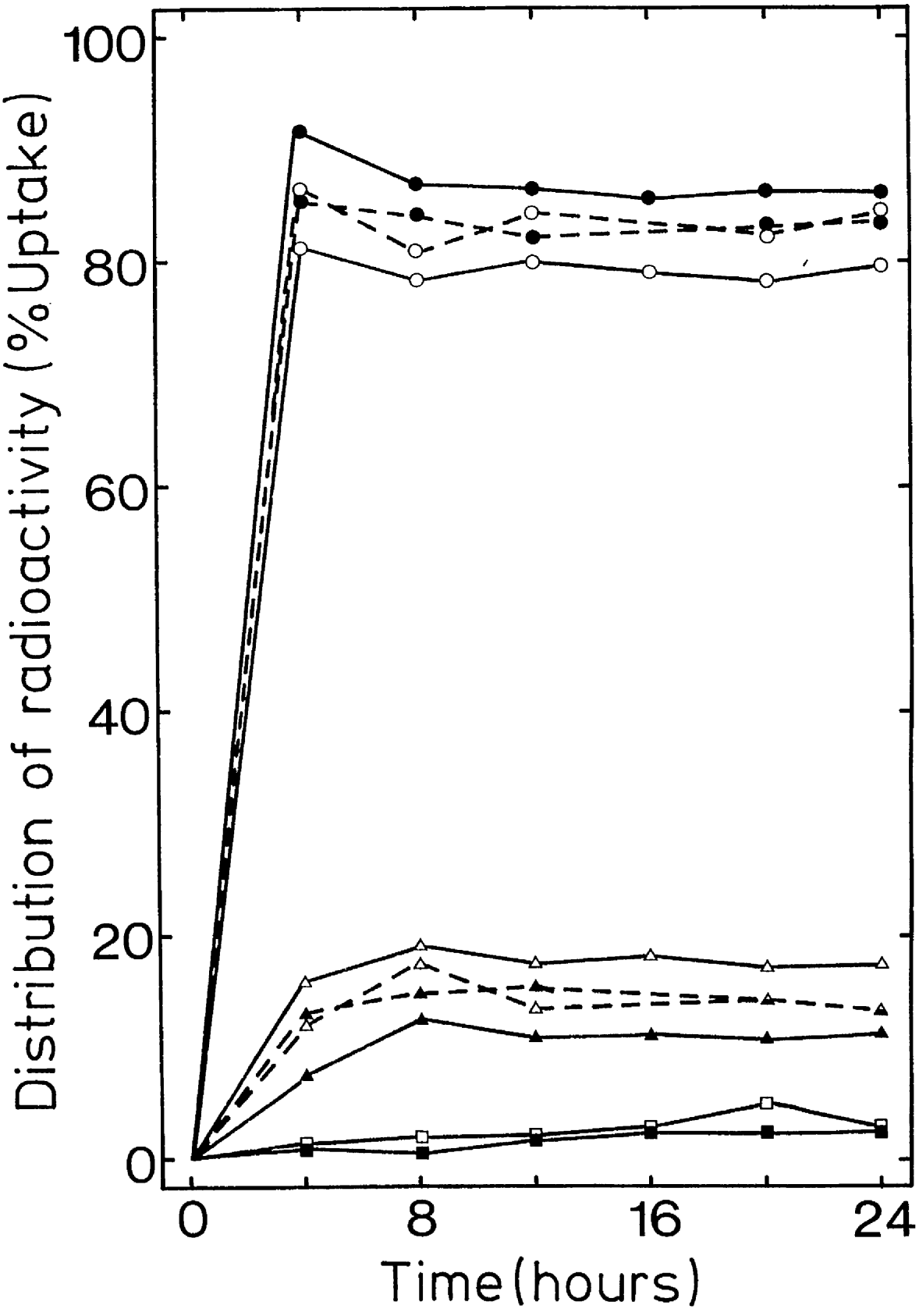

100
80
60
40
20
0
Distribution of radioactivity (%Uptake)
0
8
16
24
Time(hours)

Table 6

Time hours	Uptake % Total ± for control			Uptake % Total ± for CFM pretreatment		
	Apical donation	Basal donation	$t_{(A/B)}$	Apical donation	Basal donation	$t_{(A/B)}$
4	24.518 ± 1.082	17.034 ± 0.671	5.874***	21.306 ± 1.135	22.220 ± 1.274	-0.535^{NS}
8	27.562 ± 1.476	21.344 ± 1.288	3.173**	25.960 ± 1.180	23.258 ± 1.107	1.669^{NS}
12	26.352 ± 1.129	21.644 ± 1.291	2.744*	23.870 ± 0.707	24.808 ± 0.839	-0.854^{NS}
16	27.164 ± 1.089	21.944 ± 1.459	2.866*	25.262 ± 0.814	29.026 ± 1.542	-2.158^{NS}
20	27.704 ± 0.730	21.610 ± 1.461	3.730**	24.574 ± 1.357	24.082 ± 1.337	0.226^{NS}
24	26.776 ± 0.914	21.506 ± 1.687	2.745*	23.102 ± 1.253	23.758 ± 1.198	-0.378^{NS}

Fig. 63.

Triticum aestivum L. var. Kolibri.

The lateral movement of IAA through excised leaf sheath bases.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and held horizontally between blocks of 1.5% agar. The radioactivity in extracts from the proximal (———) and distal (— — —) halves of the sections (with respect to the donor), and that in the receiver blocks (—·—·—) was determined following donation of IAA-5-^{3}H to the upper (A) or lower (B) face of the segment.

White light 25°C.

Statistical analysis. The t test was used to test the differences in combined uptake into the second subsection and receiver following apical or basal donation.

Time h.	Uptake DPM		
	donation to top	donation to bottom	t(top/bottom)
4	164.0 ± 23.9	135.5 ± 44.4	0.592^{NS}
8	198.1 ± 35.8	119.8 ± 13.5	2.049^{NS}
12	285.2 ± 25.0	153.5 ± 43.9	2.622*
16	405.9 ± 68.6	227.3 ± 60.4	1.954^{NS}
20	334.6 ± 44.7	135.0 ± 13.0	4.292**
24	305.1 ± 40.3	153.1 ± 38.0	2.748*

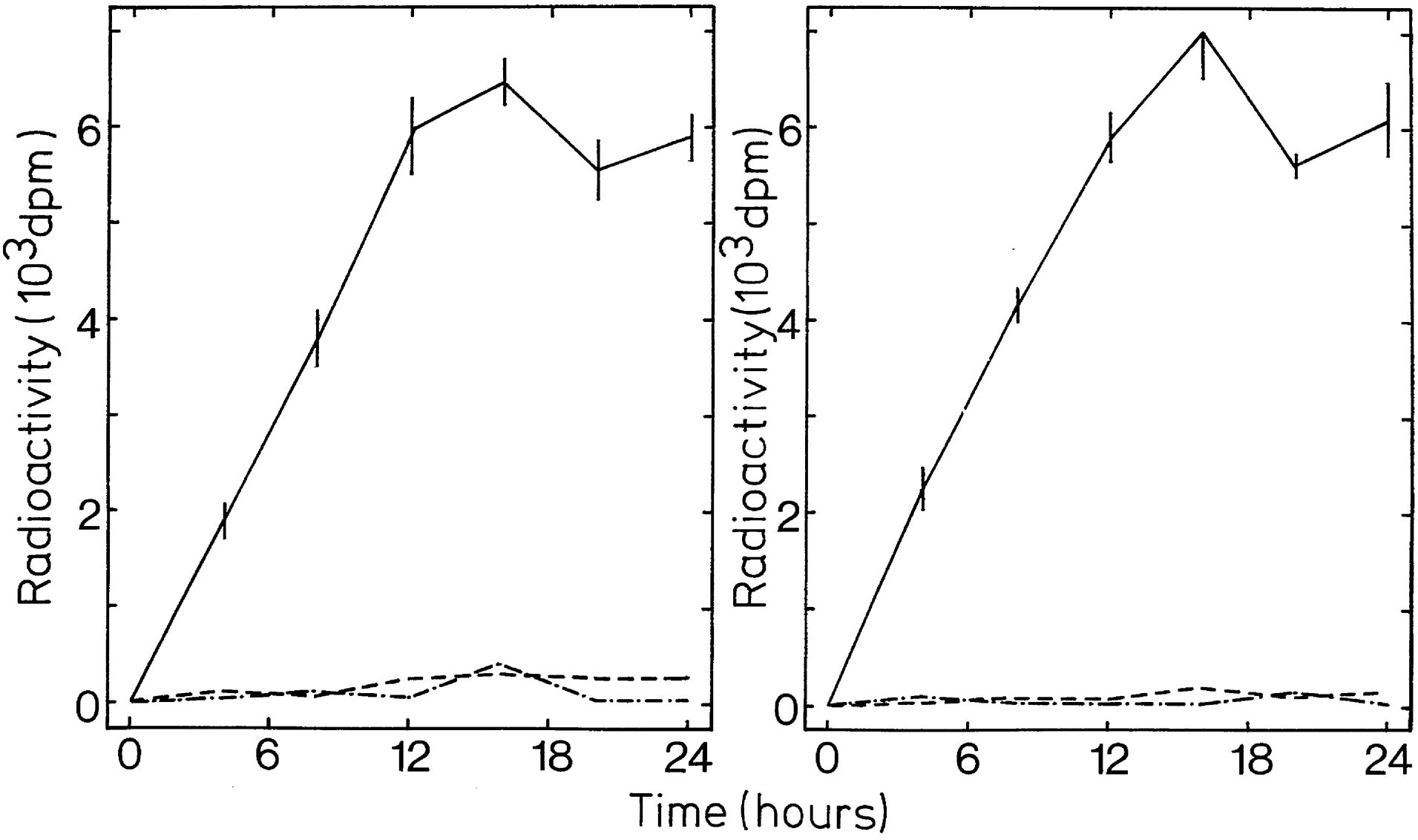

Radioactivity (10^3dpm)
Radioactivity(10^3dpm)
0
2
4
6
0
6
12
18
24
Time (hours)

Fig. 64.

Triticum aestivum L. var. Kolibri.

The effect of CFM on the lateral movement of IAA through excised leaf sheath bases.

Treatment: Portions of the leaf sheath base 2.4 mm in length were excised and pretreated by vertical submersion in a solution of 10^{-5} M CFM (broken lines) or distilled water (solid lines) for 1 h. After pretreatment they were removed and laid horizontally between blocks of 1.5% agar. The radioactivity in extracts from the proximal (circles) and distal (triangles) halves of the sections (with respect to the donor) and that in the receiver blocks was determined following donation to the upper (open symbols) or lower (solid symbols) faces of the horizontal segments.

White light 25°C.

Statistical analysis (Table 7). The t test was used to test the differences in combined uptake into the second subsection and receiver block following donation to the upper or lower sides of control or CFM pretreated segments. The percentage data are tabulated after angular transformation.†

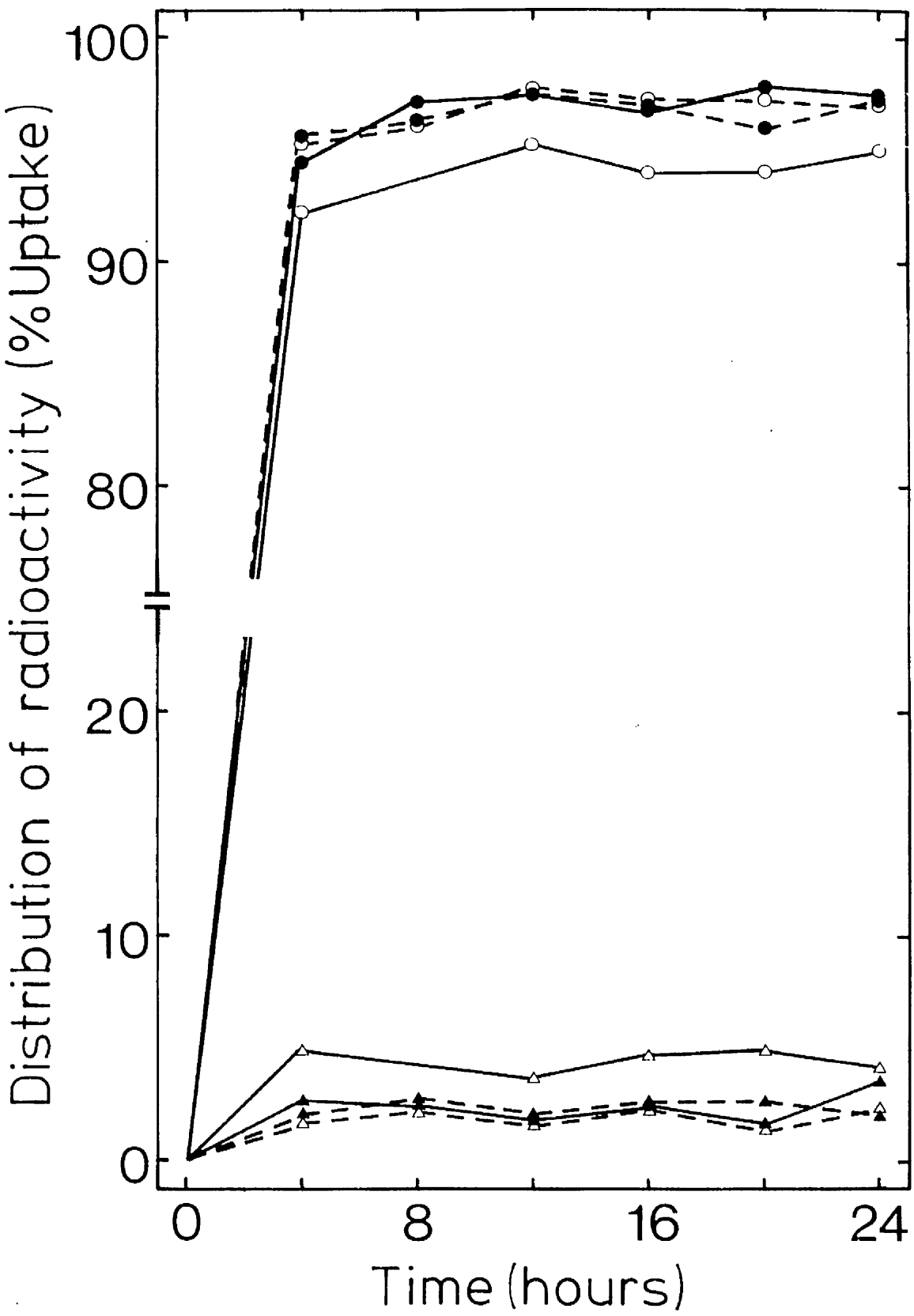

100
90
80
20
10
0
Distribution of radioactivity (%Uptake)
0
8
16
24
Time (hours)

Table 7

Time hours	Control: Uptake % of total donation to upper side	Control: Uptake % of total donation to lower side	$t_{(U/L)}$	CFM pretreatment: Uptake % of total donation to upper side	CFM pretreatment: Uptake % of total donation to lower side	$t_{(U/L)}$
4	15.718 ± 1.607	13.150 ± 1.662	1.185^{NS}	9.674 ± 0.482	9.658 ± 0.629	0.020^{NS}
8	8.206 ± 1.135	9.612 ± 0.573	-1.106	11.922 ± 0.329	11.246 ± 0.524	1.092^{NS}
12	12.350 ± 0.345	8.782 ± 1.041	3.255**	8.784 ± 0.435	9.102 ± 1.407	-0.216^{NS}
16	13.970 ± 1.224	9.938 ± 1.403	2.147*	9.776 ± 0.717	10.524 ± 0.327	-0.950^{NS}
20	14.292 ± 0.600	8.764 ± 0.412	7.603***	9.404 ± 0.777	11.382 ± 0.365	-2.305*
24	12.652 ± 0.597	9.495 ± 1.500	2.419*	9.868 ± 0.553	9.244 ± 0.771	0.658^{NS}

12. The existence of endogenous IAA in the leaf sheath base

In order to complete the investigation into the role of IAA it was felt necessary to repeat some of the diffusion and extraction experiments which have been reported by previous workers (Schmitz 1933, Van Overbeek et al., 1945) to yield positive evidence for an auxin involvement in this system.

Two assays for diffusible promoters are shown in Fig. 65, and these involve the use of the Avena straight growth assay for auxin-like activity and the Lettuce hypocotyl assay for gibberellin-like activity. No evidence for the presence of diffusible auxins or gibberellins has been forthcoming from this type of experimentation, and the inference that such substances are not involved in the geotropic response in the leaf sheath base is supported by the data presented for experiments involving barrier treatments (Fig. 13) and excised segments (Fig. 14).

Evidence for the involvement of extractable auxins in the geotropic response in the leaf sheath base is also lacking. Extractions using procedures which have been used in parallel experiments to extract IAA from various seedling organs have failed to isolate IAA from the geotropically stimulated leaf sheath base (Table 8), and little biological activity has been found in association with the acid fractions from such extracts following bioassay using the Avena straight growth test (Fig. 66).

If IAA exists in control material, then it can only be present at relatively low concentrations, because concentrations at least as low as 10^{-6}M induce straight growth. Thus if IAA is to be implicated in the geotropic response it must be concentrated at a site in response to geotropic stimulation. Several models may be advanced to explain such a process, and these may be divided into three categories.

1. Models involving IAA transport to the site.

2. Models involving IAA synthesis at the site.

3. Models involving IAA release from a bound precursor at the site.

All models will have to contain a mechanism for the rapid removal or

Fig. 65.

Triticum aestivum L. var. Kolibri.

Diffusible promotors from the leaf sheath base.

Treatment: Leaf sheath bases were excised and bisected and segments were orientated as uppers (U) or lowers (L) on blocks of 1.5% agar. Blocks were removed after a 24-h diffusion period for assay against untreated control blocks (C).

1. Auxin assay. (fig. A) The agar blocks were applied apically to Avena coleoptile segments and the segments were measured after a 24-h treatment period.

Darkness 25°C.

2. Gibberellin assay. (fig. B) Germinated lettuce seedlings (var. Arctic King) were placed on the agar blocks and the lengths of the hypocotyls were determined after a 72-h treatment period.

White light 25°C.

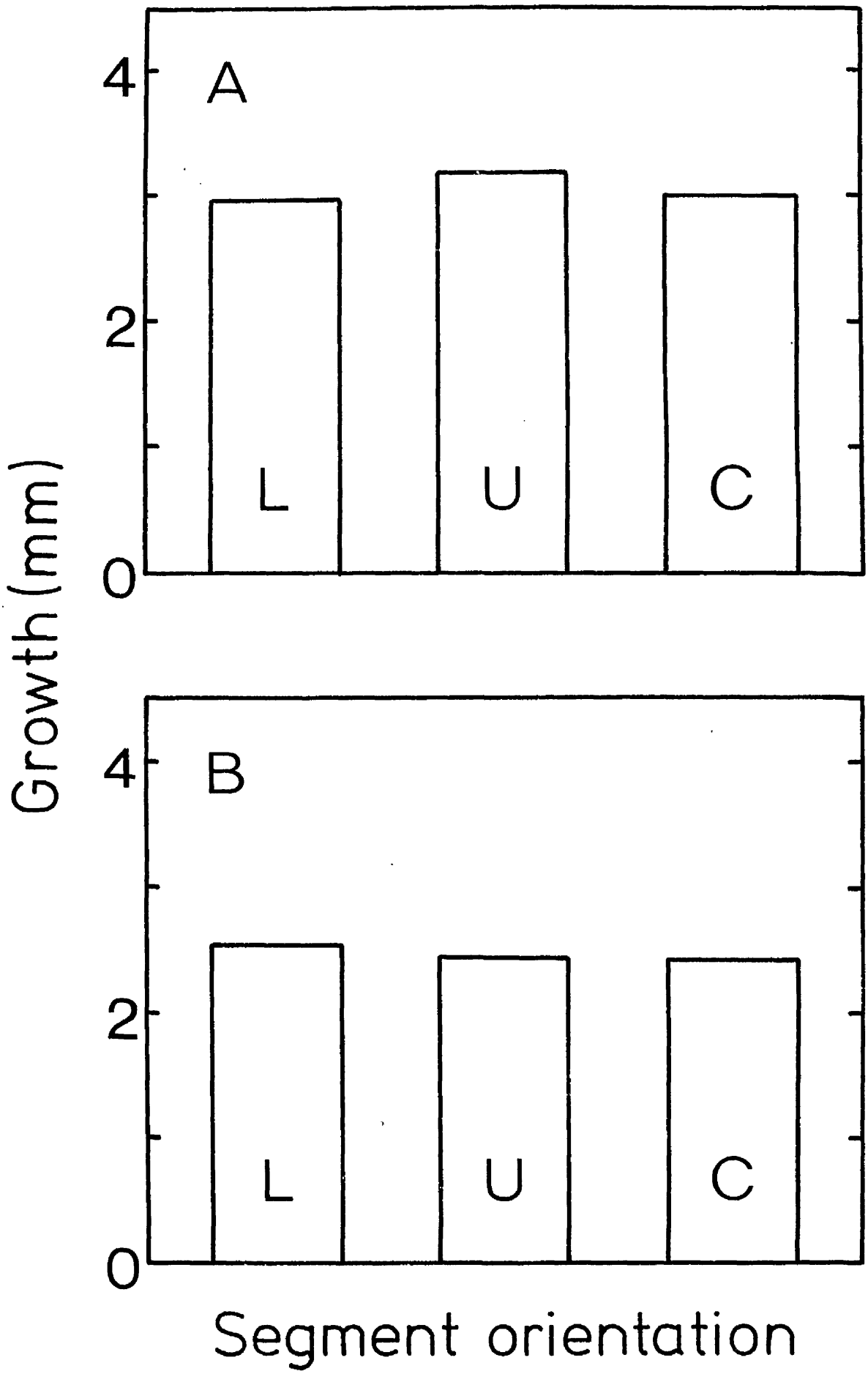

A
B
4
2
0
L
U
C
Growth (mm)
Segment orientation

Table 8.

Triticum aestivum L. var. Kolibri.

The occurrence of indoles in the leaf sheath base.

Treatment: Crude extracts were reduced to small volume and streaked on Whatman 3MM chromatography papers. Aliquots of several known standards were also applied separately along the starting lines and the papers were developed through 300 mm using either the basic solvent 10 : 1 : 1 isopropanol : ammonia : water or the acid solvent 5 : 1 : 2.2 butanol : acetic acid : water. Chromatograms were dried and sprayed with Ehrlich's reagent and colours were allowed to develop in an oven at 95°C.

Table 8A

Standard	R f	
	solvent (a) 10 : 1 : 1	solvent (b) 5 : 1 : 2.2
Indole acetic acid	0.484	0.872
Indole glyoxylic acid	0.324	0.798
Indole lactic acid	0.438	0.798
Indole proprionic acid	0.483	0.872
5 hydroxy indole acetic acid	0.250	0.723
Indolyl acetyl aspartate	0.106	0.761
Tryptophan	0.364	0.461

Table 8B

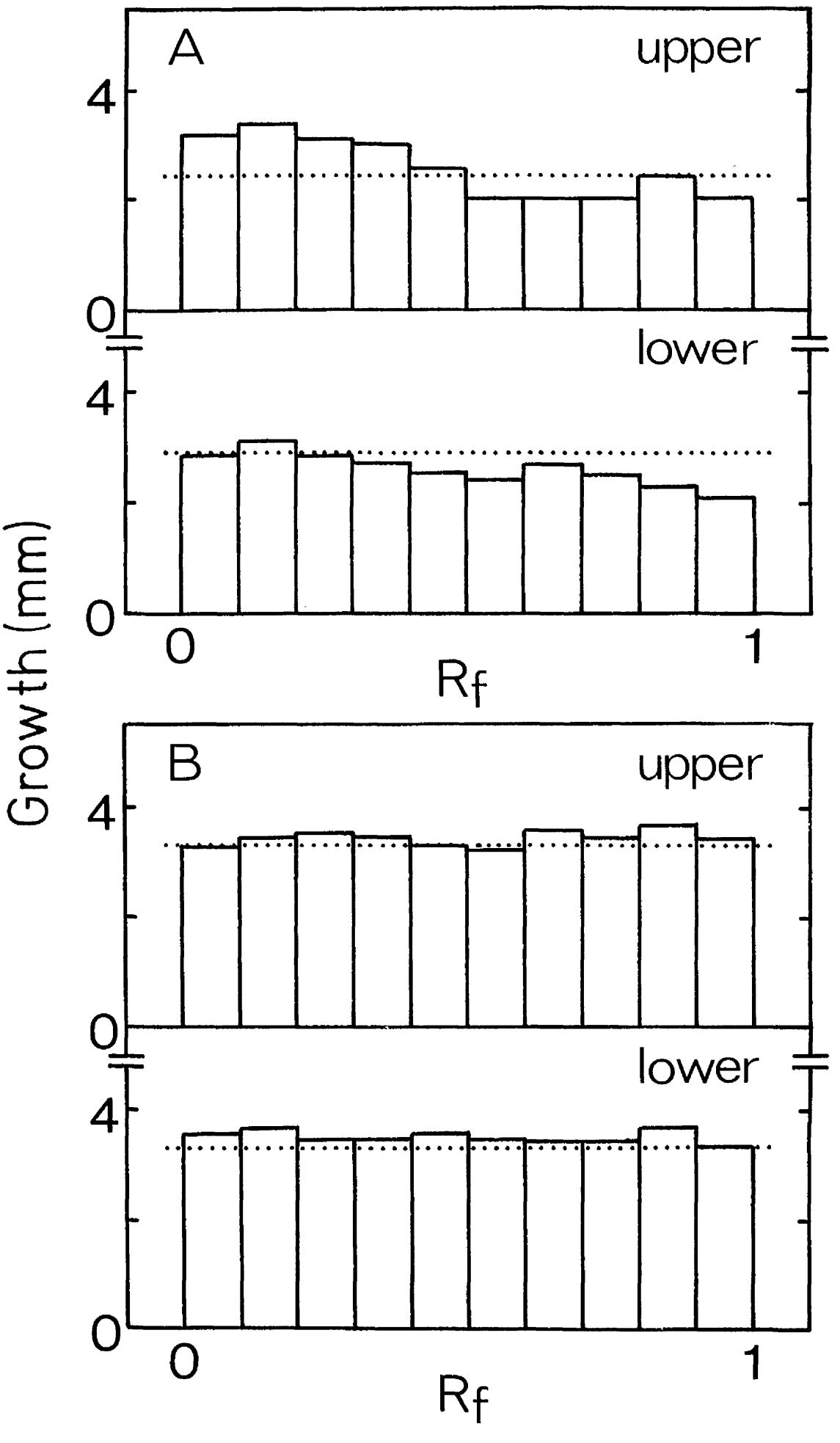
Ehrlich positive Unknowns	R f	
	solvent (a) 10 : 1 : 1	solvent (b) 5 : 1 : 2.2
A	0.362	0.461
B	0.146	0.720

Fig. 66.

Triticum aestivum L. var. Kolibri.

The occurrence of IAA in leaf sheath bases.

Treatment: Batches of 1000 stem segments were pinned horizontally and leaf sheath bases were excised and bisected after a 12-h stimulation period. Upper and lower halves were extracted in 80% methanol and extracts were partitioned with diethyl ether. The acid fraction was retained for paper chromatography using either the basic solvent system 10 : 1 : 1 isopropanol : ammonia : water (fig. A) or the acid solvent system 5 : 1 : 2.2 butanol : acetic acid : water (fig. B). Chromatograms were divided into 10 equal units and the units were assayed for auxin activity using the Avena coleoptile straight growth test.


A
upper
4
0
lower
4
0
0
1
Rf
Growth (mm)
B
upper
4
0
lower
4
0
0
1
Rf

destruction of IAA when the stimulus is removed, if they are to explain the requirement for continuous stimulation. Radiochemical analyses of the radioactivity present in excised segments are presented in Fig. 67 for extracts taken five hours after the apical donation of IAA-5-^{3}H. Exogenously applied IAA-5-^{3}H is extensively metabolised when applied to the excised leaf sheath base and, while poor separation of metabolites is achieved by chromatography with the polar acid solvent 5:1:2.2, butanol:acetic acid: water (row C), at least five major peaks of radioactivity are resolved by chromatography on the non-polar acid solvent 95:5 chloroform:acetic acid (row B), and the basic solvent 45:35:20 methyl acetate:isopropanol:ammonia (row A). Radioactivity is lost from the tissue to both receiver and donor blocks and metabolic products are found in both at the end of the transport period. It is clear from these data that the leaf sheath base has the capacity to metabolise IAA rapidly, and this may be taken as evidence to support the feasibility of models requiring the rapid destruction of IAA. Interpretation of the transport data is complicated, however, by this rapid accumulation of metabolites, and it may even be argued that the growth promoting activity associated with applied IAA is in fact attributable to its metabolites.

Models involving the transport of IAA to the site of the response are unacceptable, firstly because the leaf sheath base shows a very limited capacity for polar transport and, secondly, because the response remains unaffected by the barrier treatments designed to prevent transport. Failure to isolate IAA, or, indeed, any auxin-like activity, from geotropically stimulated leaf sheath bases may be taken as evidence against all three groups of models, but it may always be argued that the controlled synthesis or release of IAA at the site in quantities sufficient to permit the response without allowing the establishment of a pool of free auxin, could explain both the response and the failure to detect IAA by chemical means. The question of controlled synthesis is extremely difficult to investigate

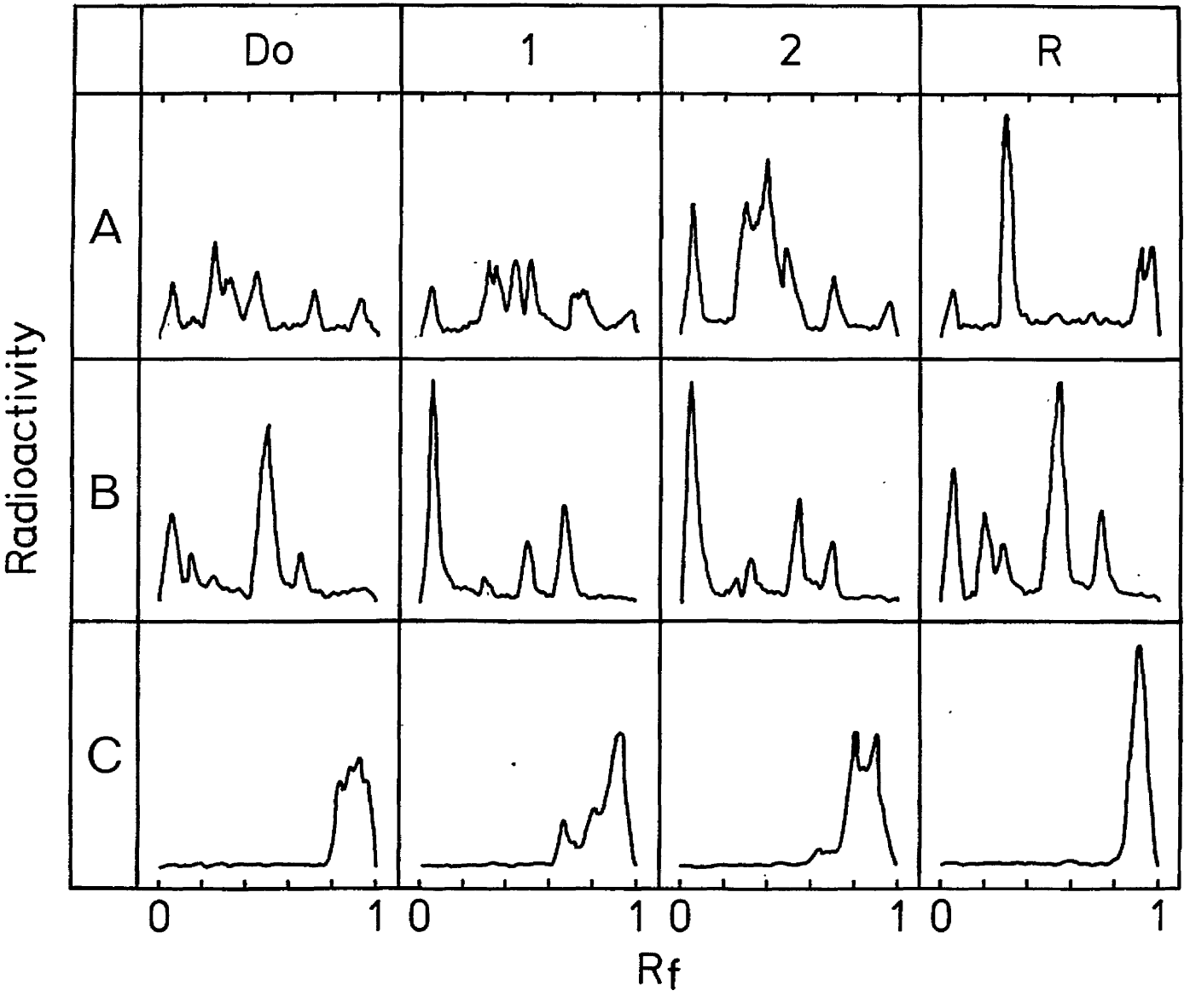
Fig. 67.

Triticum aestivum L. var. Kolibri.

Metabolism of applied IAA in excised leaf sheath bases.

Treatment: The radioactivity moving basipetally through 2.4 mm excised leaf sheath bases during the first 5 hours following apical donation of IAA-5-^{3}H was analysed by thin layer chromatography, on silica coated plates with the basic solvent 45 : 35 : 20 methyl acetate : isopropanol : ammonia (A) and the non-polar acid solvent 95 : 5 chloroform : acetic acid (B), and on cellulose coated plates with the polar acid solvent 5 : 1 : 2.2 butanol : acetic acid : water (C). Represented are chromatograms of methanolic extracts from the proximal (1) and distal (2) halves of the segments (with respect to the donor), the used donors (Do) and the receiver blocks (R).

IAA ran to Rf 0.4 on solvent (A), 0.55 on solvent (B) and 0.9 on solvent (C).


Do
1
2
R
A
B
C
Radioactivity
0
1 0
1 0
1 0
1
Rf

experimentally, and further consideration of this possibility is deferred to the Discussion. A preliminary search for bound auxins has revealed two substances which give Ehrlich positive reactions, and both of these substances are present in relative abundance (see Table 8). One of these substances has been identified by co-chromatography as tryptophan (Table 9), and the second has been partially characterised, though not actually identified (Table 9). It has no growth promoting ability and is not hydrolysed with dilute HCl. A purified extract has been subjected to mass spectroscopy, and its mass spectrum is presented in Fig. 68 together with the spectra for IAA and IAA-aspartate. The molecular ion has an m/e ratio of 330, indicative of a molecular weight of 330, and the fragmentation spectrum is not comparable with any published spectra for indoles (Jamieson and Hutzinger 1970).

Table 9.

Triticum aestivum L. var. Kolibri.

Identification of unknown indoles.

Unknown A was soluble in water and 80% ethanol, but insoluble in diethyl ether. It co-chromatographed with tryptophan (Table A).

Unknown B was soluble in water and 80% methanol and sparingly soluble in diethyl ether. It did not co-chromatograph exactly with IAA-aspartate or 5-OH IAA (Table B), and it did not hydrolyse when subjected to 1N HCl at 95°C for 24h (Table C).

Table 9

A

Substance	Number of Ehrlich positive spots	R f	
		solvent (a) 10 : 1 : 1	solvent (b) 5 : 1 : 2.2
Tryptophan	1	0.364	0.460
Tryptophan + Unknown A	1	0.364	0.461

B

Substance	Number of Ehrlich positive spots	Rf solvent (a) 10 : 1 : 1	Number of Ehrlich positive spots	Rf solvent (b) 5 : 1 : 2.2
Indolyl acetyl aspartate	1	0.106	1	0.760
5-OH Indole acetic acid	1	0.250	1	0.723
Indolyl acetyl aspartate + Unknown B	2	0.106 0.145	2	0.761 0.720
5-OH Indole acetic acid + Unknown B	2	0.250 0.146	1	0.722

C

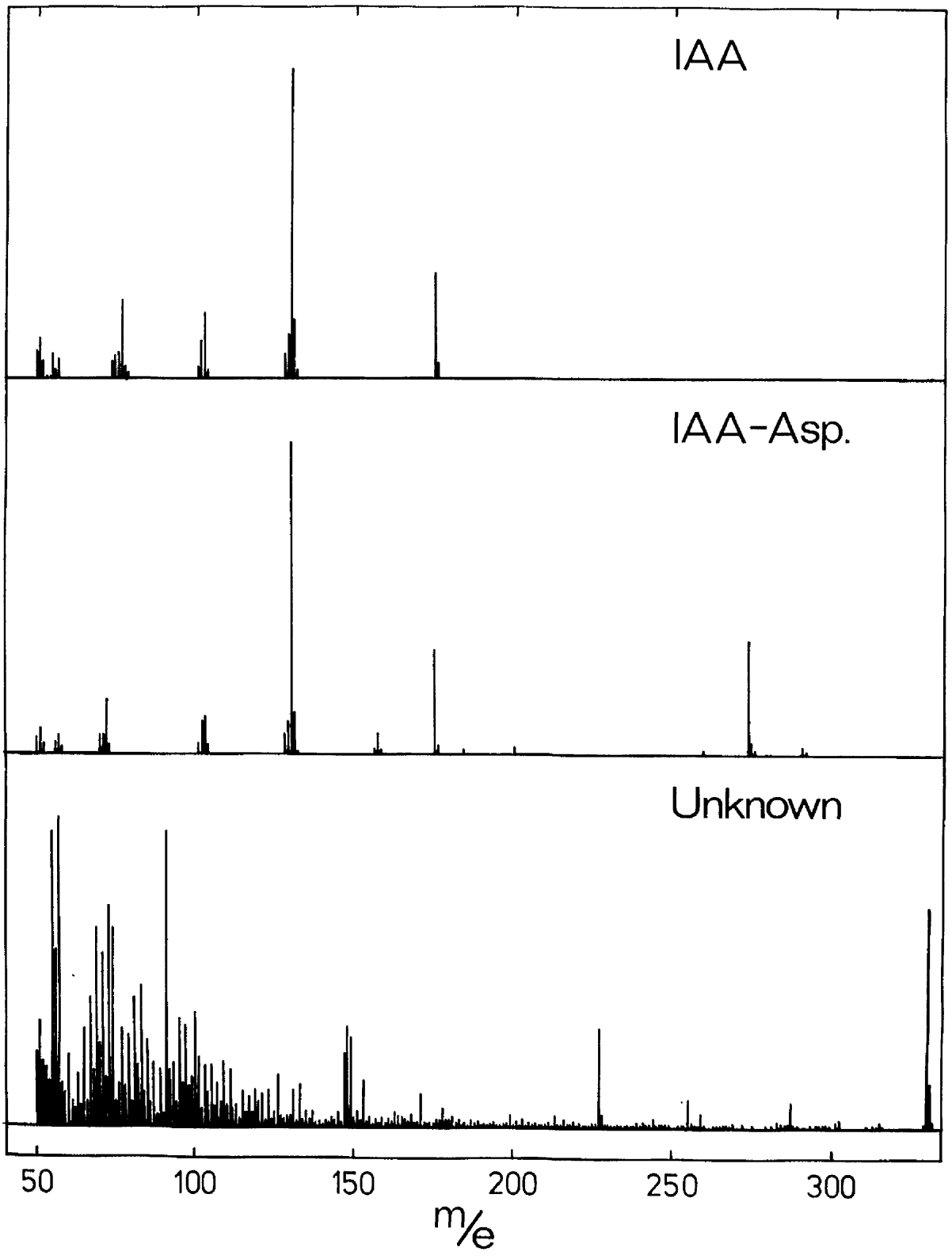
Substance hydrolysed	Rf	
	Number of spots	Solvent (b) 5 : 1 : 2.2
Indolyl acetyl aspartate	2	0.760 0.870
Unknown B	1	0.700

Fig. 68.

Triticum aestivum L. var. Kolibri.

Mass spectroscopy of unknown B.

Treatment: Unknown B was purified by paper chromatography using acid (5 : 1 : 2.2), basic (10 : 1 : 1) and acid (5 : 1 : 2.2) solvent systems, and the Ehrlich positive region was eluted from the final chromatogram for mass spectroscopy. The mass spectrum is compared with the spectra obtained for IAA and IAA-aspartate.


IAA
IAA-Asp.
Unknown
50
100
150
200
250
300
m/e

13. The turgor requirement for geotropically induced growth

The geotropic response is brought about by an increase in cell volume on the lower side of the leaf sheath base, and the expansion may be explained theoretically in terms of an increase in either cell wall extensibility or cell turgor.

The observation that growth is induced in excised segments when these are orientated as 'lowers' may be taken as evidence to preclude the requirement for transport of osmotically active substances into the lower half of the organ, but it does not preclude their production *in situ*, indeed the demonstration of an increase in the molar concentration of reducing sugars in the lower half of the organ (Arslan and Bennet-Clark, 1960) renders this a distinct possibility.

The changes in reducing sugar levels which occur in the leaf sheath base during a 24-h period of geotropic stimulation are shown in Fig. 69. Reducing sugar levels in the upper half of the organ are little affected by the treatment, but levels in the lower half increase by between 300 and 400%. This increase is greater than the increase in water content on the lower half of the organ, and the actual increase in *concentration* is of the order of 150%. Qualitative paper chromatography of extracts from the leaf sheath base indicates the presence, in readily detectable quantities, of three sugars, and these are characterised in Table 10. The three sugars have R_G values of 80, 100 and 111 when chromatographed using the solvent system 140:20:40, isopropanol:butanol:water, and they have been identified as sucrose, glucose and fructose respectively by co-chromatography with standard sugars.

A time course for the changes in sugar levels in leaf sheath bases taken from geotropically stimulated stem segments is shown in Fig. 70. Sucrose levels remain constant throughout the experimental period, but glucose and fructose levels show similar increases with stimulation time.

A comparison between the increases in reducing sugar levels in 100 mm stem segments and 2.4 mm segments excised from the leaf sheath base is

Fig. 69.

Triticum aestivum L. var. Kolibri.

Reducing sugar levels in the upper and lower halves of leaf sheath bases after a 24-h period of horizontal stimulation.

Treatment: Stem segments 100 mm in length were pinned either horizontally or vertically for 24 h. The leaf sheath bases were then excised and bisected and the upper (U) and lower (L) halves (left and right for vertical controls) were extracted in 80% ethanol. The reducing sugar contents of the extracts were determined as functions of the initial (fig. A) and final (fig. B) fresh weights of the tissues.

White light 25°C.

Sugar Realisation. Somogyi-Nelson Method.

Statistical Analysis. The t test was used to test the differences in reducing sugar levels between lower and upper halves (left and right halves in controls).

Fig. A. $t(0\ h) = 1.3^{NS}$ $t(24\ h) = 10.8^{***}$

Fig. B. $t(0\ h) = 1.3^{NS}$ $t(24\ h) = 6.4^{***}$

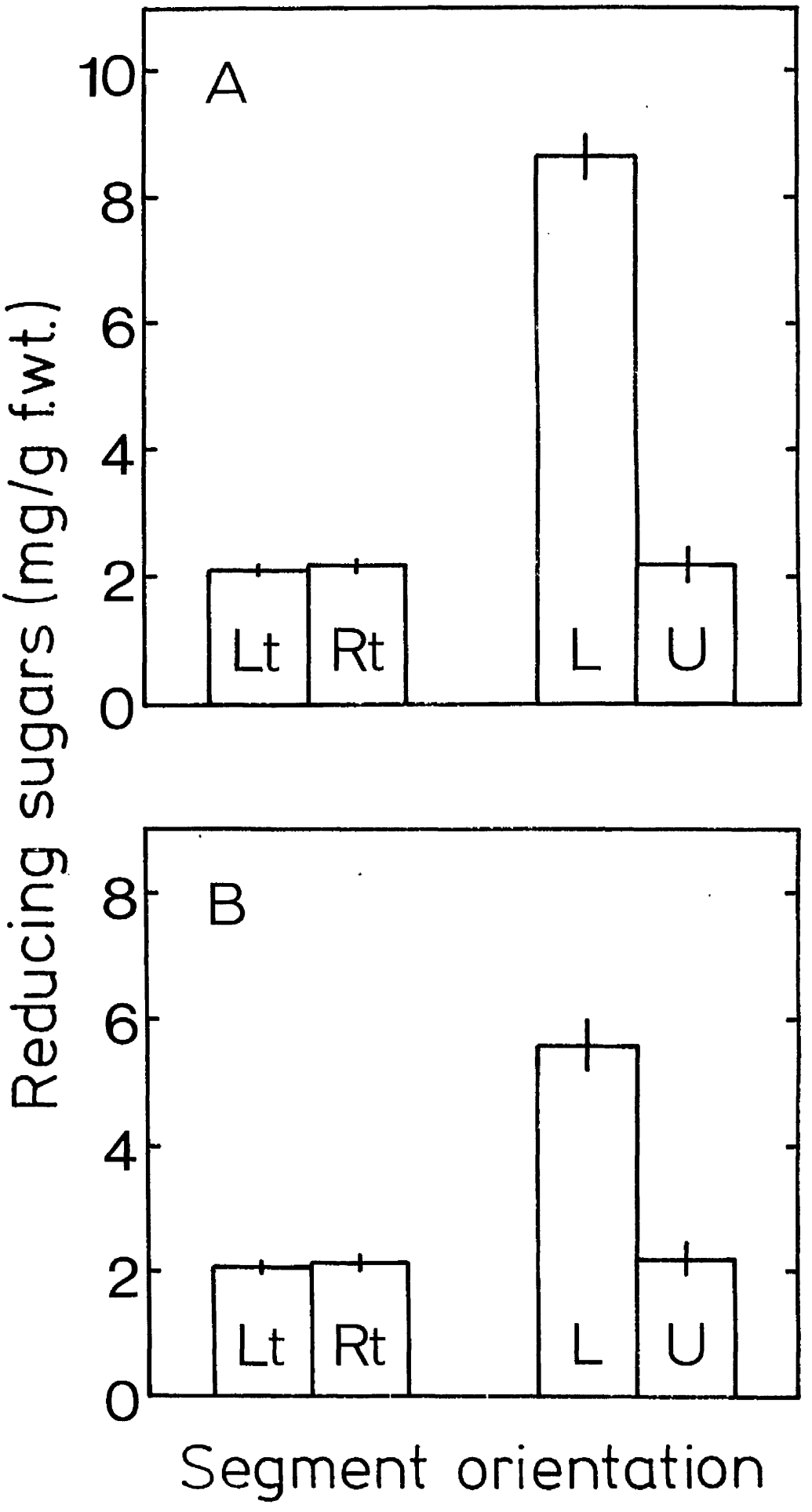

A
B
10
8
6
4
2
0
Lt
Rt
L
U
Reducing sugars (mg/g f.wt.)
Segment orientation

Table 10.

Triticum aestivum L. var. Kolibri.

Qualitative chromatography of sugars soluble in 80% ethanol.

Treatment: Stem segments 100 mm in length were pinned horizontally for a measured period of time. Leaf sheath bases were excised and bisected and the upper and lower halves were extracted in 80% ethanol. The extracts were streaked on to Whatman No. 1 chromatography papers which were chromatographed with the solvent 140 : 20 : 40 isopropanol : butanol : water for 36 h, air dried and dipped in aniline-diphenyl amine reagent. The colour reaction was allowed to develop in an oven at 95°C.

Table 10

Sample	R_G	Colour
Glucose	100.0	Grey
Fructose	111.0	Brown
Sucrose	80.0	Brown
Zero time extract	100.0	Grey
	110.0	Brown
	79.5	Brown
Lower halves after 24 hours geotropic stimulation	100.0	Grey
	110.0	Brown
	81.0	Brown
Upper halves after 24 hours geotropic stimulation	100.0	Grey
	110.0	Brown
	81.0	Brown

Fig. 70.

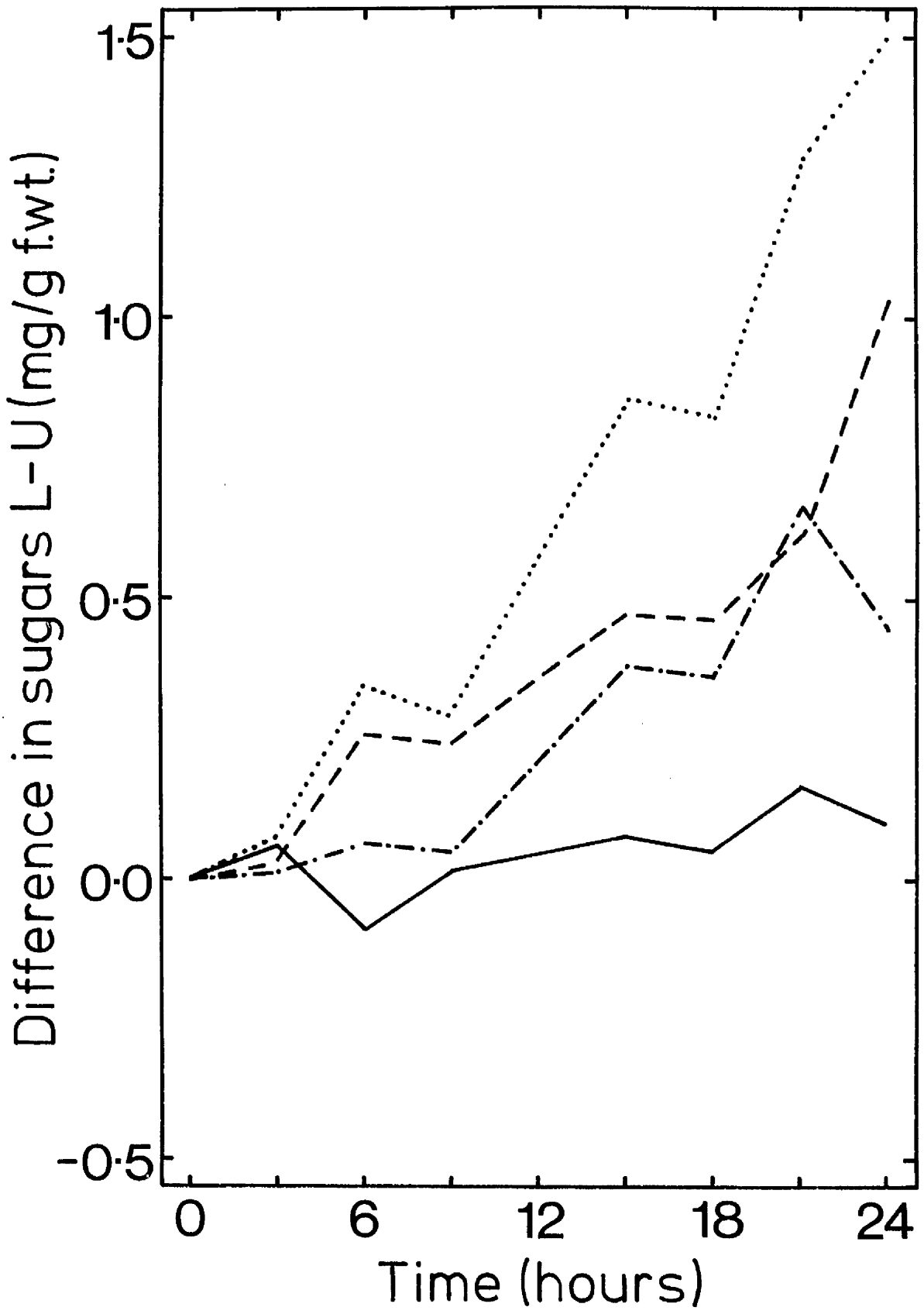
Triticum aestivum L. var. Kolibri.

The development of the asymmetry in sugar levels with time -- sugar levels as a function of initial fresh weight.

Treatment: Stem segments 100 mm in length were pinned horizontally and leaf sheath bases were excised and bisected after a measured stimulation period. The upper (U) and lower (L) halves were extracted in 80% ethanol and sugars were separated by paper chromatography. The differences in glucose (·····), fructose ($\frac{L}{U}$ — — —), sucrose (——————) and total reducing sugar levels (·-·-·) between lower and upper halves were calculated.

White light 25°C.

Sugar Realisation. Phenol-sulphuric acid method.


1·5
1·0
0·5
0·0
-0·5
Difference in sugars L-U (mg/g f.wt.)
0
6
12
18
24
Time (hours)

provided in Fig. 71. The data are calculated in terms of the percentage difference in total reducing sugars between lower and upper tissue orientations and they show that, while the reducing sugar levels in the lower halves of organs taken from 100 mm stem segments show a steady increase with time, the levels in the excised segments show only an initial 50% increase followed by a steady decline to control (uppers) levels by the end of the experimental period. The changes in fresh weight which develop during these two treatments are shown in Fig. 72. Growth proceeds at a uniform rate throughout the experimental period and, although the rate is clearly lower in the case of excised segments, the fact that it is maintained when sugar levels are in decline is evidence against the involvement of sugars in the promotion of growth through enhancement of turgor.

A time course for the changes in sugar levels in segments excised from the leaf sheath base prior to geotropic stimulation is shown in Fig. 73. Fructose levels show a small increase, but glucose levels remain virtually constant, and sucrose levels fall rapidly.

The changes in reducing sugar levels during geotropic stimulation must be considered in conjunction with the changes in other sugar levels when assessing a possible osmotic involvement for sugars. The effects of geotropic stimulation on the concentration of sugars in the upper and lower regions of geotropically stimulated leaf sheath bases are shown in Figs. 74 and 75. Although the quantities of sucrose in the upper and lower halves of the leaf sheath base remain virtually constant when 100 mm stem segments receive geotropic stimulation, the increase in volume on the lower side of the organ means that the actual concentration on the lower side falls below that on the upper side. The drop in sucrose molarity in the lower half of the organ is offset by an increase in reducing sugar levels, and the total concentration of osmotically active sugars shows an increase of about 25% over the 24-h experimental period. This increase, although considerable on a percentage basis, never represents more than a few micromoles per gram on

Fig. 71.

Triticum aestivum L. var. Kolibri.

The development of the asymmetry in reducing sugar levels in leaf sheath bases excised before (B) and after (A) geotropic stimulation.

Treatments: (A) Stem segments 100 mm in length were pinned horizontally and leaf sheath bases were excised and bisected after a measured stimulation period.

(B) Leaf sheath bases were excised and quartered and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of distilled water. Tissues were extracted in 80% ethanol and the differences in reducing sugar content between lower (L) and upper (U) tissue regions were calculated for leaf sheath bases which were excised before (-O-) and after (-●-) geotropic stimulation.

White light 25°C.

Sugar Realisation. Somogyi-Nelson Method.

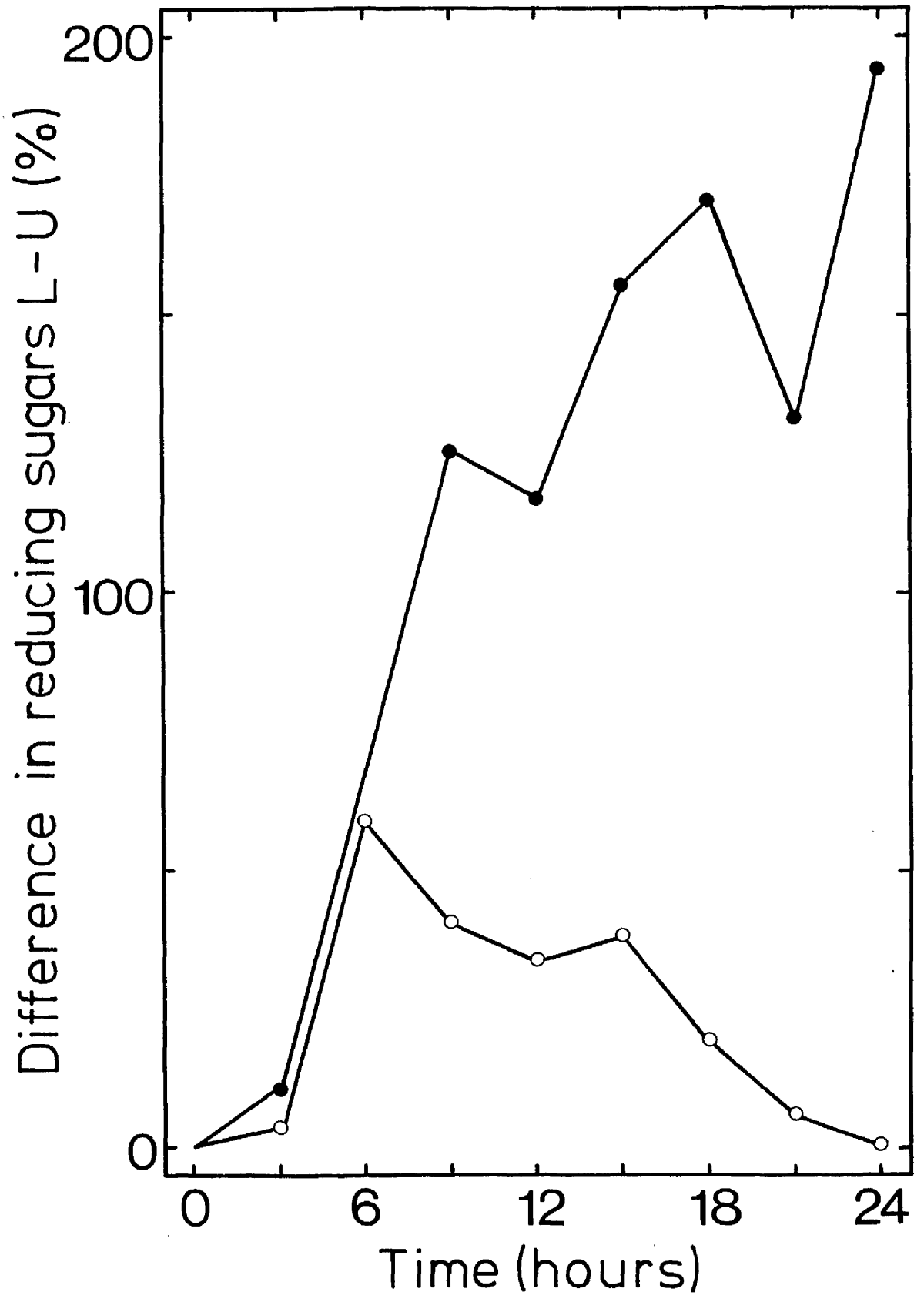

200
100
0
Difference in reducing sugars L-U (%)
0
6
12
18
24
Time (hours)

Fig. 72.

Triticum aestivum L. var. Kolibri.

The development of asymmetry in fresh weight with time. A comparison between the effects in leaf sheath bases excised before and after geotropic stimulation.

Treatments: Treatments were as in the previous figure (fig. 71), but the differences in fresh weight between the lower and upper tissues of intact leaf sheath bases (100 mm stem segments ——●——) and 'lower' and 'upper' segment orientations (excised segments ——○——) were considered.

White light 25°C.

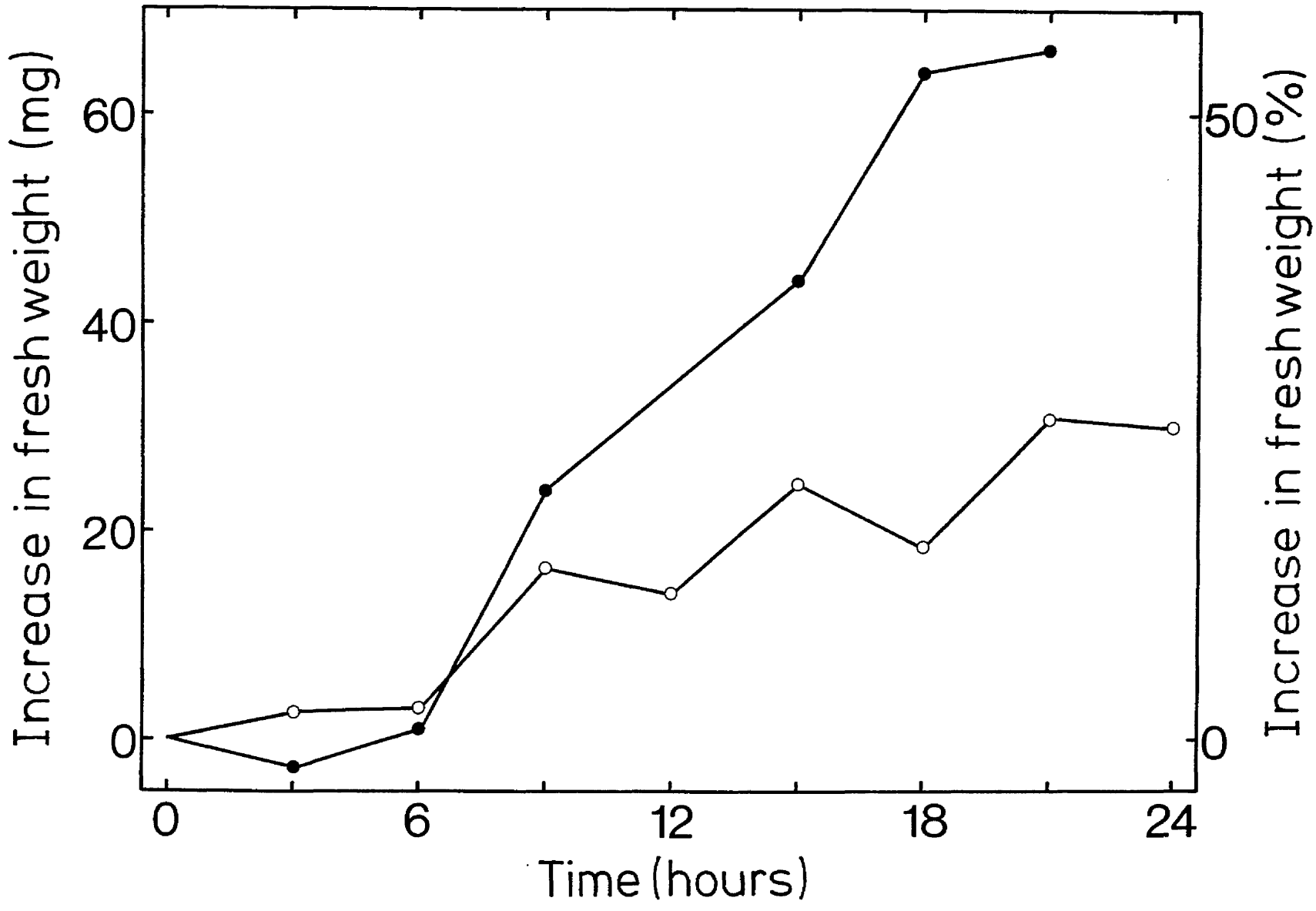

Increase in fresh weight (mg)
60
40
20
0
Increase in fresh weight (%)
50
0
0
6
12
18
24
Time (hours)

Fig. 73.

Triticum aestivum L. var. Kolibri.

The development of the asymmetry in sugar levels with time - sugars as a function of initial fresh weight.

Treatment: Leaf sheath bases were excised and quartered and batches of 20 quadrants were orientated as 'uppers' or 'lowers' in 90 mm petri dishes containing moistened filter paper. At the end of the appropriate stimulation period segments were extracted in 80% ethanol and sugars were separated by paper chromatography. The differences in glucose (—·—·—), fructose (– – – –), sucrose (———) and total reducing sugar levels (·····) in 'lower'(L) and 'upper' (U) segments were calculated in terms of the initial fresh weights of the segments.

White light 25°C.

Sugar Realisation. Phenol-Sulphuric acid Method.

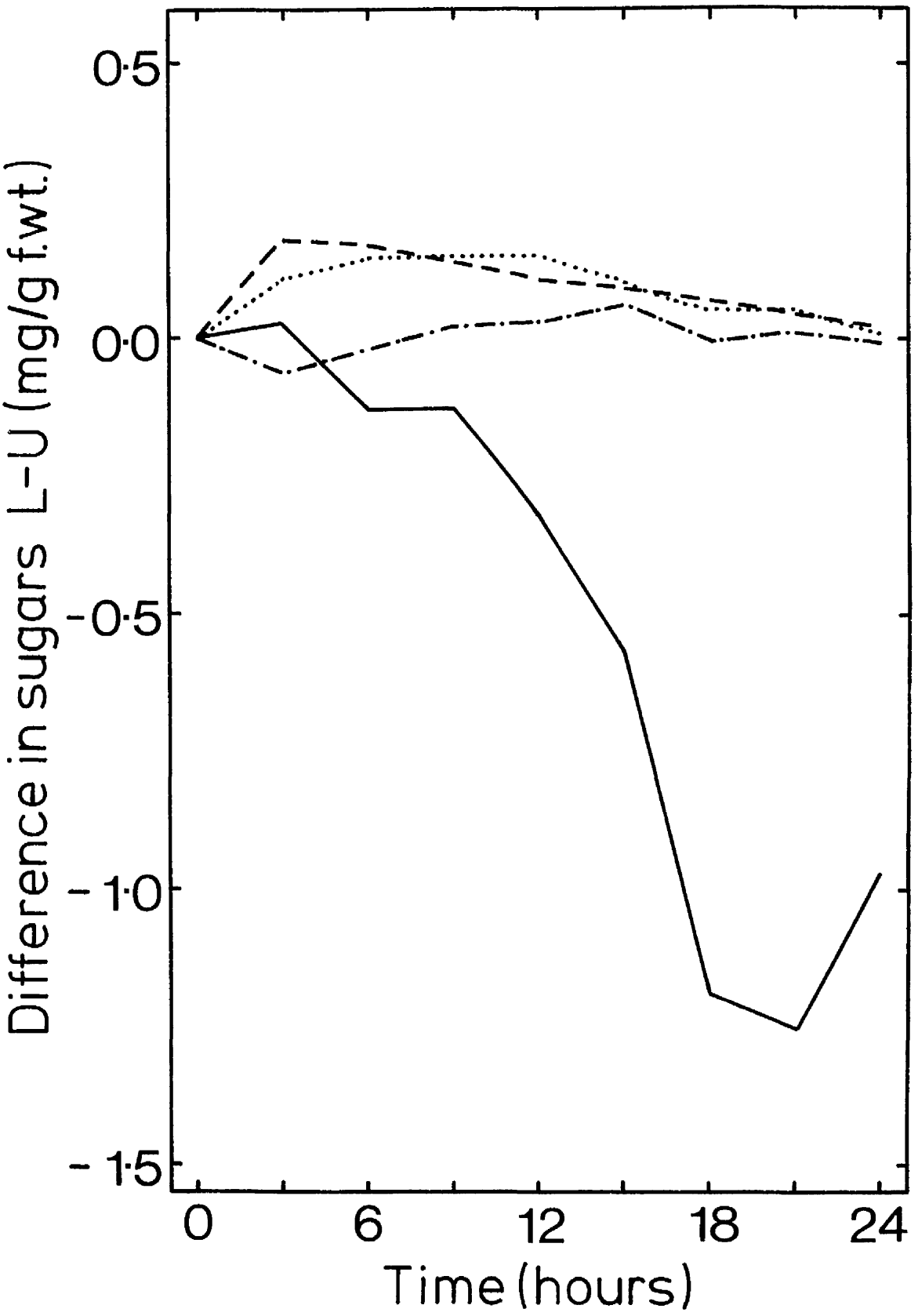

0·5
0·0
-0·5
-1·0
-1·5
Difference in sugars L-U (mg/g f.wt.)
0
6
12
18
24
Time (hours)

Fig. 74.

Triticum aestivum L. var. Kolibri.

The development of the asymmetry in sugar levels with time - sugar levels as a function of final fresh weight.

Treatment: Stem segments 100 mm in length were pinned horizontally and leaf sheath bases were excised and bisected after a measured stimulation period. The upper (U) and lower (L) halves were extracted in 80% ethanol and the sugars were separated by paper chromatography. The differences in sucrose (—o—) and total reducing sugar levels (—●—) between lower and upper halves were calculated in terms of final fresh weights of the tissues.

White light 25°C.

Sugar Realisation. Phenol-Sulphuric acid Method.

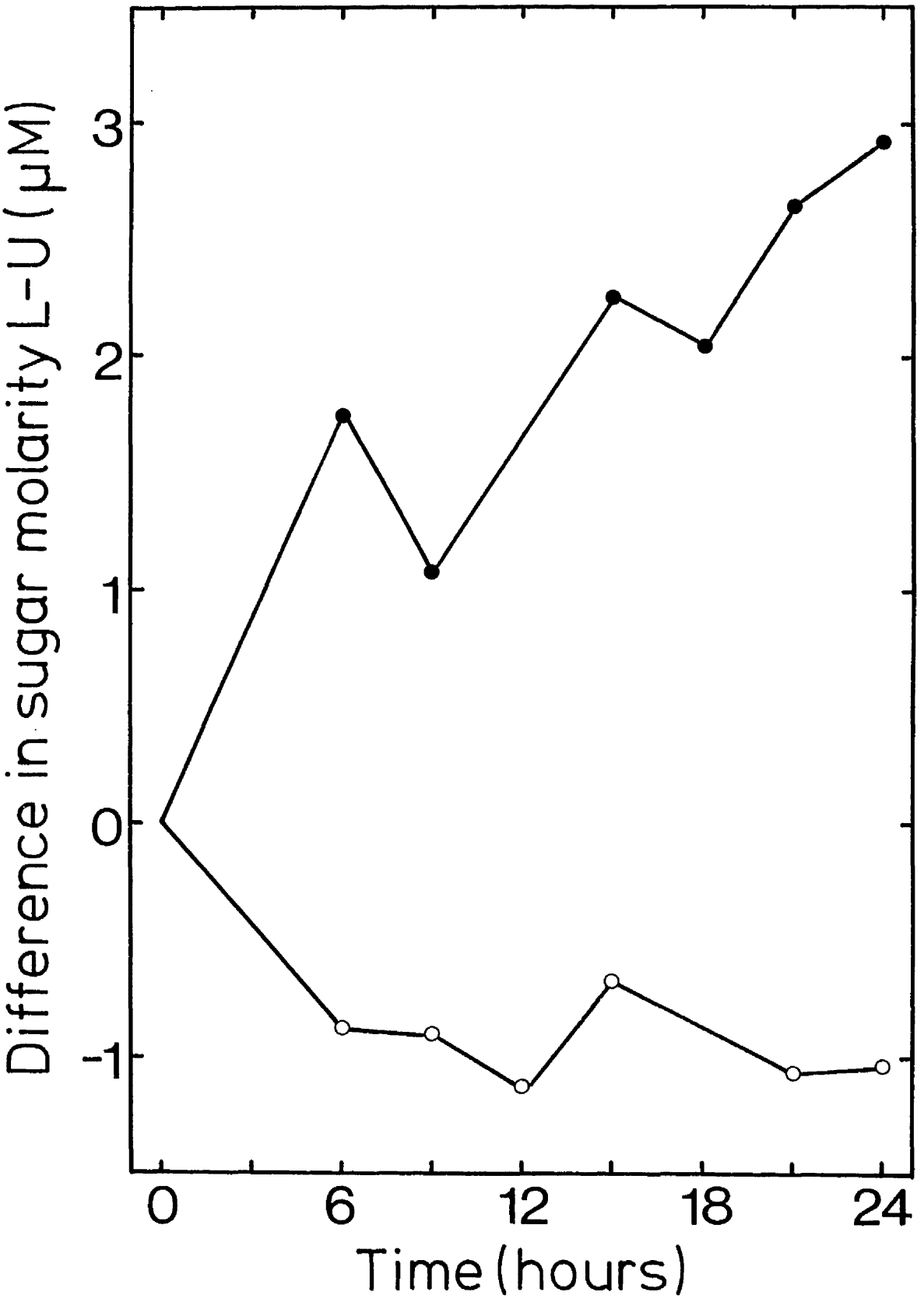

Difference in sugar molarity L-U (μM)
3
2
1
0
-1
0
6
12
18
24
Time (hours)

Fig. 75.

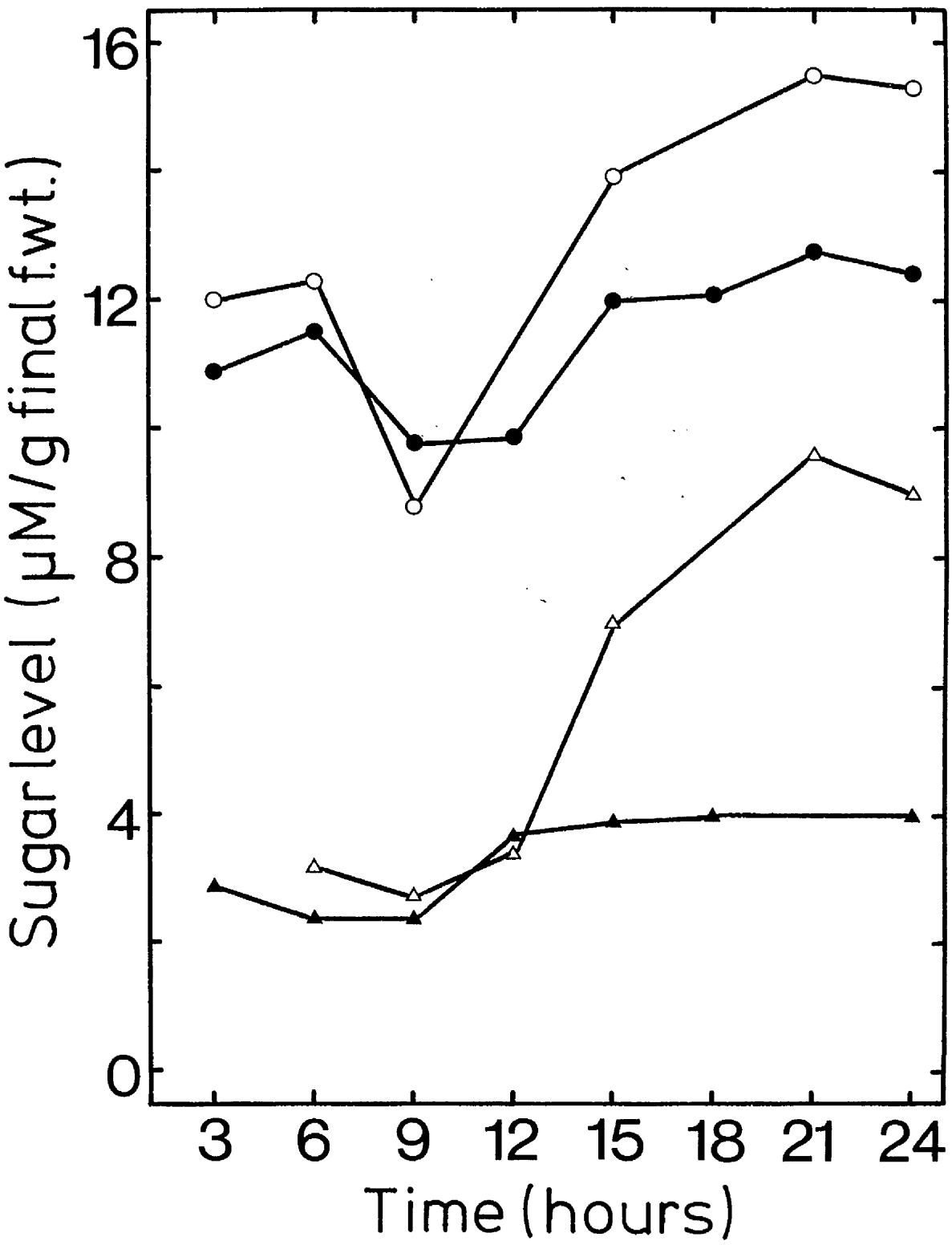
Triticum aestivum L. var. Kolibri.

The total molarity of osmotically active sugars.

Treatment: Stem segments 100 mm in length were pinned horizontally and leaf sheath bases were excised and bisected after a measured stimulation period. The upper and lower halves were extracted in 80% ethanol and the sugars were separated by paper chromatography. The total sugar molarities (circles) and reducing sugar molarities (triangles) in the upper (closed symbols) and lower (open symbols) halves were calculated.

White light 25°C.

Sugar Realisation. Phenol-Sulphuric acid Method.


16
12
8
4
0
Sugar level (μM/g final f.wt.)
3
6
9
12
15
18
21
24
Time (hours)

a fresh weight basis, and it is probably insignificant in terms of cell turgor.

The effect of geotropic stimulation on sugar concentration in segments excised prior to stimulation is shown in Figs. 76 and 77. The sucrose supply in transit from the flag leaf is eliminated when segments are excised from the leaf sheath base prior to the start of the stimulation period, but segments are still able to grow in response to geotropic stimulation. Sucrose molarity shows a rapid decline, and glucose and fructose molarities also fall. The free sugars present in the incubation media at the end of a 24-h experimental period are quantified in Table 11. The loss to the medium is of the order of 2μM per gram of tissue regardless of segment orientation, and a loss of this order is too small to account for the reduction in total sugar concentration, even in control segments. The total sugar concentration drops by about 75% in geotropically stimulated segments compared with 45% in control segments, and these losses can only be assumed to represent a metabolic requirement.

Since the fresh weight of the geotropically stimulated segments continues to rise despite the reduction in sugar concentration, the concept of growth by enhanced turgor seems unlikely. It may, however, be argued that the gross depletion of sugar reserves in excised segments is not representative of the situation in the growing cells. Further evidence against an increased turgor requirement may be taken from certain manipulative experiments which are summarised in Fig. 78. The geotropic stimulus is retained for only 45 min on returning a horizontal organ to vertical, and during this lag period the magnitude of the response is considerably reduced (see Fig. 32). Three hours after returning a horizontal organ to vertical the recovery response is proceeding at a maximal rate of $1.5°h^{-1}$, but the difference in reducing sugar levels between right and left sides is not significantly different from that between the upper and lower sides at the time of righting (compare histograms 1 and 2 in Figs. 78A and B). The geotropic response is also

Fig. 76.

Triticum aestivum L. var. Kolibri.

The development of the asymmetry in sugar levels with time - sugar levels as a function of final fresh weight.

Treatment: Leaf sheath bases were excised and quartered and batches of 20 quadrants were orientated as 'uppers' or 'lowers' in 90 mm petri dishes containing moistened filter paper. After a measured stimulation period the segments were extracted in 80% ethanol and sugars were separated by paper chromatography. The differences in sucrose (—o—) and total reducing sugar levels (—●—) in 'lower' (L) and 'upper' (U) segments were calculated in terms of final fresh weights of the segments.

White light 25°C.

Sugar Realisation. Phenol-Sulphuric acid Method.

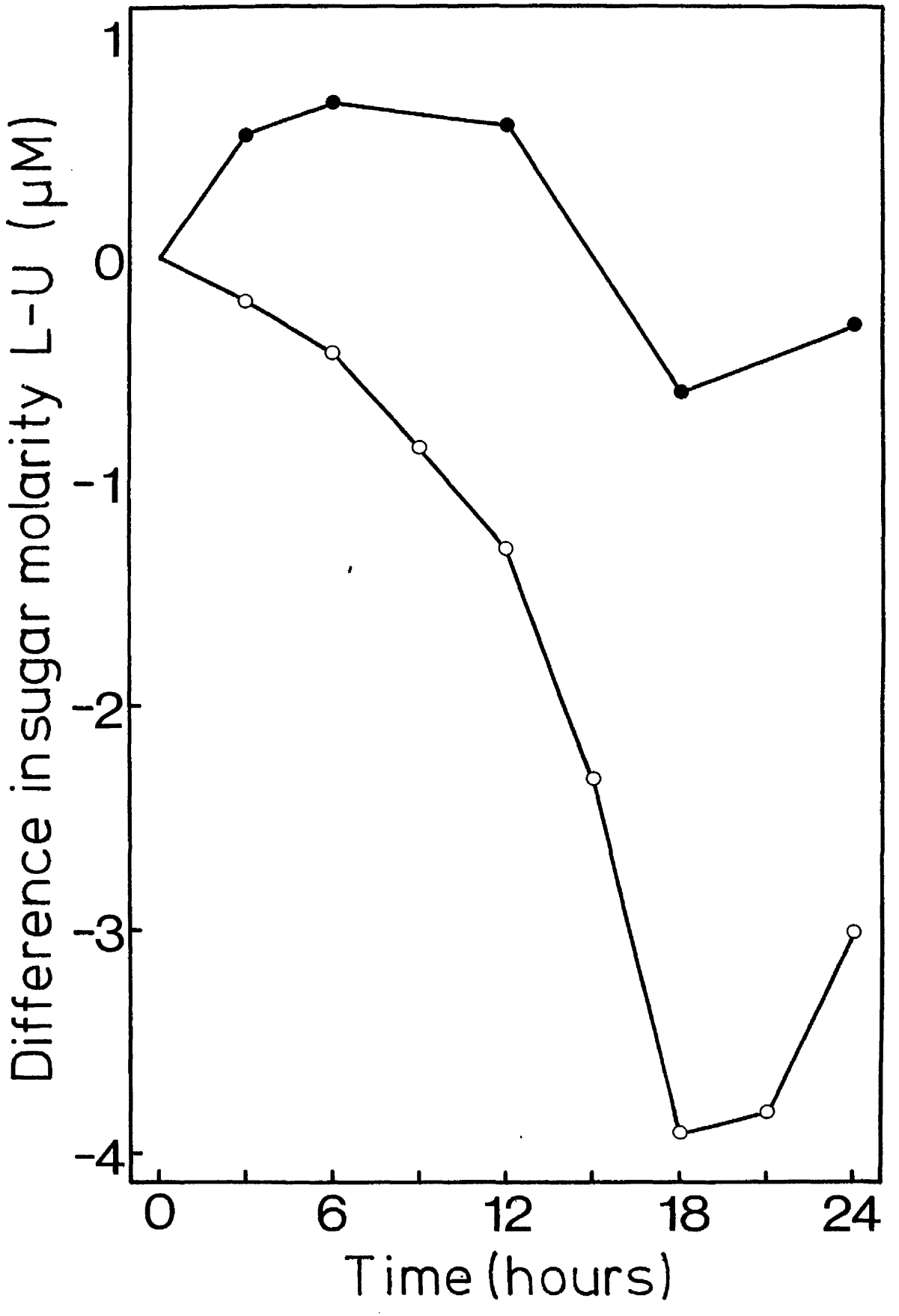

1
0
-1
-2
-3
-4
Difference in sugar molarity L-U (μM)
0
6
12
18
24
Time (hours)

Fig. 77.

Triticum aestivum L. var. Kolibri.

The total molarity of osmotically active sugars in excised segments.

Treatment: Leaf sheath bases were excised and quartered and batches of 20 quadrants were orientated as 'uppers' or 'lowers' in 90 mm petri dishes containing moistened filter paper. At the end of the appropriate stimulation period segments were extracted in 80% ethanol and sugars were separated by paper chromatography. The total sugar molarities (circles) and reducing sugar molarities (triangles) in 'upper' (closed symbols) and 'lower' (open symbols) segments were calculated.

White light 25°C.

Sugar Realisation. Phenol-Sulphuric acid Method.

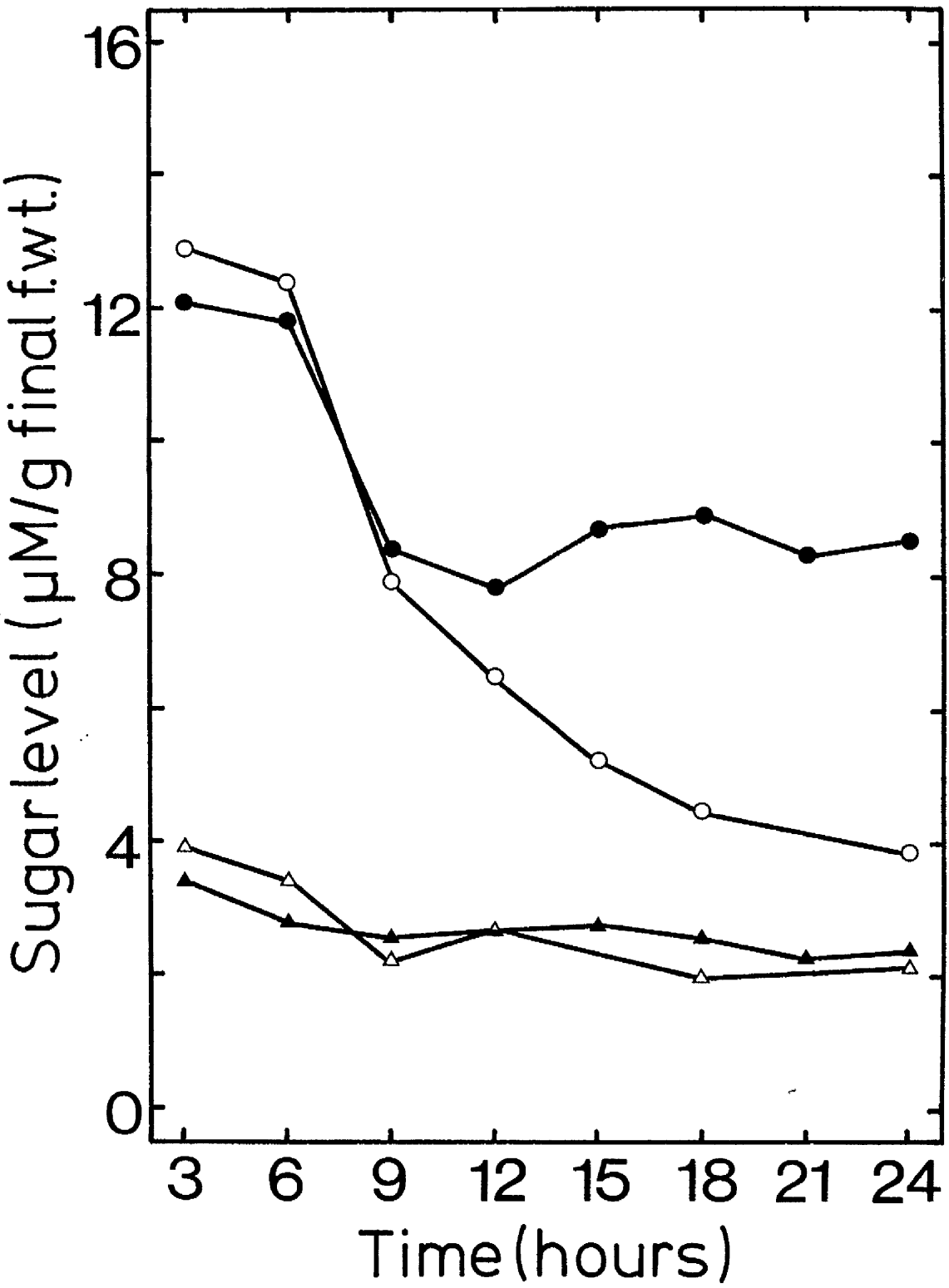

16
12
8
4
0
Sugar level (μM/g final f.wt.)
3
6
9
12
15
18
21
24
Time (hours)

Table 11.

Triticum aestivum L. var. Kolibri.

Sugars in solution after a 24-h incubation period.

Treatment: Leaf sheath bases were excised and quartered and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of distilled water. The incubation media were analysed for sucrose and reducing sugars at the end of the 24-h treatment period.

White light 25°C.

Sugar Realisation. Reducing sugars : Somogyi-Nelson Method.

Sucrose : Phenol-Sulphuric acid Method after paper chromatography.

Table 11

Treatment	Sugar in Solution µM/g f. wt. tissue	
	Sucrose	Reducing Sugars
Lowers	0.927	1.177
Uppers	1.387	1.156

Fig. 78.

Triticum aestivum L. var. Kolibri.

The effects of various post-treatments on the reducing sugar asymmetry developed during a 24-h period of geotropic stimulation.

Treatment: Stem segments 100 mm in length were pinned horizontally for 24 h. They then received a post-treatment, after which the leaf sheath bases were excised and bisected into upper and lower halves for extraction in 80% ethanol. Reducing sugar levels were determined as functions of the initial (fig. A) and final (fig. B) fresh weights of the tissues.

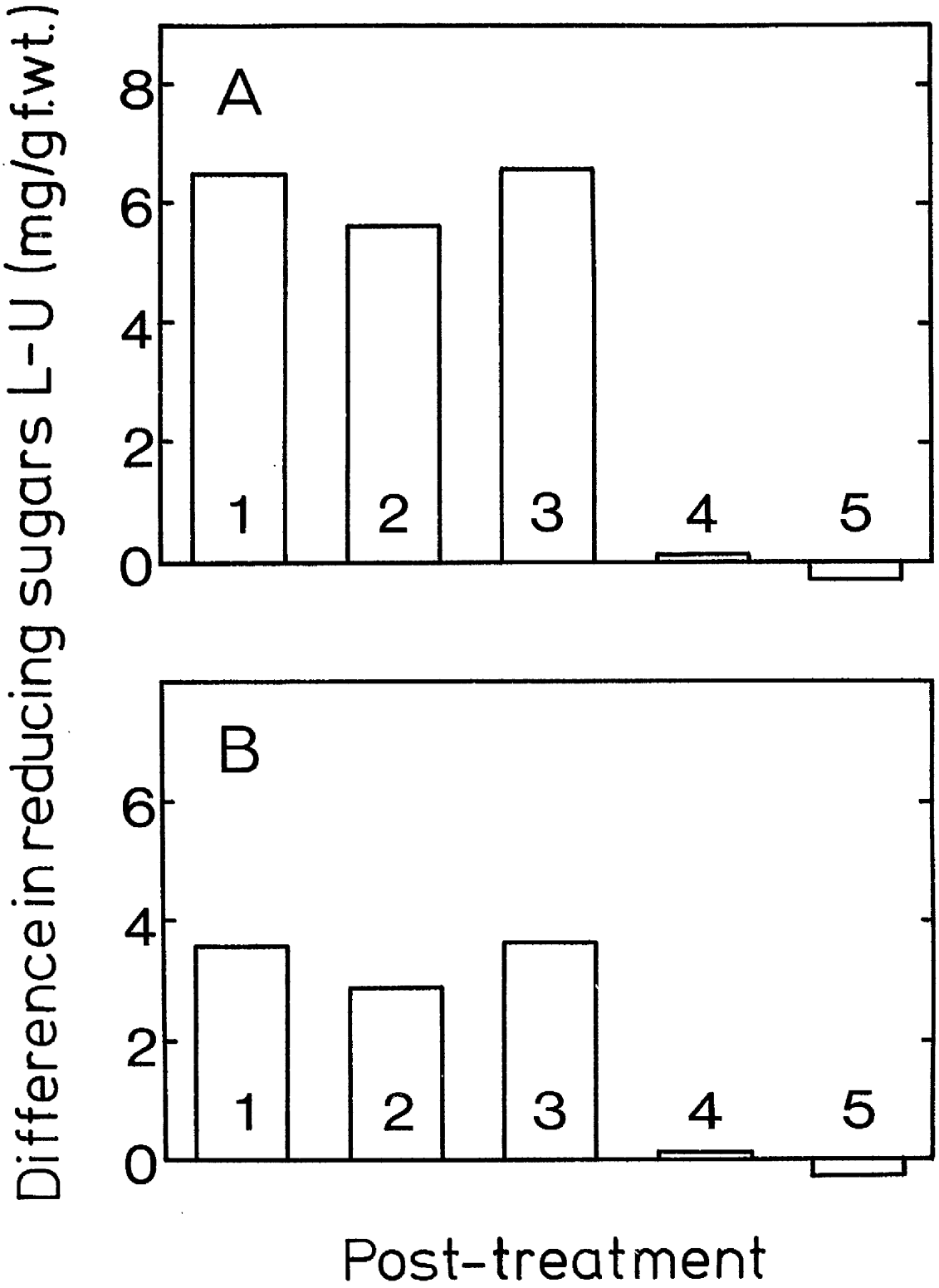
Post-treatments
(1) No post-treatment [control].
(2) 3 h in the vertical position.
(3) 3 h in the horizontal position under nitrogen.
(4) No post-treatment, but treatment run under nitrogen.
(5) No post-treatment, but treatment run under nitrogen after an initial 3 h in air.

White light 25°C.

Statistical Analysis. The t test was used to test the differences in the reducing sugar content of the lower halves of organs before and after post-treatments (2) and (3).

$t[(1)/(2)] = 1.54^{NS}$

$t[(1)/(3)] = 0.15^{NS}$


Difference in reducing sugars L-U (mg/g f.wt.)
A
B
0
2
4
6
8
1
2
3
4
5
Post-treatment

dependent on aerobic metabolism. Curvature may be terminated by removing the experimental material to an atmosphere of nitrogen (see Fig. 29), but this treatment has no effect on the sugar asymmetry which persists for at least 3 h in an atmosphere of nitrogen (compare histograms 1 and 3 in Figs. 78A and B).

The reaction sequences of both the latent period and the subsequent response are oxygen dependent. Exposure to oxygen during the latent period only does not evoke the response (see Fig. 30) and the asymmetry in reducing sugar levels does not develop in material which is stimulated under nitrogen, even when oxygen is supplied during the latent period (Figs. 78A and B histogram 4 and 5).

14. The significance of the reducing sugar asymmetry

The conditions required for the development of the geotropic response and the reducing sugar asymmetry appear to be similar. Both require continuous geotropic stimulation and aerobic metabolism but, since curvature may be suspended and even reversed without effect on the reducing sugar asymmetry, any concept involving the production of sugars to give cell expansion by enhanced turgor is unacceptable. The changes in sugar metabolism may be connected with the primary response sequence, but they may equally represent the onset of secondary metabolism and timing is of critical importance when evaluating these two possibilities. The reaction time for the geotropic response is 2h-20 min at 25°C (see Fig. 28) and any metabolism connected with the primary reaction sequence ought therefore to be evident within the first few hours following the onset of geotropic stimulation. Secondary metabolism, on the other hand, might only be expected to develop when the primary reaction is well underway.

The changes in reducing sugar levels in the upper and lower regions of leaf sheath bases which were removed from 100 mm stem segments at intervals during a 5-h period of geotropic stimulation are shown in Fig. 79. The asymmetry appears to develop within the reaction time, but the values obtained during this period are not significantly different from the control values obtained for left and right halves in vertical material.

Differences in fresh weight between lower and upper halves of the organ do not begin to develop rapidly during the first 6 h of geotropic stimulation (Fig. 72), but the reducing sugar asymmetry does develop during this period, and this development may be taken as evidence for an involvement with the primary reaction sequence.

Interpretation of data concerning changes in reducing sugar levels is complicated firstly by the initial presence of glucose and fructose at basal levels and, secondly, by the dependency of changes on the availability of sucrose reserves. The production of glucose and fructose in similar

Fig. 79.

Triticum aestivum L. var. Kolibri.

The development of the asymmetry in reducing sugars with time.

Treatment: Stem segments 100 mm in length were pinned horizontally and leaf sheath bases were excised and bisected after a measured period of stimulation. The upper and lower halves were extracted with 80% ethanol and the differences in reducing sugar content were calculated in terms of the initial fresh weights of the tissues.

White light 25°C.

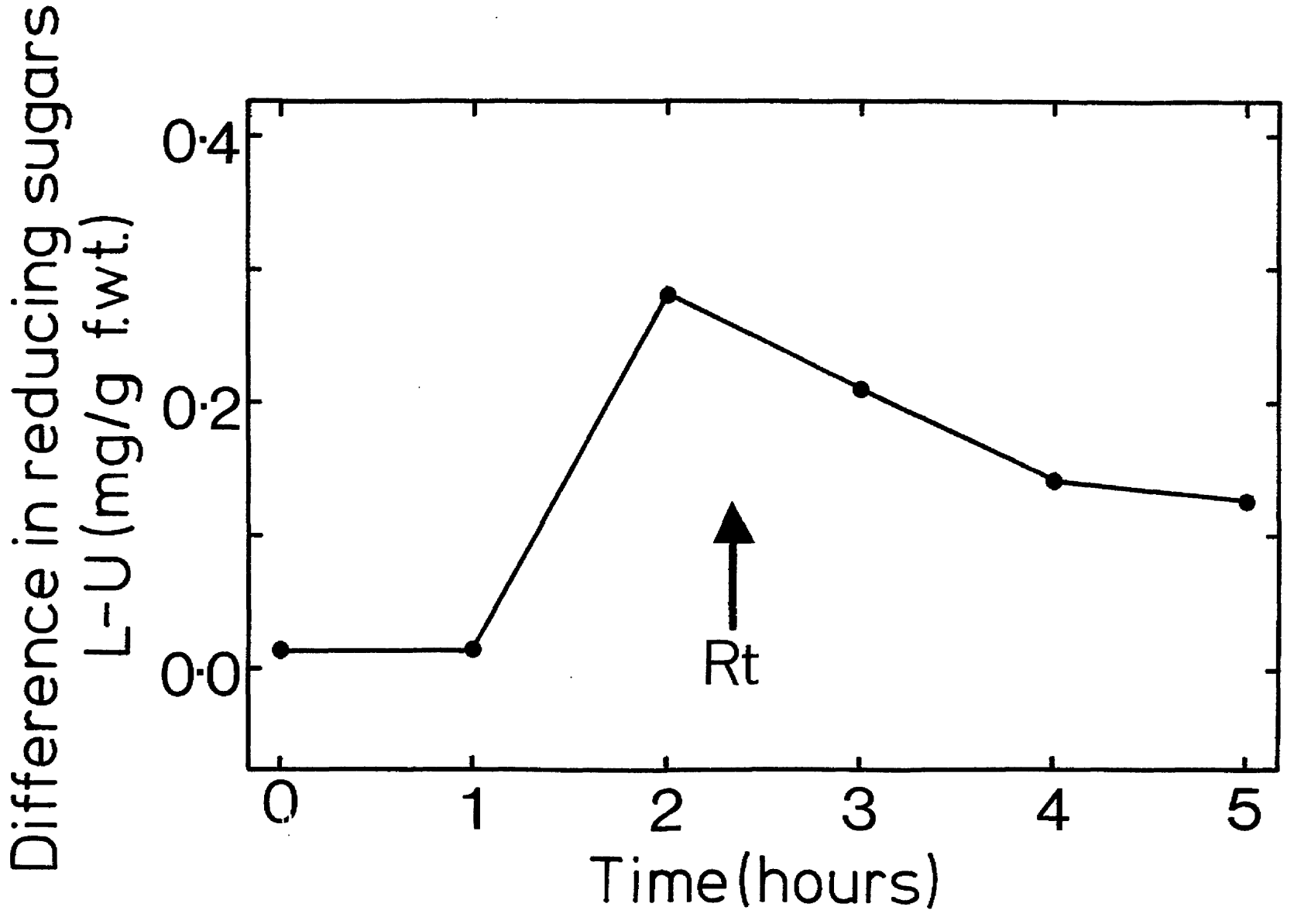
Sugar Realisation: Somogyi-Nelson Method.

Reaction time for curvature: The reaction time for curvature (see fig. 27) is marked by the vertical arrow and the symbol Rt.

Statistical analysis: The t test was used to test the differences in reducing sugar levels between the lower and upper halves of the organ after periods of 2 and 4 h geotropic stimulation.

$t\ 2h = 1.05^{NS}$

$t\ 4h = 1.60^{NS}$


Difference in reducing sugars
L-U (mg/g f.wt.)
0·4
0·2
0·0
Rt
0
1
2
3
4
5
Time (hours)

quantities with concomitant loss of sucrose is indicative of a reaction involving the inversion of sucrose, and the effect of geotropic stimulation on invertase activity in the leaf sheath base has therefore been examined. Data are presented in Table 12 for invertase activity in homogenates prepared from leaf sheath bases excised both before and after geotropic stimulation. Invertase activity increases in response to geotropic stimulation, and activity in homogenates prepared from the lower halves of stimulated leaf sheath bases shows an increase of some 300% over the 24-h stimulation period. A small increase of about 30% is also observed in homogenates from the upper halves of the organs, but this latter increase may be associated with inversion in side tissues. No increase is observed in homogenates prepared from excised segments when these are orientated as uppers following excision, but inversion proceeds at an increased rate when segments are orientated as lowers, and the increase is comparable with that observed in preparations from the lower halves of stimulated intact organs.

The failure to maintain increased reducing sugar production in excised segments must therefore be attributed to a shortage of sucrose rather than a decline in invertase activity.

Invertase activity in homogenates taken from the upper and lower halves of the leaf sheath base after 0, 3, 6 and 24-h periods of geotropic stimulation is shown in Fig. 30. The data suggest a tendency towards increased inversion on the lower side of the organ following 3 and 6-h periods of geotropic stimulation, but the large values obtained for invertase activity in the control material make these differences difficult to analyse.

The effect of physiological age on the changes in invertase activity following geotropic stimulation is shown in Table 14. The ability to bend in response to geotropic stimulation is determined by the age of the leaf sheath base, and at the time of sampling the curvature developed at the third node was only 8 per cent of that developed at the apical node. Invertase activity shows a much smaller decline with physiological age and, although

Table 12.

Triticum aestivum L. var. Kolibri.

Invertase activity in homogenates from the leaf sheath base.

Treatments: A. Stem segments 100 mm in length were pinned horizontally. Leaf sheath bases were excised and bisected after a 24-h stimulation period, and the upper and lower halves were crushed in 10 ml aliquots of 0.05M sucrose.

B. Leaf sheath bases were excised and quartered. Quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of distilled water. Segments were crushed in 10 ml aliquots of 0.05M sucrose after a 24-h treatment period.

Homogenates were incubated at 25°C for 3 h, boiled to terminate the reaction, and analysed to determine the reducing sugar content.

Sugar Realisation. Somogyi-Nelson Method.

Table 12.

A Leaf sheath bases excised after stimulation.

Treatment	Reducing Sugar production µM/h/½ leaf sheath base	% Control	Reducing Sugar production mM/h/gm fresh weight
Control	3.877	100	0.2422
time = 0	± 0.1525		± 0.0089
Lowers	12.396	319	0.7746
time = 24 h	± 0.1390		± 0.0070
Uppers	5.065	130	0.3161
time = 24 h	± 0.0863		± 0.0040

B Leaf sheath bases excised before stimulation.

Treatment	Reducing Sugar production µM/h/½ leaf sheath base	% Control	Reducing Sugar production mM/h/gm fresh weight
Control	5.0600	100	0.2780
time = 0	± 0.2318		± 0.0145
Lowers	11.8225	245	0.6806
time = 24 h	± 1.3166		± 0.0757
Uppers	3.9077	80.9	0.2249
time = 24 h	± 0.1534		± 0.0081

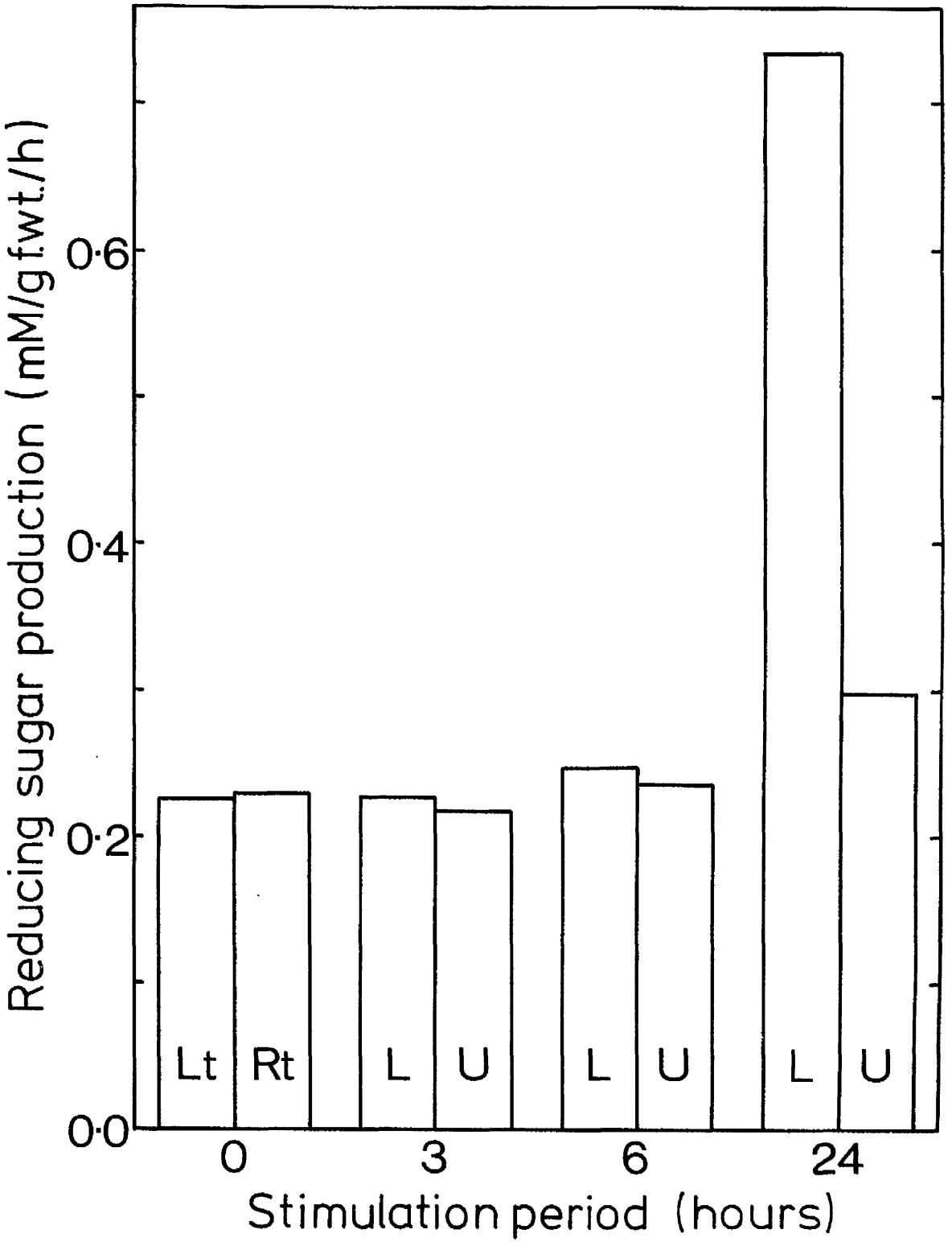
Fig. 80.

Triticum aestivum L. var. Kolibri.

Invertase activity in homogenates from the leaf sheath base.

Treatment: STem segments 100 mm in length were pinned horizontally and the leaf sheath bases were excised and bisected after a measured stimulation period. The upper (U) and lower (L) halves were crushed in 10 ml aliquots of 0.05M sucrose and homogenates were incubated at 25°C for 3 h. The homogenates were boiled to terminate the reaction and analysed to determine the reducing sugar content.

Sugar Realisation. Somogyi-Nelson Method.


Reducing sugar production (mM/g f.wt./h)
0·0
0·2
0·4
0·6
Lt
Rt
L
U
L
U
L
U
0
3
6
24
Stimulation period (hours)

activity in the third leaf sheath base is only 60% of that in the apical leaf sheath base, the percentage increase in invertase activity in the lower halves of horizontal organs remains equivalent following a 24-h period of geotropic stimulation, irrespective of the age of the organ. Since the third leaf sheath base from the apex is no longer able to bend in response to geotropic stimulation, the increase in invertase activity cannot be connected with secondary metabolism initiated in response to growth, and must instead be connected with the primary response to geotropic stimulation.

The loss with age of the ability to bend in response to geotropic stimulation cannot be connected with a reduction in the capacity for gravi-perception because, if this was the case, the changes in invertase activity would not have been detected in the older leaf sheath bases. The availability of sucrose may, however, be a contributory factor. The effect of physiological age on the extent of sugar reserves in the leaf sheath base is shown in Table 15. There appears to be a small increase in the reducing sugar content with increasing age, but the sucrose reserve in the third leaf sheath base is only 25% of that in the apical leaf sheath base. The growth of segments excised from the leaf sheath base is clearly susceptible to endogenous sucrose concentration (see Fig. 14) and it is reasonable to suppose that growth will also be affected by the endogenous concentration, but the importance of the thickening of cell walls in the leaf sheath base and surrounding tissues must not be overlooked when considering the factors affecting curvature at a particular node (see Figs. 35 and 99 and Table 3).

Hormone levels are known to affect the activity of certain enzymes, and it is interesting to note the considerable increase in invertase activity in the unstimulated segments ('uppers') following treatment with 10^{-4} M IAA (Table 13). The data presented give invertase activity after a 24-h incubation period and do not in themselves demonstrate the direct involvement of auxin in reducing sugar production. The effect could also be produced in response to growth, and further experimentation is required in order to

clarify this situation.

Growth is induced by exposure to acid buffers, but this treatment does not increase invertase activity. The effect does not appear to involve metabolism, and exposure to buffers of low pH results in a considerable reduction in the capacity for sucrose inversion.

Table 13.

Triticum aestivum L. var. Kolibri.

The effects of IAA and low pH on invertase activity in the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were placed in 50 mm petri dishes containing 2.5 ml of solution. Quadrants were orientated either as 'lowers' in water (E) or 'uppers' in water (A), 10^{-4}M IAA (B), 0.025M citrate : phosphate buffer at pH3 (C) or 0.1M glycine : HCl buffer at pH3 (D). Segments were crushed in 10 mls of 0.05M sucrose after a 24-h treatment period (12h at 25°C for the faster acid responses) and homogenates were incubated at 25°C for 3 h. The tubes were boiled to terminate the reaction and the reducing sugars were analysed.

Sugar Realisation. Somogyi-Nelson Method.

Table 13

Treatment	Reducing sugar prod. µM/h/½ leaf sheath base	% Control	Reducing sugar prod. mM/h/gm f. wt.
(A) Water Control 24 hr	3.9077 ± 0.1534	100	0.2249 ± 0.0081
(B) 10^{-4}M IAA 24 hr	9.6055 ± 0.3260	246	0.5529 ± 0.0185
(C) Phosphate:Citrate buffer (pH_3) 12 hr	1.2636 ± 0.0300	32.3	0.0727 ± 0.0013
(D) Glycine + HCl buffer (pH_3) 12 hr	2.0960 ± 0.1770	53.6	0.1207 ± 0.0102
(E) Water/geotropic stimulation (lowers) 24 hr	11.8225 ± 1.3166	300	0.6806 ± 0.0757

Table 14.

Triticum aestivum L. var. Kolibri.

The effect of physiological age on invertase activity in the leaf sheath base.

Treatment: Batches of 10 stem segments containing the first, second or third node from the apex were laid horizontally for 24 h and curvature was measured. The leaf sheath bases were then excised and bisected and the upper and lower halves were crushed in 10 ml aliquots of 0.05M sucrose solution. Homogenates were incubated for 3 h at 25°C and, after boiling to terminate the reactions, the media were analysed for reducing sugar content.

Sugar Realisation: Somogyi-Nelson Method.

White light 25°C.

Table 14.

Position from apex		Initial f.wt. ½ leaf sheath base	Reducing sugar prod. mM/hour	% increase in lower half	Reducing sugar prod. mM/h/g f.wt.	Curvature developed during 24h.
Node 1	L	0.01520	0.01184	244	0.779	24.9°
	U		0.00486		0.320	± 0.9°
Node 2	L	0.01188	0.00857	230	0.721	16.2°
	U		0.00373		0.314	± 0.9°
Node 3	L	0.00641	0.00322	221	0.502	2.4°
	U		0.00146		0.228	± 0.5°

Table 15.

Triticum aestivum L. var. Kolibri.

The effect of physiological age on the sugar reserves in the leaf sheath base.

Treatment: Batches of 15 leaf sheath bases were excised from the first, second and third nodes below the apex. These were extracted with 80% ethanol and sugars were separated by paper chromatography.

Sugar Realisation. Phenol-Sulphuric acid Method.

Table 15

Position from apex	Initial fresh weight of leaf sheath base	Sucrose µg	Sucrose µM /g f. wt.	Reducing sugars µg	Reducing sugars µM/g f. wt.	Curvature developed during 24h.
Node 1	0.03473	147.07	12.38	12.37	1.980	24.9° ± 0.9°
Node 2	0.03374	66.40	5.75	17.34	2.85	16.2° ± 0.9°
Node 3	0.02362	26.39	3.27	15.09	3.55	2.4° ± 0.5°

15. The acid growth response

Buffers of low pH were found to induce growth in excised segments, and it was decided to investigate the phenomenon in detail in order to try to establish its mechanism. The effects of pH on the growth of excised segments are shown in Figs. 81 and 82. Growth is extremely sensitive to pH, and buffers of neutral pH have the effect of suppressing geotropically induced growth. Buffers of acid pH promote extensive elongation in both geotropically stimulated and unstimulated segments, and the response is maximal at pH3. The fact that similar growth promotions are induced when low pH is established with either citrate:phosphate buffer (Fig. 81) or glycine: hydrochloric acid buffer (Fig. 82) may be taken as evidence for a pH induced effect rather than an ion induced effect.

The effect of buffer concentration on acid induced growth is shown in Figs. 83 and 84. Segments excised from the leaf sheath base and orientated as uppers show a growth response which is similar in magnitude to that induced in geotropically stimulated segments (lowers) at optimal buffer concentrations, but at sub-optimal buffer concentrations the effects of low pH and geotropic stimulation appear additive. This is seen by a comparison between the dotted and solid lines at the 0.0125M point in the data for the citrate:phosphate buffer (Fig. 83), but is more apparent in the data for the glycine:HCl buffer where the effect is noticed over the range between 0 and 0.08M (Fig. 84). The citrate:phosphate buffer (pH3) is the more efficient buffer, and a ten-fold increase in concentration is required in order to achieve the same promotion with the glycine:HCl buffer.

The direction of acid induced growth is shown in Fig. 85. There is little change in segment width during a 24-h incubation period at pH3, but the segments increase in length by up to 100% during this period. The increases in length which develop in 'upper' and 'lower' segments during a 24-h incubation period at pH3 are comparable with the increase in the length of the lower side of the intact organ during an equivalent period of

Fig. 81.

Triticum aestivum L. var. Kolibri.

The effect of pH on the growth of segments excised from the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (— — — —) or 'lowers' (————) in 50 mm petri dishes containing 2.5 mls of 0.025M citrate : phosphate buffer, and segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between means for control and buffer treatments.

'Lowers'		'Uppers'	
$t(H_2O/pH3)$	$= 9.385^{***}$	$t(H_2O/pH3)$	$= -0.275^{NS}$
$t(H_2O/pH3)$	$= -5.807^{***}$	$t(H_2O/pH3)$	$= -11.547^{***}$

t(pH3 lowers/uppers) $= 0.796^{NS}$

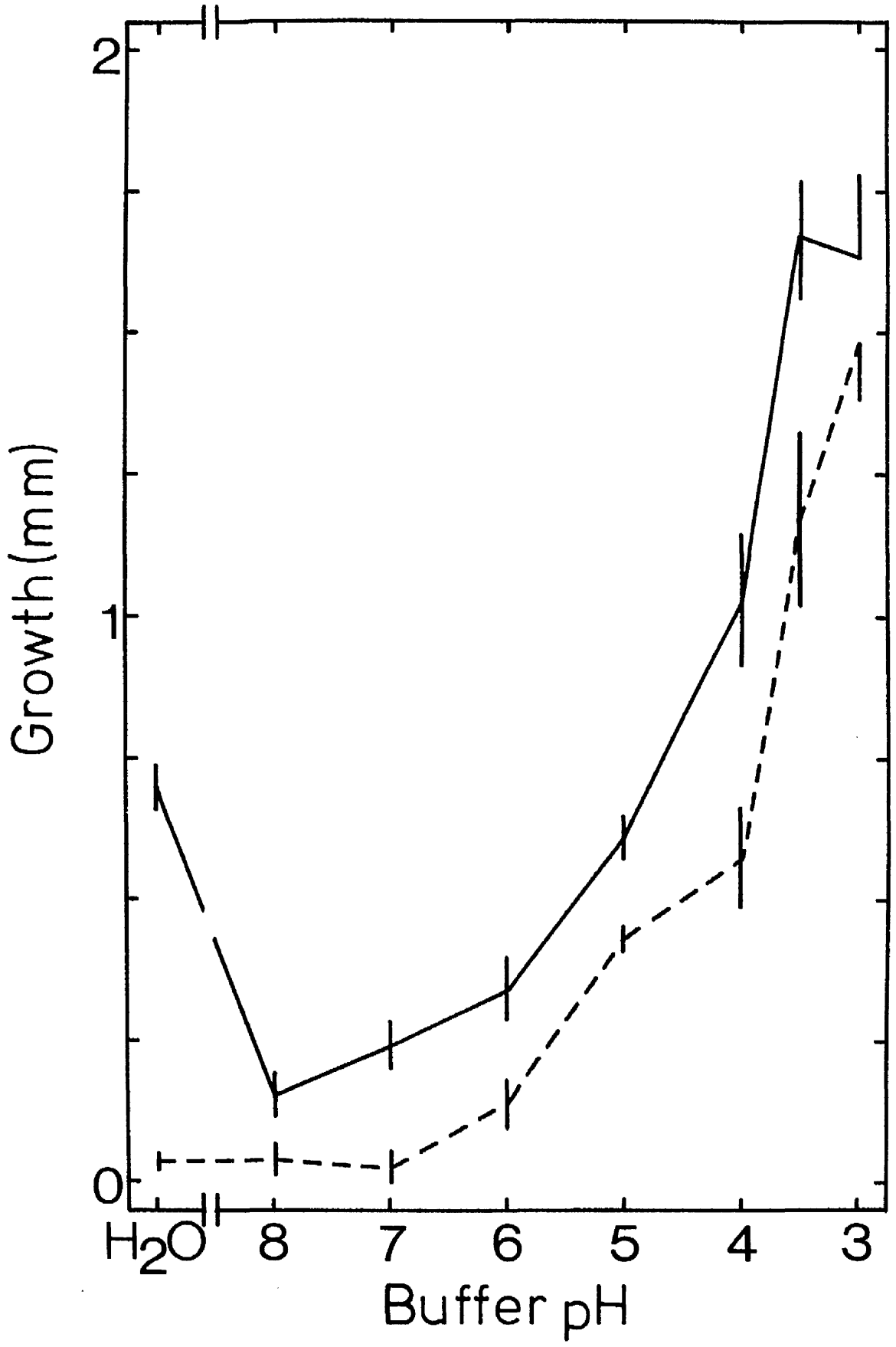

2
1
0
Growth (mm)
H2O
8
7
6
5
4
3
Buffer pH

Fig. 82.

Triticum aestivum L. var. Kolibri.

The effect of pH on the growth of segments excised from the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (- - - -) or 'lowers' (————) in 50 mm petri dishes containing 2.5 ml of 0.1M glycine : hydrochloric acid buffer, and segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

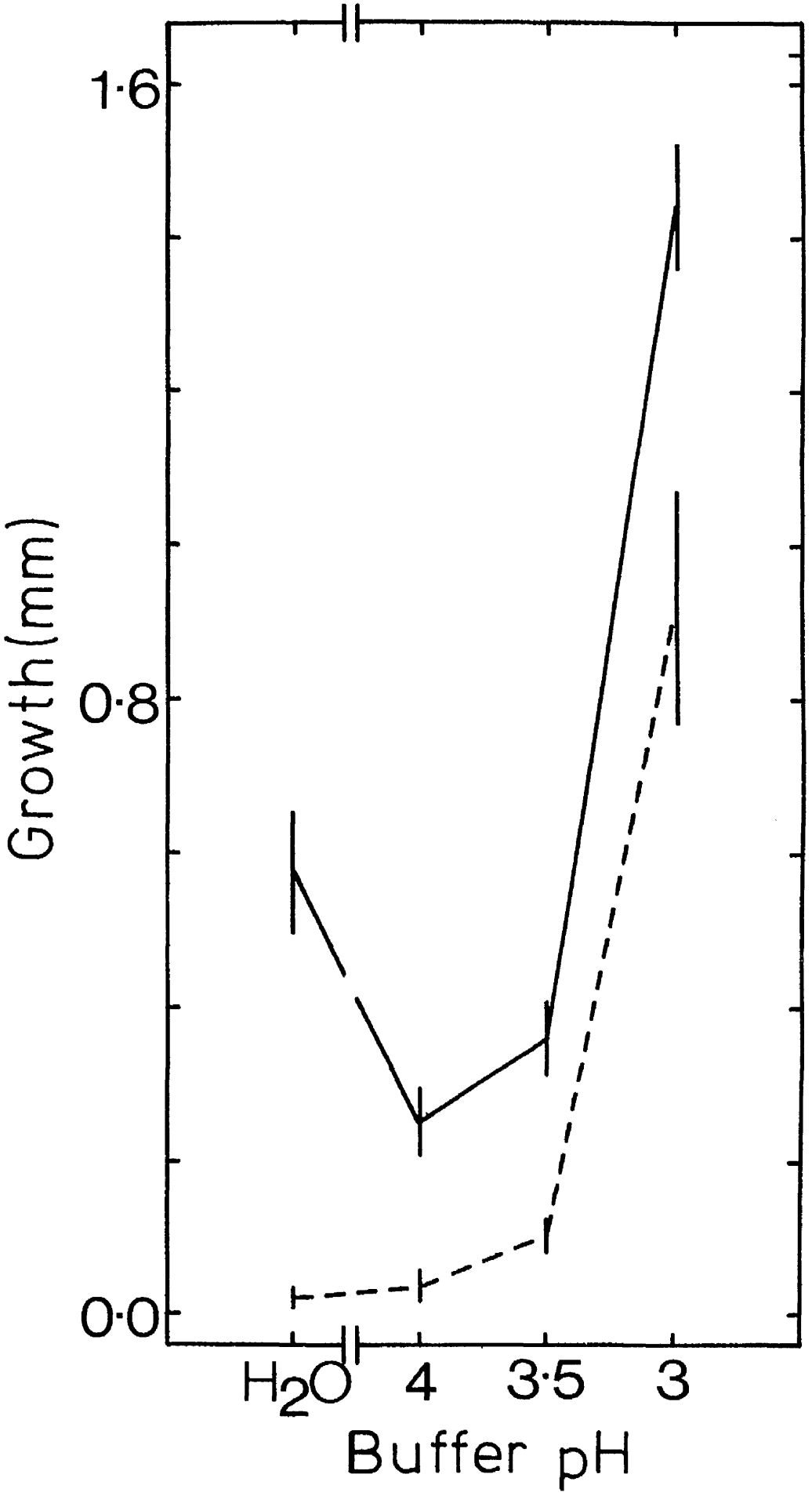

1·6
0·8
0·0
Growth(mm)
H2O
4
3·5
3
Buffer pH

Fig. 83.

Triticum aestivum L. var. Kolibri.

The effect of citrate buffer concentration (pH3) on the growth of segments excised from the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (– – –) or 'lowers' (———) in 50 mm petri dishes containing 2.5 ml of citrate : phosphate buffer (pH3) and segments were rhabdographed after a 24-h treatment period.

White light 25°C.

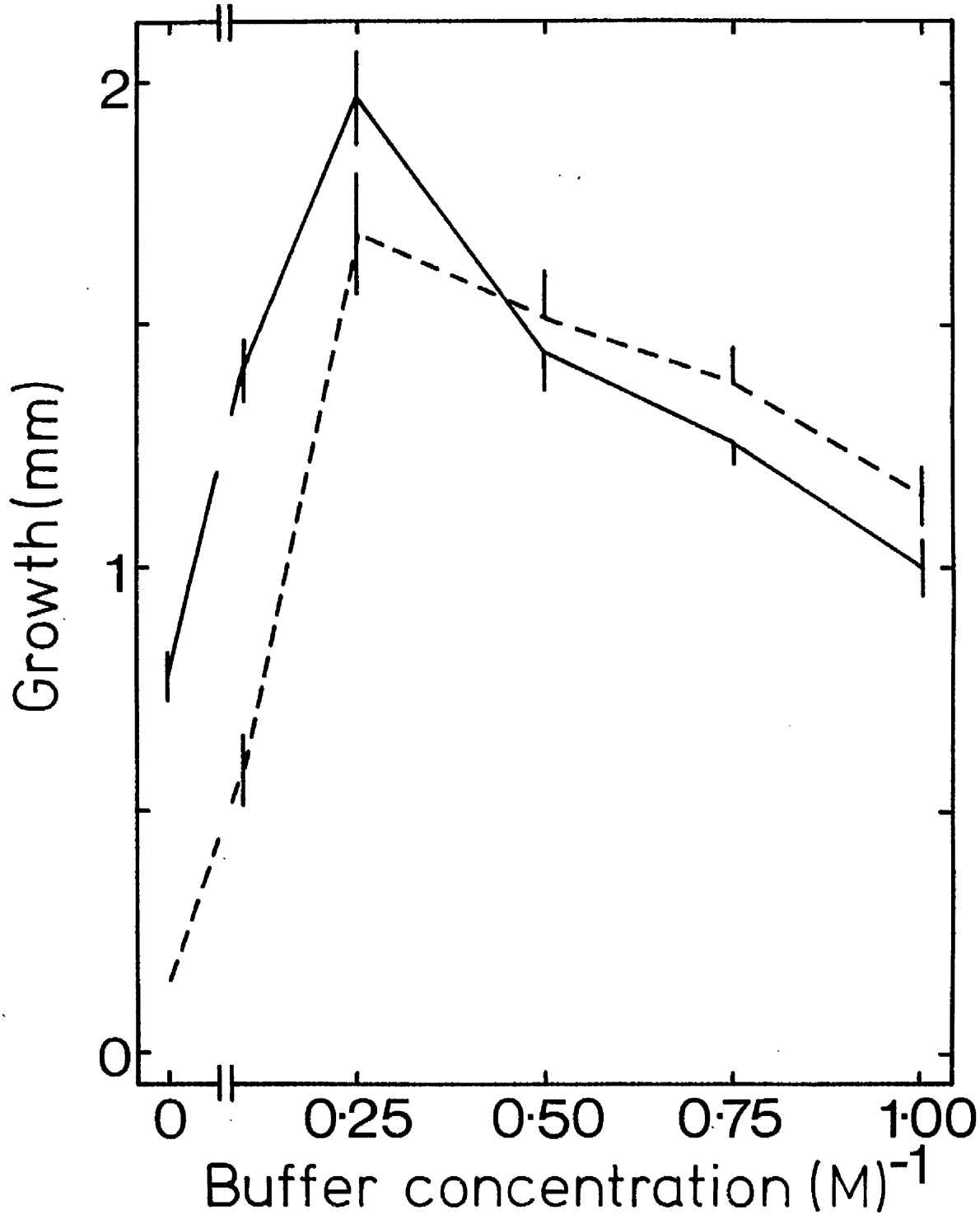

Growth (mm)
2
1
0
0
0·25
0·50
0·75
1·00
Buffer concentration (M)$^{-1}$

Fig. 84.

Triticum aestivum L. var. Kolibri.

The effect of Glycine buffer concentration (pH3) on the growth of segments excised from the leaf sheath base.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (— — —) or 'lowers' (————) in 50 mm petri dishes containing 2.5 ml of Glycine : HCl buffer (pH3), and segments were shadowgraphed after a 24-h treatment period.

White light 25 °C.

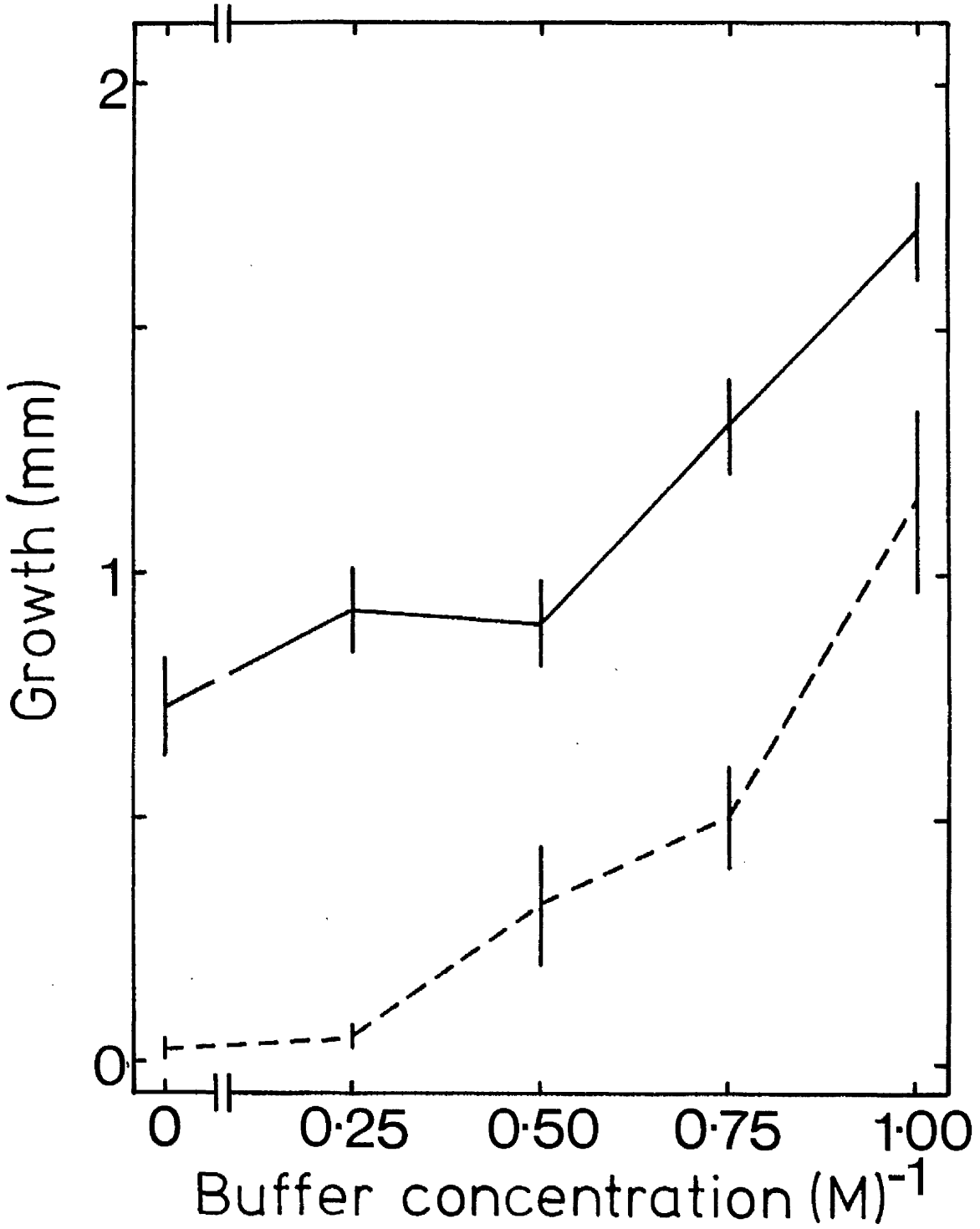

Growth (mm)
2
1
0
0
0·25
0·50
0·75
1·00
Buffer concentration (M)$^{-1}$

Fig. 85.

Triticum aestivum L. var. Kolibri.

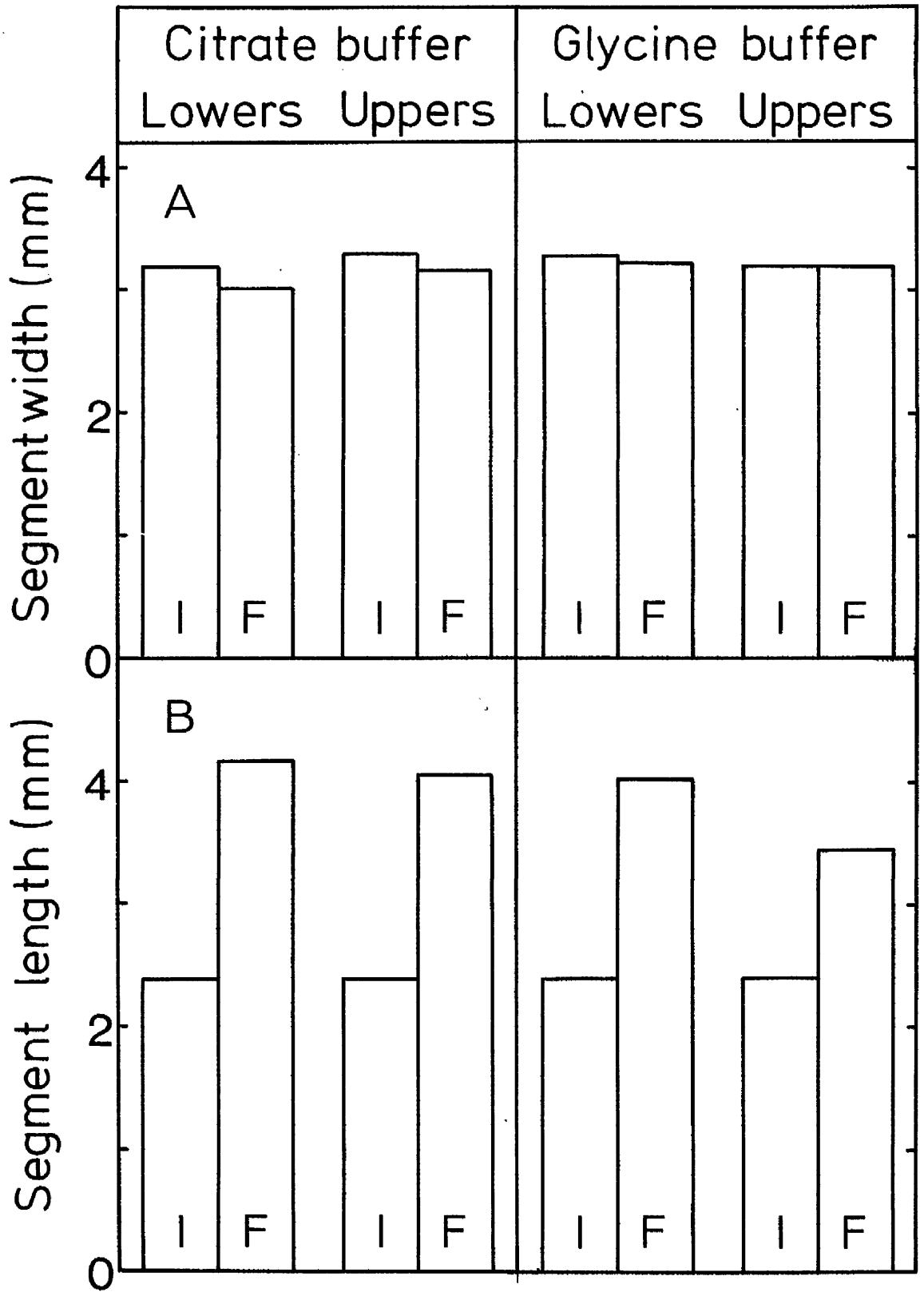
The plane of the acid induced response.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were shadowgraphed and orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing either 0.025M citrate : phosphate buffer (pH3) or 0.1M glycine : HCl buffer (pH3). Segment widths (fig. A) and lengths (fig. B) were determined before (I) and after (F) the 24-h buffer treatment.

White light 25°C.

Statistical Analysis. The t test was used to test the differences in segment width during the 24-h treatment.

Segment orientation	Buffer (pH3)	
	Citrate : phosphate	Glycine : HCl
Lowers	$t(w) = 1.565^{NS}$	$t(w) = 0.527^{NS}$
Uppers	$t(w) = 1.720^{NS}$	$t(w) = 0.052^{NS}$


Citrate buffer
Lowers Uppers
Glycine buffer
Lowers Uppers
A
B
Segment width (mm)
Segment length (mm)
4
2
0
I
F

geotropic stimulation (Fig. 86), but the acid response is clearly different from the geotropic response because, unlike the former which persists for only 8 to 10 h (Fig. 87), the latter shows a steady rate of development over at least 48 h at 25°C (see Fig. 9).

The development of the acid induced response at temperatures in the physiological range is shown in Fig. 88. Unlike the geotropically induced response which exhibits a temperature dependent reaction time (see Fig. 30A), the development of the acid response is immediate over the range of temperatures tested. The duration of the acid induced response is reduced with increasing temperature (compare 30°C curve in Fig. 88 with 25°C curve in Fig. 87), but the rate of the acid induced response is increased with increasing temperature. The acid induced response rate is plotted against temperature in Fig. 89 and the Q_{10} value for the response is calculated at 1.82, a figure which is comparable with the Q_{10} for the geotropically induced response (see Fig. 31B).

Fig. 86.

Triticum aestivum L. var. Kolibri.

A comparison between the magnitudes of the acid induced and geotropically induced responses.

Treatment: Portions of the leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' (U) or 'lowers' (L) in 50 mm petri dishes containing 2.5 ml of 2% sucrose (2), distilled water (3) or 0.025M citrate : phosphate buffer (pH3) (4). Segments were shadowgraphed after 24h treatment and their growth was compared with growth induced on the upper and lower faces of the intact organ during a 24-h period of geotropic stimulation (1).

White light 25°C.

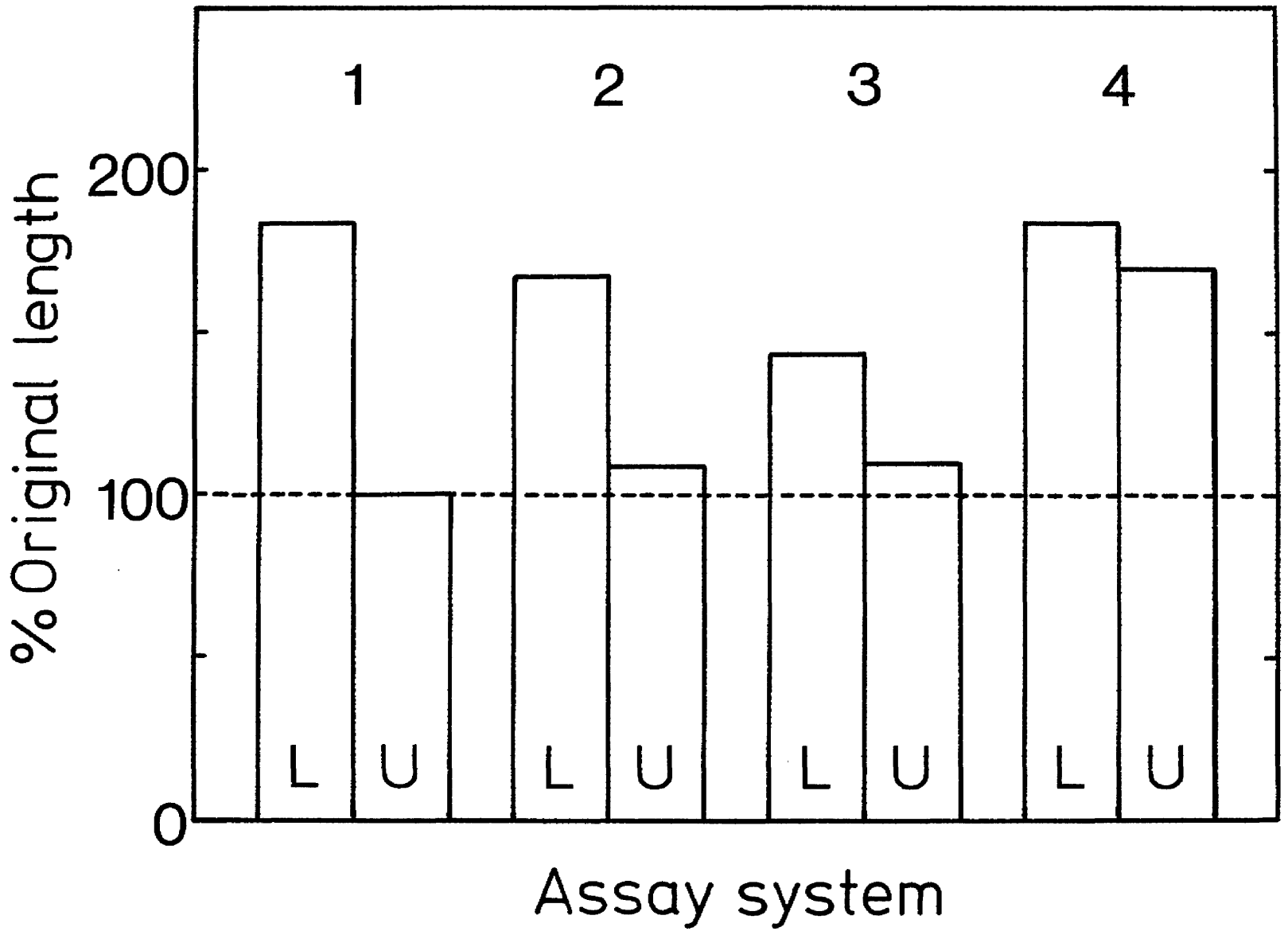

1
2
3
4
200
100
0
% Original length
L
U
L
U
L
U
L
U
Assay system

Fig. 87.

Triticum aestivum L. var. Kolibri.

The duration of the acid induced response.

Treatment: Leaf sheath bases were excised and threaded in batches of five on glass rods submerged in 0.025M citrate : phosphate buffer (pH3). The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at a chart speed of 50 mm h^{-1}.

The vertical bar indicates 1 mm growth.

White light 25°C.

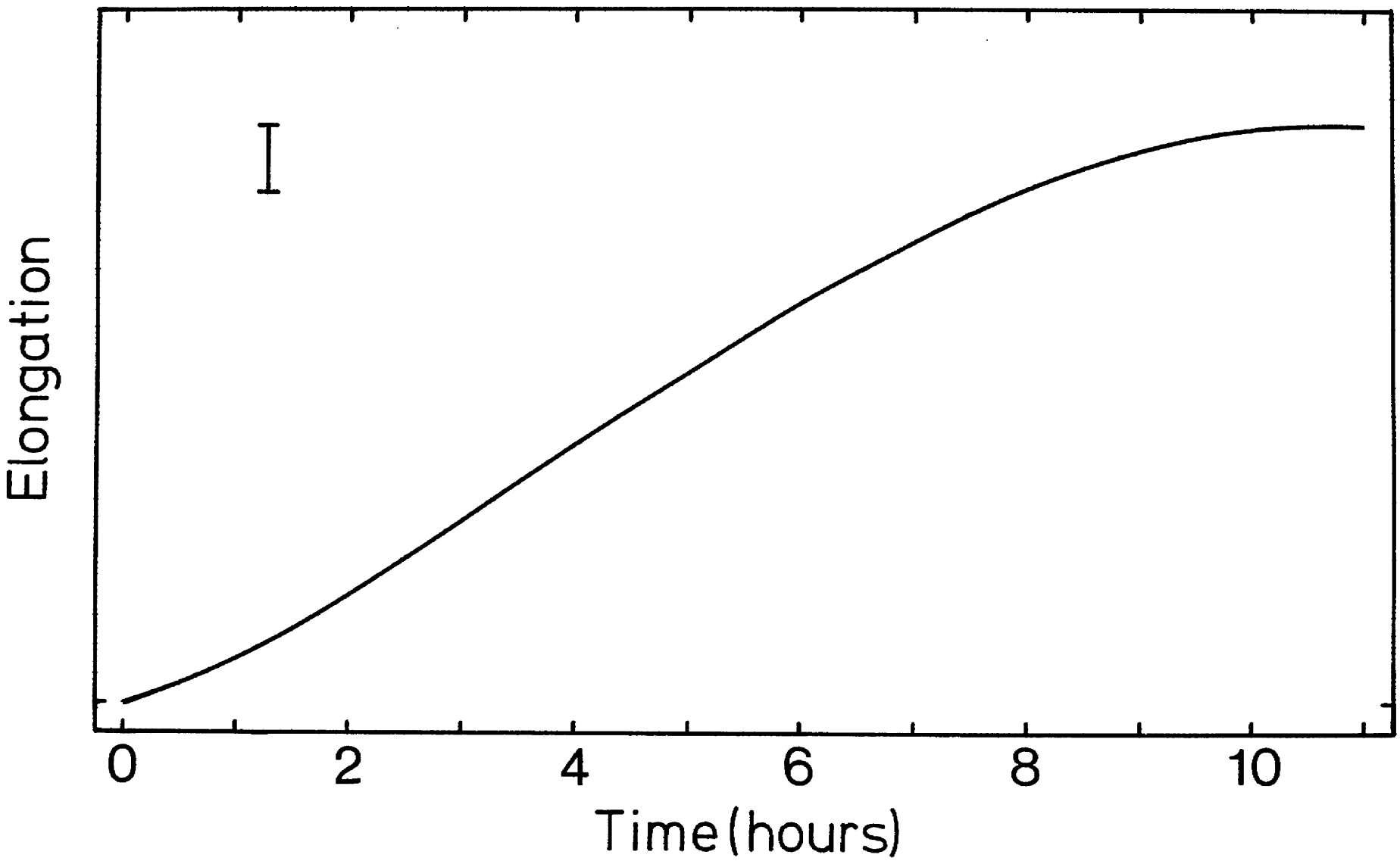

Elongation
0
2
4
6
8
10
Time (hours)

Fig. 88.

Triticum aestivum L. var. Kolibri.

The effect of temperature on acid induced growth.

Treatment: Leaf sheath bases were excised and threaded in batches of five on glass rods submerged in 0.025M citrate : phosphate buffer (pH3). The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at a chart speed of 100 mm h^{-1}.

The vertical bar indicates 1 mm growth.

White light 25°C.

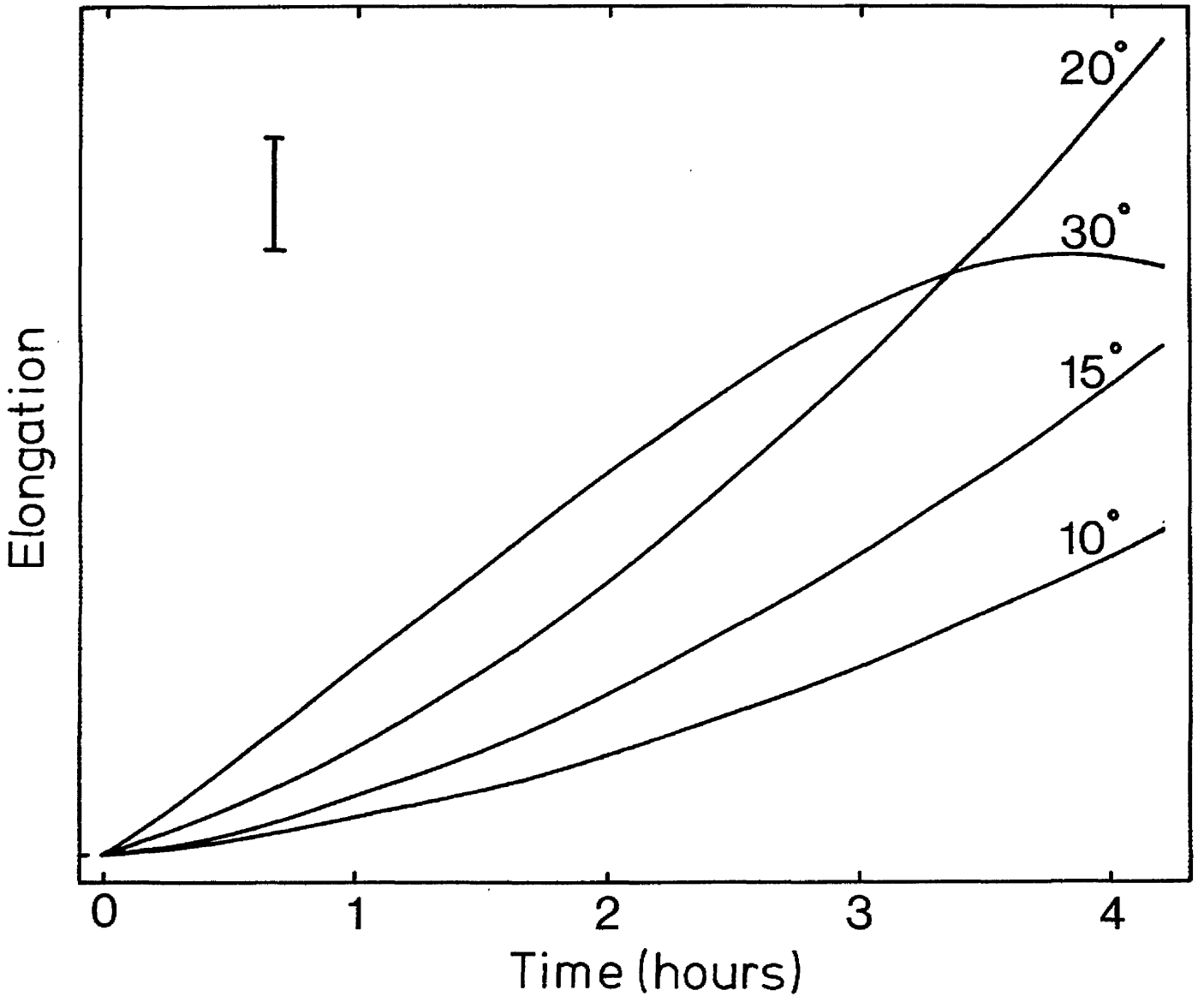

20°
30°
15°
10°
Elongation
0
1
2
3
4
Time (hours)

Fig. 69.

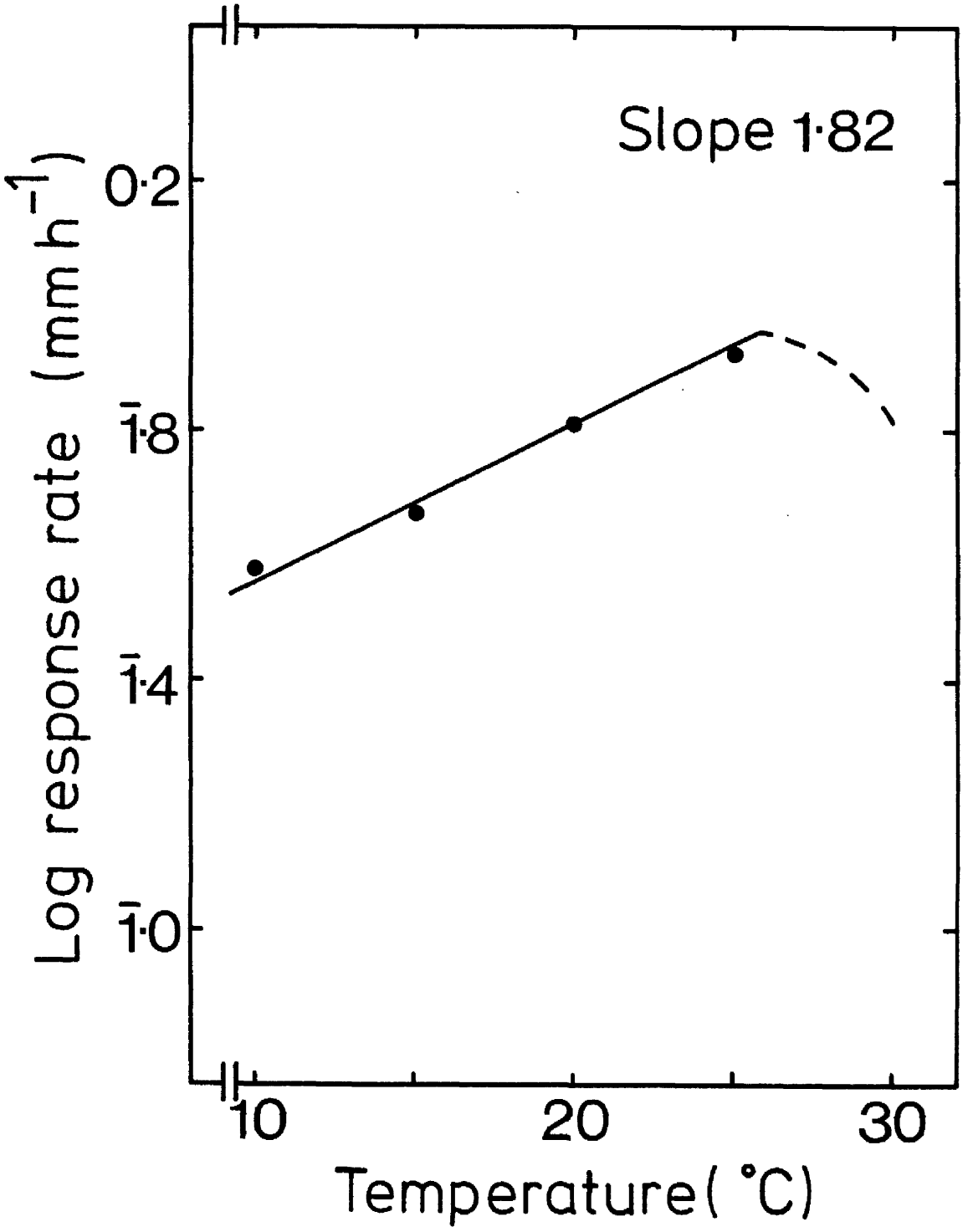
Triticum aestivum L. var. Kolibri.

The (The Q_{10} for the acid induced response.

Treatment: Leaf sheath bases were excised and threaded in batches of five on glass rods submerged in 0.025% citrate : phosphate buffer (pH3). The growth responses were magnified through kymograph levers and recorded on smoke drums rotated at chart speeds of 100 mm h^{-1}. The initial steady growth rates (t = 2 + 4 h) were calculated and plotted against temperature, and the Q_{10} value was determined from the slope of the line which was fitted through the points by eye.

White light 25°C.

Q_{10} value 1.82.


Slope 1·82
Log response rate (mm h⁻¹)
0·2
1̄·8
1̄·4
1̄·0
10
20
30
Temperature(°C)

16. The mechanism of the acid induced response

Data presented in Fig. 90 show the effect of changing pH on the development of the acid induced response. Acid induced growth may be terminated by increasing the pH of the buffer, and it may subsequently be re-initiated by lowering the pH to its original value, but the responses to these pH changes are not immediate. A lag exists for both the termination and re-initiation of the response, and the length of the lag period is determined by the difference in pH between the two buffers. The smaller the differential the longer the lag for termination, and the shorter the lag for re-initiation.

The ability to abolish the response by increasing the pH is not compatible with the concept of hormone release by acid hydrolysis because hydrolysis would not be reversible over this narrow pH range. The only plant growth regulator which has been shown to be active in this system is IAA (Fig. 35) and, as seen in Fig. 91, equivalent data are obtained when IAA is supplied in water or 0.025M citrate:phosphate buffer at pH 4 or 5. Thus, if the buffer was to act by releasing IAA into the system, the act of raising the pH from pH3 to pH4 or 5 should have no effect on the development of the response. The fact that it will abolish the acid induced response may be taken as evidence to preclude the involvement of growth regulators produced by the acid hydrolysis of bound precursors.

Further evidence to substantiate this reasoning is provided by a study of the effects of metabolic inhibitors on acid induced growth. If low pH serves not to induce growth directly but rather to initiate a chain reaction leading ultimately to growth, then the effect of inhibitors on acid induced growth ought to resemble the effects on geotropically induced growth. The effects of anoxia on acid induced, auxin induced and geotropically induced growth in excised segments are shown in Fig. 92. Both the auxin induced and geotropically induced responses are dependent on aerobic metabolism, but the acid induced response develops normally under nitrogen.

Fig. 99.

Triticum aestivum L. var. Kolibri.

The effect of changing pH on acid induced growth.

Treatment: Leaf sheath bases were excised and threaded in batches of five on glass rods submerged in 0.025M citrate : phosphate buffer (pH3). The buffer was changed to the new pH, 2 h after the start of the experiment and was returned to the original pH after a further 2 h. The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at a chart speed of 100 mm h^{-1}.

The vertical bar indicates 1 mm growth.

White light 25°C.

Lag periods for the inhibition and re-initiation of the response.
(see arrows on figs. opposite)

pH3 → pH7 transition 35.82m ± 1.9m.

pH3 → pH5 transition 65.40m ± 7.2m.

pH7 → pH3 transition 28.80m ± 3.6m.

pH5 → pH3 transition 25.00m ± 1.0m.

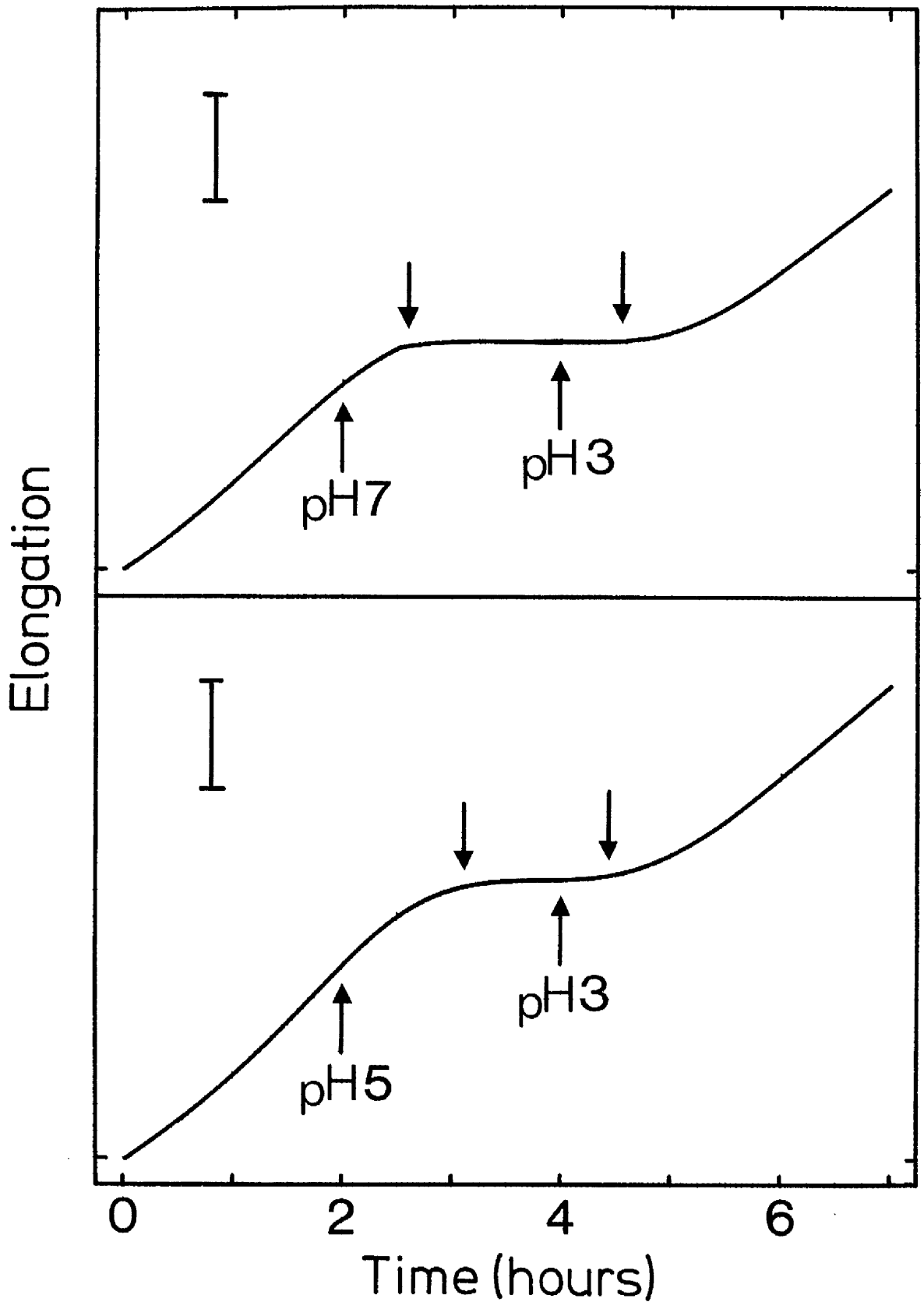

Elongation
pH7
pH3
pH5
pH3
0
2
4
6
Time (hours)

Fig. 91.

Triticum aestivum L. var. Kolibri.

The effect of pH on auxin induced growth.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' in petri dishes containing one of the following combinations of IAA and 0.025M citrate : phosphate buffer in the presence (figs. A & C) or absence (figs. B & D) of 2% sucrose. Segments were shadowgraphed after a 24-h treatment period and data are presented with IAA (figs. A & B) and pH (figs. C & D) as abscissae.

	pH		
	No buffer	3	4	5
No IAA				
10^{-5}M IAA				
10^{-4}M IAA				

White light 25°C.

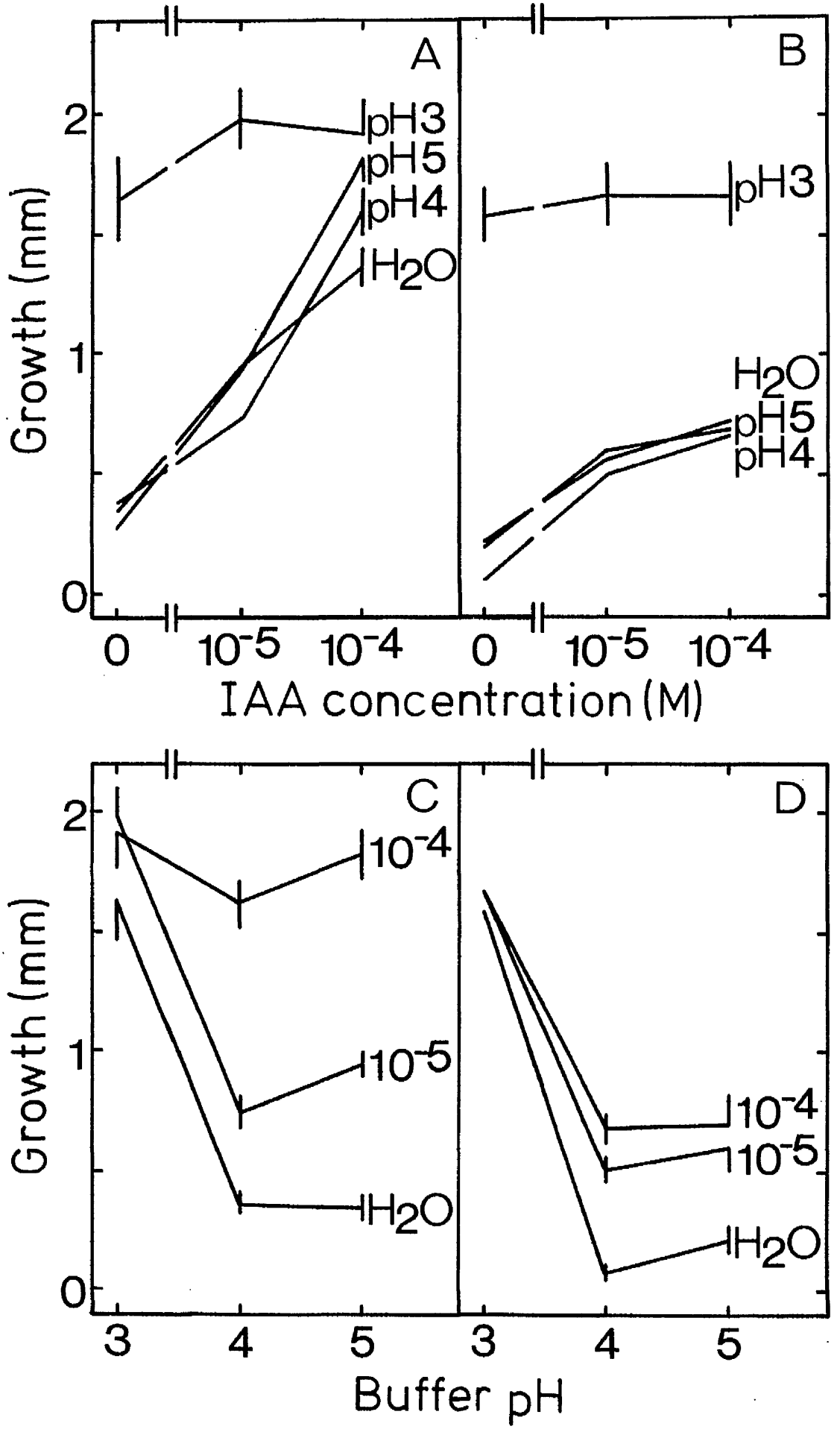

A
B
pH3
pH5
pH4
H2O
pH3
H2O
pH5
pH4
Growth (mm)
2
1
0
0
10-5
10-4
0
10-5
10-4
IAA concentration (M)
C
D
10-4
10-5
H2O
10-4
10-5
H2O
Growth (mm)
2
1
0
3
4
5
3
4
5
Buffer pH

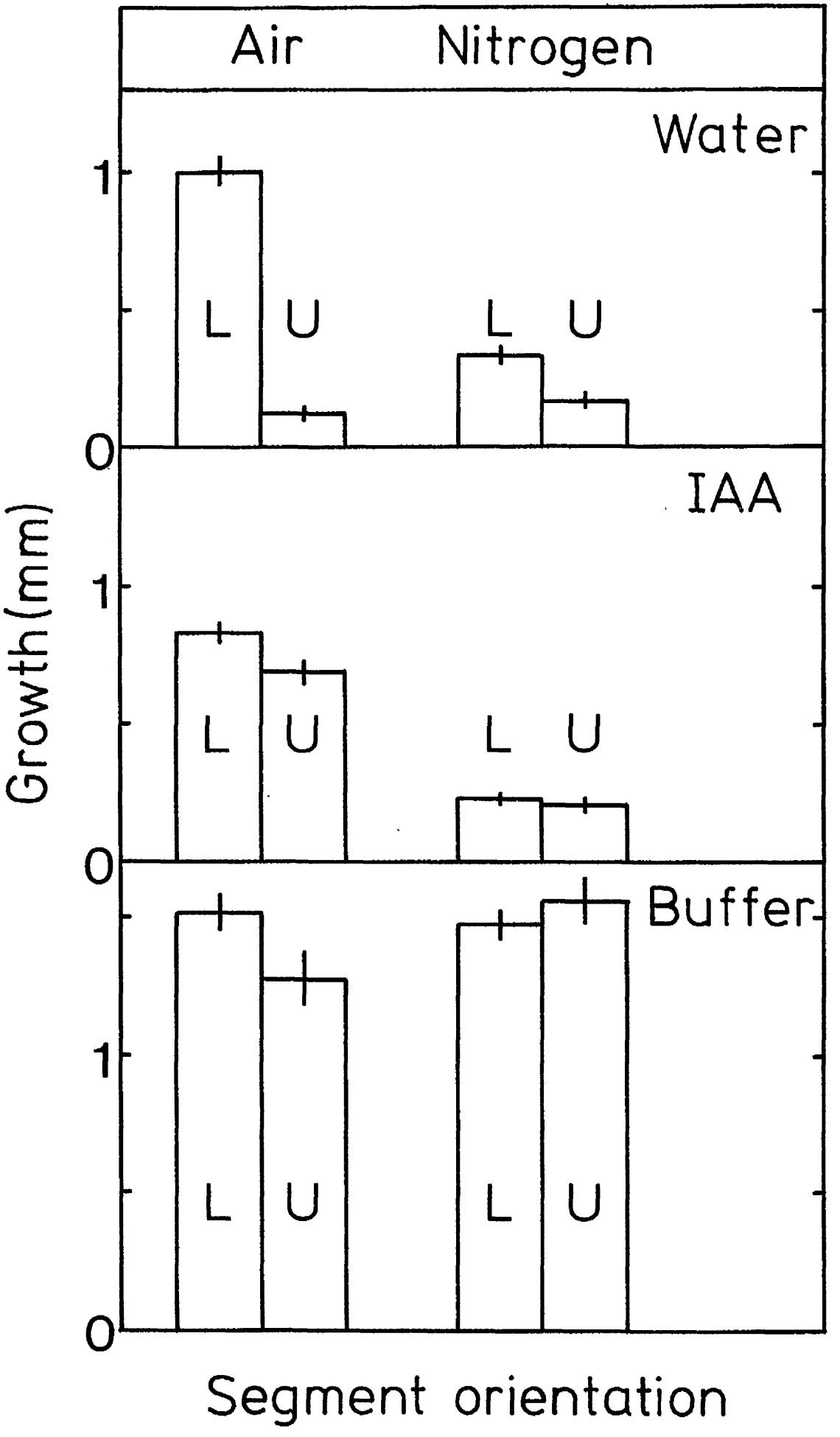
Fig. 92.

Triticum aestivum L. var. Kolibri.

The effect of anoxia on acid induced growth.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (U) or 'lowers' (L) in 50 mm petri dishes containing 2.5 ml of distilled water, 10^{-4} M IAA or 0.025M citrate : phosphate Buffer (pH3). Dishes were maintained in a stream of air or nitrogen and segments were shadowgraphed after 24h treatment.

White light 25°C.


Air
Nitrogen
Water
IAA
Buffer
1
0
L
U
Growth (mm)
Segment orientation

Respiratory inhibitors and uncouplers have also been tested, and their effects on acid induced and geotropically induced responses are shown in Fig. 93 and Table 16. The respiratory inhibitors potassium cyanide and sodium azide and the uncoupler dinitrophenol each inhibit the development of the geotropically induced response. The magnitude of the acid induced response, as measured at the end of a 24-h incubation period, is also reduced when the metabolic poisons are applied at concentrations sufficient to abolish the geotropic response, but significant growth is still induced even in the presence of the highest inhibitor concentrations tested. Data presented in Table 16 show the initial rate of the acid induced response to be unaffected by the inhibitor treatments tested above, and the inhibitory effects noted in 24-h growth assays must therefore be connected with the duration of the acid induced response in the presence of these substances.

The toxic effects of the metabolic poisons are immediately apparent from the bleaching which occurs in segments used in 24-h growth assays, and a time course for the bleaching induced in excised leaf sheath bases during incubation with 10^{-3}M KCN is shown in Fig. 94. Chlorophyll levels begin to fall dramatically after as little as 3-h treatment when KCN is supplied at pH3, but little change is apparent to the eye after 24 h when the inhibitor is supplied in distilled water. Some bleaching is also induced after 6 h by the buffer treatment alone, and the incidence of this bleaching may be correlated with the decline in rate noted for the acid induced response in Fig. 86. The buffer treatments appear to render the membranes 'leaky' and the acceleration of this effect by the inhibitor treatments results in the diminution of the response in the presence of these substances.

The requirements for nucleic acid metabolism and protein synthesis in the acid induced, auxin induced and geotropically induced responses have been investigated, and the effects of inhibitors on these processes are shown in Fig. 95. The auxin induced and geotropically induced responses are inhibited by exposure to actinomycin D and cycloheximide, indicating a

Fig. 93.

Triticum aestivum L. var. Kolibri.

The effect of respiratory inhibitors on acid induced growth.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered, and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of inhibitor made up in either distilled water (+ 'lowers') or 0.025M citrate : phosphate buffer at pH3 (+ 'uppers'). Segments were shadowgraphed after a 24-h treatment period in the presence or the absence of the inhibitor.

White light 25°C.

Statistical Analysis. The t test was used to test the differences in growth following the application of the inhibitor to geotropically induced (lowers) or acid induced (uppers) segments.

Inhibitor	t(geoinduced/acid induced growth)
10^{-2}M KCN	-10.555***
10^{-3}M NaN_3	- 4.206***
10^{-4}M DNP	- 6.500***

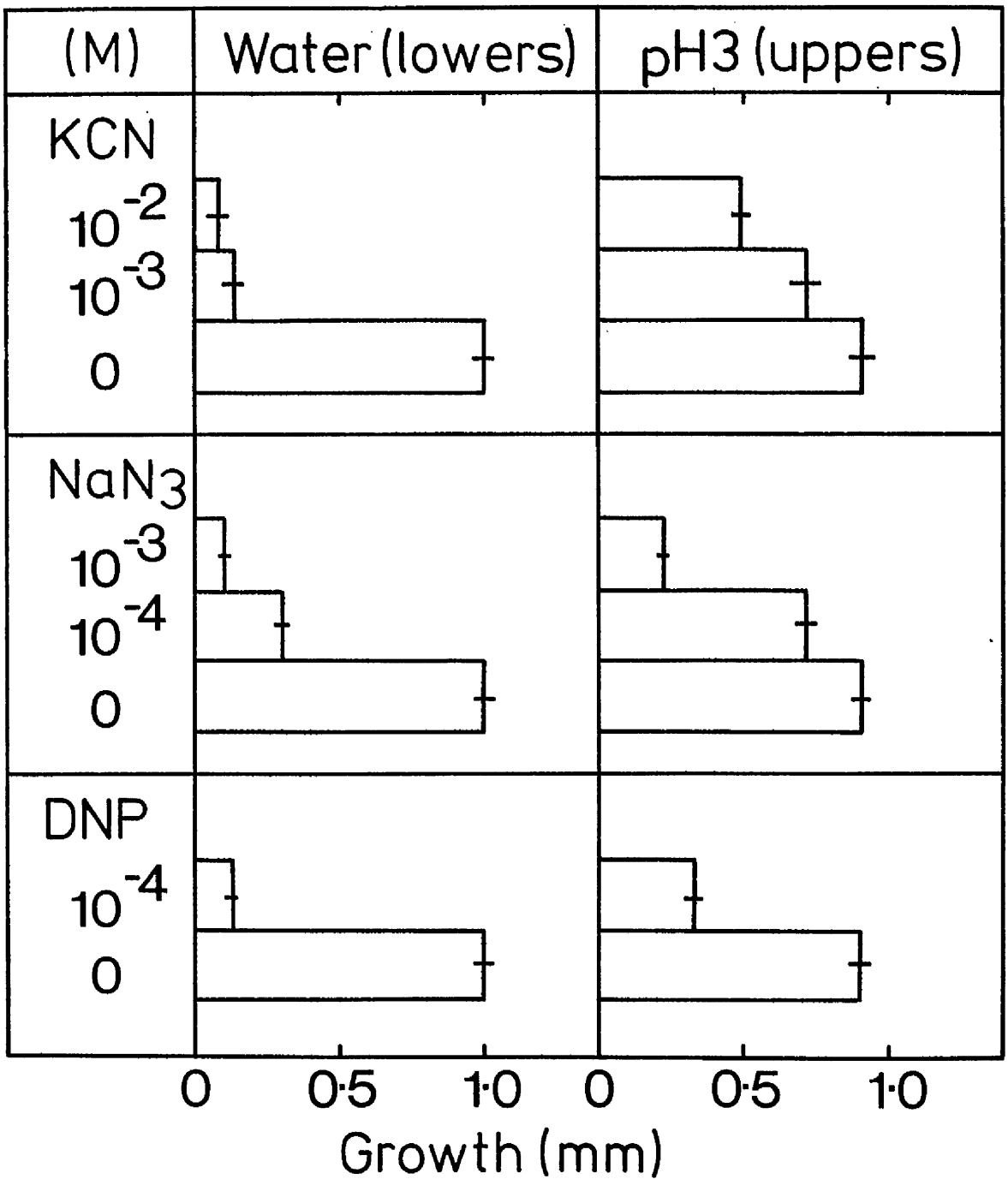

(M)
Water (lowers)
pH3 (uppers)
KCN
10-2
10-3
0
NaN3
10-3
10-4
0
DNP
10-4
0
0
0·5
1·0
0
0·5
1·0
Growth (mm)

Table 16.

Triticum aestivum L. var. Kolibri.

The effect of respiratory inhibitors on the initial rate of the acid induced response.

Treatment: Leaf sheath bases were excised and threaded, in batches of five, on glass rods submerged in a solution of the inhibitor made up in 0.025M citrate : phosphate buffer at pH3. The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at 100 mm h^{-1}.

White light 25°C.

Statistical Analysis. The t test was used to test the differences between response rates for control and inhibitor treatments.

Table 16

	Control	10^{-3}M KCN	10^{-4}M DNP	10^{-3}M NaN_3
Response Rate (mm rise on kymograph drum/hour)	26.41 ± 1.65	29.42 ± 3.33	25.64 ± 2.05	30.28 ± 1.41
t value with respect to the control treatment		0.971^{NS}	-0.237^{NS}	0.769^{NS}

Fig. 94.

Triticum aestivum L. var. Kolibri.

The effect of low pH on chlorophyll levels.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' in 50 mm petri dishes containing 2.5 ml of distilled water (—○—), 10^{-3}M KCN in distilled water (—●—), 0.025M citrate : phosphate buffer at pH3 (—□—), or 10^{-3}M KCN in 0.025M citrate : phosphate buffer at pH3 (——■——). Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

taken as a measure of the chlorophyll contents of the ethanolic extracts.

White light 25°C.

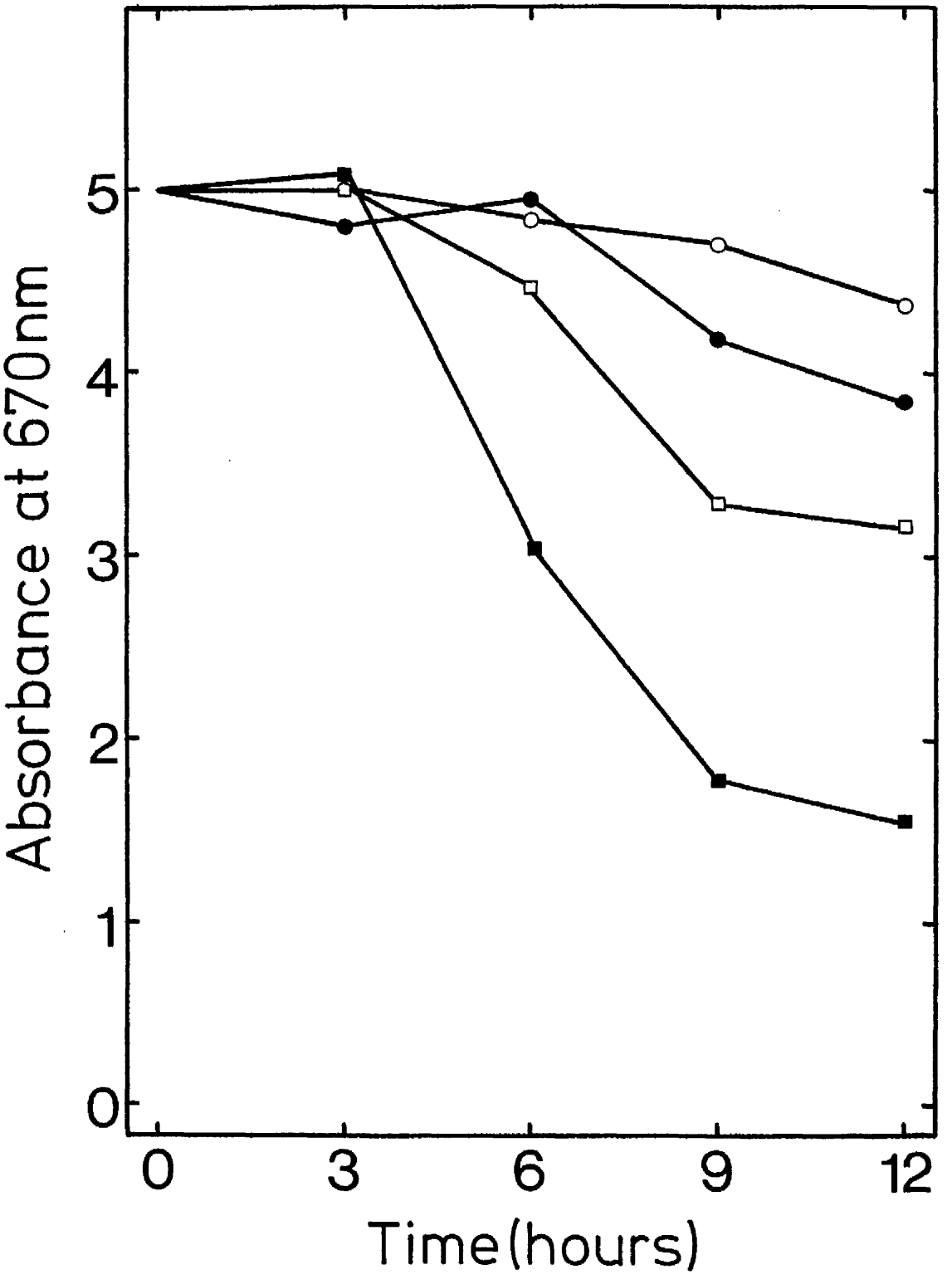

Absorbance at 670nm
0
1
2
3
4
5
0
3
6
9
12
Time (hours)

Fig. 95.

Triticum aestivum L. var. Kolibri.

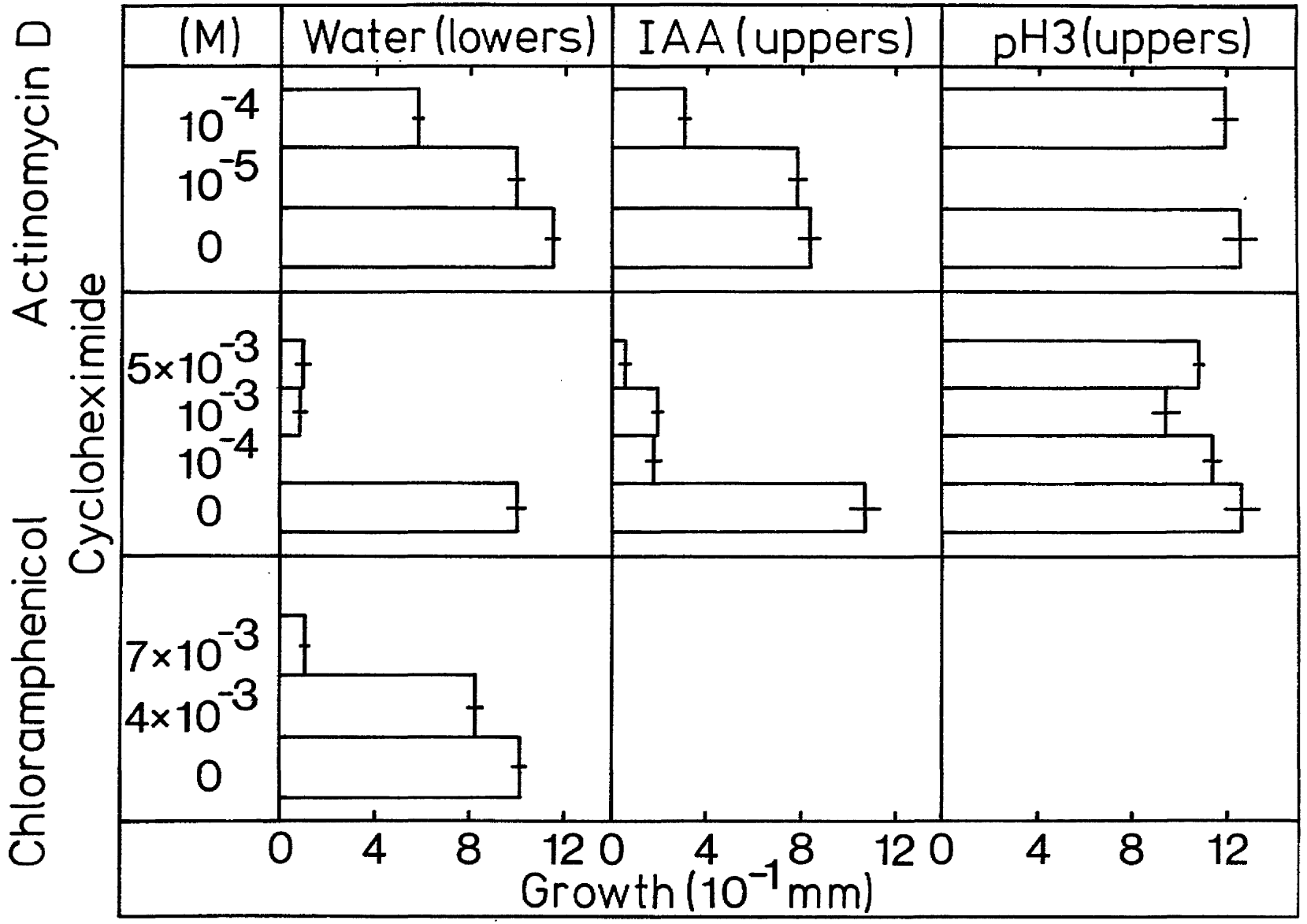
A comparison between the effects of inhibitors of transcription and translation on the acid induced, auxin induced and geotropically induced responses.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered and quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of one of the following solutions:-

	Distilled Water	10^{-4} M IAA	0.025M Citrate phosphate buffer pH3
Distilled Water	'Lowers'	'Uppers'	'Uppers'
Actinomycin 10^{-5}M - 10^{-4}M	"	"	"
Cycloheximide 10^{-5}M - 5×10^{-3}M	"	"	"
Chloramphenicol 3.5×10^{-3}M - 7×10^{-3}M	"		

Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.


(M)
Water (lowers)
IAA (uppers)
pH3 (uppers)
Actinomycin D
Cycloheximide
Chloramphenicol
10^-4
10^-5
0
5×10^-3
10^-3
10^-4
0
7×10^-3
4×10^-3
0
0 4 8 12
Growth (10^-1 mm)

requirement for the synthesis of both RNA and protein in these responses. Chloramphenicol is also inhibitory, but much higher concentrations are required, suggesting the involvement of nuclear rather than organelle DNA in the initiation of growth. Acid induced growth is insensitive to both actinomycin D and cycloheximide treatments, and is not therefore dependent on transcriptive or translative processes.

Geotropically induced growth is abolished by treatment with 10^{-4}M CPM, but auxin induced growth remains insensitive to this treatment, and the data presented in Fig. 96 show that acid induced growth is also insensitive to treatment with CPM.

The turgor requirement for the acid induced and geotropically induced growth responses are shown in Figs. 97 and 98. The requirements are similar and both responses are inhibited by increasing water stress, but considerable growth is able to occur during a 24-h incubation period under stresses at least as high as 15 atmospheres. Growth under such conditions suggests the ability to overcome water stress, and this is confirmed by a study of the effect of mannitol on the rate of the acid induced growth response (Fig. 98). When segments are subjected to water stress by the addition of mannitol to the buffer solution, the cessation of growth is immediate. Growth is terminated in the presence of 0.5M mannitol, but the effect is complete for only 30 min (Fig. 98A). Recovery is observed after this period and acid growth is proceeding at the control rate within 90 min of the addition of the osmoticum. The addition of higher concentrations of mannitol (0.7M) results in an initial contraction in the material, but recovery is again underway after 75 min and growth proceeds at a new and increased rate if mannitol is removed from the medium at this point. Growth at the new rate proceeds uniformly for at least 3 h following the removal of the osmoticum, and the phenomenon is best explained in terms of an increase in turgor pressure. A concept of 'stored growth' could explain an initial burst of growth, but a prolonged increase in growth rate would not be expected because

Fig. 96.

Triticum aestivum L. var. Kolibri.

A comparison between the effect of morphactin on acid induced, auxin induced and geotropically induced responses.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' or 'lowers' in 50 mm petri dishes containing 2.5 ml of distilled water, 10^{-4}M IAA or 0.025M citrate : phosphate buffer (pH3) in the presence (M) or absence (C) of 10^{-4}M CFM. Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

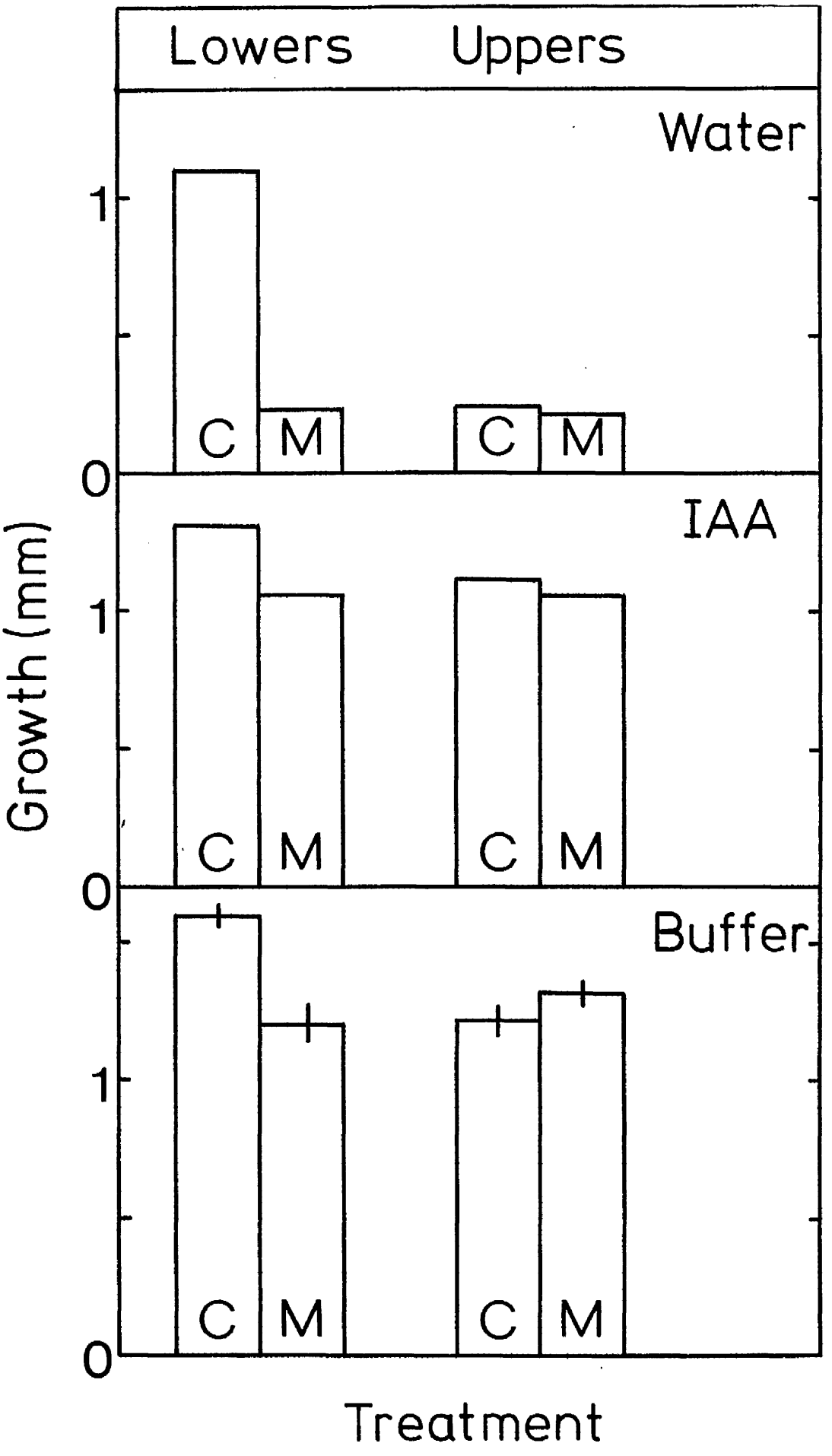

Lowers
Uppers
Water
IAA
Buffer
Growth (mm)
1
0
C
M
Treatment

Fig. 97.

Triticum aestivum L. var. Kolibri.

The requirement for cell turgor in acid induced growth.

Treatment: Portions of leaf sheath base 2.4 mm in length were excised and quartered. Quadrants were orientated as 'uppers' (– – –) or 'lowers' (———) in 2.5 ml of a solution containing mannitol made up either in distilled water (circles) or in 0.025M citrate : phosphate buffer at pH3 (squares). Segments were shadowgraphed after a 24-h treatment period.

White light 25°C.

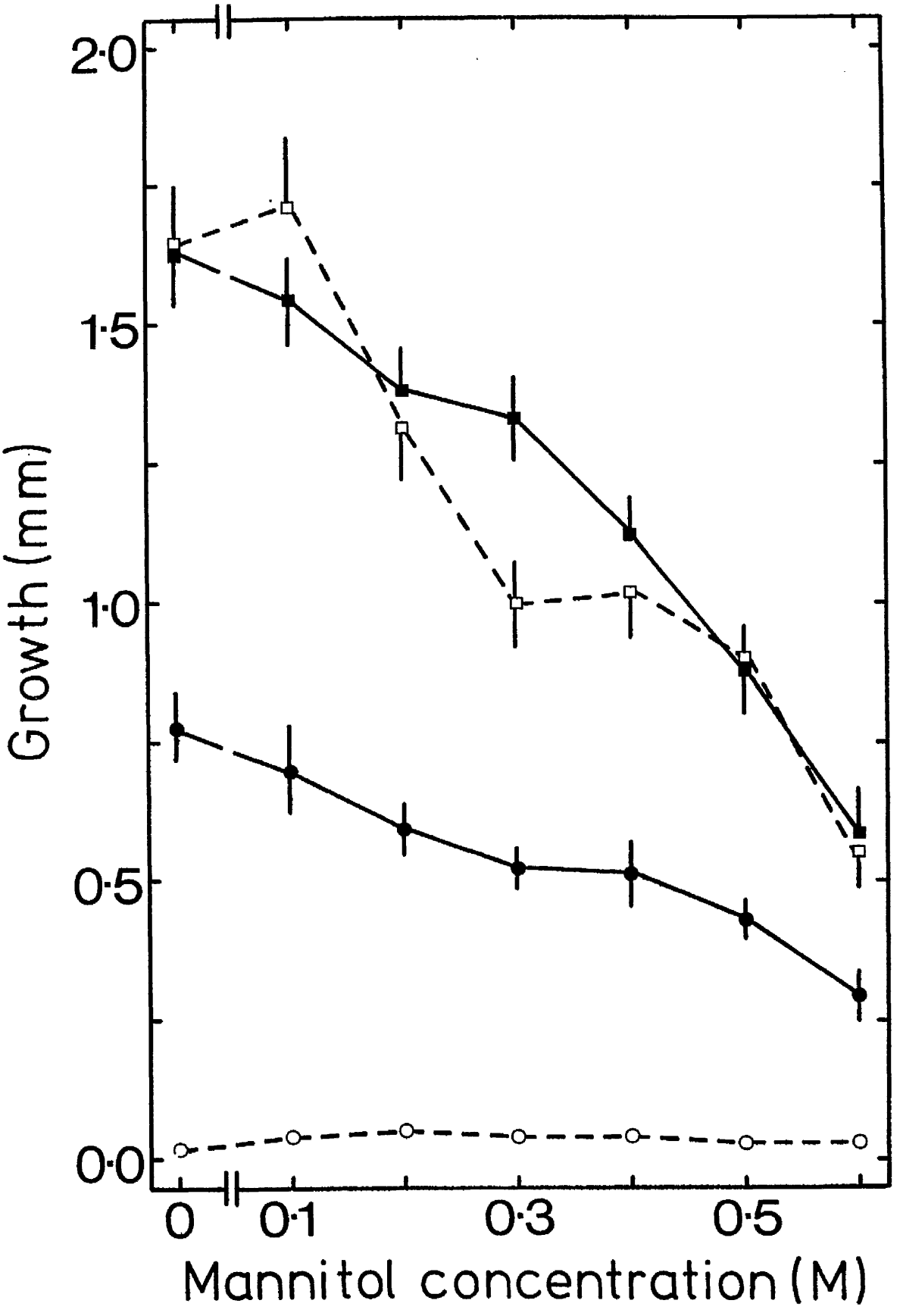

2·0
1·5
1·0
0·5
0·0
Growth (mm)
0
0·1
0·3
0·5
Mannitol concentration (M)

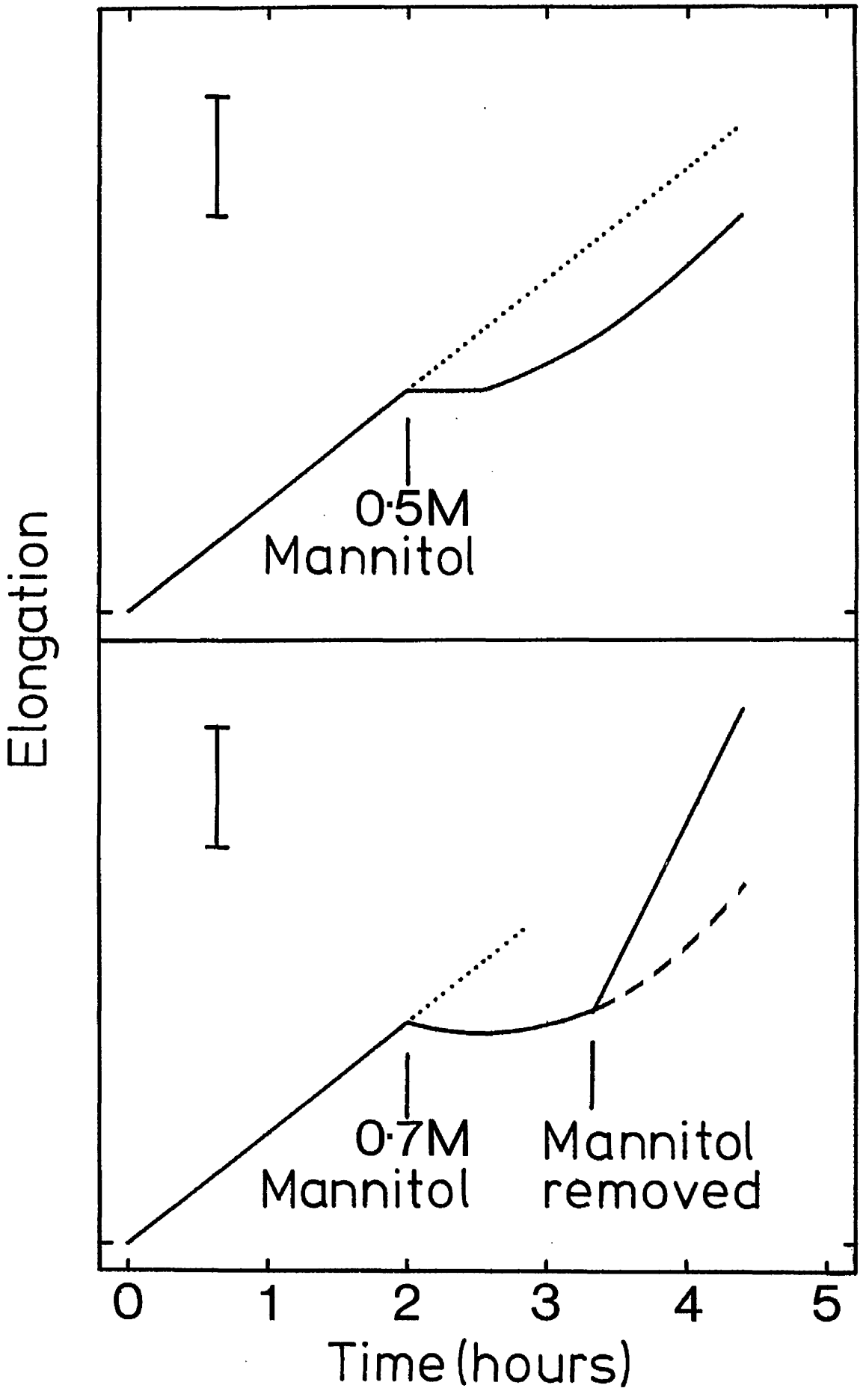
Fig. 98.

Triticum aestivum L. var. Kolibri.

The effect of water stress on the rate of acid induced growth.

Treatment: Leaf sheath bands were excised and threaded in batches of five on glass rods submerged in 0.025M citrate : phosphate buffer (pH3). The buffer was drained away 2 h after the start of the experiment and replaced by new buffer containing 0.5M (upper Fig.) or 0.7M (lower Fig.) mannitol. In some instances (lower Fig.) the second buffer was removed and replaced by the original mannitol free buffer at the end of a further 2-h incubation period. The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at a chart speed of 100 mm h^{-1}. The broken lines on the figures opposite represent the progression of the control responses, and the vertical bar indicates 1 mm growth.

White light 25°C.


Elongation
0·5M
Mannitol
0·7M
Mannitol
Mannitol
removed
0
1
2
3
4
5
Time (hours)

any such store would be utilised rapidly. The existence of a prolonged increase in growth rate on removing the mannitol, and a recovery mechanism in the continued presence of mannitol, render an explanation involving increased turgor most acceptable.

The loss with age of the ability to bend at the node has already received attention, and a concept involving physical restriction caused by the thickening of cell walls has been developed as a possible explanation for the phenomenon. The effect of physiological age on acid induced growth in the leaf sheath is therefore of interest, and data in this respect are presented in Fig. 99. The ability to respond to low pH treatment diminishes with age (Fig. 99A), and the effect can be attributed to a reduction in the response rate (Fig. 99B). Since the induction of growth by acid buffers appears not to be connected with cellular metabolism, the loss of the capacity for acid induced growth must be associated directly with the physico-chemical constitution of the cells, and the data may be taken as evidence in support of a concept involving the limitation of curvature by physical means.

Fig. 99.

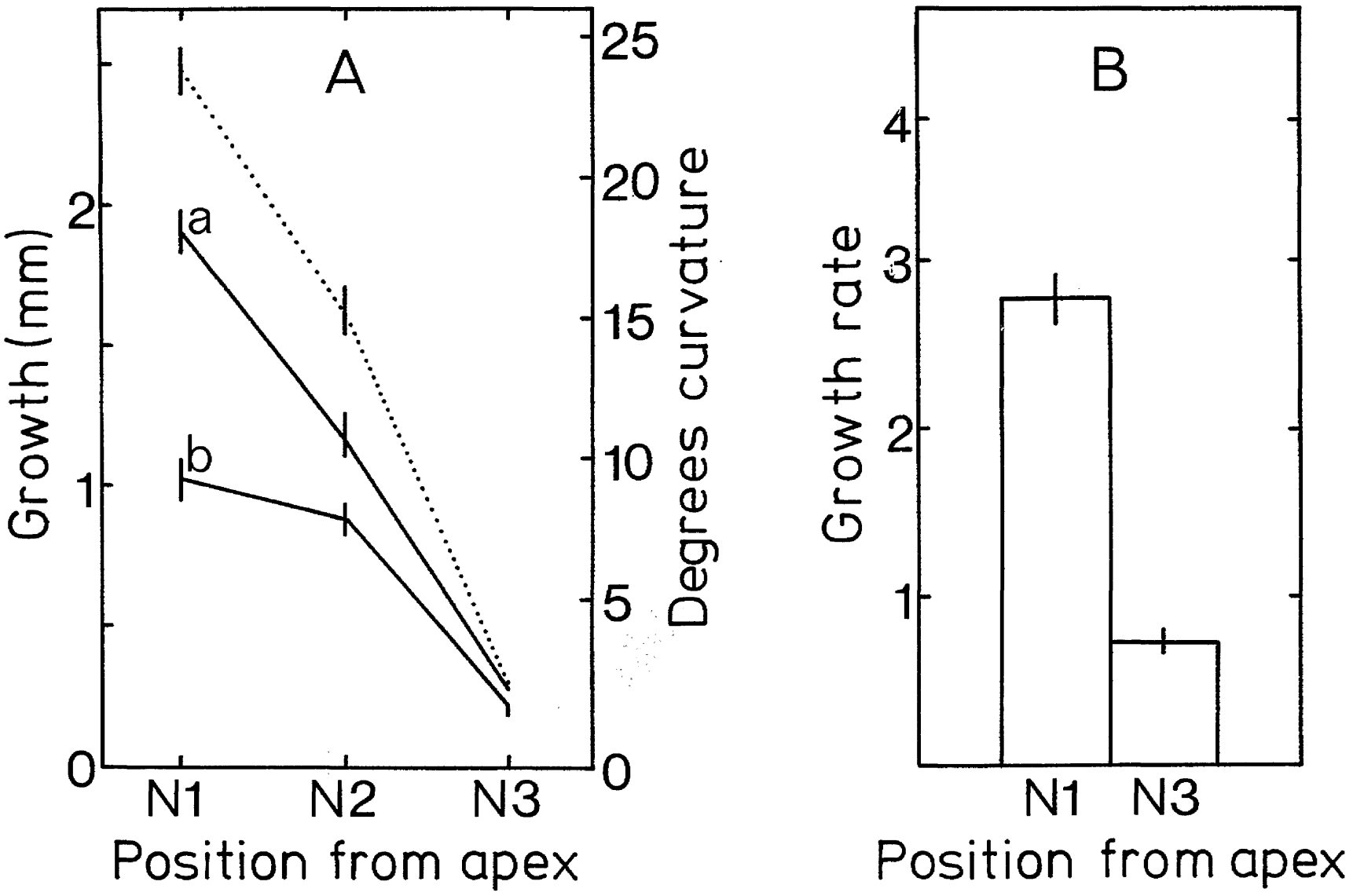
Triticum aestivum L. var. Kolibri.

The effect of physiological age on the magnitude of the acid induced response.

Treatments: Fig. A. Portions of leaf sheath base 2.4 mm in length were excised at nodes 1, 2 and 3. These were quartered and quadrants were orientated as 'uppers' in 50 mm petri dishes containing 2.5 ml of 0.025M (a) or 0.05M (b) citrate : phosphate buffer at pH3. Segments were shadowgraphed after 24 h treatment. The curvatures developed in response to a 24-h period of geotropic stimulation were determined for 100 mm stem segments prepared at nodes 1, 2 and 3 (·····).

Fig. B. Leaf sheath bases were excised at the first and third nodes from the apex and segments were threaded, in batches of five, on glass rods submerged in 0.025M citrate : phosphate buffer (pH3). The growth response was magnified through a kymograph lever and recorded on a smoke drum rotated at 100 mm h^{-1}.

White light 25°C.


A
B
Growth (mm)
Degrees curvature
Growth rate
a
b
0
1
2
5
10
15
20
25
3
4
N1
N2
N3
Position from apex
Position from apex

DISCUSSION

The leaf sheath base constitutes a specialised organ which is concerned with the execution of the geotropic response, and the anatomy of the structure is therefore of special interest. Growth results from the elongation of the parenchymatous tissues on the lower side of the displaced organ, and the individual cells in this tissue show increases of between 200 and 300% in longitudinal diameter without corresponding changes in radial or tangential diameter. The cells on the upper side of the organ do not respond to geotropic stimulation, but tend to become compressed as growth proceeds on the lower side. The parenchymatous cells of the leaf sheath base are hexagonal in longitudinal section, but they do not appear to exhibit the thick longitudinal walls reported by Arslan and Bonnet-Clark in Triticum aestivum var. Atle. This discrepancy may represent a varietal difference but, since both varieties are capable of similar geotropic responses, it seems right to question the existence of such structures. Arslan and Bonnet-Clark (1960) did not present photomicrographs in their publication and they gave no details of the anatomical techniques used in their study. It may be that the differences represent artifacts arising during the preparation of material for sectioning but this possibility cannot be tested in the absence of details relating to the anatomical techniques employed.

An alternative explanation for the discrepancy may be that extensive growth on the lower side of the organ leads to compression on the upper side, which in some instances results in the folding reported by Arslan and Bennet-Clark. Increases in dry weight do not keep pace with increases in fresh weight, and it is therefore reasonable to assume that some wall stretching is involved, but the concept of a specialised structure in which 'the microfibrillar material of the wall is folded like the sides of a concertina' (Arslan and Bennet-Clark, 1960) is difficult to equate with the findings of this investigation. Instead, an elongation process more akin to that found in growing organs is envisaged.

The structure of the vascular bundles is modified in the leaf sheath base to reduce resistance to curvature. The heavily lignified bundle sheaths of the leaf sheath are replaced in the leaf sheath base by large bundle caps which arise on the outsides of the vascular bundles. The bundle caps are composed of angular cells which pack together tightly. The cells are elongate in longitudinal section and their end walls are tapered. They retain active protoplasts and their walls are unlignified, but walls become lignified and the protoplasts disappear towards the extremities of the organ. Certain authors (Esau 1961) have referred to these unlignified cells as collenchyma, but this description seems to the present author to be incorrect. Collenchyma and sclerenchyma differ from one another mainly in wall structure and the condition of the protoplast at maturity. The constituent cells in the bundle caps of geotropically active leaf sheath bases show characteristics which are diagnostic of collenchyma, but the organs have not reached maturity at this stage. The dry matter content of the leaf sheath base increases as the organ ages, and this increase represents wall thickening. Lignification occurs in the bundle caps and the cells come to resemble sclerenchyma. Thus it seems reasonable to suppose that the bundle caps are composed of sclerenchymatous cells which experience a prolonged state of immaturity.

The internode plays no part in the geotropic response, but is bent

passively through the angle described by the leaf sheath. The intercalary meristem is situated at the base of the internode and the tissues in this region are unlignified when the leaf sheath base is in its most geotropically reactive state. The soft internodal tissues in the region of the meristem provide a flexible fulcrum whilst stem elongation is in progress, but when elongation is complete, wall thickening occurs and flexibility is lost.

Lignification does not generally occur in young cells, and the high concentration of chemical growth regulators in growing regions has been suggested as a possible cause for this restriction (Brown 1961). Parish (1968) has shown that IAA will inhibit lignification in the lower two-thirds of the wheat internode, and this observation, coupled with the many observations implicating IAA in other geotropic systems, renders models involving auxin in this geotropic response especially attractive.

The mechanism of gravi-perception in plants remains uncertain, but the consensus of opinion falls in favour of the statolith theory. There are two types of potential statolith in the leaf sheath base and both have been described in detail by Prankard (1920). The carbohydrate store in grass stems is mainly in the form of fructosans, but sedimentable starch grains are found in the leaf sheath bases in the cells lying adaxial to the vascular elements. Crystal inclusions are also found, and these are distributed throughout the parenchymatous cells of the leaf sheath base. Prankard (1920) believed that both bodies were involved in gravi-perception, but her evidence was based solely on ability to sediment. Experiments designed to remove starch preferentially from the leaf sheath base, using the destarching procedure of Pickard and Thimann (1966), have questioned the involvement of crystals as statoliths. Starch grains may be removed completely from the statenchyma during a 3-day incubation period in a solution of 5×10^{-5}M GA_3 + 5×10^{-5}M kinetin at 30°C, and this treatment has the effect of abolishing geotropic sensitivity. It may always be argued that such treatments affect other parameters in addition to starch content, but the fact that gravi-

sensitivity returns with the resynthesis of starch grains may be taken as strong circumstantial evidence in favour of the involvement of starch grains and not crystals in the gravi-perception mechanism.

Any assessment of the threshold acceleration which an organ can detect is difficult because the force of gravity can never be eliminated in the laboratory. The clinostat may be used to compensate for the effects of gravity, and an elaborate apparatus designed to compensate for the effects of gravity whilst applying a measured acceleration has been developed by Shen-Miller et al. (1968). The apparatus involves rotation about twin axes, rotation about a horizontal axis providing gravity compensation, and rotation about a vertical axis providing centrifugal acceleration, but the logic behind the design of the apparatus is open to serious criticism. The acceleration induced by horizontal rotation is greater than many of the accelerations induced by vertical rotation, and the stimulus received in the direction of the resultant acceleration cannot be equated with that received when the statoliths sediment in a plane perpendicular to the tangential cell wall. Experiments reported in this thesis have involved rotation about a single horizontal axis and have indicated a 'threshold acceleration' of between 1/10,000 x g and 1/1,000 x g for a response over a 24-h period. Since reciprocity appears to hold for varying g forces (Shen-Miller 1970), the threshold acceleration may be expected to vary with time, and a threshold product (gxt) may be a more meaningful measure. The threshold product in the wheat system would thus be of the order of 8.6 to 86 gs.

The absolute threshold acceleration determined on the clinostat may be taken to represent the minimum acceleration required to move the statoliths towards the receptor. At lesser accelerations the particles will tend to fall under the influence off gravity and the effect of rotation will be to constantly lift them. The statoliths will always fall vertically, but because the cell is rotating the trajectories described by the statoliths will constitute circular closed figures, and the diameters of these trajectories will be governed by the speed of rotation. As the rotation

speed is reduced the diameter of the trajectory will increase until eventually it equals the diameter of the statocyte, and at this point the statoliths will roll round the statocyte walls.

Rotation at speeds which cause the statoliths to revolve in a minimum volume of cytoplasm, without causing displacement of the trajectory by centrifugal force, ought to be most suitable for clinostat rotation. Dedolph and Dipert (1970) have utilised this reasoning to calculate the optimum angular velocity for rotation on the clinostat, and they have computed a value of 1.73 RPM. Because their calculation is independent of the size and density of statoliths, and the density and viscosity of cytoplasm, a value so calculated should be applicable to any geotropic system, and the finding that similar compensation may be achieved in satellites and on clinostats rotated at angular velocities between 1 and 3 RPM (Lyon 1970), may be taken as verification of this reasoning.

Rotation at speeds between 1/6 RPM and 5 RPM about a 20 mm radius has no effect on the external appearance of unstimulated wheat leaf sheath bases, but material which is already bending is straightened by rotation at 2 RPM, and responses fail to develop in straight material which is stimulated prior to clinostating. The effect on bent material may be explained in terms of the deviation of the apical regions from horizontal, but this explanation seems unlikely because perception is confined to the leaf sheath base, and the leaf sheath base is not displaced from its position with respect to the axis of rotation. Thus, whilst the plane of the statolith trajectories may change slightly during curvature, their radii must remain unaffected, and it is difficult to see how a stimulus to straighten can be imparted.

The straightening of bent material, and failure to bend of stimulated material, may be explained if the clinostat is assumed to achieve its effect through the provision of omnilateral stimulation because the application of a diffuse stimulus may be expected to suppress the expression of a unilateral stimulus of the same kind. This point has been illustrated by Larsen (1953)

who has shown, using Artemisia and Lepidium seedlings, that, when omnilateral stimulation involving horizontal rotation at 1 revolution per 32 mins. is applied following horizontal stimulation, the effect is to produce straightening, whilst when compensation is applied, by horizontal rotation at 2 RPM, the effect is to support curvature. Rotation at speeds of the order of 2 RPM ought, however, to be optimal for gravity nullification in the leaf sheath base if the calculations of Dedolph and Dipert (1970) are credible. Moreover, omnilateral stimulation results in the induction of straight growth in the leaf sheath base and, because such a process is not induced by rotation at 2 RPM, the stimulus at this speed cannot be thought to be omnilateral.

Our understanding of the mechanism of clinostat action is further complicated by reports in the literature concerning changes in metabolic capacity and hormone balance during periods of clinostating. A shoot will sag when tilted to the horizontal, and this effect will pass round the organ with rotation on the clinostat. Thus, physical distortion could explain the changes in respiration, but Dedolph et al. (1967) have shown, using starch grains as an index of statolith sedimentation, that the enhanced respiration observed in Avena coleoptiles during periods of clinostating may be induced, maintained, discontinued and re-induced by treatments which concomitantly result in the introduction, maintenance, discontinuation and re-introduction of a more uniform statolith distribution in the statocytes. They have concluded that 'gravity sensing by plants is broadly based on the physico-chemical relationship between particle distribution and the expression of respiratory metabolism as growth within the limits of auxin economy'. In roots, where they believe auxin availability is supra-optimal, an increase in respiration can be converted to growth, and hence explain the growth and straightening responses observed on the clinostat, whilst in shoots where auxin availability is held to be sub-optimal, concentration by lateral transport is required before increased growth is observed in the form of curvature following stimulation. Schmitz (1933) has noted an increase in

'auxin' content in the vicinity of the grass node following geotropic stimulation, and Shen-Miller and Gordon (1967) have reported an inhibition of basipetal auxin transport in coleoptiles during periods of horizontal rotation on the clinostat. Whilst these data are compatible with a hypothesis requiring an increase in auxin concentration for growth on the clinostat, the demonstration that auxin concentrations in Bean roots are not supra-optimal (Audus & Brownbridge, 1957), and the doubt surrounding the involvement of IAA in grass nodes (Arslan and Bennet-Clark, 1960; and Results sections 11 & 12 in this thesis), may be taken as evidence against the hypothesis. Further evidence against the hypothesis stems from the fact that the regions of perception and response are physically separated in roots. In the root perception is confined to the root cap (Juniper et al. 1966; Gibbons and Wilkins, 1970), whilst growth is confined to the root apex, and it is difficult to see how a process involving increased metabolism by virtue of statolith randomization can explain growth during horizontal rotation. A similar argument may be advanced for the coleoptile and leaf sheath base, where the presence of statoliths in the growing regions is restricted to the cells lying adaxial to the vascular bundles.

Growth induced in the leaf sheath base during periods of horizontal rotation can be explained satisfactorily in terms of the statolith theory. Growth is induced at rotation speeds slower than 10 RPH, and at these speeds the resultant between the downward force of gravity and the upward force from rotation will cause the statoliths to revolve in trajectories of greater diameter than that of the cell. Because the size of the trajectories is limited by the diameter of the cell, the statoliths will tend to settle at the lowest point in the cell under the dominant influence of gravity, and geotropic stimulation will occur as the cell walls revolve past these effectively stationary statoliths. Rotation is too slow to distribute the statoliths at random in the cytoplasm and, consequently, growth cannot be explained in terms of an increase in metabolism resulting from such a

dispersion. Instead it is suggested that all regions of the leaf sheath base are stimulated systematically as the organ rotates, and the demonstration that curvature develops spontaneously in the plane of the gravitational field when rotation is terminated must be taken as evidence to corroborate this hypothesis. The normal reaction time for the geotropic response in the leaf sheath base is 2h 20m at 25°C, and the optimal angular velocity for straight growth is one revolution in approximately four times this period. Thus the statoliths will be in contact with the outer tangential surface of each cell wall for exactly the reaction time.

Because rotation about a horizontal axis has proved unsuitable for gravity nullification, it has not been possible to determine a presentation time for this response. This failure may be attributed to defects in the clinostat technique, problems concerning the long development period required to initiate a measurable response or genuine requirements for continuous stimulation during the development of the response. A discussion of this latter possibility will follow presently, but further discussion of the former points must await further experimentation with more sensitive equipment. It is, however, of interest to note the connection between straight growth and reaction time, and this phenomenon, coupled with the fact that growth is only induced in excised segments when these are orientated with the outer epidermis facing downwards, would seem to indicate the restriction of the receptor mechanism to the outer tangential cell wall.

Information concerning the distribution of the gravi-receptor mechanism has been obtained from experiments involving the induction of straight growth by rotation about axes at various displacements from vertical. Because gravity is the dominant influence in these experiments, the statoliths will sink to the lowermost point in the cell, and the walls will revolve past them at a fixed angular velocity which is independent of orientation. The total stimulus per orbital will always be equal and, because the application of the stimulus is perpendicular to the wall, it is possible to

determine the receptivity at specific points on the wall. Experiments of this type show that the growth induced is proportional to a function of the sine of the angle of displacement. The response curves reach a plateau at displacements between 45° and 135° from vertical, and the plateau may be taken to represent the uniform distribution of receptors in this quadrant (i.e. the outer tangential wall). The tailing towards zero growth at 0° and 180° displacements from vertical will indicate the absence of receptor mechanisms at these points. The argument is, of course, dependent on the application of reciprocity in this system because, although the total stimulus per orbital (gt) is the same regardless of orientation, the diameter of the orbital, and hence the total stimulus received at a point, is dependent on the shape of the statocyte and the plane of rotation. The apparent unsuitability of clinostat rotation as a means of gravity nullification has prevented an appraisal of the operation of the reciprocity law in this organ, but the observations concerning curvature in response to small centrifugal accelerations may be taken to indicate some proportionality between the quantity of stimulus and the subsequent response. Similarities between the development of the straight growth response during periods of slow rotation in various orientations and the development of first and second quadrant curvatures in base held and tip held preparations, may also be taken as evidence in this respect, because the organs used in these latter experiments receive equivalent stimuli (gt) at a point rather than over an orbital.

It is important at this point to make a distinction between experiments of the type discussed above and experiments of the type designed by Dedolph, Gordon and Oemick (1966) to simulate low gravity environments by rotation at clinostat speeds about axes fixed at various displacements from vertical. The logic behind these latter experiments is rather unclear because it appears to be based on the intention to apply a stimulus at a point, and at the same time prevent the application of that stimulus by rotation at 2 RPM.

The idea is that rotation will only nullify a portion of the directional component of the gravity force vector, and that 'similar to the "sine-law" relationship the fraction not nullified will equal the sine of the angle of inclination of the rotational axis' (i.e. the fraction nullified will change with the cosine). In the opinion of the present author, the statoliths must always fall vertically, and the effect of rotation at an angle to vertical can only be to alter the value of rotation as a means of preventing this process. The value of rotation as a means of gravity compensation will vary with the sine of the angle of displacement, and the effective component will be given by the formula 2 sin Θ RPM, where Θ is the angle of displacement from vertical. The statolith trajectories will increase as Θ approaches zero and contact will eventually be made with the cell wall. When this occurs, the region of contact will receive a stimulus equal to gt and, depending on the distribution of the gravi-perception mechanism in the cell, this may or may not be equivalent to the application of the force component g sin Θ at a point in the centre of the outer tangential wall (the locus for normal stimulation at 90° displacement). Dedolph et al. interpret their data as being indicative of the absence of a threshold gravity force, and claim the production of maximal responses at forces of 0.06g (roots) and 0.08g (coleoptiles), whilst the present author interprets the data as indicative of the development of normal responses when the effective rotation for gravity compensation drops to values of less than 0.12 and 0.16 RPM respectively. Rotation is almost vertical at these points, and the statoliths must be restricted to small areas in the apical (inverted coleoptiles) or basal (inverted roots) regions of the cells. The failure to abolish the response at 180° displacement must therefore be taken to indicate the presence of receptor mechanisms in these regions and not, as inferred by Dedolph et al., the absence of a threshold force requirement for curvature.

There are typically four leaf sheath bases per plant in the spring wheat

var. Kolibri, and the combined curvatures at these growth centres return the apex of the displaced culm to vertical. The total capacity for curvature at these 4 leaf sheath bases exceeds 90°, but curvature is restricted to 90° in base held preparations. Such restrictions are often attributed to the tonic influence of gravity, and several workers are of the opinion that these tonic influences can be explained in terms of growth substance economy. Orientation can certainly affect the polar transport of IAA in coleoptiles (Little and Goldsmith 1967, Wilkins and Cane 1970), and Miehe (1902) has provided a strong case for the involvement of auxin in the nodal responses in Tradescantia fluminensis.

Arslan and Bennet-Clark (1960) have noted a similar situation at grass nodes and they have suggested that the tonic involvement is conditioned by the amount of leaf sheath remaining above the leaf sheath base. The analogy with the situation in Tradescantia is not complete, however, because the leaf sheath does not provide living continuity between nodes. The internode provides the necessary continuity, but its involvement requires the provision of a mechanism for basipetal transport between nodes, followed by a mechanism for acropetal transport back into the leaf sheath base.

Concepts involving chemical co-ordination between wheat nodes are dispelled by the demonstration that living continuity can be broken without affecting the development of individual responses. Experiments involving the use of 2 node preparations show that it is curvature at the physically upper node which is limited, and the effect can best be explained in terms of the changing angle of exposure to gravity at this locus. Experiments designed to investigate the effect of the angle of displacement on the development of geotropically induced curvature, and growth, provide conclusive evidence to show that the geotropic response is conditioned by the angle of stimulation, and it is suggested that both tonic and tropic influences of gravity can be explained in terms of a concept involving physical co-ordination in which the responses are determined by the distribution of

receptor sites in the individual leaf sheath bases.

Tip held and base held 1 node preparations prepared from the culms of the spring wheat var. Kolibri produce equivalent curvatures in response to horizontal stimulation, but data provided by Arslan and Bennet-Clark (1960) show a greater capacity for curvature in tip held preparations when preparations are taken from culms of Bromus sterilis.

The difference in magnitude between the asymmetries noted in the response curves for tip held and base held wheat preparations when these preparations are stimulated at increasing displacements from vertical can be explained in terms of a gradient in either perceptive or reactive capacities in the leaf sheath base, but the presence of the asymmetries must be taken to indicate the distribution of the perception apparatus throughout the organ (see Fig. 18 and associated explanations on page 60). Thus, although reactivity as measured by growth in the apical, central and basal regions of tip held and base held preparations, is greatest in the apical regions of the leaf sheath base, the curvatures developed in tip held and base held preparations remain equivalent because increased growth in the apical regions is coupled with decreased growth in the basal regions. If the perception mechanism was absent from the basal regions of the leaf sheath base, then growth would not be expected in this region unless information was transmitted from the more apical regions of the organ, and in either instance the response would be governed by the quantity of stimulus perceived in this apical region. Responses would be greater in tip held organs because the apical regions of these organs would remain horizontal, and a situation such as that reported in Bromus sterilis (Arslan and Bennet-Clark 1960) would be expected.

The tonic effect of gravity can thus be explained without reference to the involvement of chemical growth regulators outside the leaf sheath base, but their possible involvement within the leaf sheath base and the effects of such an involvement on the operation of a physically co-ordinated system, remain to be considered. Evidence from barrier experiments suggests that

lateral transport of growth regulators within the leaf sheath base is not required, and this inference is substantiated by the demonstration that growth may be induced in small segments cut longitudinally from excised leaf sheath bases. This latter observation is thought to be particularly significant because the response is controlled entirely by orientation, and growth is only observed when the segment is orientated with its outer epidermis facing downwards.

Several plant growth regulators have been tested for activity in the leaf sheath base, but only IAA has been found to be active. Indole acetic acid is unable to stimulate growth in geotropically stimulated segments (lowers), but it is able to induce considerable growth in unstimulated segments, and the possibility arises that IAA may indeed be involved in the induction of this response. The question of an auxin involvement was first raised in connection with the suppression of lignification in the leaf sheath base, but such a rôle must be considered unlikely when growth is induced in response to exogenously applied IAA. The geotropic induction of growth may be explained if auxin concentrations are increased locally in response to geotropic stimulation, but auxin can only be present initially at extremely low concentrations if the absence of growth in the vertical organ is to be explained satisfactorily. Processes involving auxin synthesis or release could allow localised increases in IAA concentration to occur and, although the data obtained from experiments involving barrier insertion and segment excision render processes involving transport unlikely, the inhibitory action of the morphactins in this geotropic system may be construed as evidence to implicate the involvement of such a transport system.

Although there is reasonable evidence in the literature to suggest that the morphactins interfere with auxin economy, their exact mode of action is by no means clear, and an investigation of their action in cereal coleoptiles has therefore been undertaken. It is clear from this investigation that the principal effect is on the basipetal transport of IAA. Basipetal

transport is inhibited by 10^{-7}M CFM and inhibition is complete at 10^{-5}M. Acropetal movement is unaffected over transport periods of short duration, but a significant increase in flux may arise with time. Acropetal movement is generally recognised to be a physical process, anaerobic conditions having little effect on its magnitude (Goldsmith 1966a, Wilkins and Martin 1967, Wilkins and Whyte 1967), but it is plausible that an interaction with basipetal transport will tend to reduce its magnitude. Evidence of such an interaction has been provided by Goldsmith (1966b), and it seems probable therefore that the acropetal movement of IAA in segments pretreated with CFM represents the true rate for diffusion of IAA through the tissues. The downward lateral movement of IAA in horizontal coleoptile segments is also inhibited by CFM pretreatment, but the pretreatment has no effect on movement against gravity, and the suggestion that CFM may be capable of redirecting transport (Parups 1971) must therefore be considered incorrect.

The possible effects of CFM on the synthesis of endogenous auxins have not been investigated, but it is evident from the chromatographic analyses that the metabolism of applied IAA remains unaffected by the treatment. Where transport occurs, the radioactivity is mainly confined to the IAA molecule, and it seems reasonable to assume that the effect of CFM is to prevent the polarised transport of this molecule. The question of how the morphactin achieves this effect must now be considered. Strong correlations exist between the effects of CFM on both longitudinal and lateral auxin transport, and the effect on curvature in horizontal Zea coleoptile segments, and a similar correlation may be drawn between polar auxin transport and straight growth in Avena coleoptile segments. These correlations may be interpreted in favour of processes involving competition with IAA for response sites, but such concepts are incompatible with the data obtained for straight growth in Zea coleoptile segments. Highly significant growth promotion may be obtained in Zea coleoptile segments in the presence of CFM at concentrations which inhibit the auxin transport systems, and this

promotion cannot be ascribed to an effect on auxin transport, because significant promotion can be achieved in the absence of an auxin source. The promotive effects of CFM and IAA on straight growth in Zea coleoptile segments appear to be additive and, because the addition of either at optimal concentrations will significantly increase the maximal extension produced by the other, the two cannot interact at the same site. Several workers have mused on the operation of morphactins as gibberellins, but the idea seems to be based solely on superficial resemblances between the two molecules when portrayed in two dimensional drawings. Gibberellin has little effect on the growth of coleoptiles, and factorial experiments show that the morphactins do not act like gibberellins when promoting growth in this system.

Similar antitropistic effects have been ascribed to naphthylthalamic acid and tri-chlorobenzoic acid, and Lembi et al. (1971) have shown that naphthylthalamic acid binds to the plasma membrane where it may interfere with sites concerned with auxin transport. It is therefore attractive to speculate on the action of morphactins at the plasma membrane where they may inhibit the processes responsible for the maintenance of polarised auxin transport. An explanation of this kind is especially attractive because, by connecting the inhibition with the establishment of the transport system, it permits the extension of the concept to root geotropism without presupposing that the inhibitor involved is IAA. It does at the same time, however, require that inhibition by these antitropistic reagents can no longer be taken as evidence for an auxin involvement in geotropism, and it may be reasonable to suppose that these effects merely indicate the existence of a polarisable system.

Nevertheless, it is fair to assume that the inhibition of geotropism in cereal coleoptiles results from an effect on polarised auxin transport, and the extent to which the system is analagous with that in the leaf sheath base must now be considered. The morphactins are powerful inhibitors of

the geotropic response in the leaf sheath base, but inhibition is overcome completely in the presence of relatively low concentrations of IAA, and auxin induced growth in unstimulated segments (uppers) remains unaffected by CFM. Whilst these observations cannot be taken to preclude the involvement of auxin in this geotropic response, they can, when considered in conjunction with the data obtained from experiments involving barrier treatments and segments excision, be taken as conclusive evidence to preclude the involvement of auxin in the type of co-ordinated reaction sequence found in the coleoptile.

The dosage response curves obtained in the presence of CFM for the inhibition of root, coleoptile and node geotropism are very similar, and the similarity may be taken as evidence for the existence of a common site of action in all three systems. The sedimentation of starch grains in the coleoptile does not appear to be affected by the CFM pretreatment, and this information, coupled with the reported extension of inhibition to other tropisms, must render an effect on the initial purely physical stage of perception extremely unlikely. An effect on the development of polarity at the statocyte membrane could, however, be advanced to explain both the universal inhibition of tropic responses and the inhibition of polarized transport in coleoptiles, and such a suggestion may also explain the very marginal effect of CFM on the movement of IAA in the leaf sheath base.

Auxin transport in leaf sheath bases is very slightly polar, but the distribution patterns observed for both acropetal and basipetal movements resemble diffusion gradients. Lateral movement is even less polarized, but significantly more radioactivity is found in the lower half of the organ after a 12-h transport period. The reaction time for the response is only 2h 20m, however, and the delayed lateral polarity may represent movement towards a metabolic sink created by growth on the lower side of the organ. In either event, the transport capacity is wholly inadequate as a means of auxin concentration in response to geotropic stimulation.

The absence of a transport requirement constitutes a major deviation from the response sequence envisaged in the Cholodny-Went theory of geotropism, and it places a restriction on the mechanisms available to explain transverse polarisation in the leaf sheath base. Hypotheses involving differential secretion from statocytes are unable to explain the development of a transverse polarity in the absence of a transport requirement, but concepts involving the critical positioning of receptor sites may still remain viable. The restriction of the response in excised segments to those segments orientated with their outer epidermis facing downwards suggests the operation of gravity as a unilateral stimulus and, because the effect on statolith sedimentation can only be directional, it seems appropriate to attach special significance to the outer tangential walls of the statocytes, and to anticipate the existence of receptor mechanisms at these sites. The action of the perception mechanism may be to initiate hormone production in stimulated cells, and production of IAA in response to stimulation has been suggested by Schmitz (1933) as a means of explaining the response in the leaf sheath base. Experimental evidence for this possibility is subject to serious criticism, however, firstly because it relates only to biological activity associated with crude diffusates and, secondly, because little significance can be attached to such diffusates in a system which is not dependent on hormone movement.

No evidence for the existence of IAA in stimulated material has been forthcoming from this investigation, but it may always be argued that the controlled synthesis or release of IAA at a site in quantities sufficient to permit the response but prevent the establishment of a free auxin pool, will explain both the response and the failure to detect IAA by chemical means. Evidence concerning the persistence of the stimulus suggests that substances of short half life are involved in the induction of growth and, if these substances include auxin which is released from a precursor, then a pool of precursor of sufficient magnitude to allow continuous release

will need to be available. It ought to be possible to detect such a pool by chemical means, but no such pool has been isolated, and the inference must be that auxin release from precursors is not involved. This leaves the possibility of synthesis in response to geotropic stimulation, and the negative evidence obtained in this respect is extremely difficult to interpret. Such evidence may be taken to indicate the absence of an auxin involvement, but it may also indicate a lack of sophistication in the experimental technique, and because a technique can never be proved perfect, it is difficult to see how evidence of this kind can ever be taken as conclusive.

The reaction time for the geotropic response is 2h 20m at 25°C and, although this period may appear adequate for gene induction when compared with the induction periods observed in bacteria, it is short for induction in plants. The shortest induction period measured in plants is that for nitrate reductase in maize, and the value in this instance in 2h (Afridi and Hewitt 1964). Auxin is thought to act as a messenger, and its ultimate function may only be to induce the production of further substances. If this is the case, then the production of auxin in a system where the regions of perception and response are common may be considered superfluous, and production may be questioned on the grounds of plant economy. Critical growth regulating proteins are known to be involved in growth (Cleland 1971) and the production of these substances in a system where growth is initiated by the stimulus is likely to involve transcription. The reaction time appears short to encompass these events but our understanding of the kinetics of the development of these processes may once again be restricted by the lack of sophistication in experimental techniques.

A more rewarding line of enquiry would appear to involve an investigation of alternative mechanisms which could explain growth in the absence of a hormonal involvement, and in order to carry out such an enquiry an appreciation of the growth mechanism in the leaf sheath base is necessary.

Growth is extremely sensitive to pH and buffers of neutral pH have the effect of suppressing geotropically induced growth. Extensive growth promotion occurs during incubation with acid buffers, however, and a maximal response is obtained at pH3. The response occurs in both stimulated (lowers) and unstimulated (uppers) segments, and the response characteristics are similar to those reported by Rayle and Cleland (1970, 1972) for the acid induced response in the Avena coleoptile. Acid growth develops without a lag, and its development remains unaffected by treatment with respiratory poisons and inhibitors of transcription and translation. The response is of short duration, but it persists for considerably longer than the response in the Avena coleoptile, and is therefore more amenable to experimentation.

Acid induced responses in Avena coleoptiles can be propagated over very much longer periods when an external driving force is used to replace the cell turgor which is drastically reduced during the buffer treatment (Rayle and Cleland 1972), and the increased duration of the response in the leaf sheath base may be interpreted in terms of a slower loss of turgor from this organ. Time courses for the incidence of bleaching in response to acid buffers are in accordance with this hypothesis.

Acid induced growth is terminated by increasing pH, and this observation is especially significant because it is not compatible with the postulated action of acid buffers as triggers for auxin release (cf. Bonner 1934). Equivalent data are obtained when IAA is supplied to the leaf sheath base in water or buffer at pH4 or 5, but an increase in buffer pH from 3 to 4 or 5 is sufficient to abolish the acid response completely. The concept of reversible hydrolysis of auxin precursors is therefore untenable, and the acid response must be interpreted as a direct response to H^+ ions.

If growth is induced by H^+ ions, then re-application of acid buffers ought to reinduce the response, and this is found to be the case in the leaf sheath base. Uptake of solute molecules and turgor susceptibility to acid buffers may be advanced as possible explanations for the absence of

re-initiation in Avena coleoptiles apparent in the data of Rayle and Cleland (1970), and it ought to be possible to begin to differentiate between these possibilities with the aid of an external force to replace cell turgor. The responses to pH changes are not immediate, but exhibit lag periods which appear to be related to the differences in pH between the two buffer treatments. Lags for re-initiation of the response are directly proportional to the pH differential, whilst those for inhibition are inversely related. The lags for inhibition could be interpreted as evidence for the activation of a wall loosening enzyme by protons, but the lags for re-initiation cannot be explained on this basis. The effect can perhaps best be ascribed to the uptake of buffer into the segments.

Equivalent maximum growth rates are observed for the auxin induced and acid induced responses in Avena coleoptiles, but the acid induced response far exceeds the auxin induced response in magnitude in the wheat leaf sheath base. Rayle and Cleland (1972) suggest that normal growth occurs in response to the hydrolysis of acid labile bonds in the cell wall, and auxin may be responsible for the initiation of biochemical processes which lead to the provision of the H^+ ions required for such hydrolyses. The question arises as to whether IAA is the only stimulus capable of initiating this activity, and data provided by Ganot and Reinhold suggest that it is not. Ganot and Reinhold (1970) have found that treatment with acid buffer, but not auxin, can restore gravi-sensitivity in Helianthus hypocotyls which have been etiolated or starved prior to treatment. They suggest that the geotropic stimulus may bring about a 'physiological asymmetry in the tissue apart from the asymmetrical distribution of auxin', and that this physiological asymmetry may cause the differential response to acid buffer.

Certain aspects of the data presented by Ganot and Reinhold (1970) are difficult to equate with current interpretations of the process of acid induced growth. They find, for example, that their tissues can be conditioned to yield a response in water by pretreatment with acid buffers. This is distinct from the situation found in the wheat leaf sheath base and oat coleoptile, where the continued presence of buffer is required for growth, but the apparent ability to maintain this response in the presence of inhibitory concentrations of various poisons suggests that the effect is indeed independent of metabolism. The buffering capacity and, by inference, the H^+ ion concentration must become dissipated during transference from buffer to water, and an explanation involving acid hydrolysis would appear untenable unless it is assumed that the organ is able to 'store' growth.

Susceptible bonds in the cell wall would have to be hydrolysed permanently in order to store growth. Growth would then proceed in the absence of H^+ ions until all were extended, but the response would be reversible, and biochemical changes would be needed to render it permanent. Recent research suggests that the hydrolyses are reversible and that growth is irreversible. Rayle and Cleland (1972) have shown that identical irreversible acid induced responses are obtained in living and frozen-thawed wall preparations from Avena coleoptiles when an external force is used to replace cell turgor as the driving force, and they have extended this work to show that the response in frozen-thawed wall preparations is completely independent of any residual enzyme activity which may be associated with the wall preparations. Irreversibility in the absence of biochemical changes suggests that the acid induced hydrolyses are not permanent and that growth is not stored. This inference is in keeping with evidence from *in vivo* experiments concerning the effect of acid buffers during periods of water stress. Acid induced growth in the leaf sheath base is prevented during periods of water stress, but evidence is presented to show that water stresses may be overcome with time. This recovery is coupled with the re-initiation

of acid induced growth, and if the osmoticum is removed at this point the acid response is found to proceed at a new and increased rate which remains linear for at least 3 h. An initial burst of growth could be explained in terms of stored growth, but the existence of a more permanent increase in rate when the osmoticum is removed, and a recovery mechanism when the osmoticum is maintained, must be taken to represent an increase in the turgor driving force. Essentially similar findings have recently been reported for Avena coleoptiles (Cleland and Rayle 1972), but in this instance an automatic recovery from water stress was not observed. The segments were removed from the osmoticum after periods of between 10 and 60 mins. treatment and were found to respond at control rates in acid buffer.

The initial response rate for the acid induced response in the wheat leaf sheath base is not affected by inhibitor treatments designed to inhibit metabolism, but the response rate slows as membrane permeability becomes affected. Extension is not reversed as the turgor declines, and the acid growth response in this organ must therefore be considered irreversible.

Thus the acid induced responses in oat coleoptiles and wheat leaf sheath bases appear to involve the reversible hydrolysis of acid labile bonds by H^+ ions. In the absence of a driving force the bonds will reform in their original positions, preventing stored growth, whilst in the presence of a driving force the bonds will reform in new positions to allow consolidated growth.

The index of curvature used by Ganot and Reinhold (1970) in their study of the acid growth mechanism and geotropism in Helianthus hypocotyls involved the measurement of the radius of the inner surface of the bending hypocotyl and the computation of the ratio $^{d}/_{r}$, where d is the diameter of the segment and r is the radius of its inner surface. The ratio is reputed to take account of both symmetrical and asymmetrical aspects of growth during treatment, and measurements of this parameter were used to establish the existence of enhancement between acid induced and geo-induced curvatures in

the absence of added auxin. Measurement of segments in 'upper' and 'lower' tissue orientations allows determination of these same parameters for the response in the leaf sheath base, but the latter technique is thought to be superior because the tissue orientations are maintained, and the problems encountered by Ganot and Reinhold (1970), when establishing orientation after treatment on a shaker, do not arise. Larger responses are observed in 'lower' segments when buffer concentrations are sub-optimal, and the geo-induced and acid induced responses are additive under these conditions. At optimal buffer concentrations the responses in 'upper' and 'lower' segments are identical, and the absence of enhancement at optimal concentrations may be taken to indicate the saturation of a site which is common to both geo-induced and acid induced growth responses. The site must exist at the end of the response sequence if the absence of a metabolic requirement for acid induced growth is to be explained satisfactorily, and it is therefore suggested that the ultimate step in the response in the leaf sheath base involves the hydrolysis of acid labile bonds in the cell wall.

Growth may be explained in terms of an increase in either cell wall extensibility or cell turgor and, whilst growth in response to H^+ ions may be taken to favour the former, reports of an increase in sugar concentration in response to geotropic stimulation (Arslan and Bennet-Clark 1960) may be taken as evidence for the involvement of the latter. Reducing sugars are produced by inversion of sucrose during periods of geotropic stimulation, and the reducing sugar concentrations in the lower halves of intact leaf sheath bases may increase by 150% during a 24-h stimulation period. Sucrose levels decline, however, and the actual increase in osmotically active sugars in the lower halves of intact leaf sheath bases is only of the order of 25%.

When segments are excised from the leaf sheath base the sucrose supply from the flag leaf is eliminated, and the molar concentration of osmotically active sugars shows a marked decline. The total concentration of osmotically active sugars falls by 75% in segments orientated as 'lowers'

and 45% in segments orientated as 'uppers', but growth is still induced and maintained in segments orientated as 'lowers'. A 300% increase in invertase activity is observed in homogenates prepared from excised segments which have been orientated as 'lowers' during a 24-h stimulation period, but no increases are observed when homogenates are prepared from segments which have been orientated as 'uppers'. Essentially similar changes are found in the intact organ, and a 300% increase in invertase activity is again observed in homogenates prepared from the lower regions at the end of a 24-h stimulation period. Thus the failure to maintain increased reducing sugar production in excised segments must be related to the depletion of sucrose reserves, and not to a decline in the capacity for sucrose inversion, nor by inference the activity of the gravi-perception mechanism.

Changes in invertase activity may be expected in association with growth, but the demonstration that similar increases are obtained in older leaf sheath bases which fail to grow in response to geotropic stimulation, suggests the existence of a rather more fundamental connection with the gravi-perception mechanism. The fact that growth is maintained in excised segments when sugar levels are in decline is evidence against the involvement of sugars in growth promotion through turgor enhancement, but the gross depletion of sugar reserves may not relate directly to the situation in the growing cells. Further evidence against an increased turgor requirement may be taken from the relationship between the persistence of the reducing sugar asymmetry and curvature on terminating stimulation. Curvature proceeds for about 45 minutes after returning a horizontal organ to vertical, but the curvature achieved during this period is only of the order of 0.25° compared with an initial response rate and final recovery rate of 1.5° h^{-1}.

The abrupt termination of the response on returning the organ to vertical suggests the existence of a system in which growth is tightly controlled by the perception mechanism, but the control cannot be mediated via an effect on cell turgor because the sugar asymmetry is maintained for at least 3 h and the recovery response is proceeding at a maximal rate against the sugar

gradient within this period.

The response requires continuous stimulation and is dependent on aerobic metabolism at all times. If growth was dependent on turgor enhancement, the stimulus would only be needed as a trigger, and growth would presumably proceed under anoxia until all excess turgor was dissipated. The continuous requirement for oxygen, and the close parallel between the effects of nitrogen on the development of curvatures and reducing sugar asymmetries, suggests that the two processes are closely linked, but growth cannot control the reducing sugar changes because the reducing sugar asymmetry develops in the absence of growth in older leaf sheath bases, and the reducing sugar asymmetry cannot control growth because growth can be terminated, and indeed reversed, in the presence of a large sugar asymmetry.

The changes in invertase activity may be controlled either directly by the gravi-perception mechanism, or indirectly by linkage to some critical intermediary stage in the main geotropic response sequence. In either event, the changes in activity are controlled without the involvement of hormonal growth regulators, and the activation of an enzyme in the absence of a hormonal involvement must be considered as a new and potentially significant mechanism for the environmental control of growth.

The products of inversion may be thought to have one of two fates. They may be utilised as building blocks during cell wall synthesis, or they may be consumed oxidatively to provide energy for growth. The former possibility cannot be ruled out, but it must be considered unlikely because the slight changes in dry weight associated with curvature suggest that the consolidation of growth by wall synthesis is of only marginal significance in this response. The susceptibility to respiratory inhibitors, and the postulated requirement for an endogenous supply of H^+ ions during growth, may be taken as evidence to implicate an enhanced respiratory requirement for curvature, and the following working hypothesis is advanced to explain the response. The initial stage in the gravi-perception mechanism concerns

the sedimentation of statoliths, presumably starch grains, and an interaction with specifically located receptors explains the localised induction of growth in segments in response to orientation. Metabolic changes then occur accounting for susceptibility to various poisons, and these changes lead to the production of H^+ ions required for wall loosening. The perception mechanism not only triggers the reaction sequence, explaining the latent period, but also exerts a constant control over it, presumably by regulation of one or more rate limiting events. The rate limiting events may concern production of unstable proteins or regulation of membrane characteristics and, although their exact nature remains uncertain, their location is established towards the final stages of the reaction sequence by the shortness of the lag periods observed for the termination of curvature.

The postulates are especially attractive when seeking a hypothesis to explain the induction of growth in terms of the localised activation by H^+ ions, but verification of the hypothesis will not be complete until the respiratory requirements for curvature have been investigated in detail. The hypothesis is based on the assumption that acid labile bonds are ruptured by H^+ ions in a simple chemical reaction, but wall bound enzymes may be involved in the rupture of these bonds *in vivo*, and it may be that incubation at low pH acts in parallel with this normal effect. If this is the case, the changes in activity of enzymes responsible for such hydrolyses must also be controlled by the gravi-perception mechanism.

The invertase involvement may be explained in terms of a feed-back reaction linked to the primary geotropic response sequence, or a secondary geo-induced reaction geared to meet an increased demand for substrate, and control mechanisms involving the *de novo* synthesis of the enzyme, activation of the enzyme, or removal of an inhibitor of the enzyme, may be envisaged as potentially operative. Alternatively, the increase in reducing sugar levels may represent the activation of wall bound invertases on exposure to low pH. Such an activation would only be possible if growth was induced by H^+ ions

expelled into the cell wall, however, and cell fractionation and kinetic studies designed to establish in particular the effects of pH on potential invertase isoenzymes will be required in order to differentiate between these various possibilities.

Correlation between nodes has been explained in terms of a physical co-ordination system in which the responses at individual growth centres are determined by the quantity of stimulus perceived. This explanation is able to account for the reduction in the response observed at the physically upper node of a two-node preparation, but it is not able to explain the restriction, or even elimination, of the responses at the lower nodes. If all leaf sheath bases were equally responsive and co-ordination was entirely restricted to the physical system outlined above, then straightening would be expected at the upper nodes in response to the continual lifting resulting from curvature at the lower nodes. Straightening is occasionally observed at the uppermost node in multinode preparations, but the magnitude of this latter effect is rarely in excess of 5°. A further constraint must therefore be envisaged, and such a constraint may be thought to involve a reduction in the capacity for either perception or response. The demonstration of equivalent geotropically induced changes in invertase activity in organs of all ages may be taken as evidence to preclude an effect on the perception mechanism, and an effect on the ability to respond must therefore be envisaged. Sugar availability may exert a modifying influence on the development of the response, but the reduced responses observed in segments excised from older leaf sheath bases when these are incubated in 2% sucrose solutions suggests that this is not a major factor.

A much more important factor appears to be the state of secondary wall development in the tissues. The stem is forcibly bent by the differential growth response in the leaf sheath base, and lignification of the basal regions of the internode, when elongation in this organ is complete, has the effect of markedly reducing the efficiency of this fulcrum. This is not

the only effect of thickening, however, because the magnitude of the acid induced response is also restricted in older leaf sheath bases. The restriction is connected with response rate, and not duration, and it seems right to connect the restriction with a change in the ratio of susceptible bonds which occurs as the cell walls become lignified.

Thus the concept of physical co-ordination may be extended to explain growth correlation between nodes in terms of the quantity of stimulus perceived and the mechanical resistance to growth. Differences in the relationship between the extent of secondary wall development at the various growth centres will account for the differences in their contributions towards total curvature, and this will explain the different patterns of curvature observed in grasses in the field.

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