

"STUDIES ON GROWTH INHIBITORS IN THE ROOT TIPS OF
ZEA MAYS L. AND THEIR DISTRIBUTION DURING GEOTROPIC STIMULATION"

by

Kalyan Kumar Kundu

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

September, 1975
Bedford College,
Regent's Park,
London, N.W.1.



ProQuest Number: 10098276

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10098276

Published by ProQuest LLC(2016). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to Professor L.J. Audus for his supervision, advice and valuable criticism throughout the investigations. I also wish to thank Dr. T.A. Mansfield for supplying the seeds of Commelina communis and Dr. H.F. Taylor for a generous gift of authentic xanthoxin.

My thanks are also due to Miss S. Argent and Mrs. D. Kundu for their necessary suggestions and active help during preparation of the manuscript.

ABSTRACT

A micro-assay based on the growth inhibition of root segments of Zea mays roots has been designed to investigate the root growth inhibiting substances present in the root tips of Zea mays. Two major growth inhibitors are present in the acid fraction of the extracts of root tips of maize. One of them is principally located in the root cap. This inhibitor, from its chromatographic properties as well as antitranspirant activity is suspected to be ABA. This inhibitor cannot move far beyond the meristematic zone. The other major inhibitor is predominantly present in the meristem and absent in the cap. The chemical nature of this inhibitor is unknown. Neither of these substances is IAA, whose presence in meristems is sometimes indicated by small inhibitions (or stimulations) at the Rf of marker IAA. The third inhibitor which is comparatively less active but appears in the acid and neutral fractions of the tip extract is also present in the meristem and absent in the cap. This inhibitor also possesses the anti-transpirant property like the cap inhibitor and is suspected as xanthoxin.

Production of this ABA-like cap inhibitor is dependent on light and its production is maximal in the root tips stimulated in light. In the dark greater concentrations of these two major inhibitors are found in the lower half of a stimulated root. On the other hand, when roots are stimulated in the presence of light the cap inhibitor moves in the upper half whereas the inhibitor of the meristem is restricted in the lower half of the stimulated tips. The implications of these findings for the geotropic responses of roots is briefly discussed.

CONTENTS

Chapter I	Introduction	6
Chapter II	Materials and methods	15
	Growth and harvesting of the root materials	15
	Diffusates	19
	Fractionation of extract	19
	Chromatography	22
	Assay	26
	Growth conditions for maximum sensitivity	29
	Stomatal-aperture test	44
	Experiments on geotropic stimulation	48
	Preliminary detection of the growth regulating substances using some spraying reagents	57
	Presentation of the results	58
Chapter III	Results	60
	Analysis of the acid ether fraction in the methanolic extract of different root samples	60
	A. Root cap	60
	B. Root tip	60
	C. Root tip without cap	69
	D. Serial 2 mm segments	69
	E. Diffusates	75
	F. Chromatogram of the root tip extract using solvent I	90
	G. Chromatogram of the root tip extract using solvent II	90
	H. Chromatogram of the root tip extract using solvent III	94

I.	Result of dilution response curve of ABA	104
J.	Results of stomatal-aperture assay	104
	Analysis of the neutral ether fraction in the methanolic extract of root tips		113
	Analysis of the primary ether fraction in the methanolic extract of different root materials	124
A.	Root cap	124
B.	First 2 mm segments	127
C.	Second 2 mm segments	127
D.	Third 2 mm segments	127
E.	Fourth 2 mm segments	136
	Results of the assay of root materials after geotropic stimulation	137
A.	Stimulation in the dark	137
B.	Stimulation in light	144
Chapter IV	Discussion		177
	Summary		189
	Bibliography		191
	Appendix		201
	Published papers		206

CHAPTER I

INTRODUCTION

Darwin (1880) observed that the stimulus of gravity was perceived at the root tip and then transmitted back to the region where the unequal growth and therefore the curvature occurred. With the discovery of auxin by Went in 1926 it has been realized that this stimulus was hormonal in nature and later on the classical Cholodny-Went theory of geotropism was proposed by these two authors through their independent observations. The following mechanism of hormonal regulation in geotropically stimulated root is envisaged from the basic postulates of the Cholodny-Went theory.

The root tip produces an auxin at supra-optimal concentration for its growth. During geotropic stimulation this native auxin is laterally transported from the tip to the extending zone where it is asymmetrically distributed, thus causing the uppermost part of the root to receive less auxin (at an optimal level) and the lowermost part of the root to receive auxin at a still higher than optimal concentration. Owing to this unequal distribution of the growth factor the uppermost part of the root tip grows faster than that of the lowermost resulting in positive curvature.

The Cholodny-Went theory is apparently simple, logical and has gained much of its support from the observations of Hawker (1932) and Boysen-Jensen (1933). Hawker found a greater positive curvature in the unstimulated root of Vicia faba by applying auxins extracted from the lower half of a geotropically stimulated root tip than from that of the upper half of the same root tip, thus indicating a greater accumulation of auxin in the lower half of the root. In a more or less similar experiment Boysen-Jensen

extracted auxins on agar blocks separately from the upper and lower halves of the horizontal roots of Vicia faba and assayed their growth activities by placing unilaterally on decapitated Avena coleoptiles. The block that was previously in contact with the lower half of the stimulated root produced a greater curvature than that of the upper half.

Considering the wide ranging literature so far, there seems to be little doubt that a gradient of net growth regulating activity is set up between the upper and lower halves in horizontal roots. But the conflicting evidence of the presence of auxin at supra-optimal level in root caused some doubts on the validity of the Cholodny-Went theory as Wilkins (1971) clearly pointed out that the theory could only be applicable to roots when auxin in supra-optimal concentrations would be firmly established.

Cholodny's own work (1926) and several subsequent observations (Bünning, 1928 ; Keeble et al., 1929, 1931) apparently support the supra-optimal concentration of auxin in roots. They observed acceleration of growth of decapitated corn root but a retarded growth when the root was reheaded with the same tip or the severed coleoptile tip. Moreover, the longitudinal growth of the root was found to be suppressed in several observations when natural and synthetic auxins were further applied externally (Nielsen, 1930; Cholodny, 1931; Kögl et al., 1934; Thimann, 1936; Lane, 1936; Marmer, 1937; Bonner and Koepfli, 1939; Åberg, 1952).

Rufelt (1957) using isobutyric acids, which are considered as auxin antagonists, particularly with indole-isobutyric acid, found cell elongation_^ was increased by more than 100% as well as a significant negative curvature of wheat roots. Rufelt's experiment equally suggests the presence of auxin at supra-optimal level. Supra-optimal concentration of auxin in wheat root has also been

observed by Lexander (1953) and Åberg (1957).

However, Younis (1954) observed that decapitation of Vicia faba root tips at 0.5, 1.0 or 2.0 mm behind the root apex did not accelerate the growth of the stump. He further noted that the growth of the stumps reheaded with their own tips was similar to that of unreheaded stumps. Vardar and Tözün (1958) also found that decapitation caused a characteristic retardation in root elongation of Lens culinaris roots. On the other hand, when treated with dilute auxin solutions a significant acceleration of growth of intact seedling roots (Pohl, 1951; Ochs, 1954) and in root sections (Audus and Garrard, 1953; Audus and Das, 1955) was observed. In case of root sections as suggested by Larsen (1961) ^{the} _A the endogenous auxin may be present at a lower level than that of the intact seedling root thus enhancing the growth but in the intact root the situation is clearly contradictory to the supra-optimal presence of auxin.

Audus and Brownbridge (1957) found that the growth rate of both the upper and lower halves of pea roots was accelerated by low concentration of IAA and inhibited by high concentrations. Assuming much more concentration of auxin in the lower half during geotropic stimulation a marked inhibition of growth was expected applying even lower concentration of IAA, instead of promotion of growth as observed by them. Audus (1959) however, has pointed out that if the concentration is assumed to be uniform throughout the root, the amount of IAA found to be present in several independent investigations would support the idea that the concentration of auxin is supra-optimal.

According to Wilkins (1971) if the auxin concentrations are supra-optimal then a lateral redistribution of auxin in the

horizontal root should decrease the growth rate in the lower half and there should be little or no overall change in the growth rate of the root. The results of Audus and Brownbridge (1957) and Bennet-Clark et al. (1959) have shown that there was a marked overall decrease in the growth rate of the root following geotropic stimulation. So, considering all the contradictory evidence available at present the occurrence of supra-optimal concentration of auxin in the roots has not yet been established.

Another important aspect of the Cholodny-Went theory is the lateral distribution of auxin in horizontal roots during geotropic stimulation. The evidences so far are too meagre and also conflicting to establish these. Pilet (1964) found a weak polar movement of applied labelled IAA towards the root tip of Lens. On the other hand, Yeomans and Audus (1964) could not find any evidence whatsoever of an active polar transport of auxin in Vicia faba roots. Konings (1967, 1968) applying labelled IAA ^{14}C to the tips of horizontal pea roots found an unequal lateral distribution of radioactive IAA in these tips. This distribution varied according to the length of the severed tip. Moreover, the application of some inhibitors such as caffeic acid, 2,4-dichlorophenol, and 2,3,5-triiodobenzoic acid prevented the geotropic curvature as well as the lateral distribution of auxin according to the degree of inhibition. From these results he concluded that the root cap controlled the transverse distribution of auxin. However, in the case of maize a strictly acropetal polarity in the movement of IAA was observed using IAA ^{14}C . The orientation of the segments with respect to gravity did not significantly affect the acropetal polar flux of IAA (Scott and Wilkins, 1968; Wilkins and Scott, 1968; Bowen et al., 1972).

Chromatographic studies, followed by some standard biological assays reveal the presence of a number of growth active substances in roots. Though the pattern of activities of some of these are fairly uniform during assay, their exact chemical natures are not yet established. Moreover, interconvertibility of some of these substances during extraction and chromatography and also during elution and rechromatography makes the situation more complex. From the complete survey of the auxins of roots three major active substances appear more or less regularly in the chromatogram studied so far. These are designated as accelerator α , inhibitor β and the IAA-like substance. The first two were first reported by Bennet-Clark and Kefford (1953) and Bennet-Clark et al. (1952) respectively and subsequently detected on several occasions.

Accelerator α moves more slowly than IAA-like substance on the chromatogram and promotes growth of coleoptile and pea root at concentrations segments roughly corresponding to those in the extracted tissue (Åberg, 1957). It had been tentatively suggested as β -indole pyruvic acid by Stowe and Thimann (1954). Housley and Bentley (1956), on the other hand, interpreted it as a neutral substance of unknown chemical nature. There is a possibility that this substance could be a tail of IAA (Terpstra, 1953).

Inhibitor β moves faster in the chromatogram, above the IAA-like substance. It inhibits both coleoptile and root segment growth. Torrey (1959) found a positive colour reaction with Salkowski's reagent in the region of inhibitor β on the chromatogram but negative reaction with Ehrlich's reagent. However, the major active substance in the inhibitor β complex is now found to be abscisic acid (ABA) (Robinson and Wareing, 1964; Milborrow, 1967).

For example, in pea root extracts the inhibitor β consists of several compounds including abscisic acid and trans-cinnamic acid (Tietz, 1971).

The third and highest activity is usually found in the chromatogram close to the Rf of authentic IAA and according to Åberg (1957) there is little doubt as to the identity of this native substance with IAA. It invariably promoted the growth of coleoptile sections and has been shown to occur in broad bean roots in concentrations inhibitory to the extension of pea root sections (Lahiri and Audus, 1960). However, Bennet-Clark et al. (1959) had used a starch column to separate the 'apparent IAA' from the roots of Vicia faba and found no evidence to identify it with IAA. Moreover, comparing the activity-dilution relationships of the IAA-like substance and the authentic IAA it was found that the activity of the former changed much more rapidly with dilution than in the case with authentic IAA (Lahiri and Audus, 1960). Burnett et al. (1965) using spectrophotofluorimetry also could not positively identify this substance as IAA in Vicia faba. Dullaart (1967, 1970) detected IAA by chromatography and fluorescence spectra in non-nodulated roots of Lupinus and Alnus in amounts ranging from 30 μg to 205 $\mu\text{g}/\text{Kg}$ fresh wt. Elliott (1971), on the other hand, identified α -N-malonyl-D-tryptophan in roots of wheat seedlings but was unable to detect IAA. Recently, considering the chromatographic properties and reactions to chromogenic sprays as well as using mass spectrometry IAA had been positively identified in Zea mays roots. However this IAA was found to be localized predominantly in the stele, whereas a less amount of IAA was found in the root tip (Greenwood et al., 1973; Bridges et al., 1973).

Whether or not this IAA-like substance is true IAA, the hormonal mechanism pertaining to geotropic response of the roots was principally concentrated around this substance. Audus and Lahiri (1961) found a marked increase in the content of 'AP(ii)' (IAA-like substance) during the onset of curvature of horizontal roots of Vicia faba. This increase was possibly not due to its increase from an inactive bound state but was either synthesized de novo or may be ^{from} another auxin present in the root. The gravity induced de novo production of an endogenous inhibitor in the extending cells of the roots had also been suspected by Audus and Brownbridge (1957). The complete independence of the growth actions of this inhibitor and of the applied auxins suggested that it might not be IAA or any similar compound.

So, considering the above observations as well as the overall depression in the growth rate of geotropically stimulated roots observed by Audus and Brownbridge (1957) and also by Bennet-Clark et al. (1959); predominant acropetal polarity of movement of IAA when applied to isolated segments, together with the recent observation of high IAA content in the stele strongly support the contention that a separate inhibitor rather than IAA is involved in connection with the geotropic response in root.

Juniper et al. (1966) observed that graviperception of maize root was lost when the root cap was cleanly dissected out without damaging the quiescent centre and the meristem. The decapped roots were able to grow horizontally and there were no overall changes in the growth rate of the intact and decapped root. The graviperception was restored when the root cap regenerated (usually after 36 hours). This remarkable finding was subsequently confirmed by Gibbons and Wilkins (1970) and Pilet (1971). However, Pilet (1972) found that the removal of

the cap significantly enhanced the growth velocity of the root, but only during the first three hours after decapping, after which the growth velocity was the same for both intact and decapped roots as observed by Juniper et al. Moreover, eliminating one half of the root cap and orientating the roots in any direction with respect to gravity a large positive curvature towards the remaining half cap was observed in each case (Gibbons and Wilkins, 1970; Shaw and Wilkins, 1973). These observations further indicate the presence of at least one growth inhibiting substance in the root cap which is concerned with the geotropic responsiveness of the root. The activity of this growth inhibitor may not be limited to Zea roots, as when the root caps were placed on the tips of intact lentil roots a decrease in the growth was observed (Pilet, 1972).

The nature of this inhibitor is still unknown. However, some useful information in this connection has been provided by Pilet (1971, 1972) who observed that the geotropic responsiveness was lost when the root cap was replaced on a decapped root after dipping in an oleic acid preparation, whereas the geotropic response was restored if the cap was replaced immediately on roots which had been dipped in dilute Ringer solution. From this experiment the nature of the inhibitor in the cap is suspected to be a water soluble compound.

Recent microsurgical experiments of the root apex of maize and pea using impermeable barriers of mica foil provided some indirect evidence of basipetal movement of this inhibitor from the apex to the extending zone as well as partly downward lateral transport of the inhibitor from the upper to the lower half of the stimulated roots (Shaw and Wilkins, 1973; Pilet, 1973).

The present investigation has been undertaken to evaluate the recent indirect evidence of the existence of an inhibitor (or inhibitors) in the root cap. It has been felt a direct approach to this problem is needed. One of the serious limitations in this context is a lack of any suitable assay method where maximum sensitivity can be achieved using minute quantities of fresh samples, thus reducing the lengthy and tedious procedure of sampling as far as is practicable. An attempt has been made to establish such a micro-assay technique and using this technique, the growth regulating substances present in the root tip as well as cap have been thoroughly investigated. The present work is a further reassessment of the observation of Audus and Lahiri (1961) as to whether gravity can induce any change in the quality and quantity of those growth regulating substances in the tip during geotropic stimulation. Another aspect of the present investigation is to establish whether any of the growth regulating substances are distributed asymmetrically during geotropic stimulation, a condition demanded by the classical Cholodny-Went theory.

CHAPTER II

MATERIALS AND METHODS

Throughout the present investigation the young seminal roots of Zea mays L. (var. Golden Bantam) were used. Maize was chosen for the following reasons.

- (1) Caryopses of maize can easily be grown under laboratory conditions and germination is fairly uniform.
- (2) The roots are of a reasonable thickness, convenient for segmentation and other purposes.
- (3) As in other grasses the roots of maize are characterized by having a discrete cap meristem and a very well-marked boundary between the cap and the root proper. Juniper et al. (1966) observed that it was possible in maize to detach the intact root cap clearly from the rest of the root tip without contamination of meristematic cells. This condition was found to be an advantage for the present investigation, where the samples of root caps as well as decapped root tips had been frequently used for extraction and assay.

Growth and harvesting of the root materials

Caryopses, after thorough washing in water, were soaked in aerated running water at 25°C for 24 hours. They were then spread on a glass half-brick (20 x 20 x 5 cm) lined with moist 2-ply tissue cloth. The glass half-brick was covered by a glass plate and incubated in the dark for 24 hours. After 24 hours when the radicles had just emerged they were arranged on moist vermiculite in a plastic tray (36 x 22 x 5 cm). Two such trays containing a hundred caryopses in each, were found sufficient for a hundred root samples. When more roots were needed the number of trays was increased accordingly. The trays were left in a dark cupboard

at a constant temperature of 25°C for another three days. When the seminal roots were approximately 6-7 cm in length a hundred (or, more when needed) healthy roots were selected. These roots were thoroughly washed in water and four types of root materials were harvested for primary investigations.

(A) Root caps - Root caps were dissected under a binocular microscope using a fine, sharp razor blade taking care as far as was practicable not to include any of the meristematic tissue of the root proper. Under a binocular microscope the cap could be easily differentiated from the fairly opaque root as a translucent region. Immediately after removal from the tip, each cap was plunged into a small polythene vial (2.5 x 1.5 cm) containing 1 ml of methanol cooled to -10°C in a freezing mixture of cardice and acetone. A hundred root caps dissected and fixed in this way were macerated under methanol using a small mortar and pestle and finally extracted in a total volume of 10-15 ml of methanol for 24 hours at -10°C in a deep freeze.

(B) Root tips - Root tips of 5 mm length including their caps were severed from the roots with the help of a specially designed guillotine (Fig. 1). One hundred root tips were collected in a polythene tube (6 x 1.5 cm) containing 5 ml of chilled methanol, as above, and were also extracted for 24 hours at -10°C in the deep freeze. In a few experiments two hundred tips were collected, fixed in methanol and extracted in a similar way.

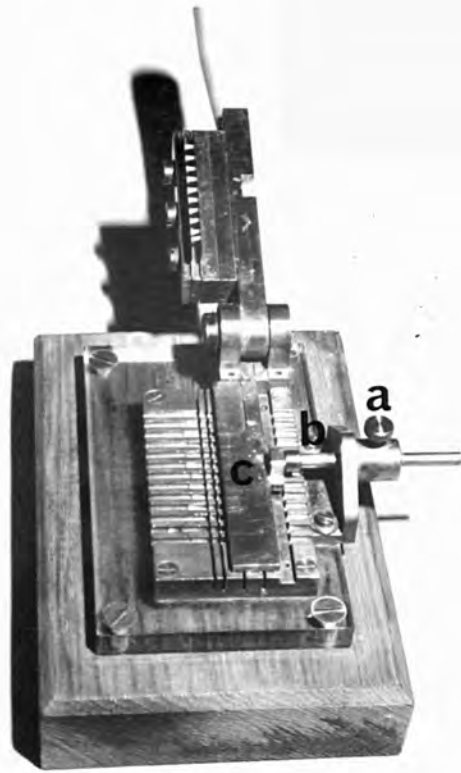
(C) Root tips without caps - One hundred root tips with their caps removed, were also harvested on several occasions. These decapped tips were also fixed in methanol at -10°C and extracted for 24 hours as above.

(D) Harvesting of root materials for successive 2 mm segments - For analyses of the extract of successive 2 mm segments, decapped roots of the same age and length as above were chosen. These

Fig. 1 A view of the guillotine employed for obtaining
root segments.

By loosening the screw 'a' bar 'b' could be
pulled in or out which eventually controlled
the distance between the plate 'c' and the cutting
blade nearest to it, thus the length of the tip to
be decapitated could be adjusted. Decapitated
segments ready for use are found to be lying
between the blades.

Fig.1



roots were serially chopped at 2 mm intervals stretching back proximally up to 8 mm from the cap/meristem junction. Careful attention was needed in sampling, in this lengthy procedure. To avoid any confusion ten roots at a time were completely segmented using the same guillotine and the segments were plunged into chilled methanol in their respective vials. Samples of a hundred segments each were similarly extracted for 24 hours at -10°C in the deep freeze.

Diffusates

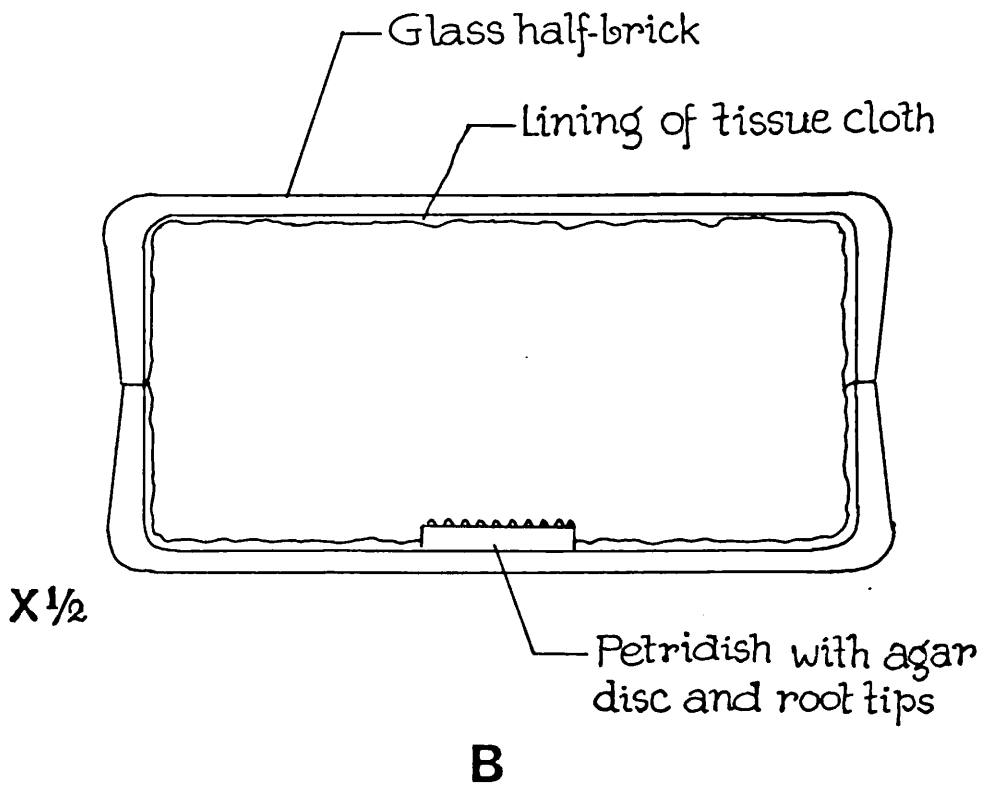
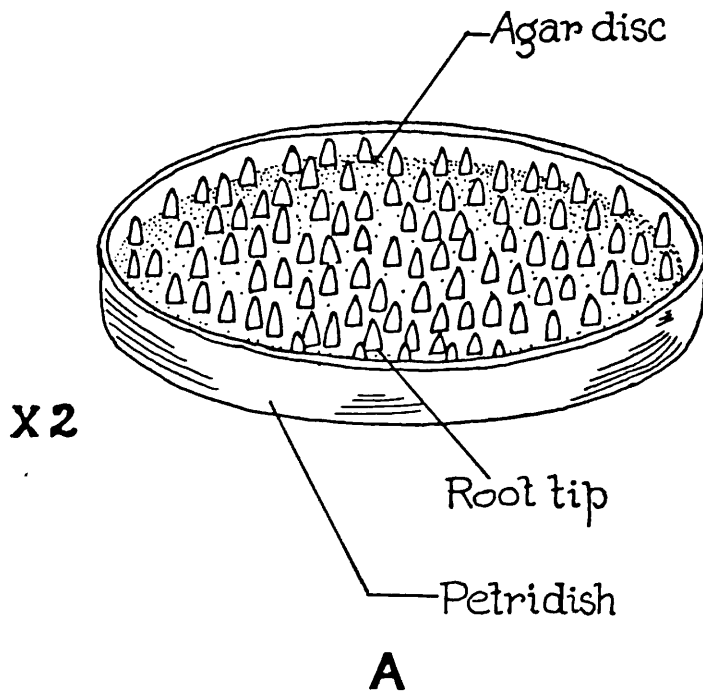
Diffusates were collected from 1 mm and 2 mm tips cut from their intact roots. They were placed, cut surface downward, on a 4 mm thick 1.5 per cent agar disc (made from 'Oxoid' ion agar No.2) of 4 cm diameter. The disc was held in a petri dish of the same diameter and the petri dish was placed centrally in a glass half-brick lined with a moist 2-ply tissue cloth (Fig.2A and B). This was covered with a similar half brick lined with moist tissue cloth. The entire unit was placed in an incubator at 25°C in the dark for 3 hours. After incubation the agar was quickly frozen with chilled methanol and the frozen agar was macerated using mortar and pestle. This was subsequently extracted in methanol as for dissected caps and tips.

Fractionation of extract

Major precautions were necessary to maintain the quality and quantity of the total extract during the period of fractionation. A possible loss of extract during fractionation could be reduced using small glass vials and avoiding unnecessary transference of the extract from one vial to the other. The fractionation was carried out away from the direct light, and where necessary, the vials were covered with black light-proof polythene sheets.

- Fig. 2 Collection of diffusates from the root tip.
- A. Petri dish containing the agar disc holding
 100 root tips.
 - B. Vertical section (diagrammatic) of incubation
 chamber. The petri dish with root tips is in
 the centre of the chamber (for description see
 the text).

Fig.2



The extract was fractionated following the conventional method of Kefford (1955). Initially this was filtered through a sintered glass funnel directly into an evaporating flask (50 ml capacity). After filtration the residue was washed with 10 ml of methanol and then with 10 ml of water. Then the evaporating flask with the filtrate (final volume between 25-30 ml) was placed in a rotary flash evaporator and the methanol was evaporated under vacuum at 35°C. After removal of the methanol, the aqueous residue was acidified to pH3 with N/10 phosphoric acid (Sp. gr., 1.75). This was shaken with three successive aliquots of ether (approximately 10-15 ml each aliquot) using a small separating funnel (100 ml capacity). As ether tends to form peroxides which destroy many auxins, peroxide-free ether was used throughout, and to prevent further oxidation one or two drops of butylated hydroxy toluene (0.1% in ethyl acetate) was added to the extract as an additional precaution. The pooled ether fractions, henceforth called the primary ether fraction, from above were then partitioned against 5% NaHCO₃ to remove acid auxins. Here also, the ether fraction was shaken three times with bicarbonate solution and the pooled bicarbonate fractions were reacidified to pH3 with phosphoric acid and repartitioned against ether as above. This ether fraction usually contains acid auxins whereas the ether fraction after shaking with bicarbonate usually contains neutral auxins. Both acid and neutral ether fractions were evaporated to dryness in vacuo and then eluted in 0.5 ml of methanol and spotted separately on chromatograms. On several occasions, the primary ether fraction was evaporated, eluted and spotted in a similar way as mentioned above.

Chromatography

Methanolic solutions of acid and neutral fractions were subjected to paper chromatography on 25 x 8 cm strips of

Whatman No.1 paper prewashed each time in the running solvent. The entire eluate was applied as a narrow transverse band 3 cm from one end of the paper and ascending, chromatography was performed. Chromatograms of marked IAA were independently run on each occasion. Three such paper strips were chromatographed at a time in a glass chromatographic tank (35 x 12 x 9 cm) (Fig. 3). The strips were hung vertically by the hooks on a horizontal bar which could be raised and lowered by means of a vertical metal rod soldered on to the horizontal bar in the middle. The vertical rod could be adjusted to any desirable length by means of a screw above the lid of the tank. Prior to each run the chromatographic tank with the paper strips was equilibrated for six hours with the running solvent.

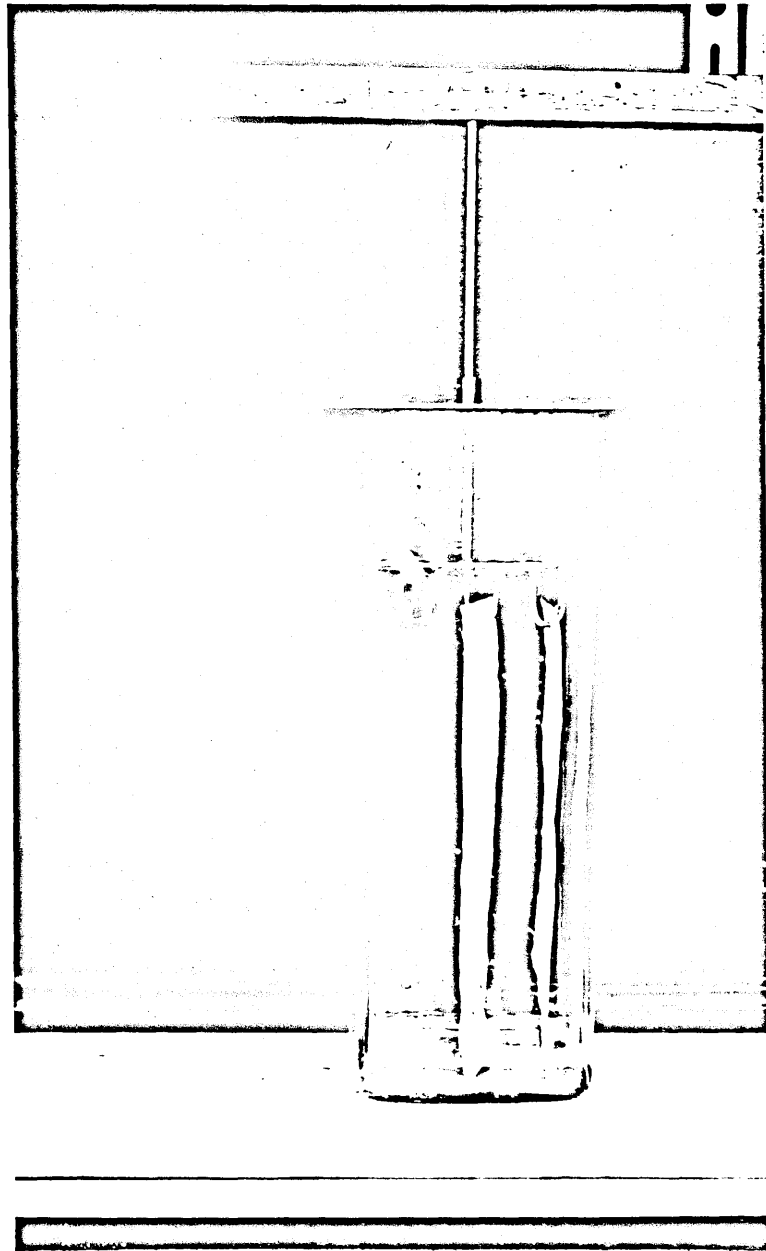
Three types of running solvent, henceforth called solvent I, II and III were used throughout the investigation. These were:
Solvent I - isopropanol:ammonia (Sp. gr.-0.88) : water (10:1:1)
Solvent II - isobutanol : methanol: water (75:10:15)
Solvent III - isopropanol : water (4:1).

Of these three, butanol solvent is less volatile than the other two, and it was found very difficult to get rid of the traces of solvent from the chromatograms. Therefore, the isopropanol solvents were preferred in most cases.

After equilibration the strips were dipped in the solvent and allowed to run exactly 10 cm from the point of extract application. After the run, each chromatogram, was dried by means of a fan in the dark, and two longitudinal central strips each 1 cm wide were cut from it (Fig. 4A). Each strip, thus contained approximately one third of the total extract of a hundred tips, loaded on the chromatogram. The strips were used as replicates in the subsequent assays.

Fig. 3 A view of the glass chromatographic tank.

Fig.3



On several occasions the entire amount of the extract was assayed. In this case the methanolic eluate was applied in a transverse band, less than 1 cm wide, 3 cm from one end of the paper and the chromatogram was run for 15 cm from the origin (for fineness of resolution). Then the entire chromatogram, containing the total extract was cut into a single longitudinal strip of approximately 1 cm wide (Fig. 4B).

In both cases the longitudinal strips were again divided into ten or fifteen equal squares (1x1 cm). Thus, each square representing 0.1 or 0.075 of an Rf unit. Each small square was separately assayed together with 10 squares (1x1 cm) of solvent-washed paper serving as controls and providing a base line to permit the calculation of confidence limit. The chromatograms were assayed immediately after drying. However, when necessary, these were stored in a vacuum desiccator, wrapped in a black polythene sheet and stored at -10°C in the deep freeze. The position of marker IAA in the chromatogram was detected by spraying it with Ehrlich's reagent.

Assay

The assay technique employed was designed (a) to use maize root segments to detect and measure their own inhibitors and (b) to reduce the volume of assay medium to a minimum, thereby increasing the final concentration of the active material and hence the sensitivity of the assay.

The assay material consisted of 2 mm segments cut 2 mm behind the tips of the 2-day old Zea mays (var. Kelvedon 33) roots. Sections from this zone showed the greatest extension of growth, approximately 2-3 times their original length, in 0.5 per cent sucrose.

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

... ..

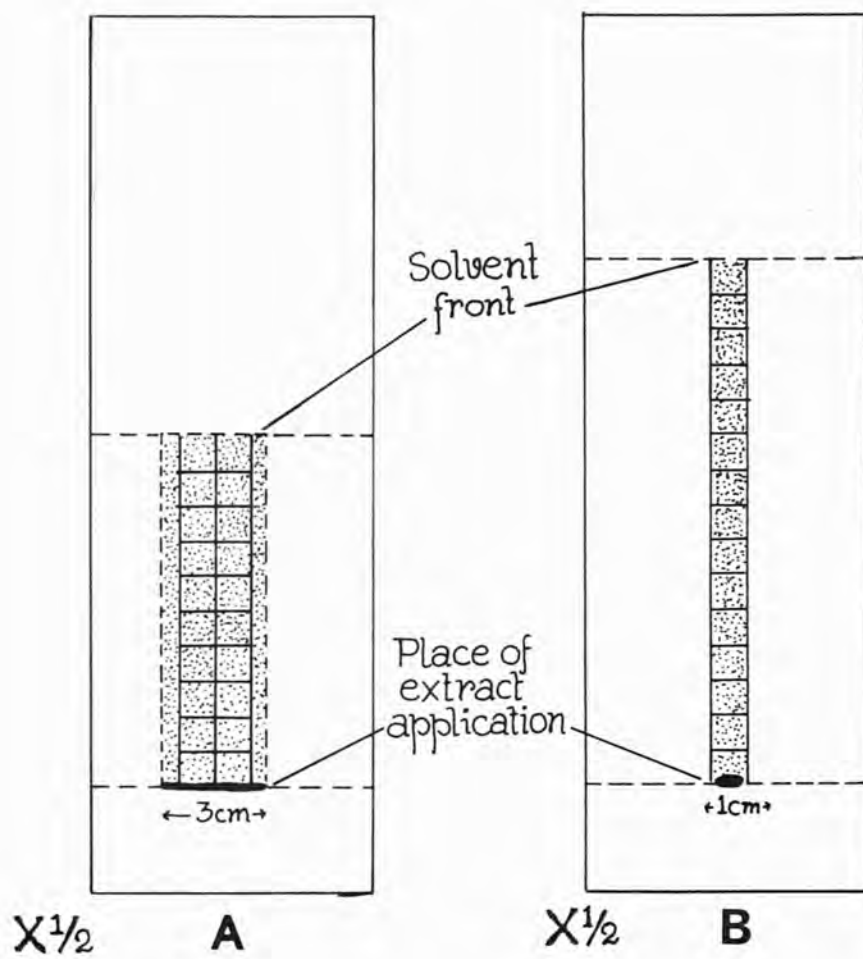
... ..

... ..

Fig. 4 Diagrams showing the position of extract application on chromatographic paper. The dotted zones represent the area covered by the extract after desired length of running.

- A. The entire extract was spotted in 3 cm band on one end of the paper and allowed to run 10 cm from the origin i.e. from the place of extract application. After the run the chromatogram was cut into twenty equal squares (as shown in the figure) thus each small square represents 0.1 Rf unit.
- B. Same except the extract was spotted in 1 cm band and the chromatogram was allowed to run 15 cm from the origin. After the run the chromatogram was cut into fifteen equal squares thus each small square represents 0.075 Rf unit.

Fig.4



Caryopses, thoroughly washed and soaked in aerated water at 25°C for 24 hours were spread on a glass half brick as usual, lined with moist 2-ply tissue. This was covered with a glass plate and placed in the incubator at 25°C in the dark. After 48 hours when the radicles were approximately 6-7 mm in length, 2 mm segments (starting at 2 mm behind the extreme tip) were cut from each of a hundred such radicles

using the same guillotine. Cut sections were collected in a petri dish on filter paper moistened with distilled water.

The container for the assay consisted of a series of rectangular cavities (1 x 1 x 0.6 cm) cut in a slab of 1 cm thick perspex. There were altogether 36 regularly arranged cavities per unit (Fig. 5A), which sufficed for the assay of the two replicated chromatogram strips of a total of twenty squares and the ten control squares (or, fifteen squares and ten control squares) at a time.

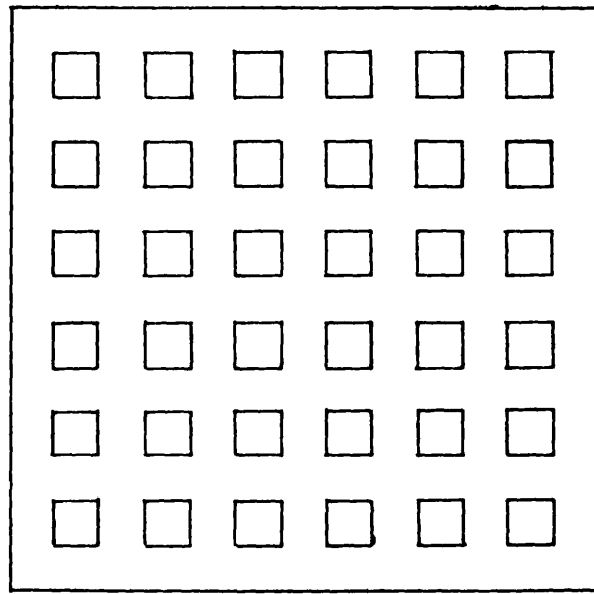
Growth conditions for maximum sensitivity

As mentioned earlier by Audus and Thresh (1953), maximum sensitivity of the root segment assay could be obtained by using relatively large volumes of sucrose, so that the availability of the growth medium would not be a limiting factor. This condition would not be applicable however, in the present investigation as very small amounts of active substances were likely to be present in a hundred root caps or root tips, and unlimited supply of growth medium increasing a greater dilution of growth factors would definitely limit the possibility of detection. On the other hand, the lengthy procedure of sampling would equally limit the use of large amounts of extractable root material for routine analyses. Hence the main objective of the present assay technique, as mentioned before, was to achieve maximum sensitivity of the root

Fig. 5 Diagrams and photograph of the apparatus used for
micro-assay of root growth inhibitors.

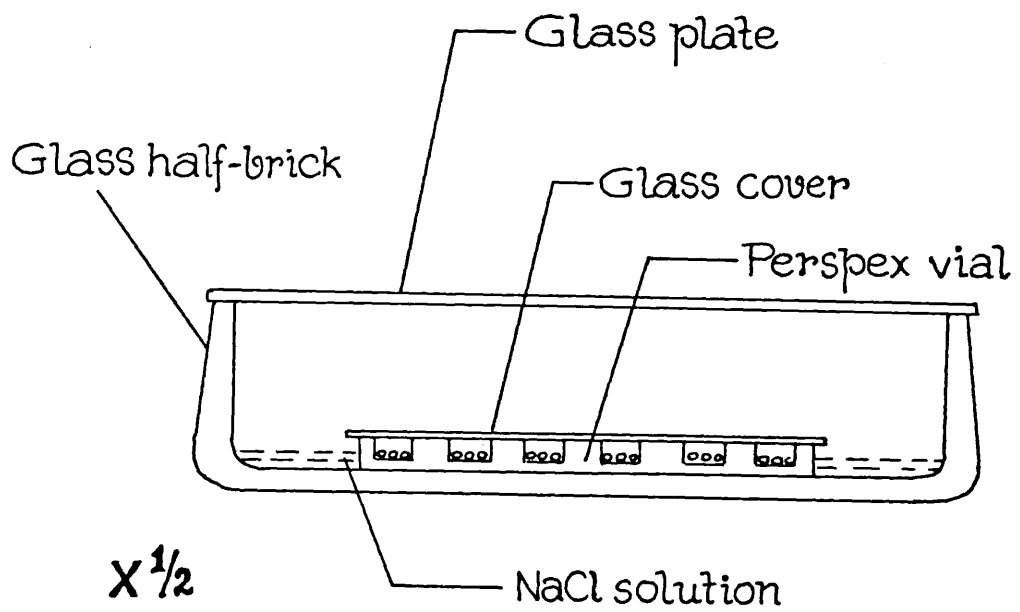
- A. Plan of perspex vial unit.
- B. Vertical section of incubation chamber and
 perspex vial unit.
- C. A view of the perspex growth vial containing
 three root segments (2 mm) in each cavity prior
 to incubation. (Top fifteen cavities containing
 the chromatogram squares and the bottom ten
 containing ten control squares from one of the
 experiments.)

Fig.5



$X^{2/3}$

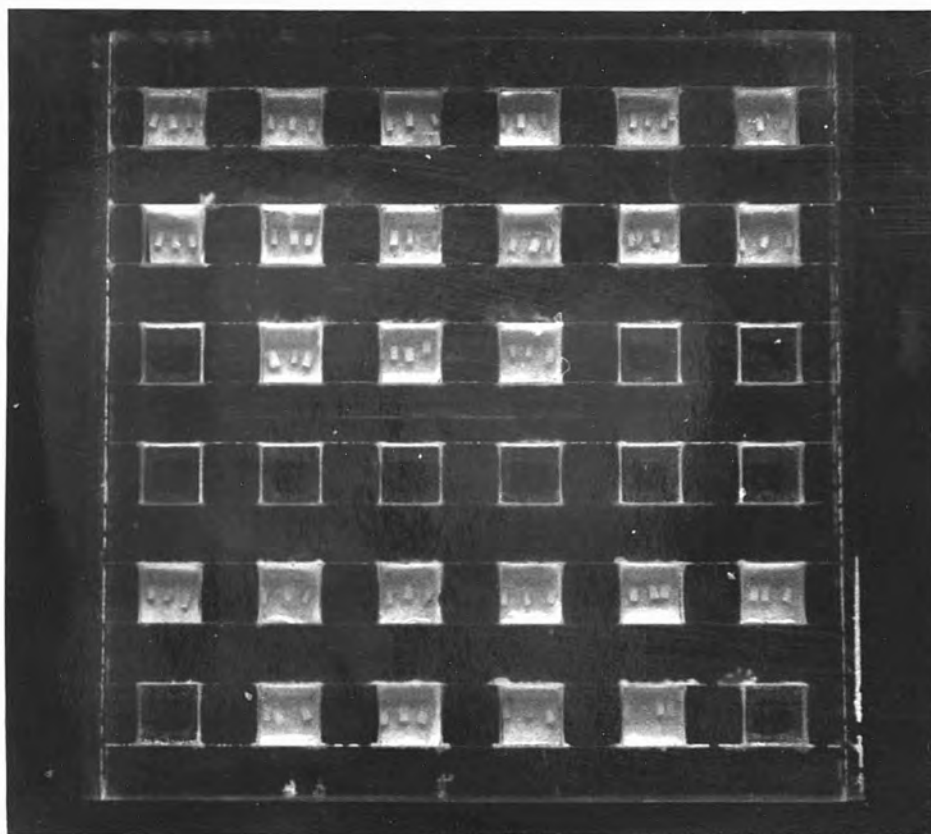
A



$X^{1/2}$

B

Fig.5



C

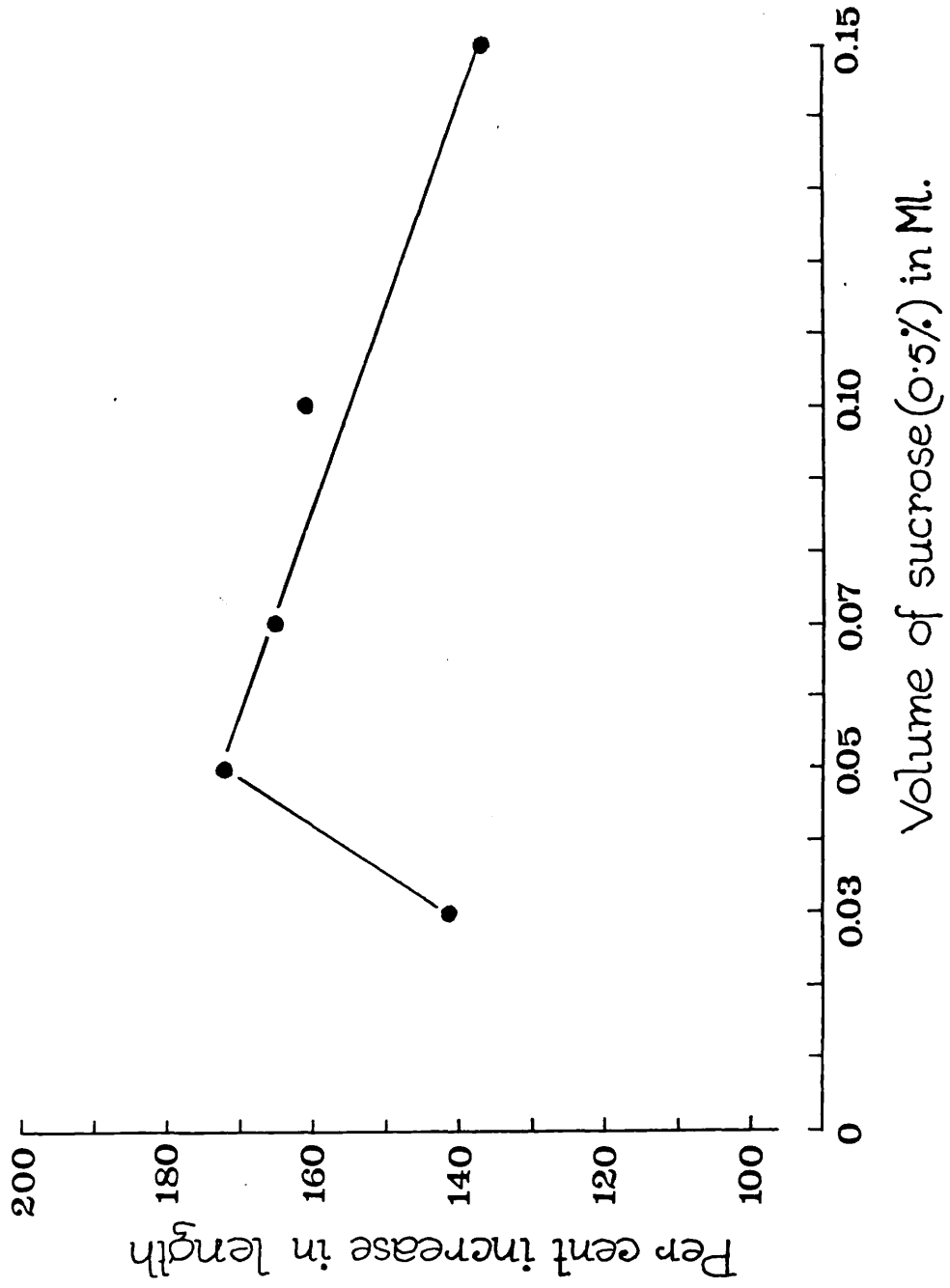
segments (used for assay) in minimum volumes of growth medium so that a dependable picture of auxin activity could be obtained using a small sample of root material. Experiments were therefore carried out to determine the minimum volume of sucrose which would support a satisfactory growth of the sections in the cavities of the perspex vial.

Five different volumes of sucrose (0.5%) starting from 0.03 to 0.15 ml were added to the squared filter^{paper} strips (1 x 1 cm) in the cavities of the perspex vial. It was observed (Walker et al, 1958) that the variance of the mean is decreased more by the number of replications (samples) than by increasing the number of sections per vial. Moreover, too many sections in the cavity of the growth vial were likely to impair growth as availability of water would be a limiting factor. In view of this, three root segments, were selected at random and were placed on filter paper strips in the cavities and the percentage increase in length was determined after 24 hours. Each volume was used five times on each occasion and the mean percentage of growth obtained from four such occasions is presented graphically in Fig. 6. The percentage increase in length on each occasion along with analysis of variance is shown in Table 1 in the Appendix.

As shown in the figure maximum growth of the root sections (about 172%) was obtained in 0.05 ml of sucrose. From this point, with the increase in volume, the growth declined, possibly due to water logging of the sections. In 0.03 ml of sucrose the availability of growth medium was a limiting factor, thus hindering the maximum response. Apparently the variations of growth from 0.05 ml up to 0.1 ml were little but significant as evidenced from the analysis of variance table (Table 1B). Here the value of the

Fig. 6 Growth curve of maize root segments in varying
 volumes of 0.5% sucrose solution.

Fig. 6



mean square variance for residual was significantly greater than that due to the sample variance indicating the fitness of the assay. The occasion variance was also significantly high, which was perhaps attributed to the biological variability of the root materials used in the assay.

In a second series of experiments the growth progress curve, over a period of 48 hours, was studied to find the optimal time interval to allow before the final recording of growth. In the perspex growth vial, three root sections with 0.05 ml of 0.5% sucrose (the optimal volume obtained from the above experiment) were placed in each cavity and the lengths were measured after 3, 6, 12, 24 and 48 hours. Five replications were run each time on each occasion. The mean percentage of growth at different time intervals on four occasions is shown in Fig.7. The mean growth data at different times on each occasion along with the analysis of variance are shown in Table 2 (Appendix).

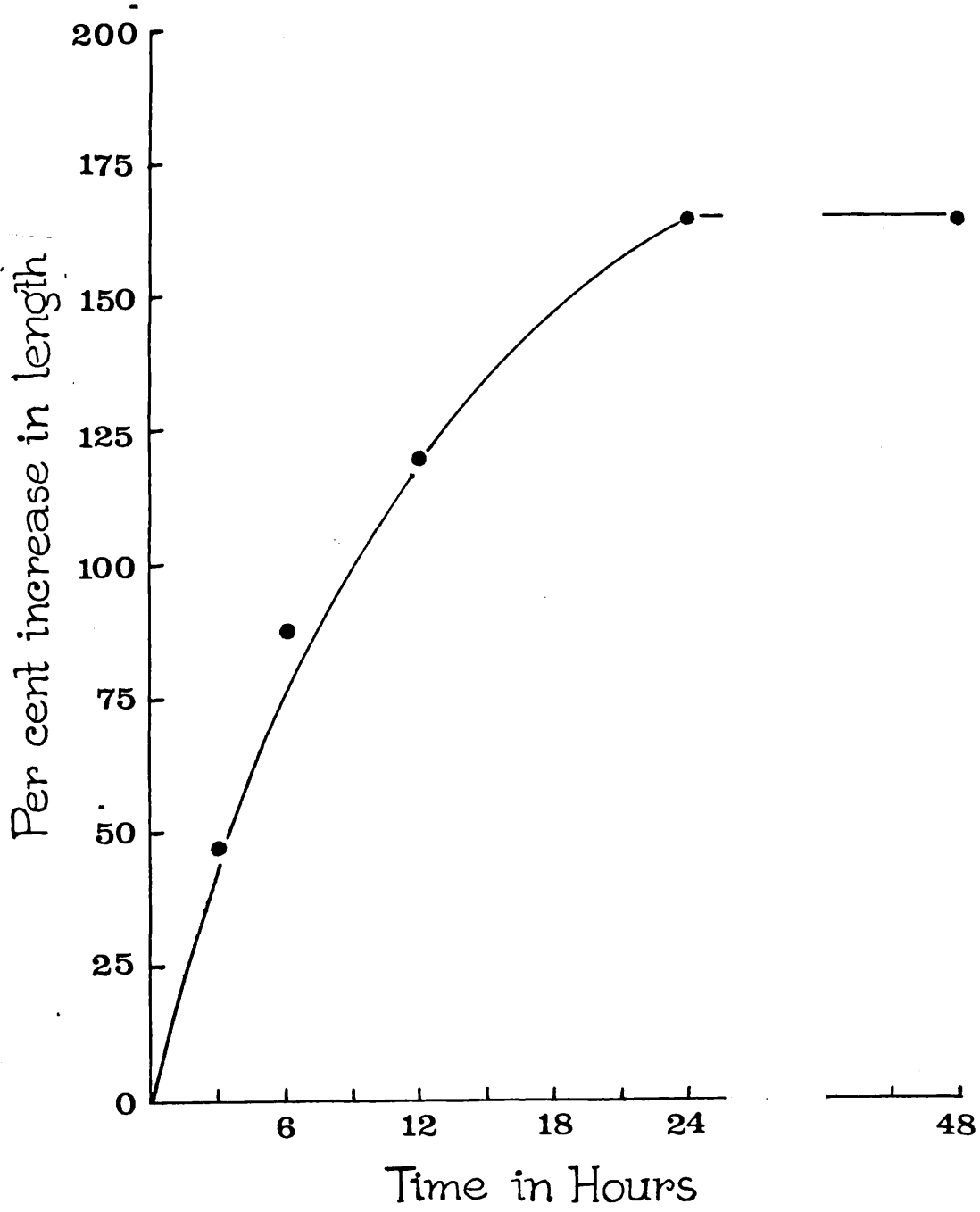
A progressive increase in growth, in a more or less linear order, was obtained over a period of 24 hours after which the growth fell to zero. Here maximum growth was obtained at 24 hours.

As a result of these two experiments, in the final assay each chromatogram strip was moistened with 0.05 ml of 0.5% sucrose and three root sections were placed on these chromatogram strips in each cavity of the growth vial (Fig. 5C).

With such small volumes of assay medium, considerable precautions were necessary to prevent volume (and hence concentration) changes due to evaporation. The perspex slab was therefore covered by a glass plate and the whole unit was placed in a glass

Fig. 7 Growth curve of maize root segments at different
time intervals in optimal volume (0.05 ml) of
0.5% sucrose solution.

Fig.7



half-brick with a ground rim, into which a quantity (approximately 100 ml) of NaCl solution of concentration isotonic with the 0.5 per cent sucrose had been poured. The glass half-brick was covered with a ground glass plate (Fig. 5B) and incubated in the dark at 25°C.

After 24 hours either the root segments were measured directly by a travelling microscope or shadow graphs of the root segments (Fig. 8) were made with a photographic enlarger and the lengths of segments subsequently measured. A significant difference in the percentage of growth over the controls was taken as indicating the presence of active substances in the chromatograms.

For the assessment of sensitivity of the present assay technique a preliminary experiment on root segment growth in a range of concentrations of IAA was performed. A series of dilutions of IAA starting from 10^{-4} M to 10^{-11} M was made up in 0.5% sucrose. Five samples of 0.05 ml quantities from each dilution were placed in the cavities of the growth vial on squared (1 x 1 cm) filter paper with three 2 mm root segments in each, as before. As there were altogether eight different concentrations of IAA two perspex vials were used (four concentrations in each vial) and the root sections of these two sets were incubated in identical conditions. The final lengths of the root sections were measured after 24 hours and expressed as a percentage of the increase obtained from the mean control growth of root segments in 0.05 ml of sucrose alone. The experiment was repeated on four occasions and the mean percentage of growth in each concentration has been brought together in Table 3 (Appendix) and the curve has been presented in Fig. 9.

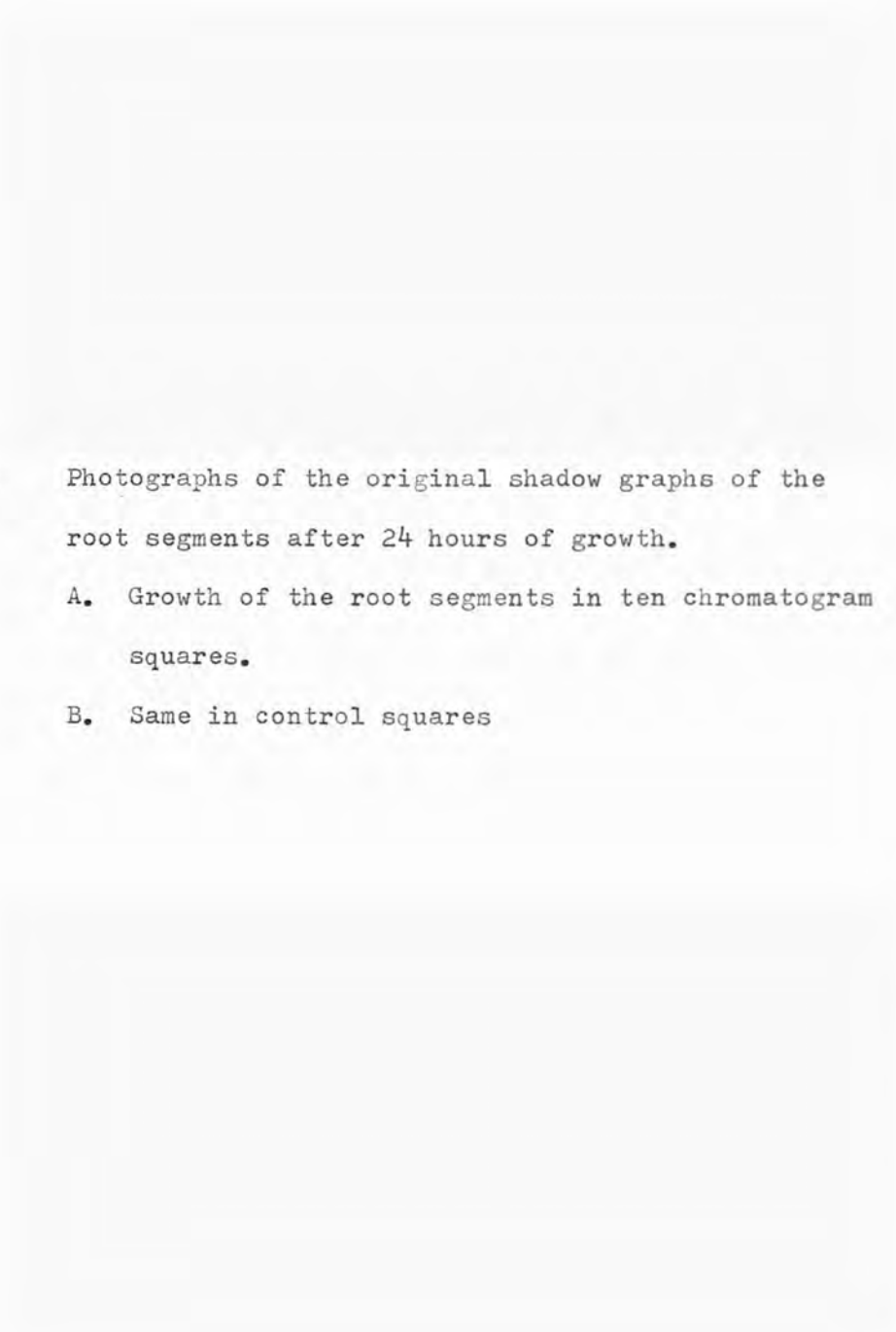
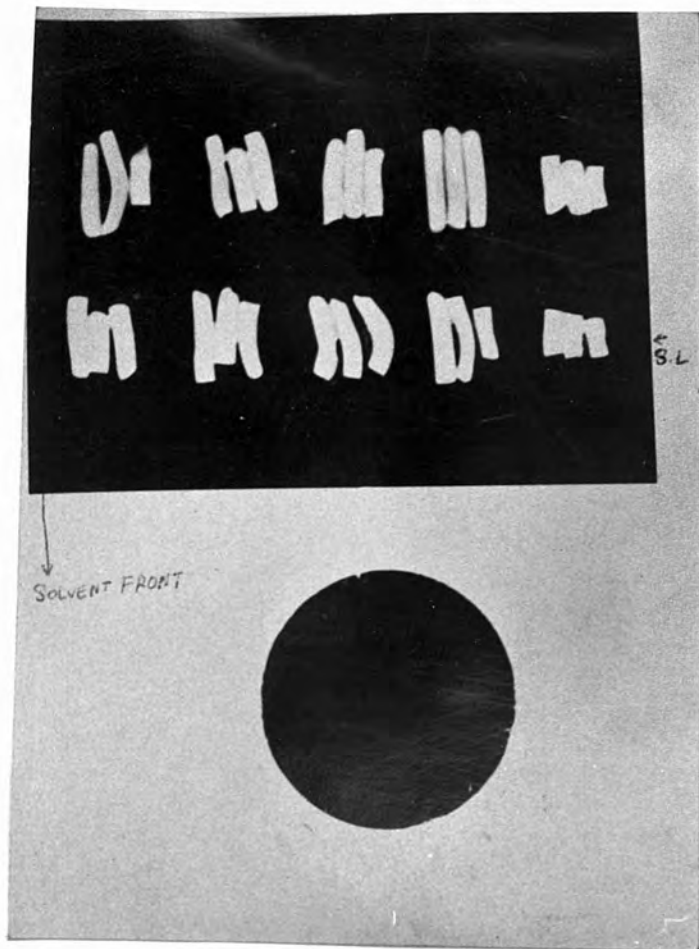
- 
- Fig. 8 Photographs of the original shadow graphs of the
root segments after 24 hours of growth.
- A. Growth of the root segments in ten chromatogram
 squares.
- B. Same in control squares

Fig.8



B

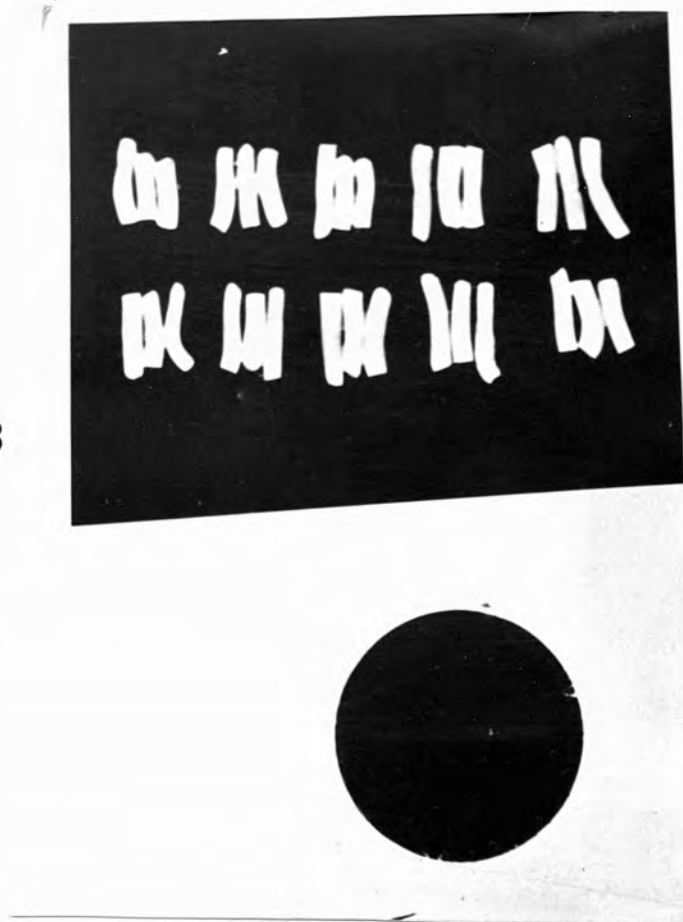
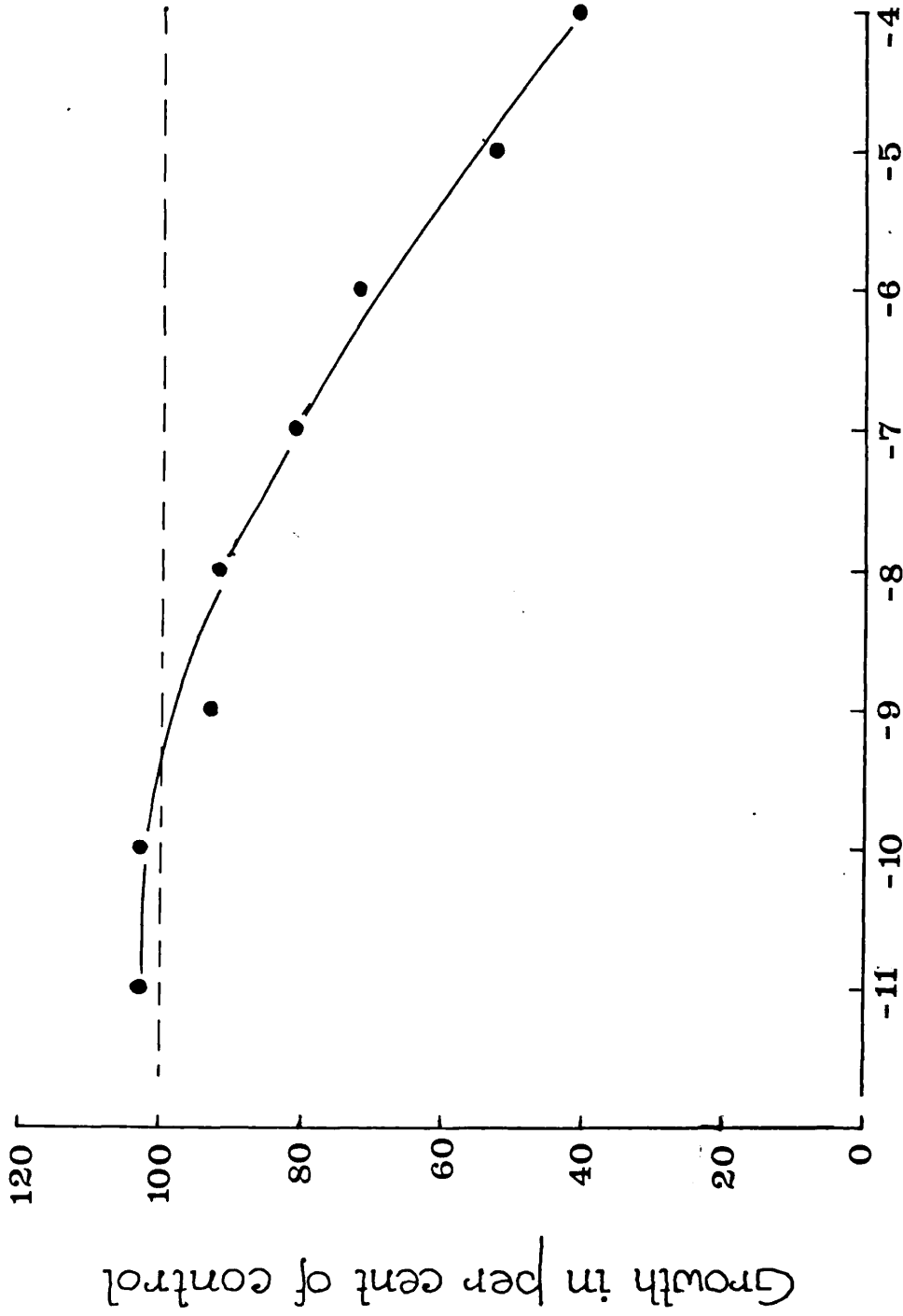


Fig. 9 The curve shows the growth (as percent of control) of maize root segments in different concentrations of IAA.



Log concentrations of IAA in Molar

Fig.9

The IAA curve showed that a little stimulation of growth was obtained in the concentrations of 10^{-11} and 10^{-10} M. Around 10^{-9} M the growth was not very consistent. Moreover, a null point of growth response might be obtained around this concentration. However, a steady and consistent inhibition of growth began at approximately 10^{-8} M of IAA and the growth fell to 40% of the normal value in 10^{-4} M. The significance of the results is evidenced from the analysis of variance table presented in Table 3B. Considering the threshold of inhibition around 10^{-8} M, it was estimated that such an assay should be capable of detecting quantities of IAA per chromatogram segment of the order of 50 pg.

Stomatal-aperture test

For preliminary detection of abscisic acid (ABA)-like compounds on the chromatogram the stomatal aperture test of Willmer and Mansfield (1969) was followed. The epidermal strips from the leaves of Commelina communis L. were used for this purpose. Commelina plants were grown in the greenhouse for 6 weeks and the healthy plants selected were kept in darkness at 25°C for 12 hours, prior to use, to ensure that the stomata were in a closed condition at the beginning of the experiment. Plants were sufficiently watered when kept in the dark to prevent any increase of ABA-level within the plant due to wilting. The epidermal strips were detached with the help of fine forceps from the abaxial surfaces of the fully expanded young leaves of more or less uniform size. Usually two to three strips were taken out from each leaf and floated on 0.01 M citrate buffer at pH 5.5 in a watchglass. All manipulations were carried out in a dim light and after stripping, the watchglass was covered with a black polythene sheet until the vials were ready for incubation (see below).

The entire acid fraction from two hundred tips (5 mm) was loaded as a 2 cm band on the chromatogram and the chromatogram was run exactly 10 cm from the point of origin in solvent I. The chromatogram was then dried and divided longitudinally into two equal strips (each containing the extract of approximately 100 tips). One strip was assayed using the root-segment assay method described above. The other strip was segmented into ten squares of 1 x 1 cm. Each segment along with corresponding controls (from the solvent washed paper) were eluted in 1.5 ml of 0.01 M Citrate buffer at pH 5.5 (Ogunkanmi et al, 1973) in a small glass vial (2 x 2 cm) for 3 hours at 5°C in the dark. Epidermal strips were incubated in the eluates for 3 hours in the inoculating chamber (see below). To allow sufficient time for measuring the stomatal aperture the entire experiment was done in two stages. In the first place, half of the chromatogram squares (from 0 - 0.5 Rf unit) with three controls were eluted, while exactly 90 minutes later, (approximately equal to the time taken for measuring the stomatal apertures of 8 strips) the elution of the second set from the remaining five chromatogram squares (from 0.6 - 1.0 Rf unit) plus controls was started. Thus the two timings were adjusted in such a way that identical conditions for the elutions and incubations at the two stages were maintained.

The inoculating chamber was a rectangular perspex box (45 x 37 x 6 cm) divided into two compartments, each 45 x 37 x 2.5 cm, by a perspex partition. Cool water was circulated in the upper compartment while a stream of air bubbled through 2N KOH solution in a series of Pettenkoffer tubes and finally through lime water was passed through the lower compartment where the vials containing the epidermal strips were placed during the period of incubation (Fig. 10). The entire box was held on a glass plate on the top of

Fig. 10 Diagrammatic sectional view of the set up for the stomatal-aperture assay (for description see the text).

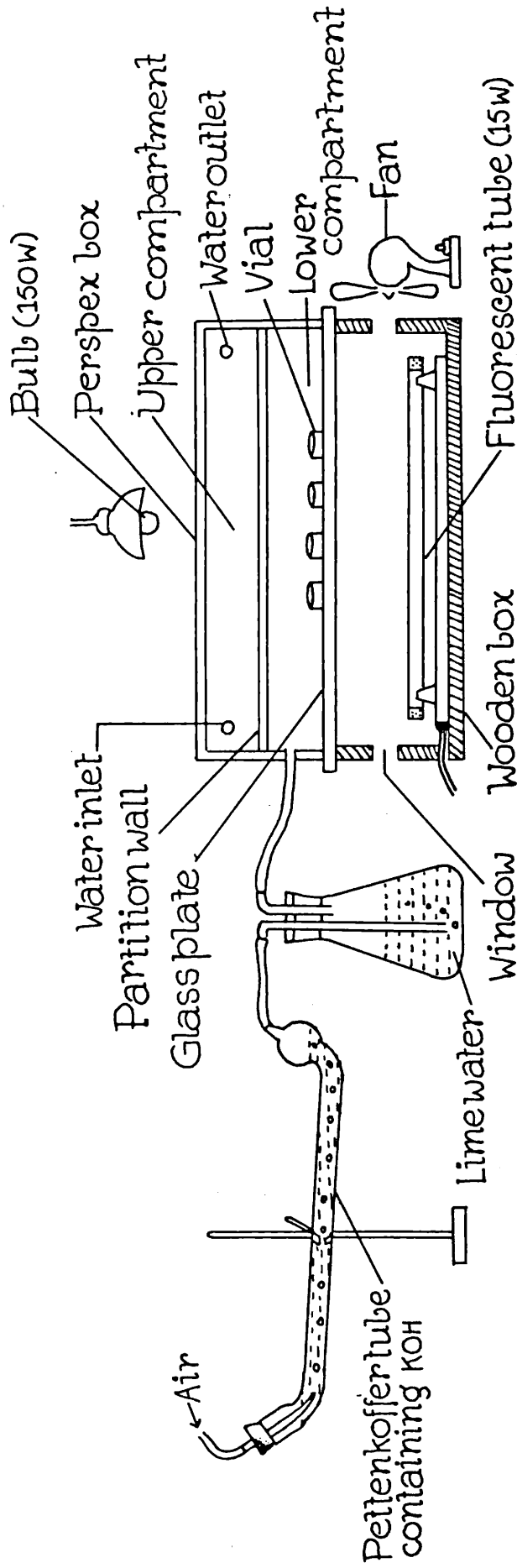


Fig.10

another wooden box having two windows in its side walls. The illumination during the period of incubation was provided by two light sources, a tungsten lamp (150W) above the perspex chamber and four 15-W warm white fluorescent tubes placed in the wooden box, beneath the glass plate supporting the chamber (Tucker and Mansfield 1971). Air was blown from the fan through the window of the wooden box to cool the bottom of the unit.

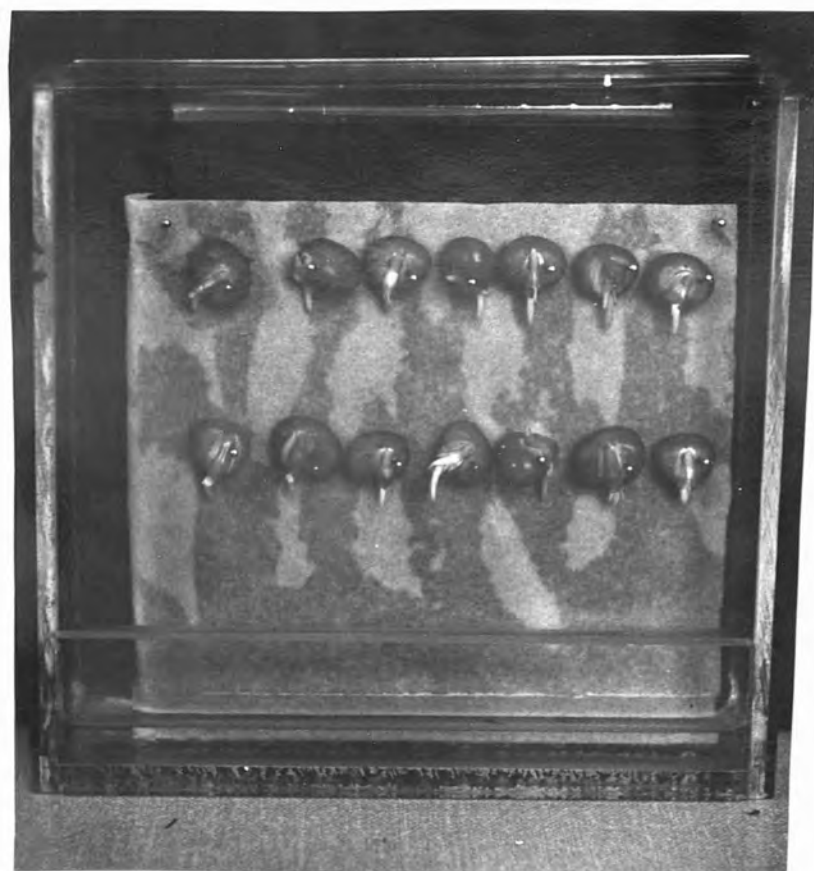
After incubation 30 stomatal apertures of one strip from each vial, selected at random were measured under oil immersion. A significant decrease in width of the stomatal aperture from that of the controls indicated the presence of ABA-like substances in the chromatogram.

Experiments on geotropic stimulation

The roots were subjected to geotropic stimulation in order to find out any qualitative and quantitative changes of the growth substances occurring under the stimulus of gravity as well as to find out any differential distribution of the growth substances in the upper and lower halves of the tips in that condition. For this purpose caryopses, thoroughly washed and soaked in aerated water at 25°C were arranged in rows on half-bricks lined with moist 2-ply tissue and incubated in the dark at 25°C for 48 hours as for the materials of assay. After 48 hours when the radicles were 4-5 mm in length, they were washed in distilled water and were arranged in two rows pinned through their endosperms on a cork mat (10.5 x 10.5 cm) lined with filter paper (Fig. 11). Each cork mat, thus held 12-14 seedlings with their radicles pointing downward they were placed vertically in a perspex box (13 x 13 x 5 cm) containing a small quantity of distilled water so that the roots were vertical.

Fig. 11. A view of the perspex box with cork mat supporting the seedlings, used for growing the seedling in a vertical position as well as ^{for} stimulation experiments. One day-old seedlings were arranged in two rows on a cork mat lined with filter paper. The cork mat was then placed in a vertical position in the box. A small amount of water in the box was used for growth of the seedlings as well as to maintain the humidity inside.

Fig.11



(A) Stimulation in the dark

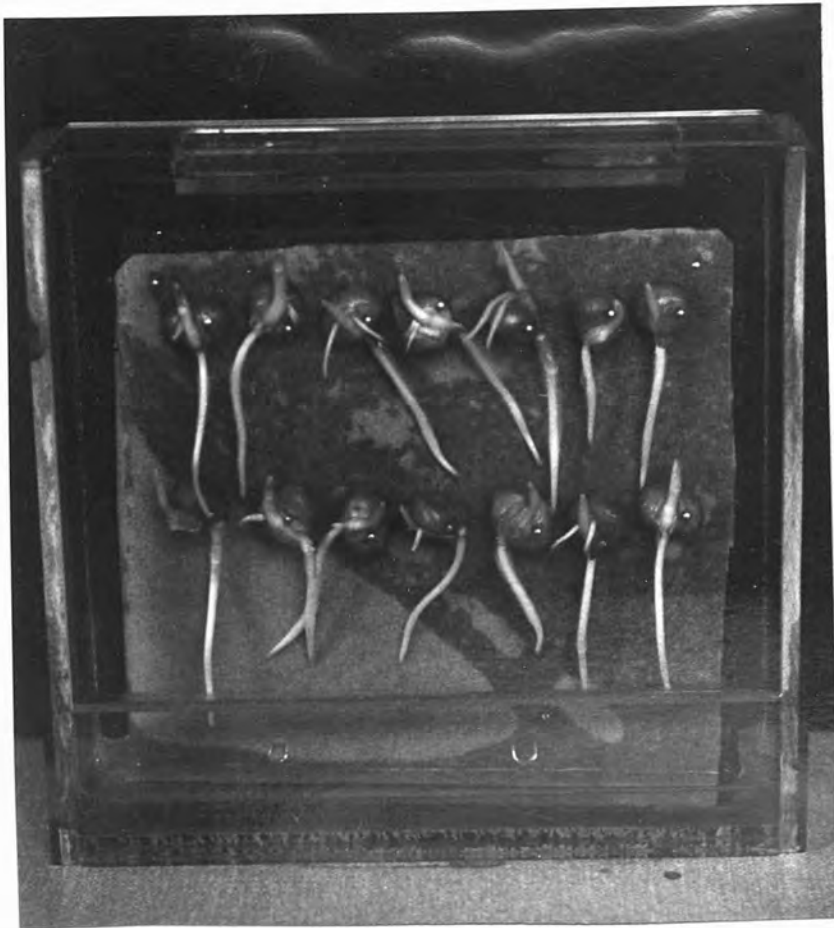
In the first series of experiments these perspex boxes with the cork mats were kept in the dark at 25°C for another 24 hours, after which the roots were ready for stimulation. It may be mentioned that in darkness a greater proportion of roots (about 60 per cent) grew in any direction even sometimes horizontally - instead of growing vertically downwards (Fig. 12A). To stimulate the roots (only those which were grown vertically) individual mats were turned through 90° in a predetermined sequence (Fig. 12C). The roots were then stimulated for 30 minutes and after stimulation three types of root material were harvested.

- (1) A hundred root tips of 5 mm length were harvested using the guillotine as before.
- (2) Root caps (100) were dissected under a binocular microscope.
- (3) A hundred root tips of 5 mm length were dissected into two equal halves - upper and lower using a sharp razor blade. It should be mentioned in this connection that among the various mechanical devices used for dissecting the tips into two equal halves, none proved entirely successful. However, this was eventually performed free-hand with a reasonable amount of accuracy by using a razor blade. Careful attention was needed so that the orientation of roots was not confused during dissection. To avoid this each root tip was dissected while attached to the cork mat in the following way.

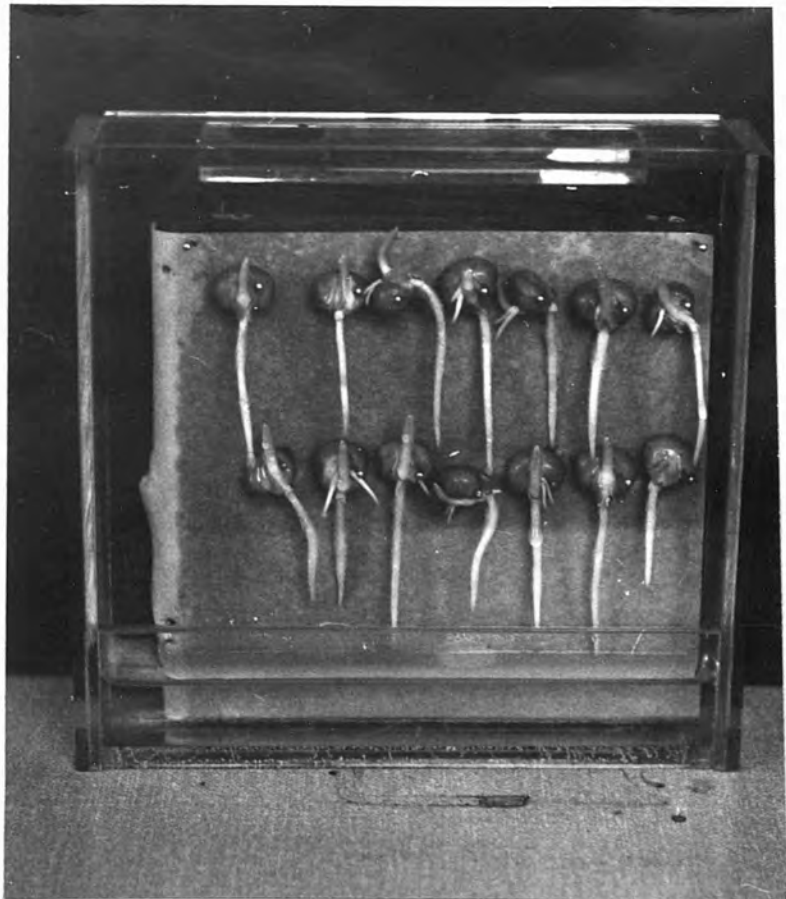
First, a microscope slide was introduced between the root and the cork mat. Then one horizontal incision was made in the middle of the root, slightly above the 5 mm tip region with a fine razor blade. The blade was then placed in the incision and pulled steadily towards the tip (Fig. 13). In this way, more or less two equal halves were obtained.

Fig. 12 Growth of the seedlings (A) in the dark and (B) in
the light. (C) For stimulation the individual cork
mat (from B) was rotated at 90°.

Fig.12

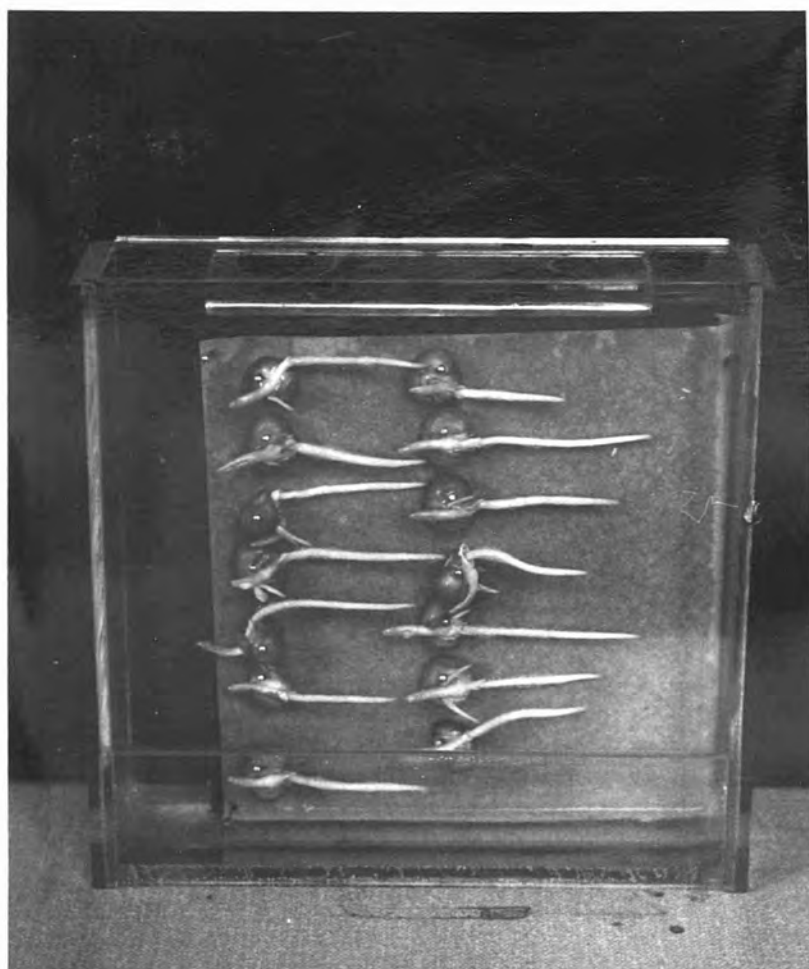


A



B

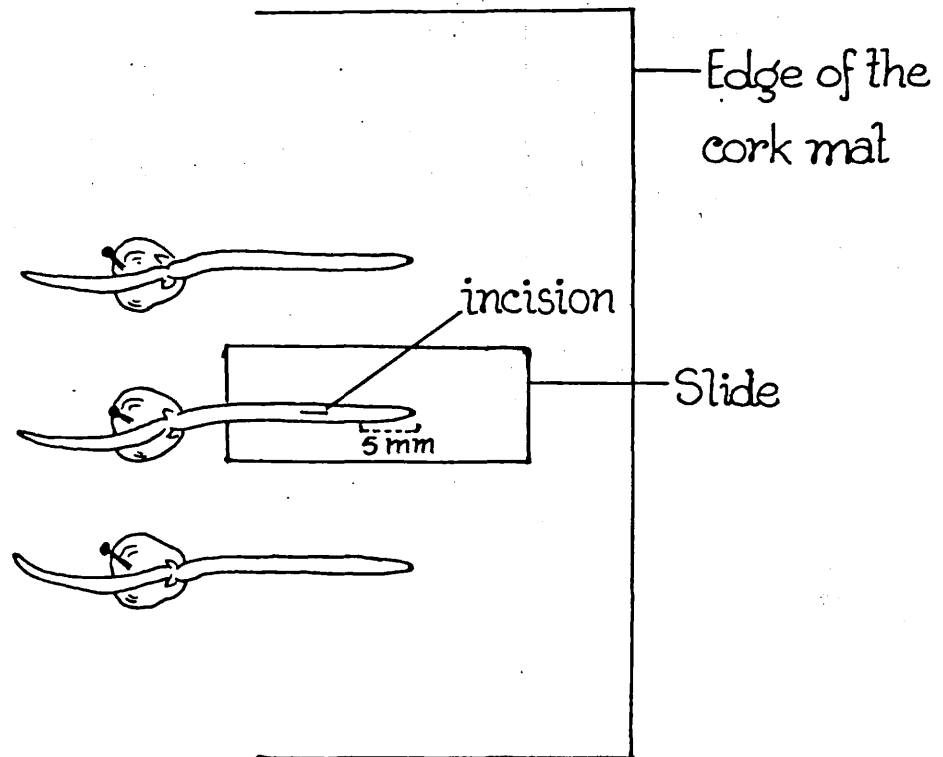
Fig.12



C

Fig. 13 Diagram showing the relative positions of the
root tip, slide and cork mat before splitting
into two halves.

Fig.13



Similar samples of root material were harvested from the roots, kept vertical in identical conditions and were used as controls. All harvestings were done in a dim diffuse light and carefully adjusting the time so that the exposure to light was minimal in each case.

(B) Stimulation in light

In a second series of experiments the perspex boxes with the cork mats holding the 2-day old seedlings were kept in diffuse light for another 24 hours. The diffuse light source was the reflection from a white surface illuminated by a 80-W fluorescent tube placed at a distance of 1 metre, so that light on the surface of the roots was approximately 5-10 foot candles. In the presence of light about 80-90 per cent of the roots were grown vertically in contrast to the dark grown roots (Fig. 12B). The roots were stimulated for 30 minutes as above and similar samples of root materials, except the root caps were harvested.

All root materials were immediately plunged into methanol at -10°C as before and extracted for 24 hours at -10°C in a deep freeze. The extractions were fractionated following the same procedure mentioned above and only the acid ether fractions were chromatographed and assayed by root segment assay technique.

Preliminary detection of the growth regulating substances using some spraying reagents

It was not intended to identify critically the nature of the active substances present in the root tip of Zea mays. However, attempts have been made for preliminary detection of some of these substances using several spray reagents. These reagents were either sprayed on paper chromatograms or on thin layer chromatograms. Where thin layer chromatograms were performed the methanolic eluates

of the active zones from the paper chromatogram were applied separately on a 20 x 5 cm thin layer plate (0.25 mm). The chromatogram was run in Benzene : Ethyl acetate : acetic acid (65:35:5) solvent. The plates were dried by means of a fan, and the following spraying reagents were used to identify the indoles and phenols.

(1) Ehrlich's reagent for the detection of indoles (used in paper chromatography)- 2 gms of p-dimethylaminobenzaldehyde in a mixture of 20 ml HCl (Sp. gr. 1.18) and 80 ml ethanol.

(2) Salkowski's reagent for the detection of indoles (used in paper chromatography) - a mixture of 1 ml of 0.5M FeCl₃ and 50 ml of 35 per cent (W/V) HClO₄.

(3) Sodium nitrite/cobalt nitrate spray for the detection of phenols (used in thin layer chromatography) - sodium nitrite (4.4 gm) was dissolved in 10 ml of water and to it, 2.6 gm of cobalt nitrate dissolved in 2 ml of glacial acetic acid was added and made up to 10 ml with distilled water. This was diluted with an equal volume of glacial acetic acid and was further diluted with an equal volume of water (Bhatia et al, 1971).

Presentation of the results

In the case of each root segment assay, the average final growth of the sections was expressed as per cent of growth over control sections simply by dividing the mean section growth of each treatment by the mean of total section growth of controls, times 100. The significance of the results was tested by determining the standard deviation (σ) of the grand mean of control samples using the formula -

$$\sigma = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

where,

\bar{x} = mean growth of sections in each control sample

n = total number of control samples

Fiducial limit, i.e., $t (2.262) \times \sigma$ expressed as per cent of \bar{x} or grand mean for all control samples was taken as the limits of significance at the 0.05 probability level. On histograms expressing the results of assay, these significant deviations are marked with black areas.

CHAPTER III

RESULTS

Analysis of the acid ether fraction in the methanolic extract of different root samples of *Zea mays*:

A. Root cap

The individual assays (Fig. 14 B-F) of the acid fraction of the methanolic extract of root caps show a uniformity of character and this has been taken as justification for combining the results in Fig. 14 A, where the sums of all significant growth responses for five such chromatograms are plotted against the corresponding Rf values. It is evident from the figures that one major active substance running between Rf 0.5 to 0.7 (in solvent I) was present in the chromatogram. This substance inhibiting the root segment growth (around 50% of controls) appeared consistently on all five occasions. This substance, henceforth called the 'cap inhibitor' was unlikely to be IAA which ran between Rf 0.2 to 0.35 in this solvent. In only one out of five extracts slight inhibition (about 25% of control : Fig. 14 D) was observed at the Rf of IAA. On two other occasions there were indications of another inhibitor between Rf 0-0.1 (Figs. 14 C and 14 D). Occasionally stimulation of growth was observed close to the 'cap inhibitor' at slightly lower Rf values (Figs. 14 E and 14 F). Otherwise, there was no indication of any growth promoting substance or 'accelerator' present in the chromatogram.

B. Root tip

Extracts of the complete 5 mm tips (Fig. 15), on the other hand, showed the presence of two major zones of constant inhibition. One of these coincided with the Rf of the 'cap inhibitor' (between 0.4 to 0.7). The other inhibitor, henceforth called the 'meristem

Fig. 14 Chromatograms of ether soluble acid fraction of the
 extracts of root caps (running solvent - isopropanol:
 ammonia:water). Approximately one third of the
 total extract of 100 caps has been assayed.

A. Composite diagram from five separate
 chromatograms showing the growth active
 regions.

B-F Chromatograms of five individual assays of
 the above.

Fig. 14

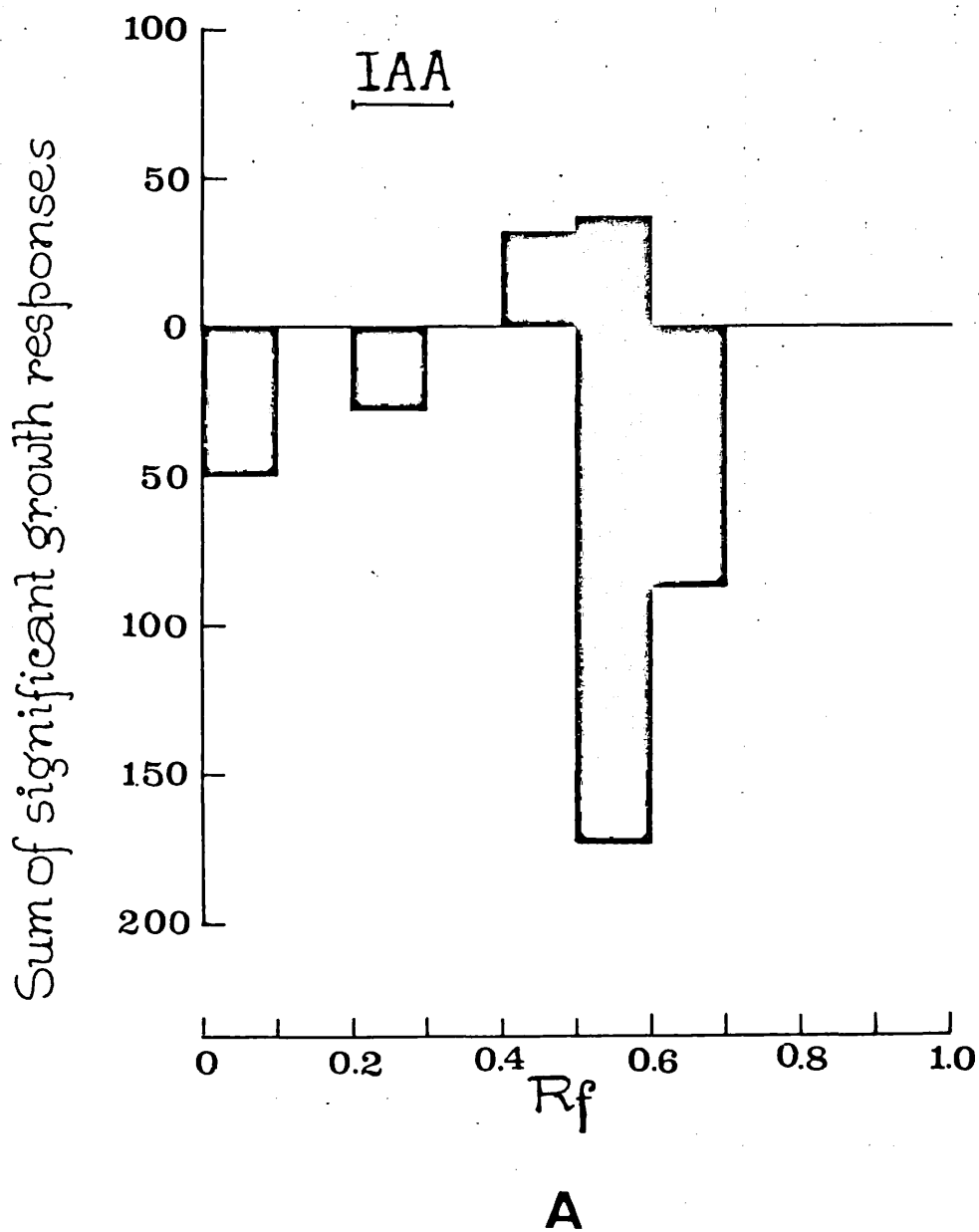


Fig.14 (contd.)

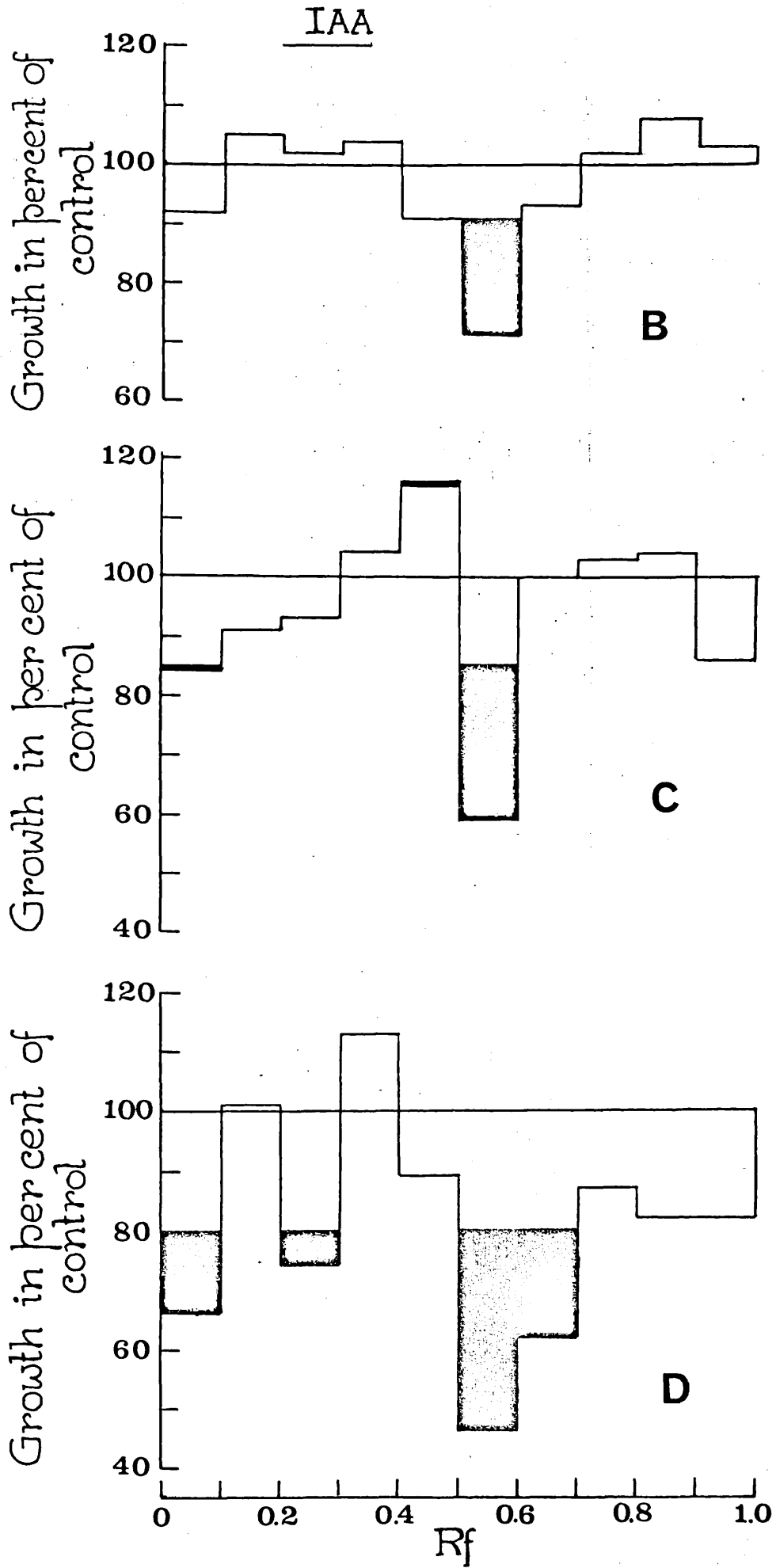


Fig.14 (Contd.)

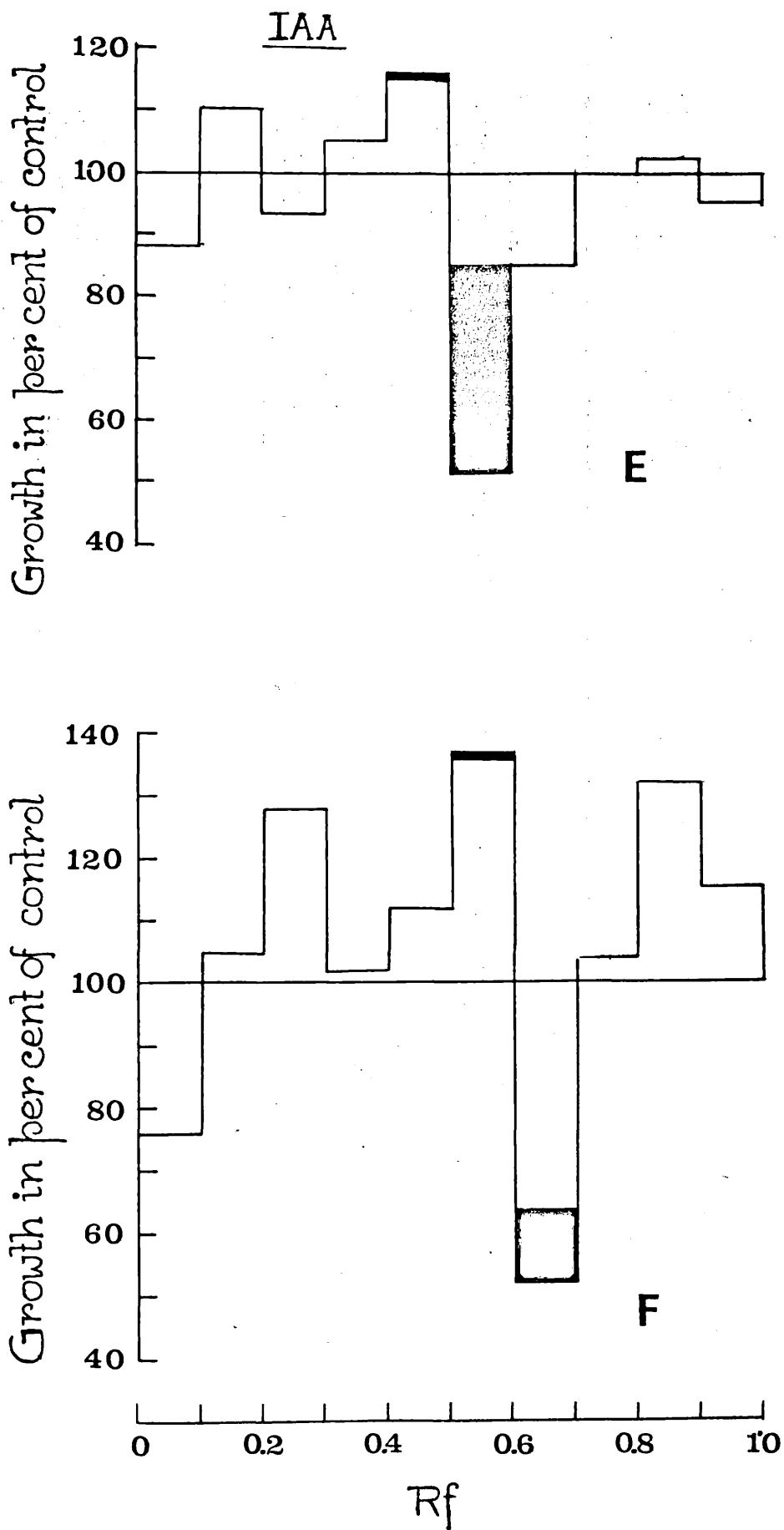


Fig. 15 Chromatograms of ether soluble acid fraction of the extracts of 5 mm root tips (running solvent - isopropanol:ammonia:water). Approximately one third of the total extract of 100 tips has been assayed.

A. Composite diagram from five separate chromatograms showing the growth active regions.

B-F Chromatograms of five individual assays of the above.

Fig.15

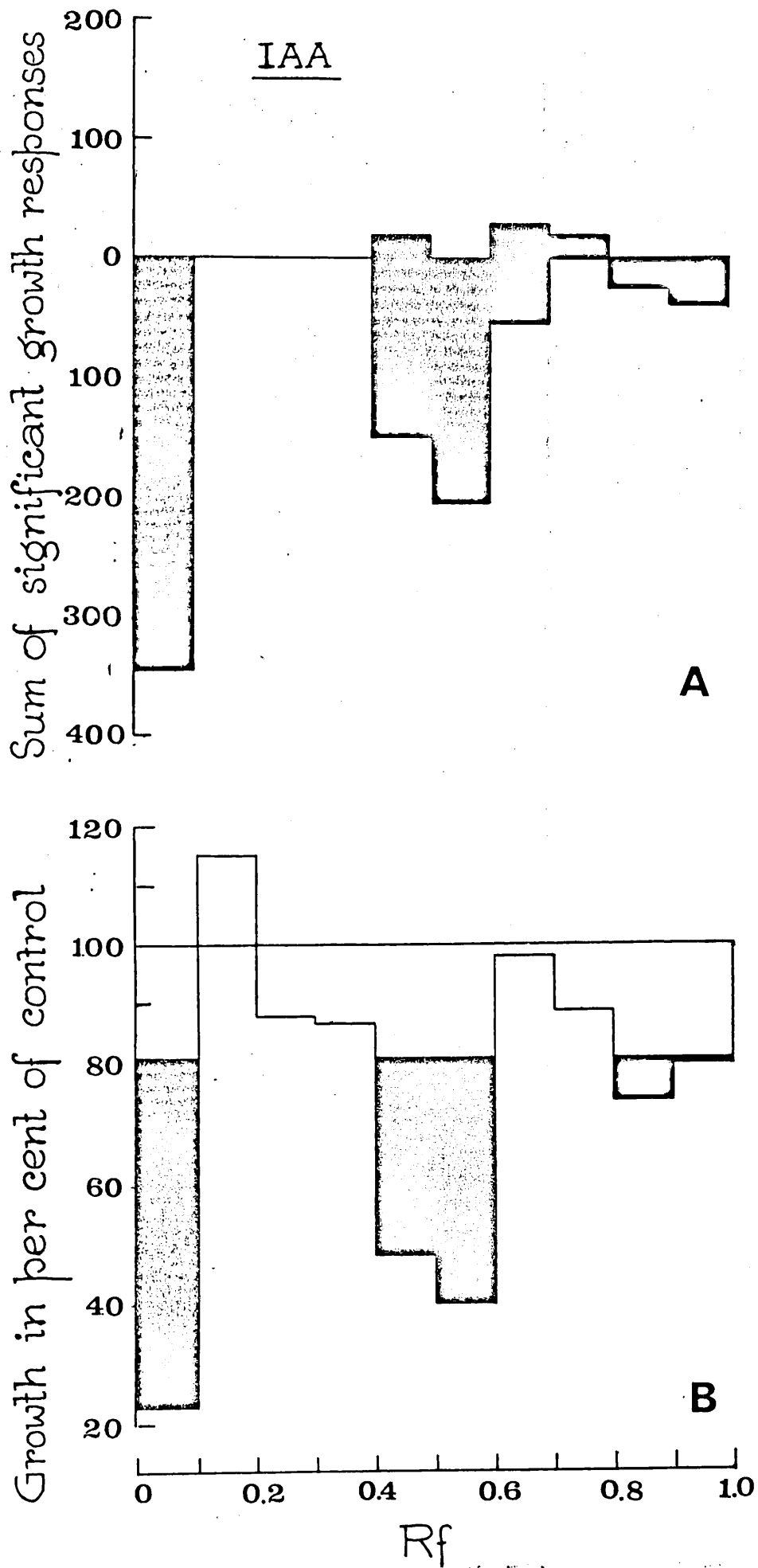


Fig.15 (Contd.)

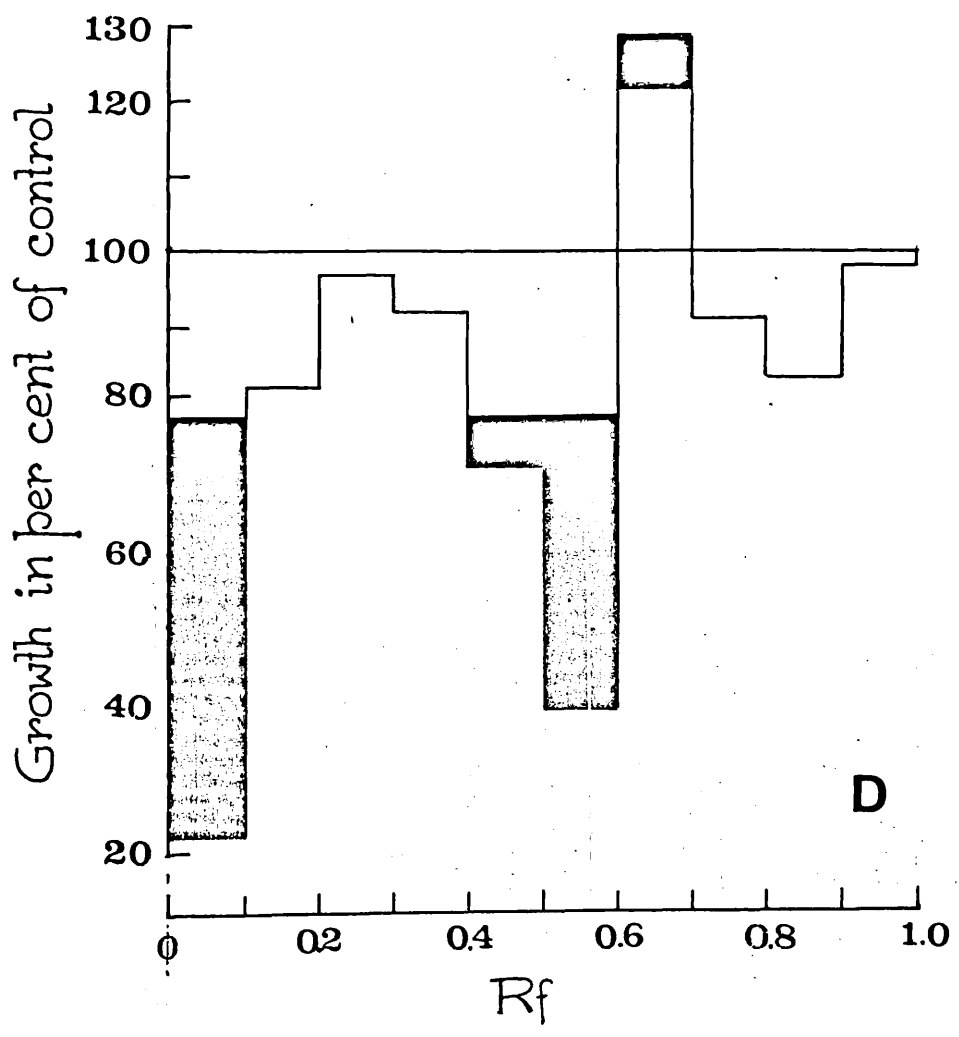
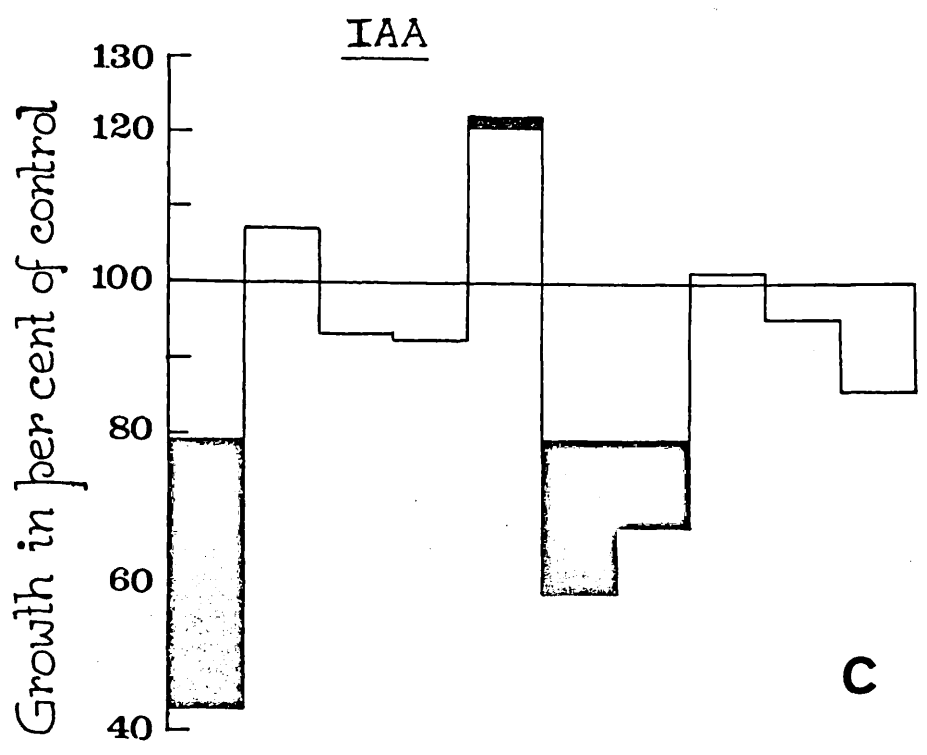
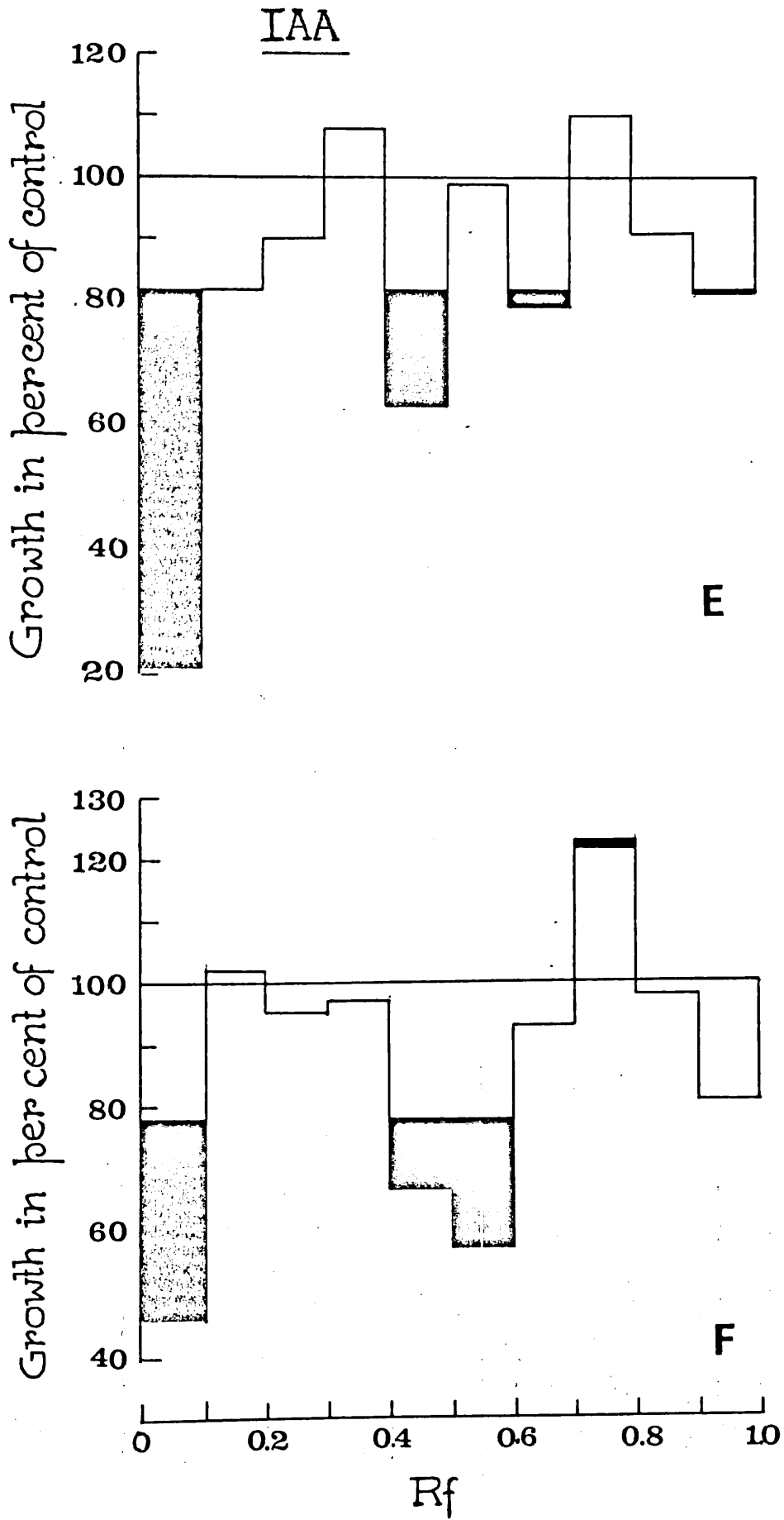


Fig.15 (Contd.)



'inhibitor' corresponded with the minor inhibitor at Rf 0-0.1 of the root cap. A third inhibitor, which will be called the 'inhibitor-3', running at the solvent front (between Rf 0.8-1.0) exerted a much smaller action and did not appear so consistently (appearing in two out of the five extracts - see Figs. 15 B and 15 E). No activity was observed corresponding to the IAA region on the chromatogram. Here also stimulation of growth was occasionally observed on either side and close to the 'cap inhibitor' (Figs. 15 C, 15 D and 15 F).

C. Root tip without cap

On three occasions extracts of decapped root tips were analysed (Fig. 16). In these extracts only the 'meristem inhibitor' was present in any amount and appeared consistently. Traces of the 'cap inhibitor' also appeared regularly on all three occasions. In only one extract little promotion of growth was observed around the Rf of marker IAA (Fig. 16 A) otherwise, no other active substances were found to be present.

D. Serial 2 mm segments

Analyses of the extracts of four successive 2 mm segments of the root, stretching back proximally from the cap/meristem junction are presented in Figs. 17 to 20. Among two figures in each number the top one (A) represents the composite diagram derived from the three successive observations for each segment and the figure below (B) is a typical chromatogram from one occasion.

In the first segment (nearest to the cap), as evidenced from the figures (17 A and 17 B), the two major inhibitors were present in significant amounts. No other active substances were found to be present except on one occasion where slight promotion of growth (Fig. 17 B) was observed close to the 'cap inhibitor'.

Fig. 16 Chromatograms of the ether soluble acid fraction
of the extract of 100 tips without cap (running
solvent - isopropanol:ammonia:water). Approximately
one third of the total extract of 100 tips has been
assayed.

A. Composite diagram from three separate
 chromatograms.

B-D Chromatograms of three individual assays of
 the above.

Fig.16

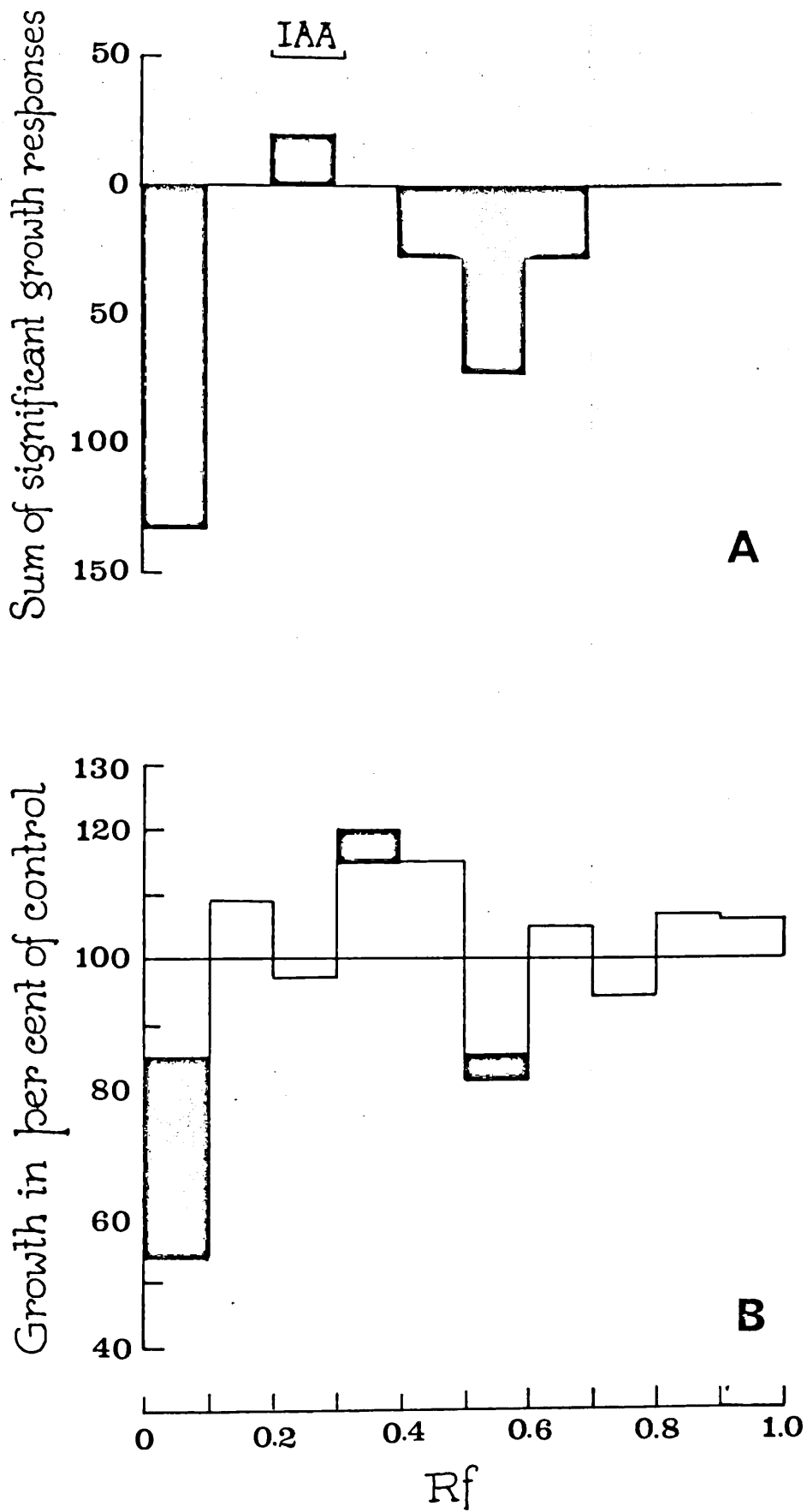


Fig.16 (Contd.)

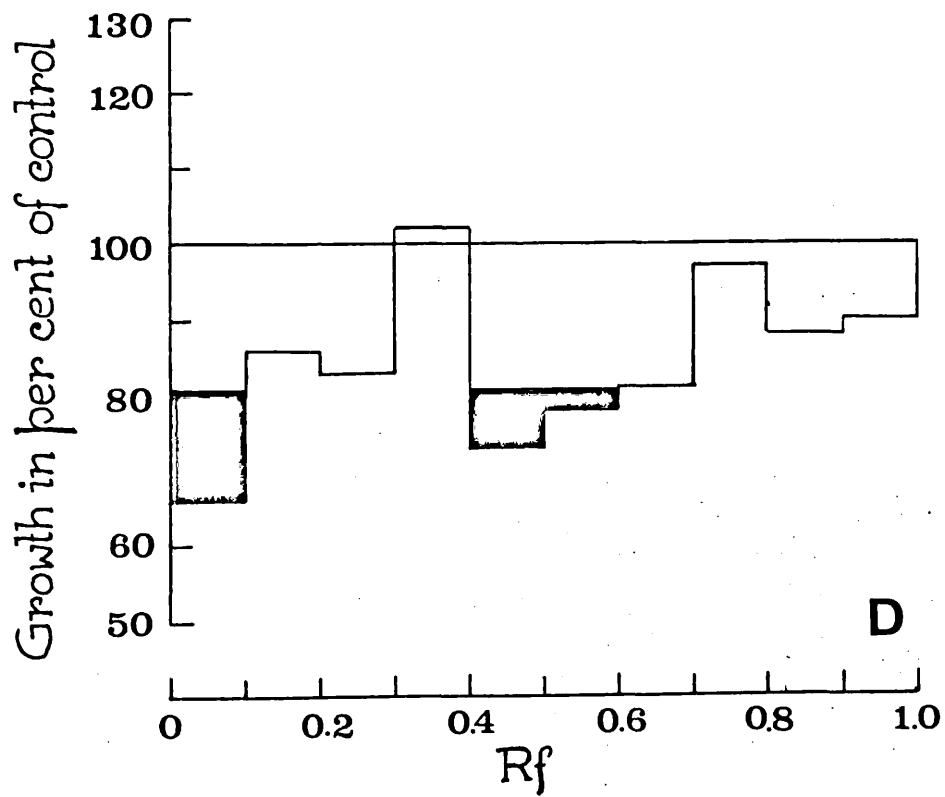
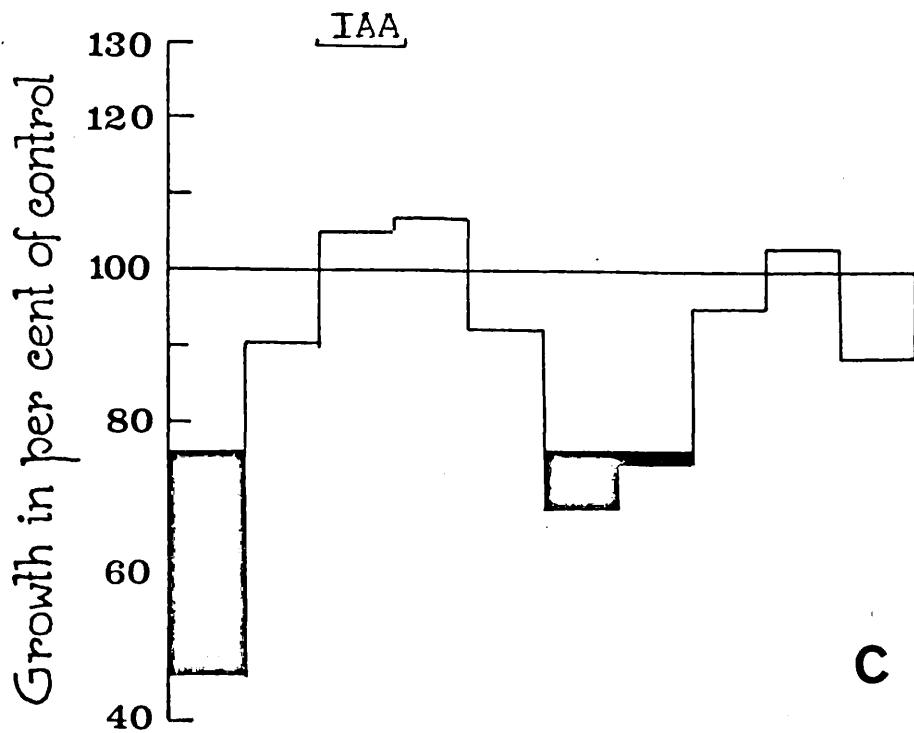
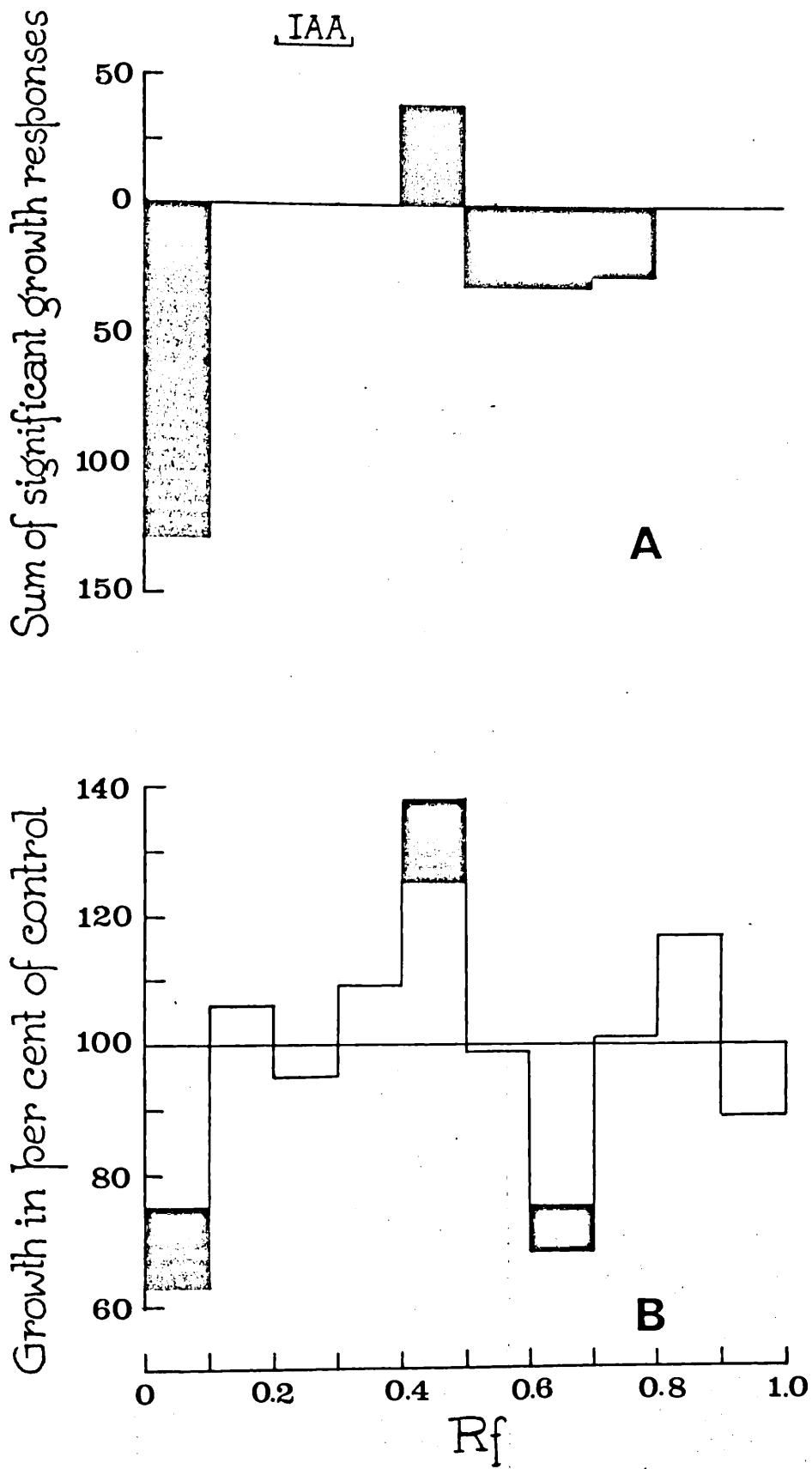


Fig. 17 Chromatograms of acid ether fraction of the extract of the first 2 mm segments (100) next to the root cap (running solvent - isopropanol:ammonia:water). One third of the total extract has been assayed.

A. Composite diagram from three separate chromatograms.

B. A typical chromatogram from one of the three occasions.

Fig.17



In the second and the third succeeding segments (Figs. 18 and 19) the 'cap inhibitor' appeared inconsistently in the chromatograms. However, in these two segments the 'meristem inhibitor' appeared regularly. Besides these two inhibitors, on one occasion, stimulation of growth was observed in the position of the 'inhibitor-3' (between Rf 0.9 to 1.0) in the extract of the second segment (Fig. 18 B). Occasionally promotion as well as inhibition of growth were noticed at the Rf of IAA in the extracts of these two segments.

In the proximal segment (about 8 mm away from the root cap) no trace of the 'cap inhibitor' was detected (Fig. 20). Here only the 'meristem inhibitor' appeared consistently. As in previous segments stimulation of growth corresponding to the Rfs of the 'inhibitor-3' and IAA were observed on several occasions.

The observations so far suggest that two major active acids, both inhibiting the root segment growth are present in the root tip of maize. Of these two the 'cap inhibitor' is predominantly present in the root cap and

the 'meristem inhibitor', on the other hand, is the main inhibitor of the meristematic tissue. Other inhibitors, i.e. the 'inhibitor-3' and one corresponding to the Rf of IAA are less active and appear sporadically. None of them seems to be present in the root cap.

F. Diffusates

Analyses of diffusates collected from the extreme 1 and 2 mm tips (including cap) are presented in Figs. 21 and 22. In diffusates from the 1 mm tip, the 'cap inhibitor' was present in significant amounts on the three occasions (Figs. 21 B, 21 D and 21 F). On the other two occasions, though the inhibition was not significant up

Fig. 18 Chromatograms of acid ether fraction of the extract of the second 2 mm segments (100) next to the first segment (running solvent - isopropanol:ammonia: water). One third of the total extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.18

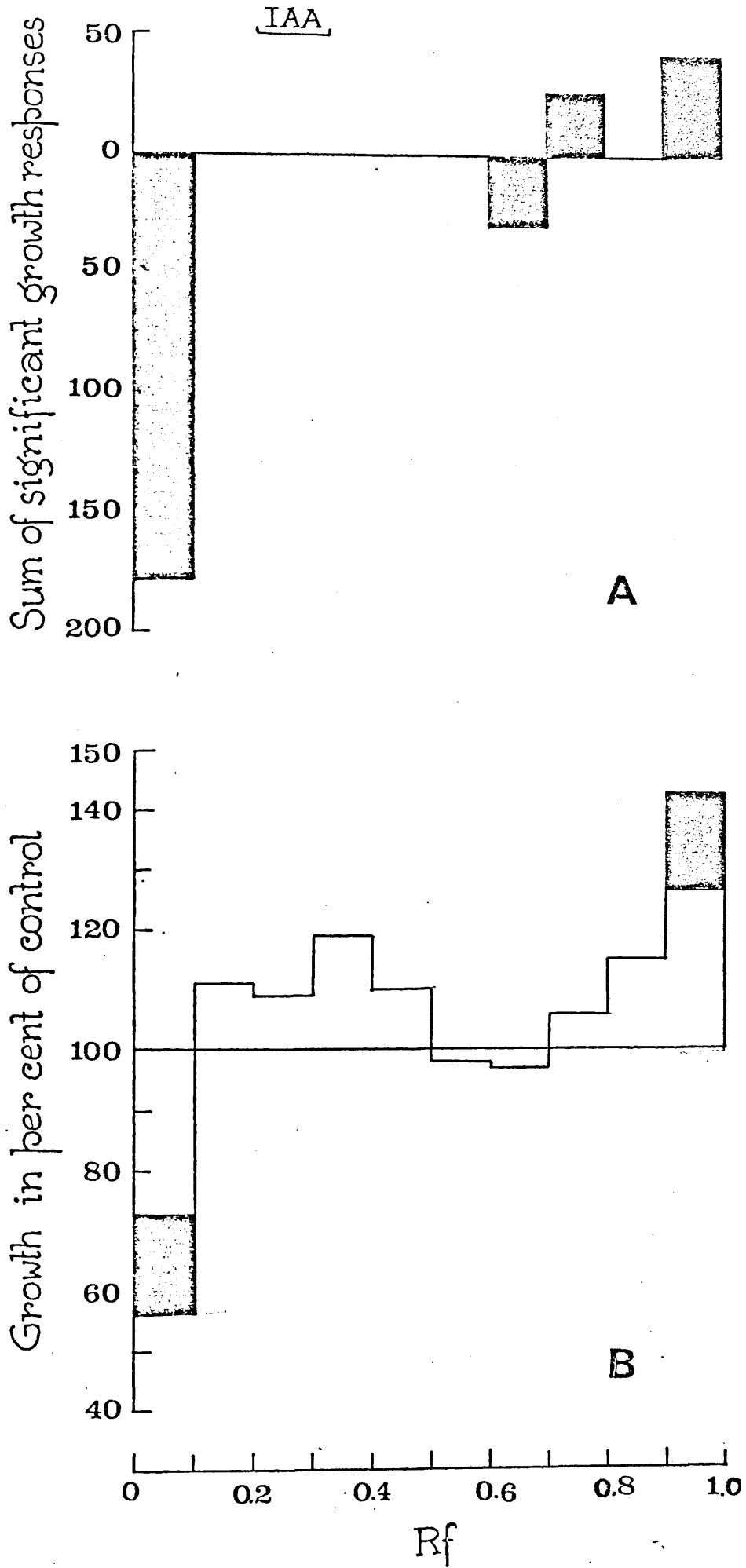


Fig. 19 Chromatograms of acid ether fraction of the extract of the third 2 mm segments (100) next to the second segment (running solvent - isopropanol:ammonia: water). One third of the total extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.19

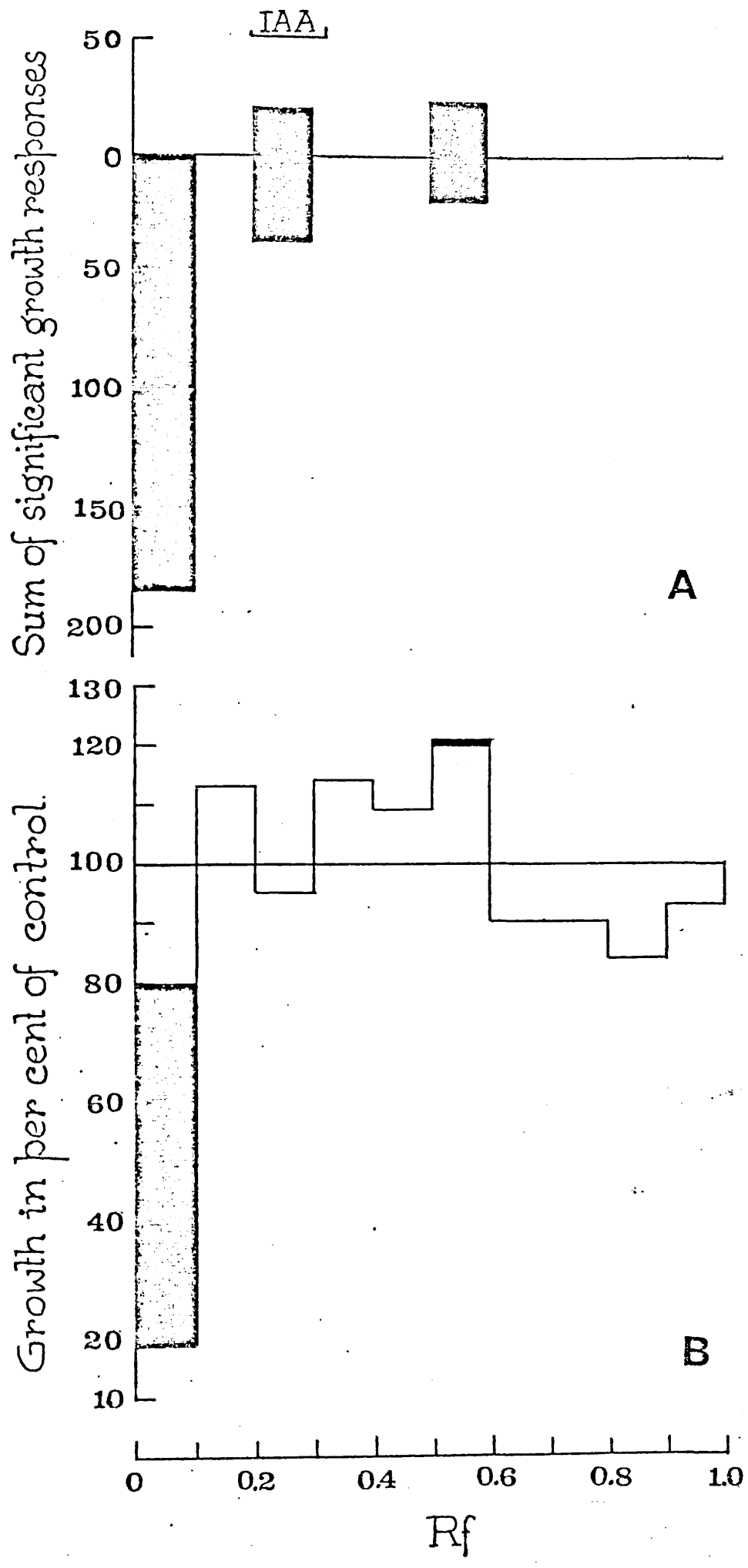
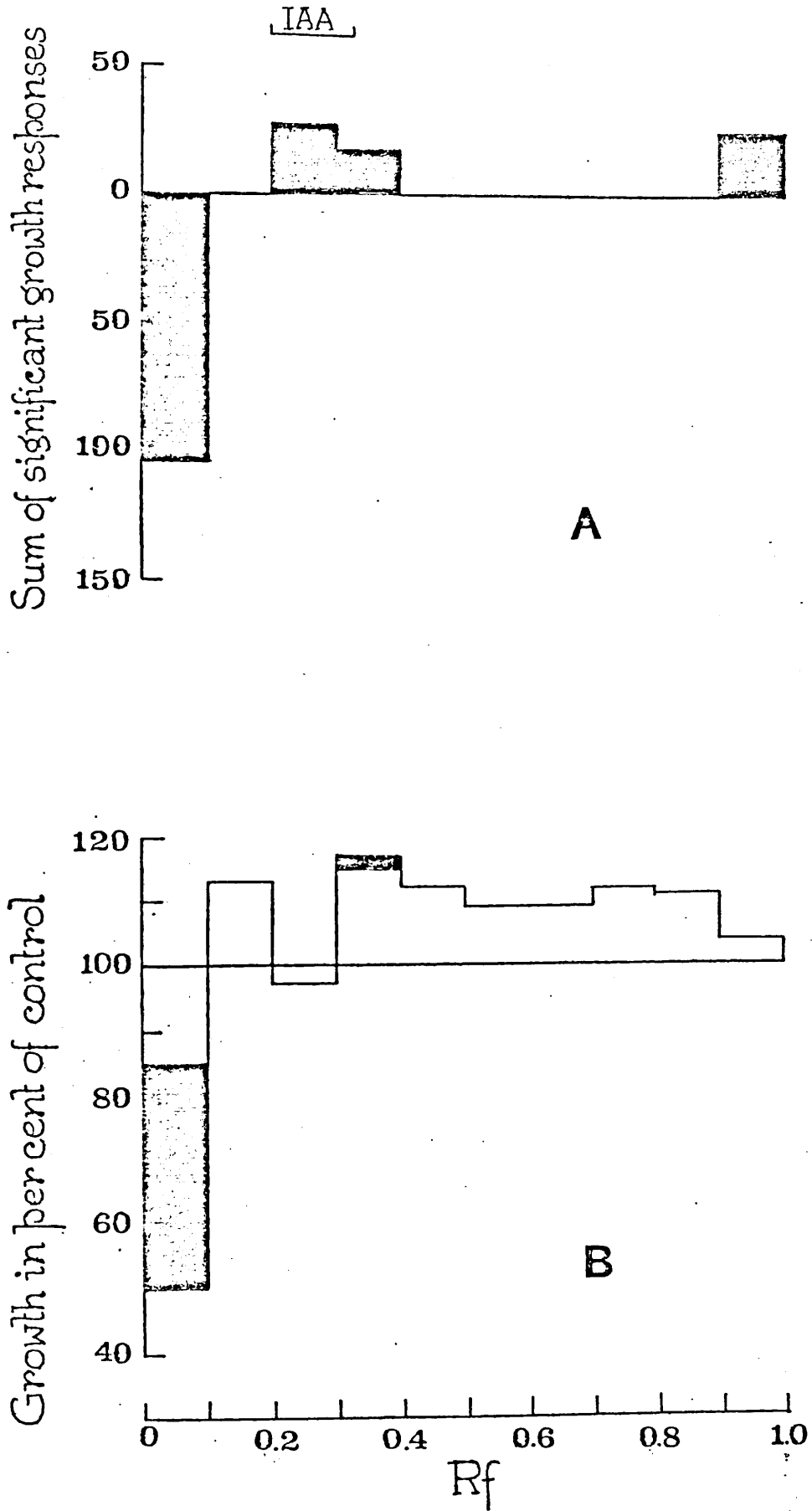


Fig. 20 Chromatograms of acid ether fraction of the extract of the fourth 2 mm segments (100) next to the third segment (running solvent - isopropanol:ammonia: water). One third of the total extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig. 20



to the limits of confidence, yet a depression of growth in the relevant Rfs indicated its existence in the chromatogram. On one occasion, (Fig. 21 E) slight promotion of growth was observed between Rf 0.4-0.5. The 'meristem inhibitor' appeared twice in the diffusates (Figs. 21 C and 21 F) while no traces of the 'inhibitor-3' was observed. However, on one occasion (Fig. 21 B) a little promotion of growth was observed between Rfs 0.9-1.0. Similarly, no growth activity was seen in the region of IAA except on one occasion where stimulation of growth was observed at slightly higher but close to the Rf of IAA (Fig. 21 F).

On the other hand, in the diffusates of 2 mm root tips where the cut surface was meristematic tissue, no traces of the 'cap inhibitor' was observed except a little stimulation of growth at Rf 0.4-0.5 on one occasion (Fig. 22 B). The 'meristem inhibitor' appeared consistently and in considerable amounts in all three chromatograms. On two occasions (Figs. 22 B and 22 C) slight inhibition was observed in the position corresponding to that of IAA. The 'inhibitor-3' was not observed on any one of the chromatograms.

Analyses of the diffusates as well as the extracts of the serial 2 mm root segments clearly indicate that the 'cap inhibitor' is mainly present in the cap but its progressive fall of concentration into the extending zone implies its basipetal movement there from production centres in the cap.

It seemed probable that several active substances may be present in the same Rf in one particular solvent system - a situation which often arises due to insufficient resolution of the chromatogram. For this, the acid ether fraction of the extract of 200 root tips was separately applied on the paper as a 2 cm band

Fig. 21 Chromatograms of acid ether fraction of the
diffusates collected from a hundred 1 mm tips
(running solvent - isopropanol:ammonia:water).
One third of the total diffusates has been
assayed.

A. Composite diagram from five separate
 chromatograms.

B-F Individual chromatograms from each
 occasion.

Fig.21

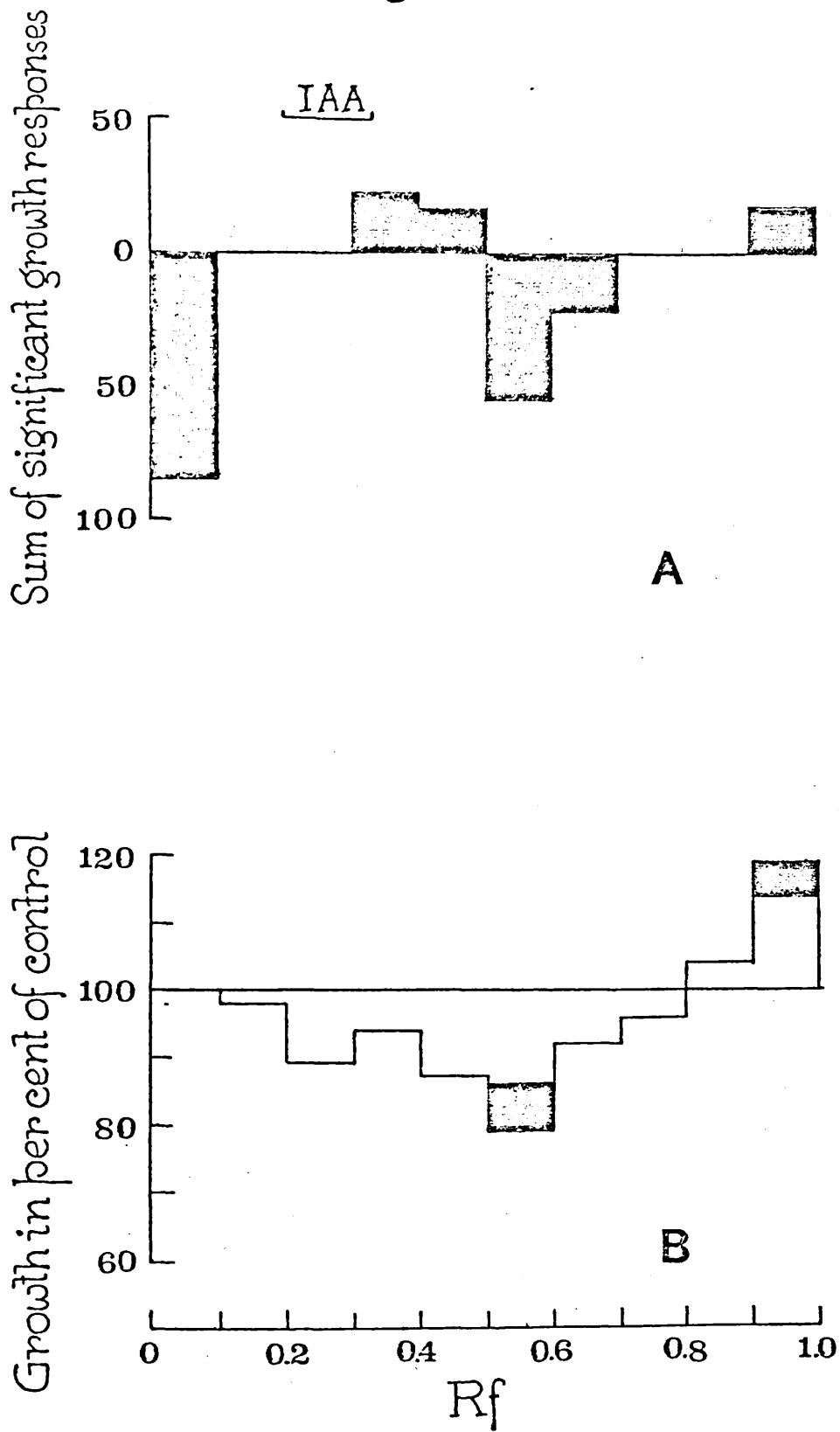


Fig.21(Contd.)

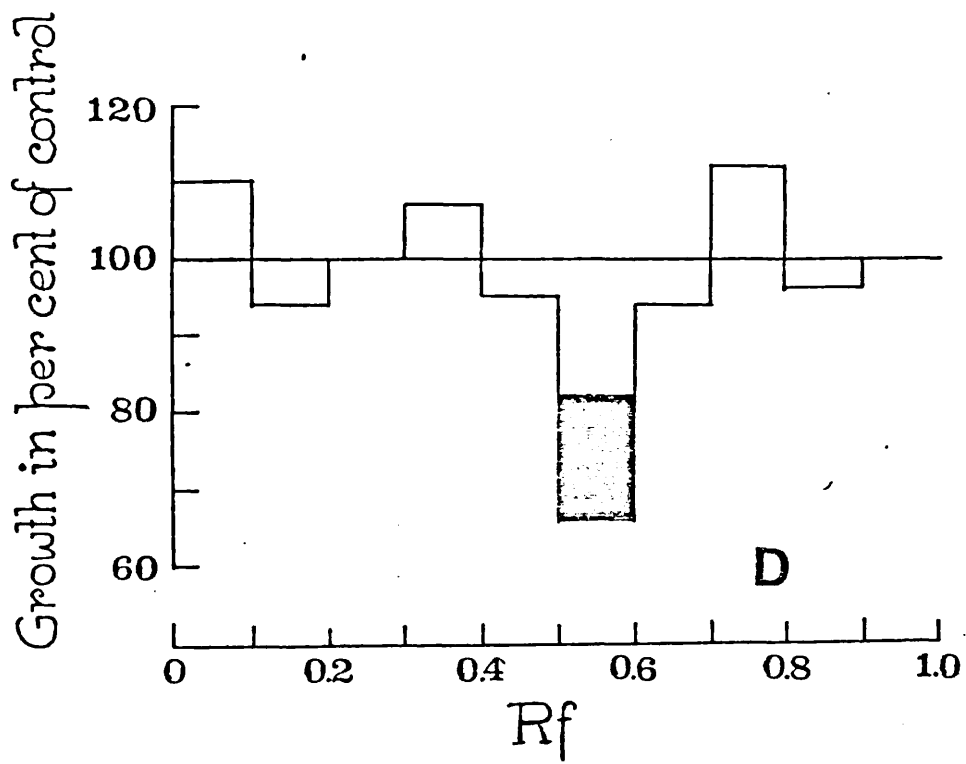
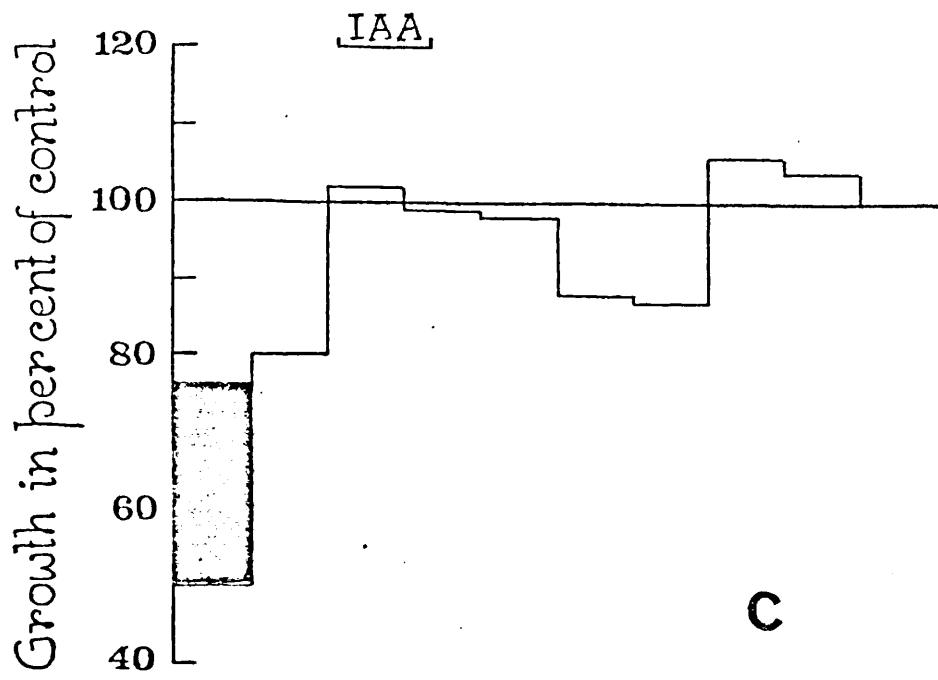


Fig.21(Contd.)

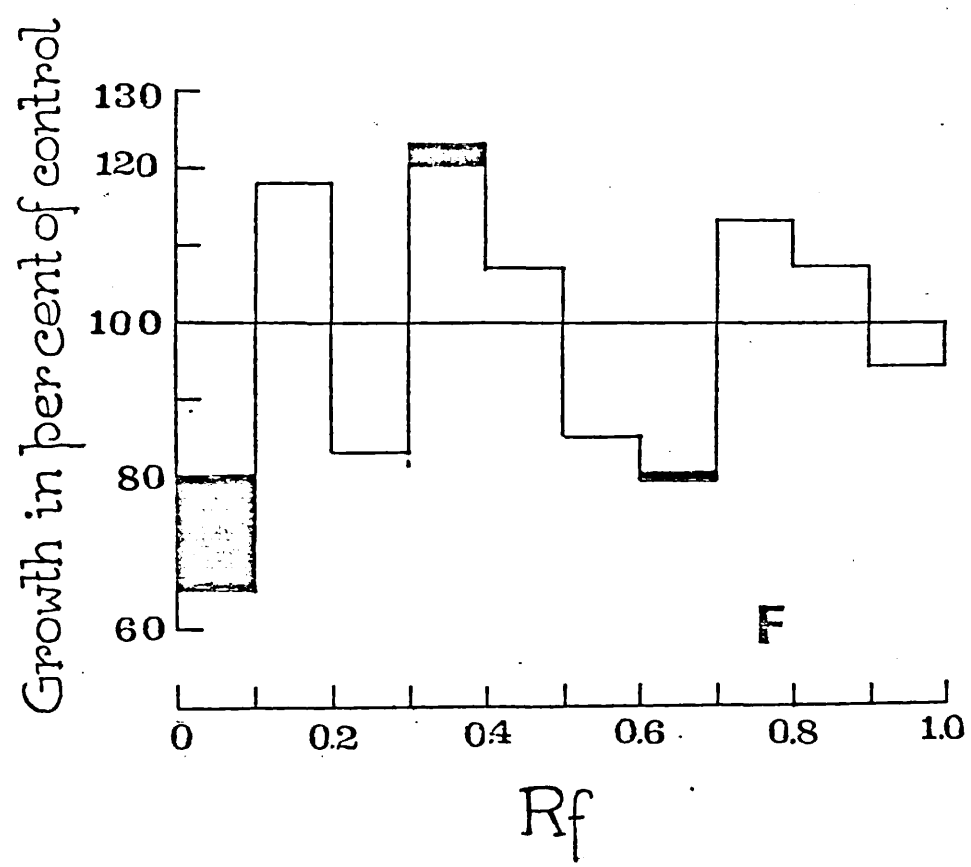
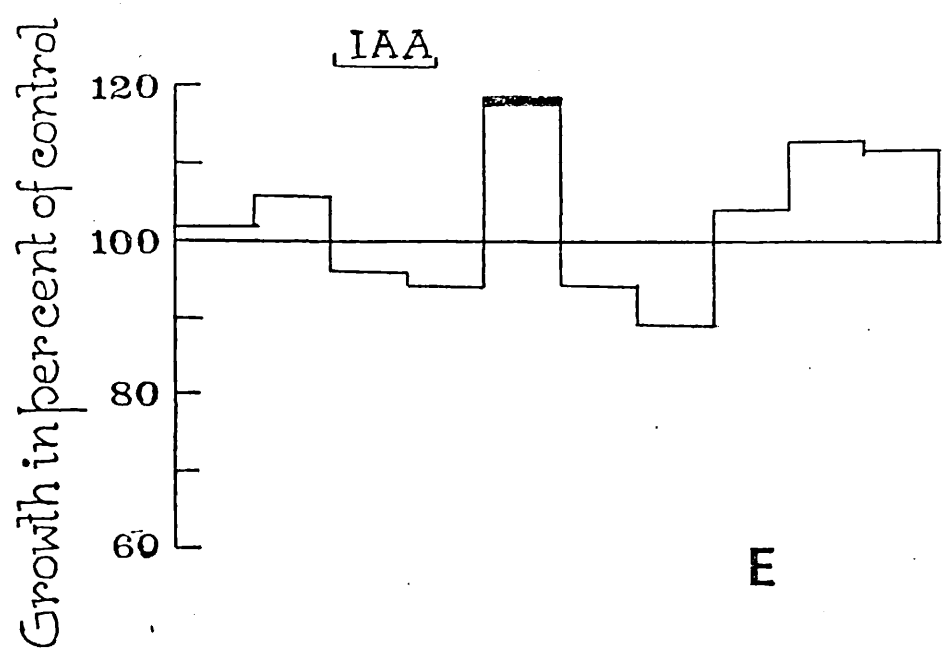


Fig. 22 Chromatograms of acid ether fraction of the
diffusates collected from a hundred 2mm tips
(running solvent - isopropanol:ammonia:water).
One third of the total diffusates has been
assayed.

A. Composite diagram from three separate
 chromatograms.

B-D Individual chromatograms from each
 occasion.

Fig.22

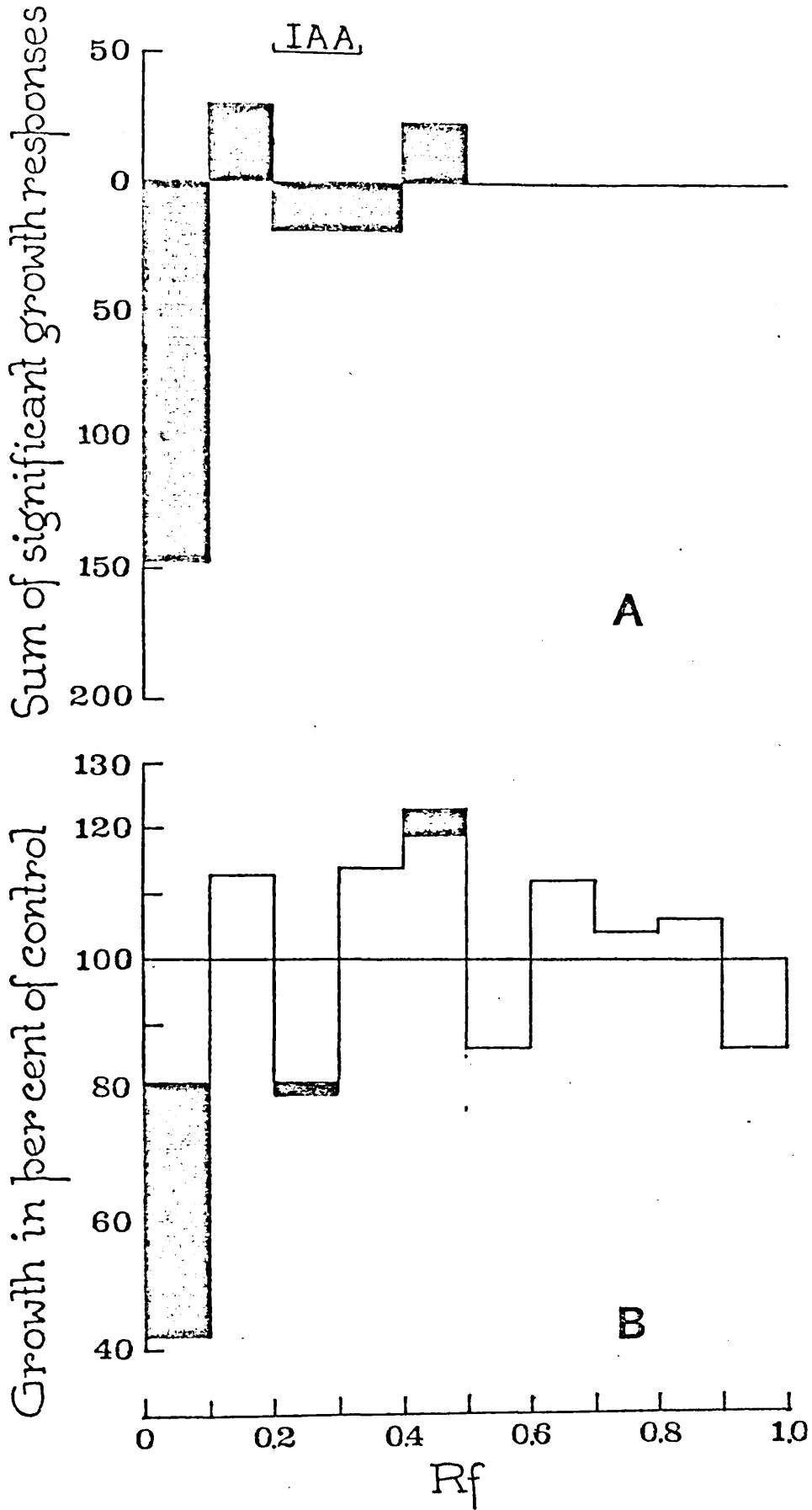
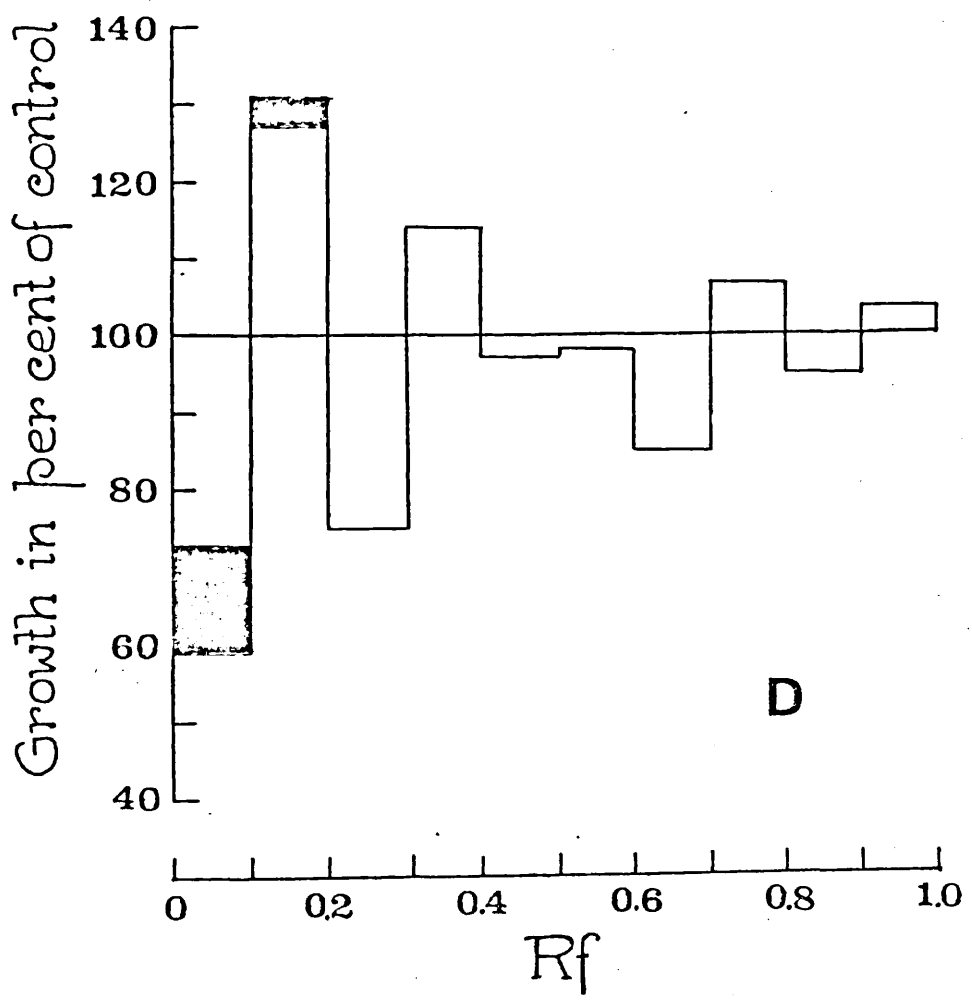
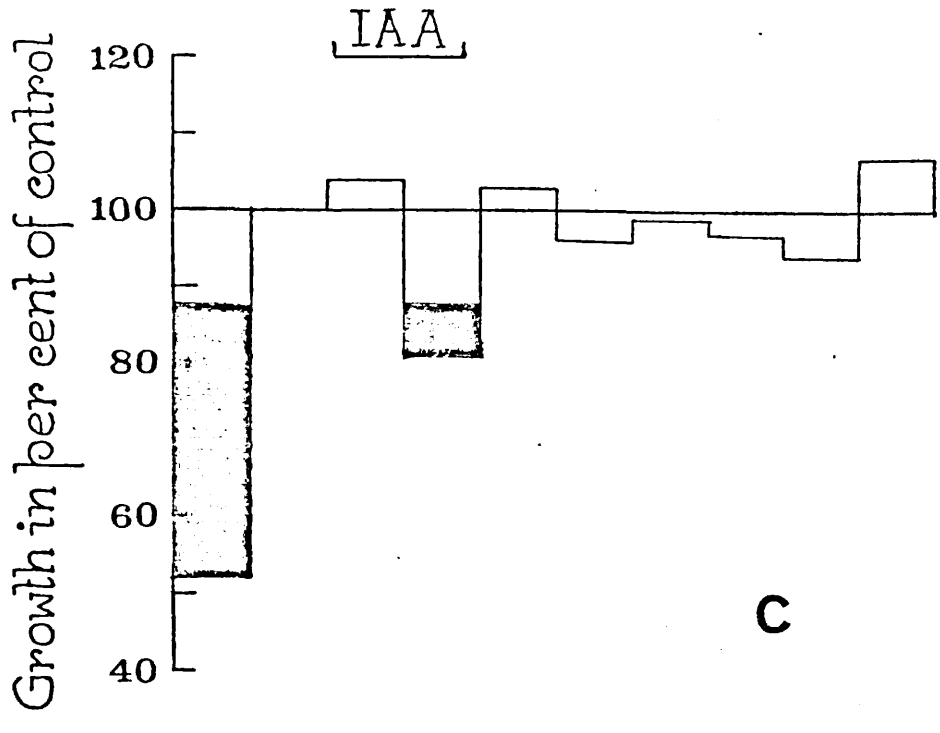


Fig. 22 (Contd.)



and allowed to run up to 15 cm in the solvents I, II and III. Each chromatogram was then longitudinally halved into two equal strips (each strip thus containing approximately 100 root tip-extract). One of the half-strips was assayed following the usual procedure while the other half was sprayed with Ehrlich's reagent on one occasion and with Salkowski's reagent on the other occasion. The results of the observations are presented below.

F. Chromatogram of the root-tip extract using solvent I.

In spite of better resolution (15 cm) and a comparatively greater amount of the extract assayed, the activity spectrum of the growth substances in the solvent I was virtually identical as before. A typical chromatogram is presented in Fig. 23 A. The 'cap inhibitor' and the 'meristem inhibitor' were always present in the extract, whereas inhibition in the region of IAA was noticed occasionally. However, 'inhibitor-3' was absent in all the observations. None of the active zones were Ehrlich-positive whereas a deep purple colour appeared in the Rf corresponding to the 'cap inhibitor' after spraying with Salkowski's reagent.

G. Chromatogram of the root-tip extract using solvent II.

In the second solvent system owing to the close Rf values of the active substances, a continuous zone of inhibition was frequently obtained from Rf 0.25 to 0.85 (Fig. 23 B). However, repeating the experiment, two major peaks of inhibition were revealed. The first active zone fell between Rfs 0.25 to 0.45, slightly below the position of IAA (marker IAA in this solvent ran between Rfs 0.4-0.5). No colour reaction was obtained in this zone and this could possibly be the 'meristem inhibitor'. The second active zone which appeared between Rfs 0.5-0.85, just above IAA and gave a positive colour reaction with Salkowski's reagent indicated its identity with the 'cap inhibitor'. On two out of three occasions, significant inhibition

Fig. 23 Chromatograms of acid ether fraction of the extract of root tips (5 mm). Chromatograms run in different solvents and the entire extract of a hundred root tips has been assayed.

- A. Running solvent - isopropanol:ammonia:water
- B. Running solvent - isobutanol:methanol:water
- C. Running solvent - isopropanol:water

Fig.23

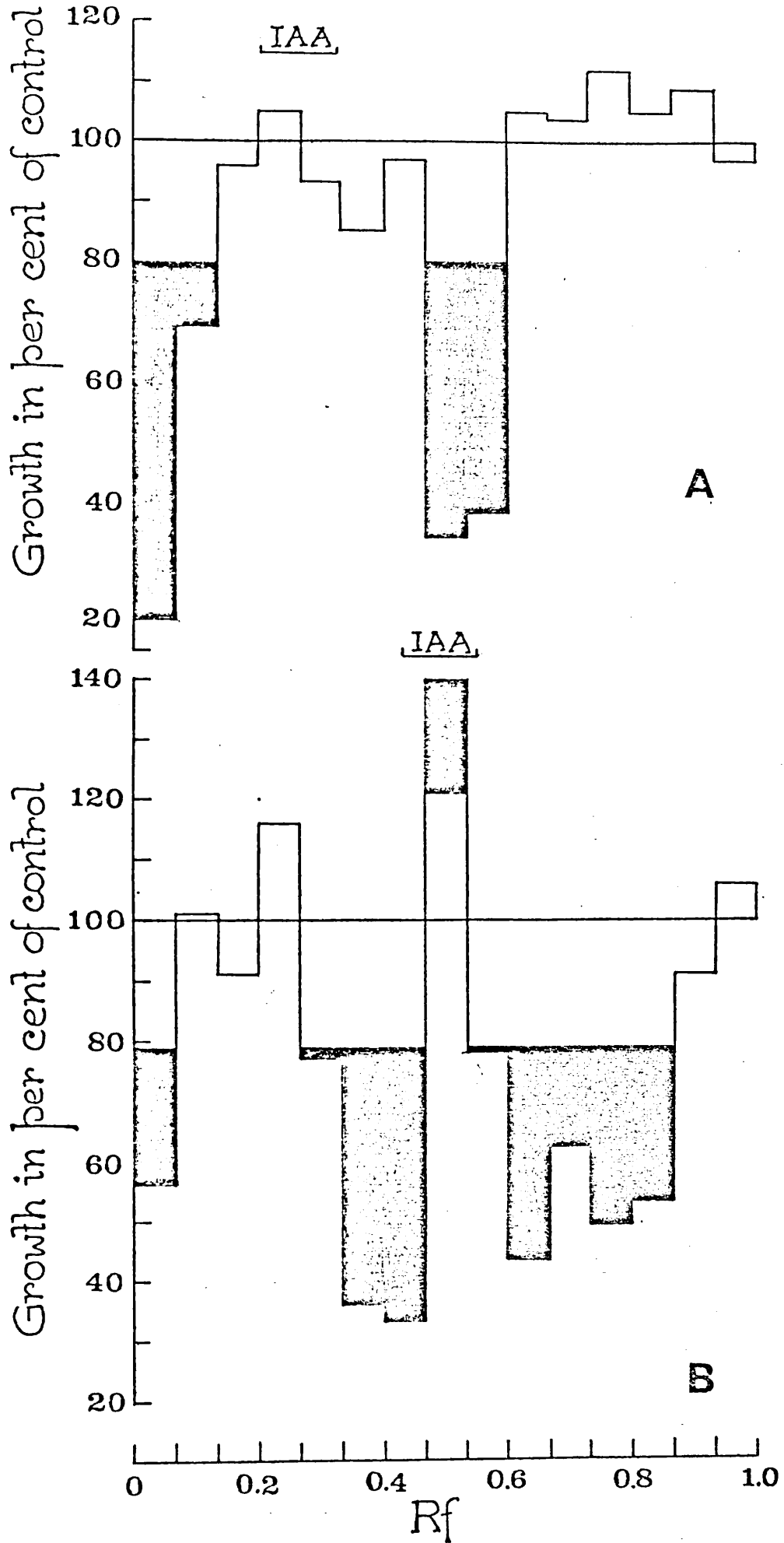
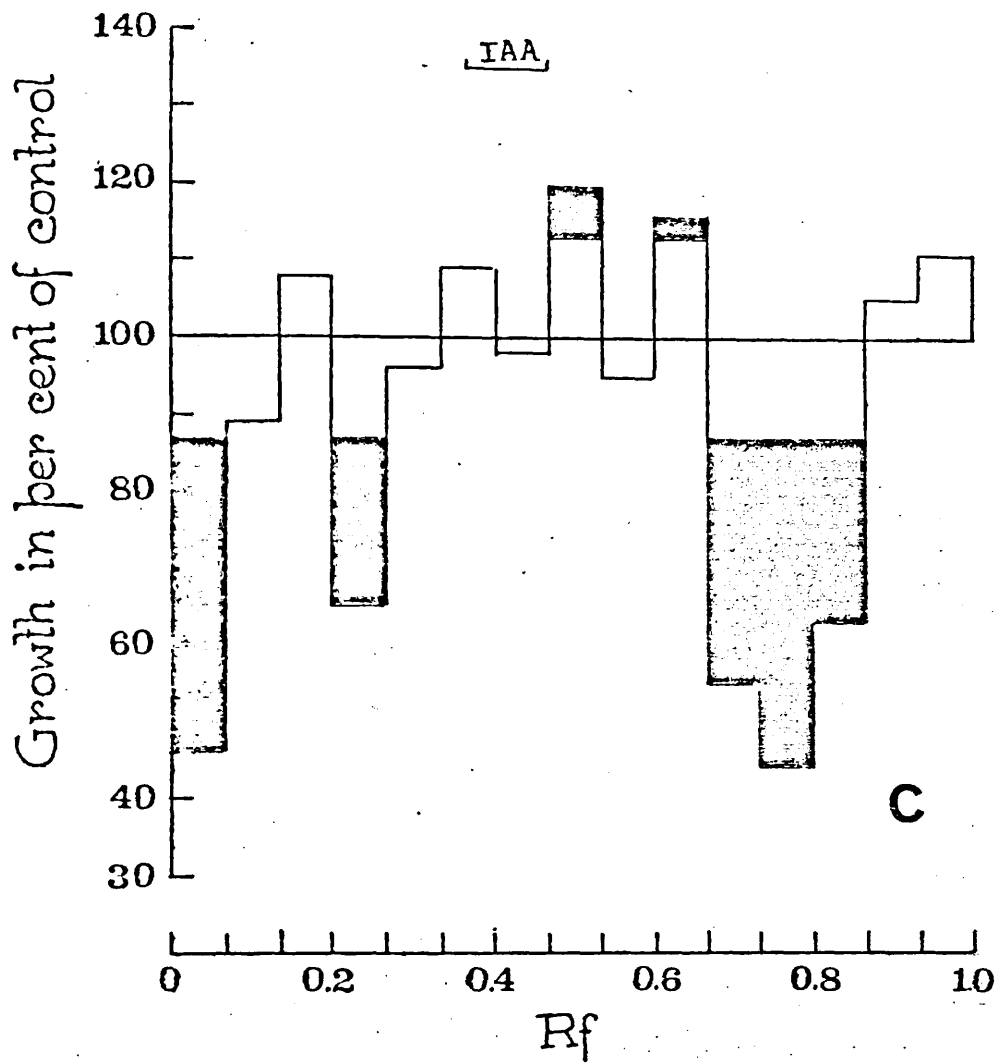


Fig.23 (contd.)



was obtained in the origin of chromatogram (between Rf 0-0.1). This was marked as the 'Inhibitor-4A'. Slight inhibition was also observed once in the solvent front (Rf 0.8-1.0). This inhibitor was probably equivalent to the 'inhibitor-3'. Due to overlapping of these two major inhibitors around the IAA region of the chromatogram, the latter, if present, is difficult to detect using this solvent system.

H. Chromatogram of the root-tip extract using solvent III.

The activity spectrum of the chromatogram obtained using solvent III was more or less identical with that obtained using solvent I, (Fig. 23 C). Here also one of the major inhibitory zones was restricted to the origin of the chromatogram between Rf 0-0.1 and this was probably identical with the 'meristem inhibitor'. The other major inhibitor, which was Ehrlich-negative but Salkowski-positive and possibly identical with the 'cap inhibitor' appeared between Rf 0.6-0.85. In this solvent system another inhibitor appeared consistently on all three occasions a little below the position of marker IAA, which ran between Rf 0.3-0.45. Comparing with the major inhibitors its activity was substantially low. This inhibitor or the 'inhibitor-4B' was presumably not IAA, as frequently stimulation of growth was observed between Rf 0.3-0.45, slightly apart from the above inhibitory zone and close to the position of IAA.

In the next experiment, the eluates from the active zones of the chromatogram in one solvent were rechromatographed in the other solvent to compare further the active zones in different solvent systems. For this, in the first place, the acid ether fraction of 200 root tips was spotted as a 2 cm band, and allowed to run 15 cm as before, in the solvent II. The chromatogram was then divided into two longitudinal strips one of which was sprayed with Salkowski's reagent. From the area of the colour reaction, which usually appeared between Rfs. 0.5-0.85, the

corresponding region of the other half was cut out and eluted in 5 ml of methanol for 24 hours at -10°C . The next major active zone, just below the coloured area (Rf 0.2-0.5), was also cut out and eluted in methanol in a similar way. These two eluates were separately rechromatographed in the other two solvents. The results were presented in Figs. 24 A and 24 B.

Rechromatography of the eluates from Rf 0.5-0.85 of solvent II indicated its identity with the inhibitors corresponding to the Rf 0.5-0.7 of solvent I (Fig. 24 A) and Rf 0.55-0.85 of solvent III (Fig. 24 C). These active substances were identical with the 'cap inhibitor' and were likely to be one single substance inhibiting root growth. On the other hand, eluates from Rf 0.2-0.5 of solvent II when rechromatographed in the solvent I contained one major inhibitor or the 'meristem inhibitor' (at Rf 0-0.1) and traces of IAA and the 'cap inhibitor' (Fig. 24 B). Whereas two different inhibitory zones, slightly apart, were obtained when the same was rechromatographed in solvent III (Fig. 24 D). The one between Rf 0-0.1 could possibly be 'meristem inhibitor' and the other at Rf 0.2-0.25 might be the 'inhibitor-4' which appeared consistently in this solvent.

A comparatively clear picture was obtained when the eluates from Rf 0-0.2 (or, the 'meristem inhibitor') and 0.5-0.7 (or, the 'cap inhibitor') of solvent I were similarly rechromatographed in solvent II. Here the 'cap inhibitor' appeared between Rf 0.5-0.7 in the isobutanol solvent (Fig. 24 F) whereas the 'meristem inhibitor' appeared between Rf 0.25 - 0.45 along with traces of IAA (identified from its promotion of growth in the position of marker IAA : Fig. 24 E). On one occasion only a slight promotion of growth was observed in the solvent front. However, in repeat experiments this was found to be absent.

Fig. 24. Rechromatography of the eluates.

- A. Eluates from Rf 0.5 - 0.85 of the chromatogram, run in isobutanol solvent is rechromatographed in isopropanol:ammonia:water solvent.
- B. Similar rechromatography of the eluates from Rf 0.2 - 0.5.
- C. Eluates from Rf 0.5 - 0.85 of the chromatogram, run in the above solvent is rechromatographed in isopropanol:water solvent.
- D. Similar rechromatography of the eluates from Rf 0.2 - 0.5.
- E. Eluates from Rf 0 - 0.2 of the chromatogram, run in isopropanol:ammonia:water solvent is rechromatographed in isobutanol solvent.
- F. Similar rechromatography of the eluates from Rf 0.5 - 0.7.

Fig. 24

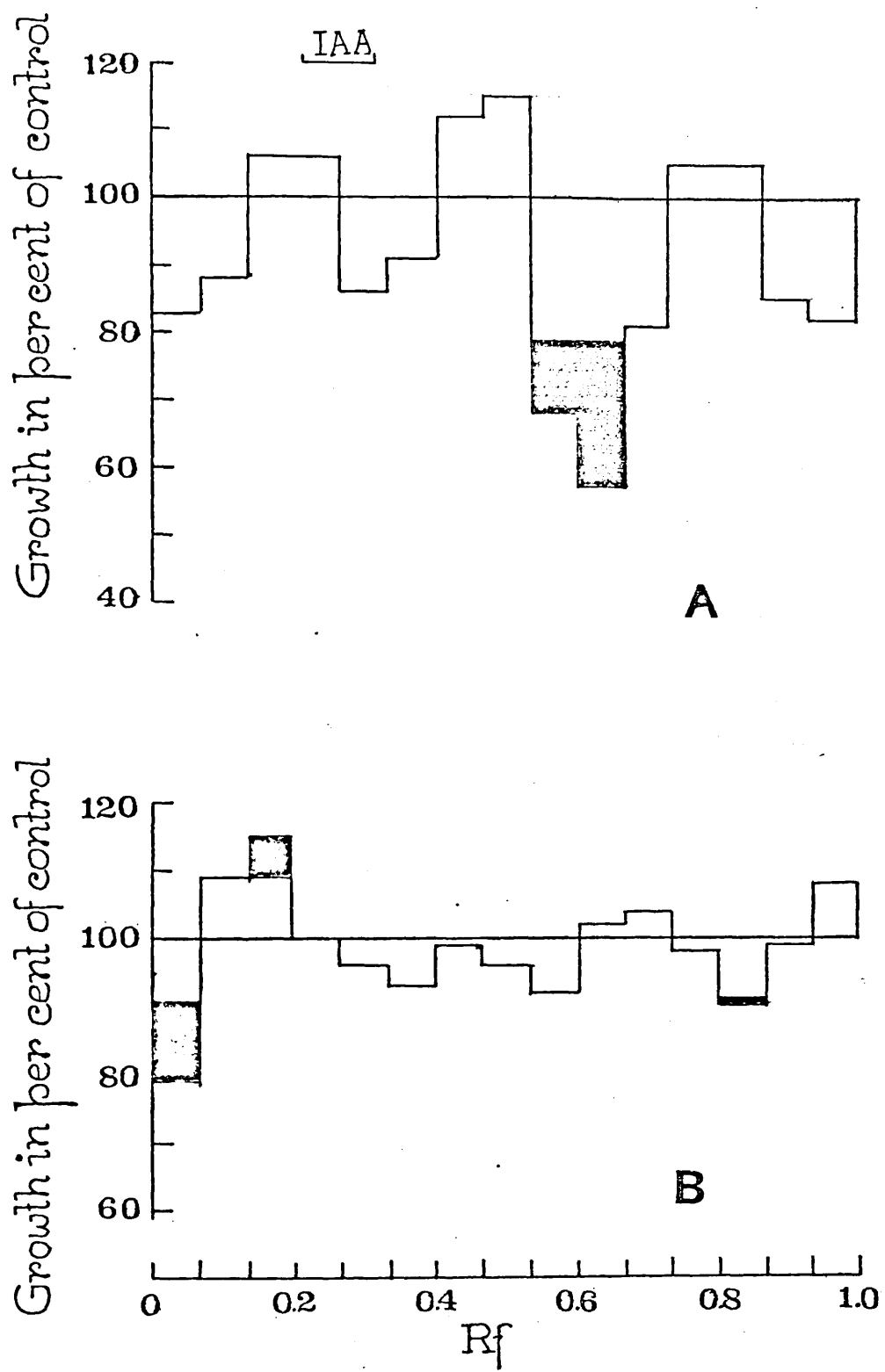


Fig.24(Contd.)

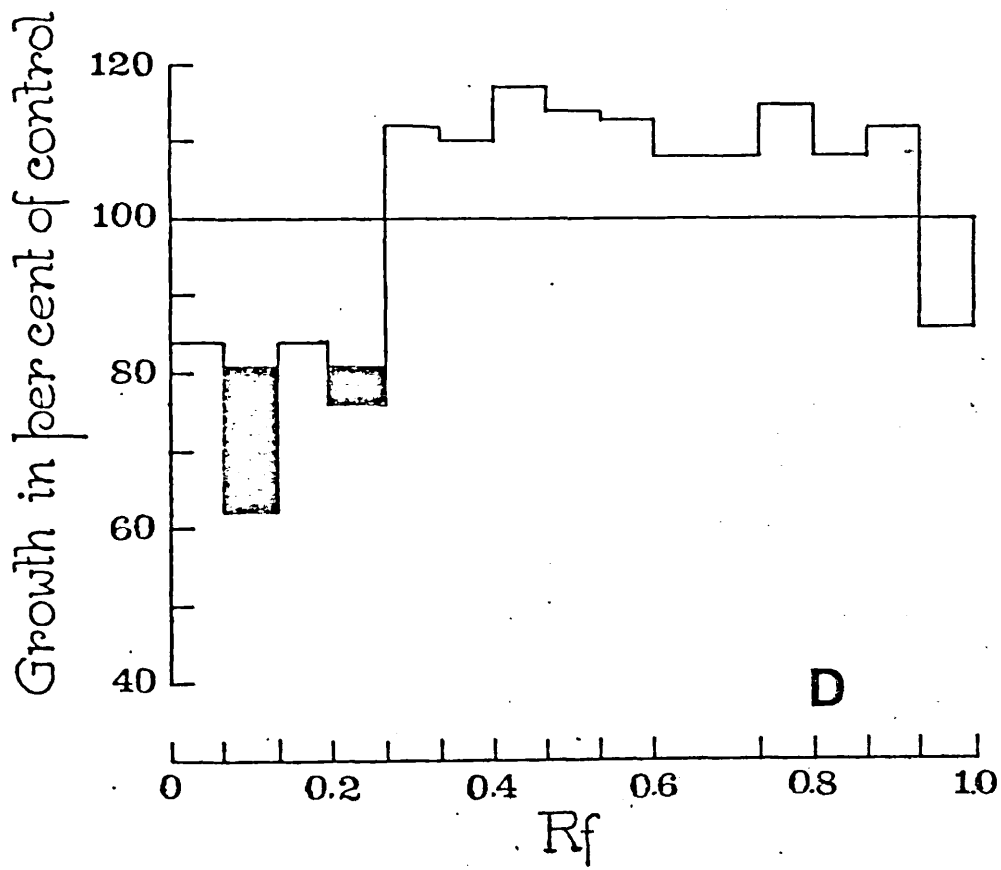
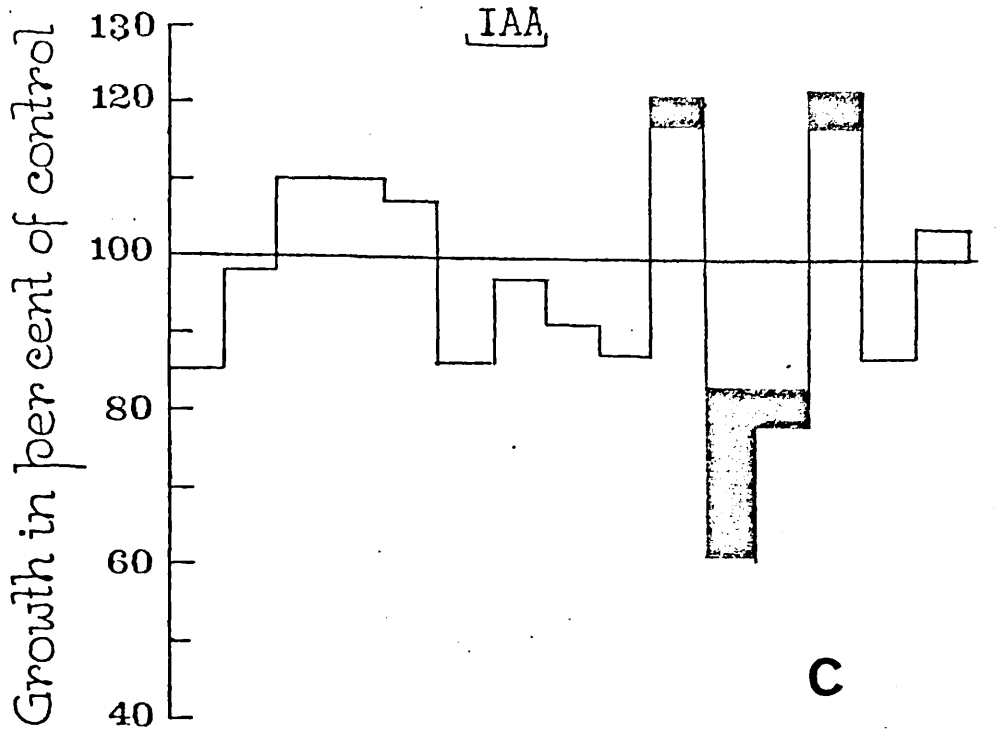
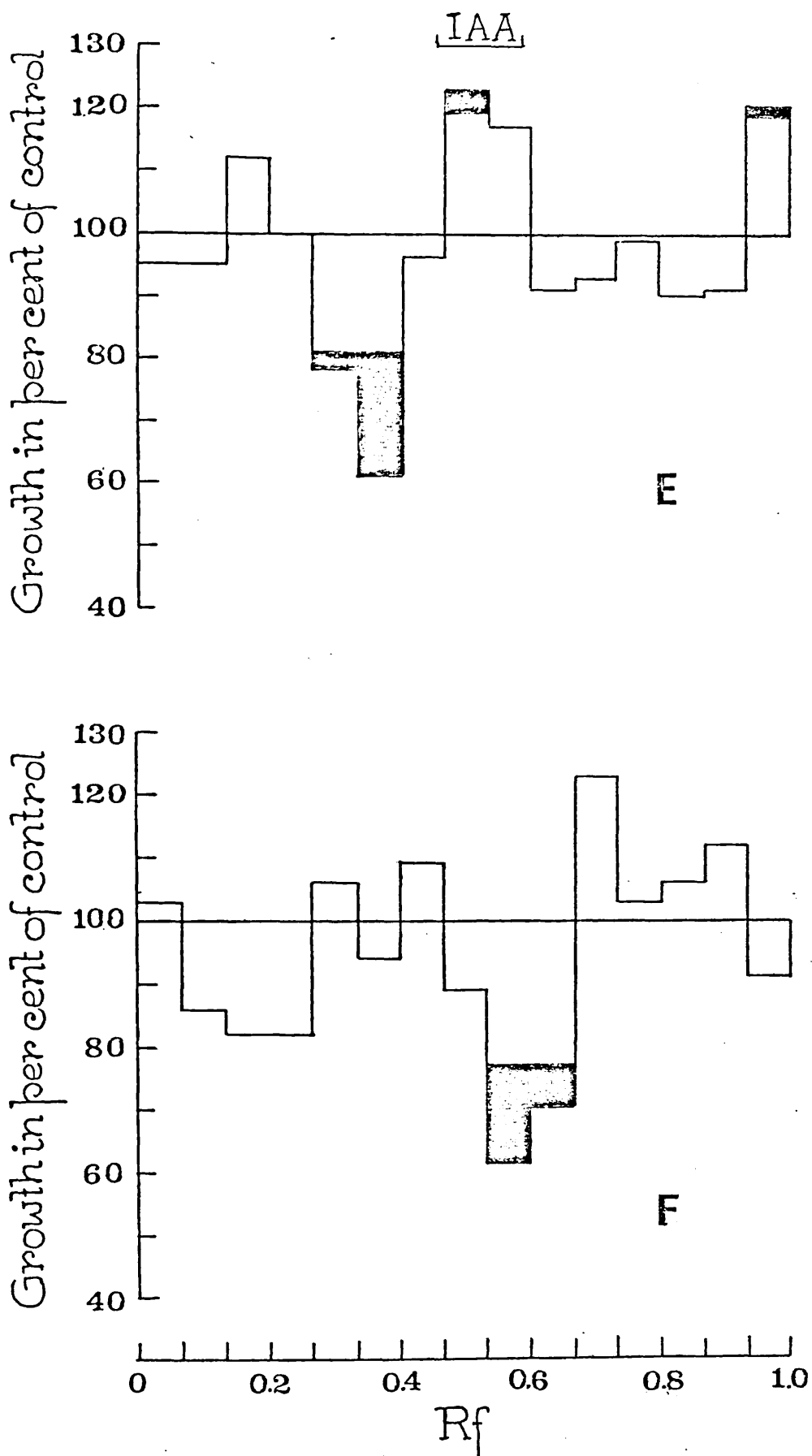


Fig.24(Contd.)



As mentioned earlier Torrey (1959) found a positive colour reaction with Salkowski's reagent in the region of inhibitor β on the chromatogram, but negative reaction was observed with Ehrlich's reagent. Along with this observation a close similarity of the Rf value of inhibitor β (Jenkins and Shepherd, 1972) with that of the 'cap inhibitor' it was suspected that the 'cap inhibitor' could possibly be inhibitor β .

Judging from the Rf alone it would not be wise to conclude anything about the occurrence of IAA on the chromatogram. It was observed that IAA could promote root growth in some species at concentrations of 10^{-5} to 10^{-4} parts/ 10^6 (Audus and Das, 1955). Here also occasional promotion as well as inhibition of growth in the position of marker IAA on the chromatogram tempted one to identify this substance as IAA. However, from its sporadic occurrence and low activity it could be concluded that IAA, if present, might be in a substantially low amount in the root tip.

Nothing like 'accelerator α ' was detected in any one of the chromatograms so far. Promotion of growth which was frequently observed around the Rfs of the 'cap inhibitor' and the 'inhibitor-3' might possibly be due to tailing of the inhibitor resulting in stimulation of growth at a low concentration as observed in the case of IAA.

At this stage the nature of the 'meristem inhibitor' was not known. No positive conclusion could be drawn whether this substance was one or more than one inhibitor overlapping in the same Rf of the chromatogram.

A summary of all the inhibitors in the root tip are presented in Table 1 below. Those which appeared inconsistently in the chromatogram are marked '*'.

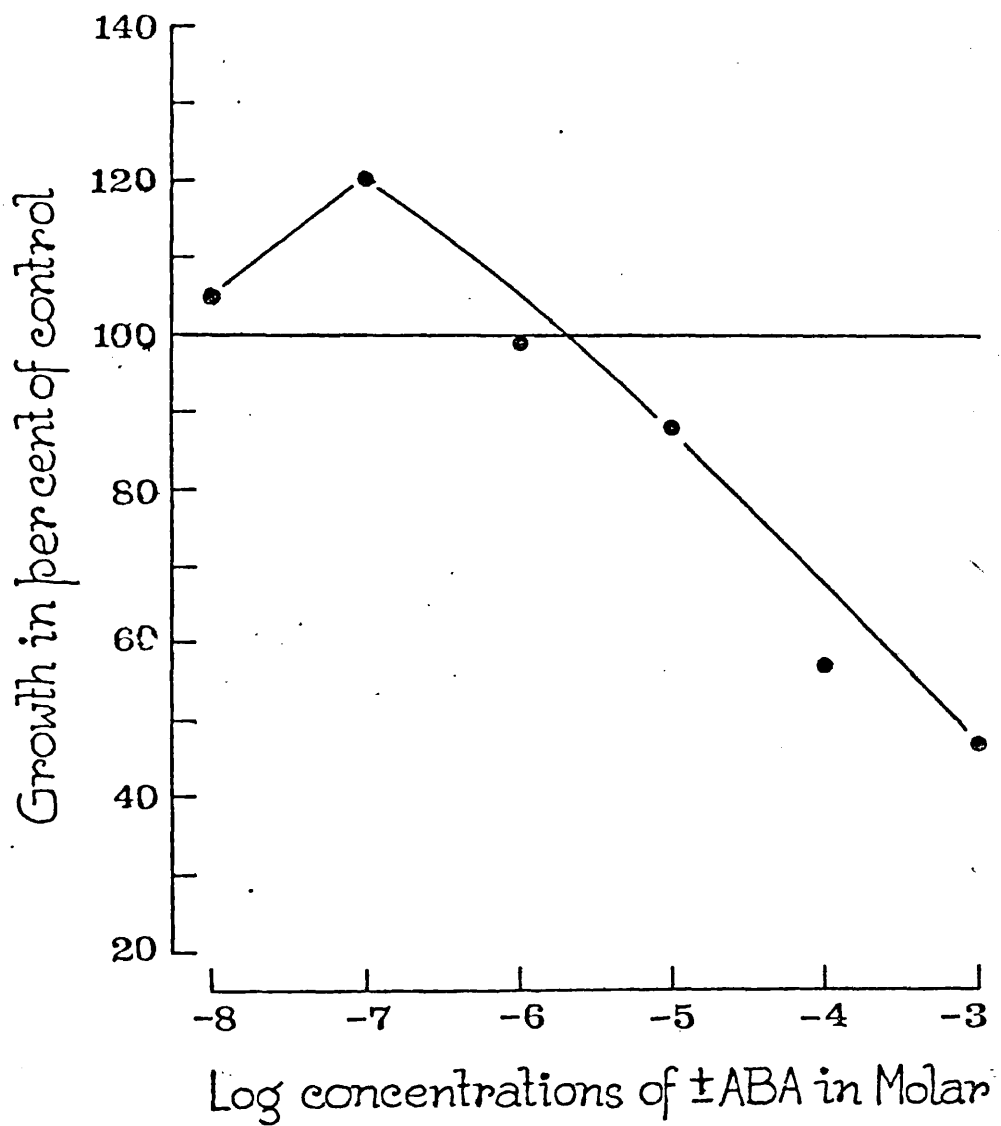
Table 1

Name	Rf in solvent I	Rf in solvent II	Rf in solvent III
Cap inhibitor	0.5-0.7	0.6-0.85	0.6-0.85
Meristem inhibitor	0-0.1	0.2-0.45	0-0.15
IAA-like substance*	0.2-0.3	0.4-0.5	0.35-0.4
Inhibitor-3*	0.8-1.0	0.8-1.0	0.9-1.0
Inhibitor-4A*	May be merged with the meristem inhibitor	0-0.1	May be merged with the meristem inhibitor
Inhibitor-4B	-	-	0.2-0.3

Since the main component of inhibitor β has been established to be abscisic acid (ABA) (Robinson and Wareing, 1964; Milborrow, 1967) and as the 'cap inhibitor', in this case, was more likely to be inhibitor β , the investigation was next directed towards the preliminary identification of the chemical nature of the 'cap inhibitor' with that of ABA. First the Rf value of authentic ABA was compared with that of the 'cap inhibitor'. For this, two separate chromatograms containing 5 μ l of 10^{-3} M \pm ABA (supplied by 'Sigma') were run in solvent I and II respectively. These two chromatograms were assayed and the activity of authentic ABA was found to be in the same position on the chromatogram corresponding to the 'cap inhibitor' (Fig. 26). Here also a significant stimulation of growth was observed at slightly higher Rf but close to the zone of inhibition further confirming that the tailing of the 'cap inhibitor' might possibly promote growth at the lower concentration as in IAA. A dilution response curve of ABA further supported this contention.

Fig. 25 Growth of maize root segments in different concentrations of \pm ABA. •

Fig.25



I. Result of dilution response curve of ABA

A series of dilutions of \pm ABA from 10^{-3} to 10^{-8} M were made in methanol. To overcome water solubility difficulties, the methanolic solutions were directly applied on squared filter paper (1 x 1 cm) which after removal of all solvents were used in the same way as in IAA. Papers similarly pretreated with methanol were employed as controls. The experiment was repeated on three occasions and the mean percentage of growth in each concentration has been brought together in Table 4 (Appendix).

A significant promotion of growth (about 120%) was obtained in the concentrations of 10^{-7} M ABA (Fig. 25). From this concentration a progressive increase in inhibition occurred and the growth fell by about 45% of the normal value at 10^{-3} M.

Thus a close similarity of growth activities of the authentic ABA and the 'cap inhibitor' prompted a more specific test for the detection of ABA-like substance in the root tip extract. For this stomatal-aperture assay (see page 44) was used.

J. Results of stomatal-aperture assay

Fig. 27 includes the results of the stomatal-aperture assay. Of the two figures on each occasion the top one represents the result of root segment assay and the bottom one for that of Commelina stomata-closure assay from the same root tip extract. Comparing the two assays it was evident that of the two major peaks of stomata-closure activity, obtained from the latter assay, one corresponded with the 'cap inhibitor' and the remaining one corresponded with the 'inhibitor-3'. These two peaks were exactly in the same Rfs corresponding with the root segment assay, indicating that these two inhibitors have similar antitranspirant properties. In Fig. 27 A the inhibition of the 'inhibitor-3' was not up to the

Fig. 26 Chromatograms of 10^{-5} molar equivalent of \pm ABA
(5 μ l) run in different solvents assayed by root
segment assay showing its relative position on
the chromatogram.

A. isopropanol:ammonia:water solvent

B. isobutanol:methanol:water solvent

Fig.26

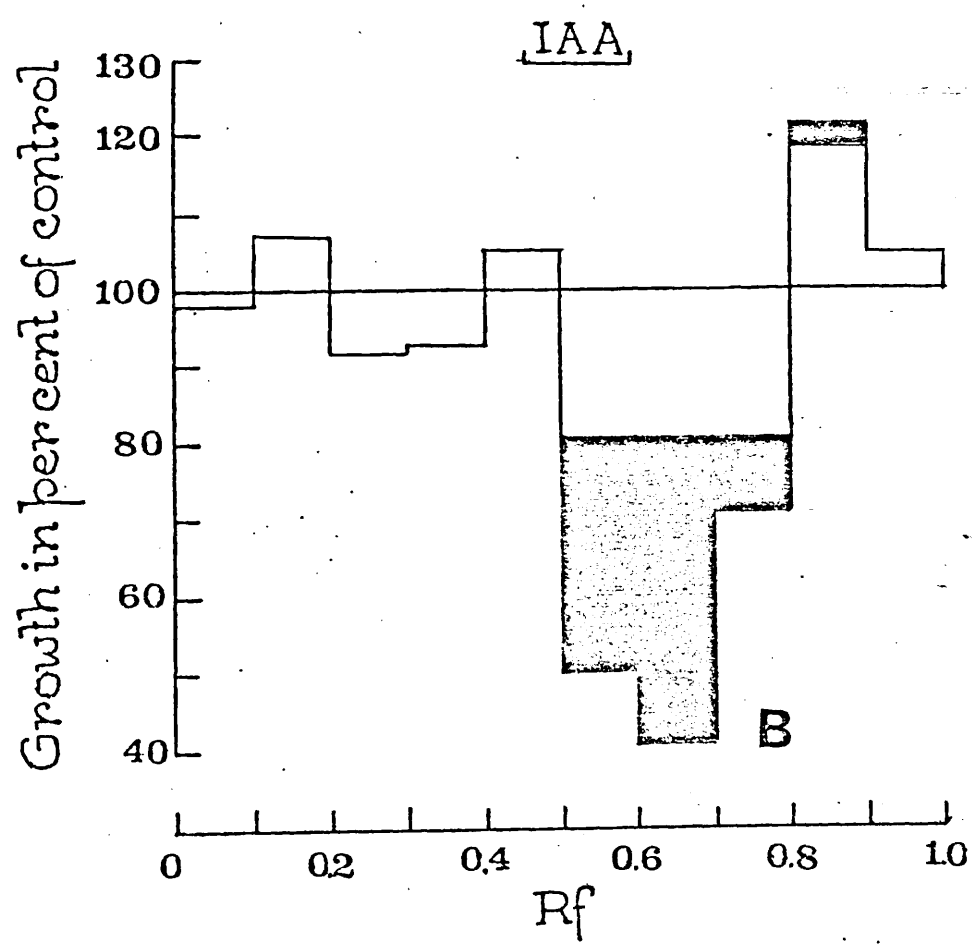
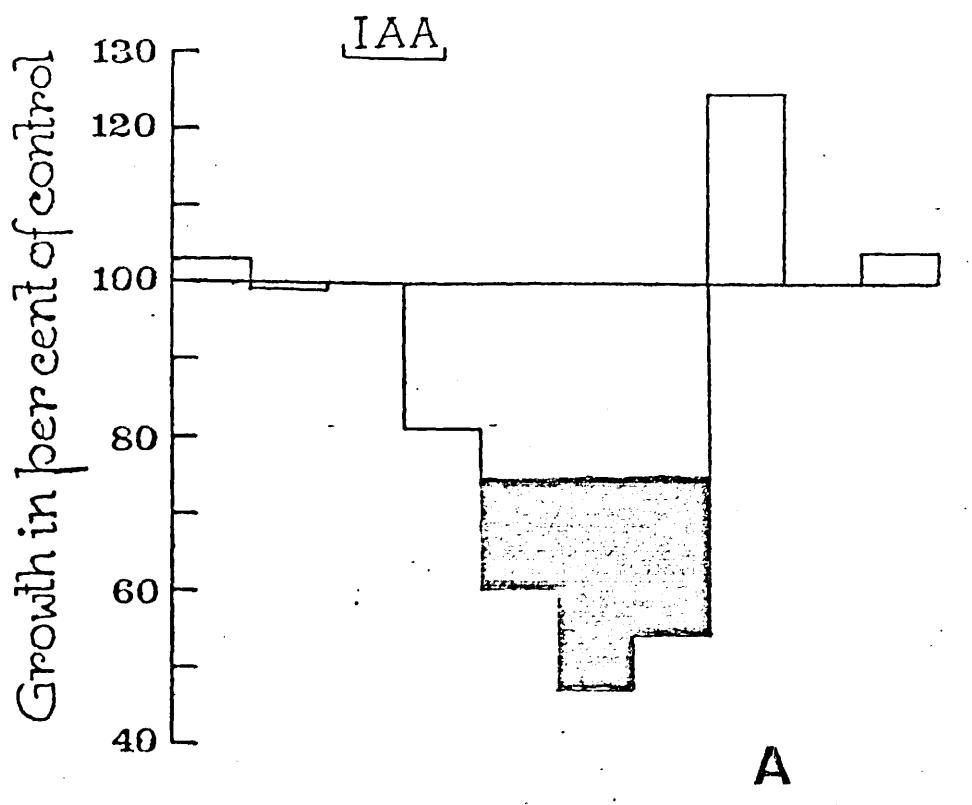


Fig. 27 Stomatal aperture assay of the chromatograms of the acid ether fraction. Extract of two hundred root tips has been applied on the chromatogram and half of the extract has been assayed by the root segment assay while the corresponding other half has been assayed by stomatal aperture assay (running solvent - isopropanol:ammonia:water).

A-C Root segment assays on three occasions.

A'-C' Corresponding stomatal aperture assays on three occasions showing the peaks of stomata closure activities on the chromatogram.

Fig.27

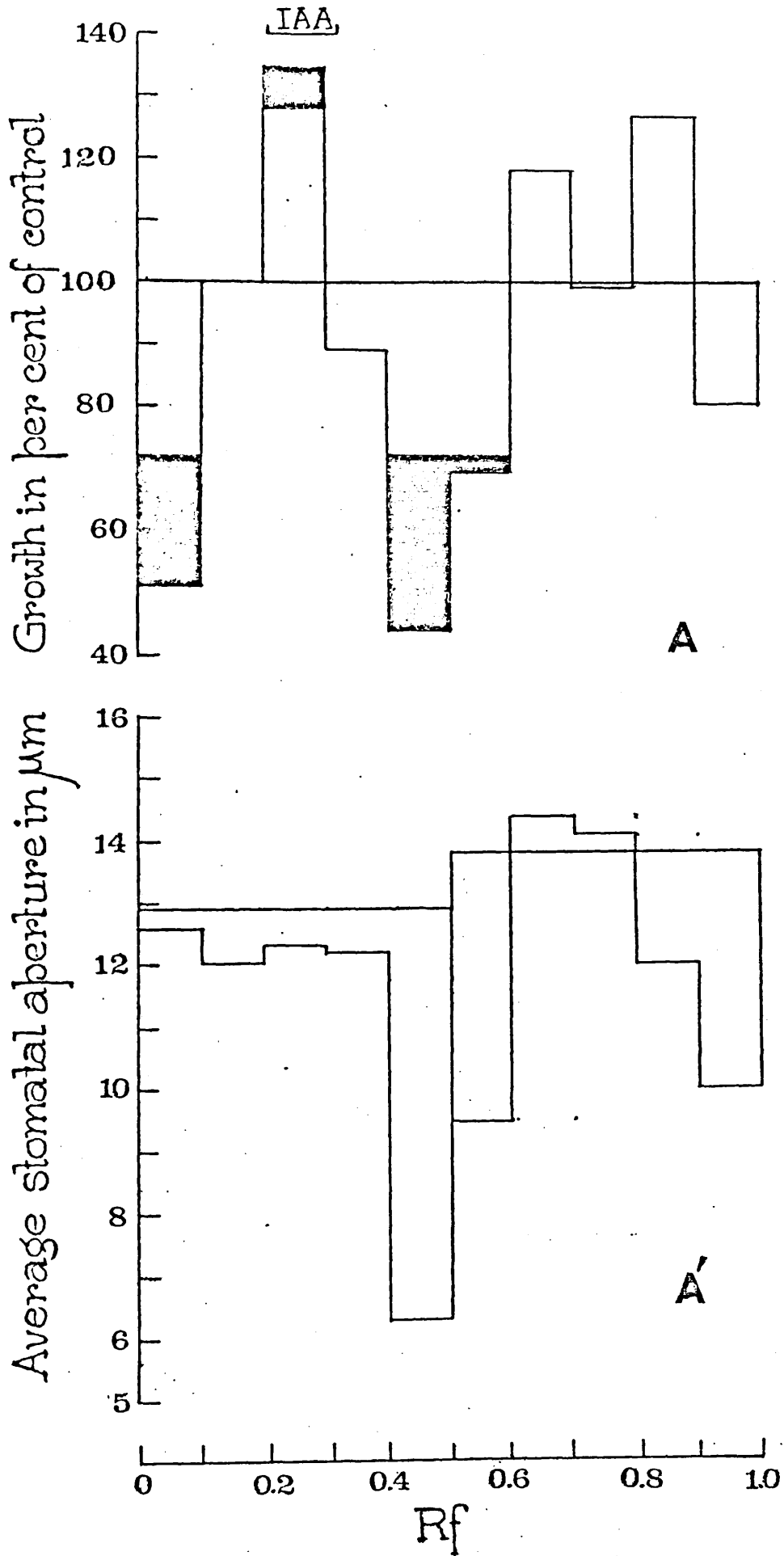


Fig.27(Contd.)

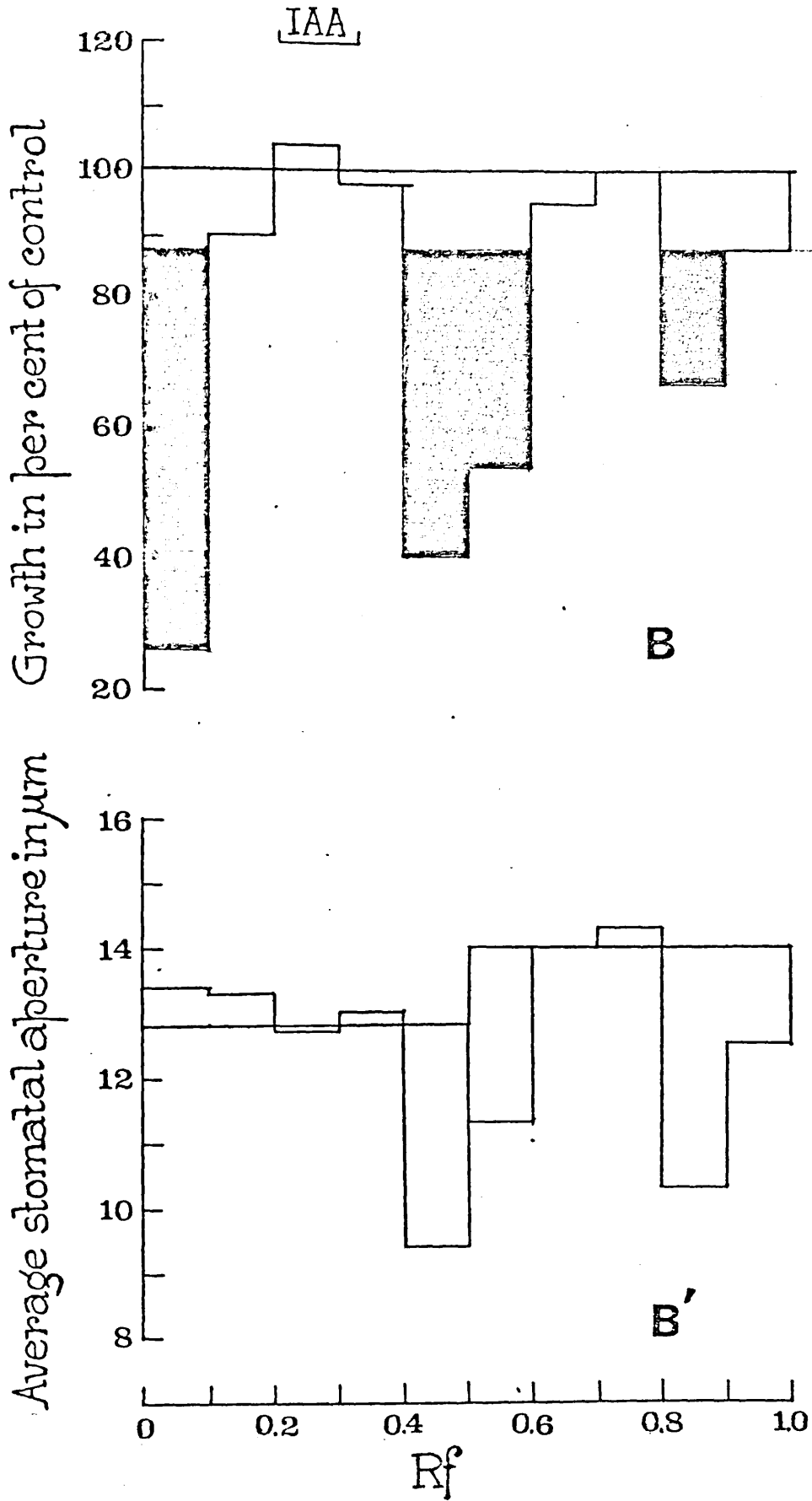
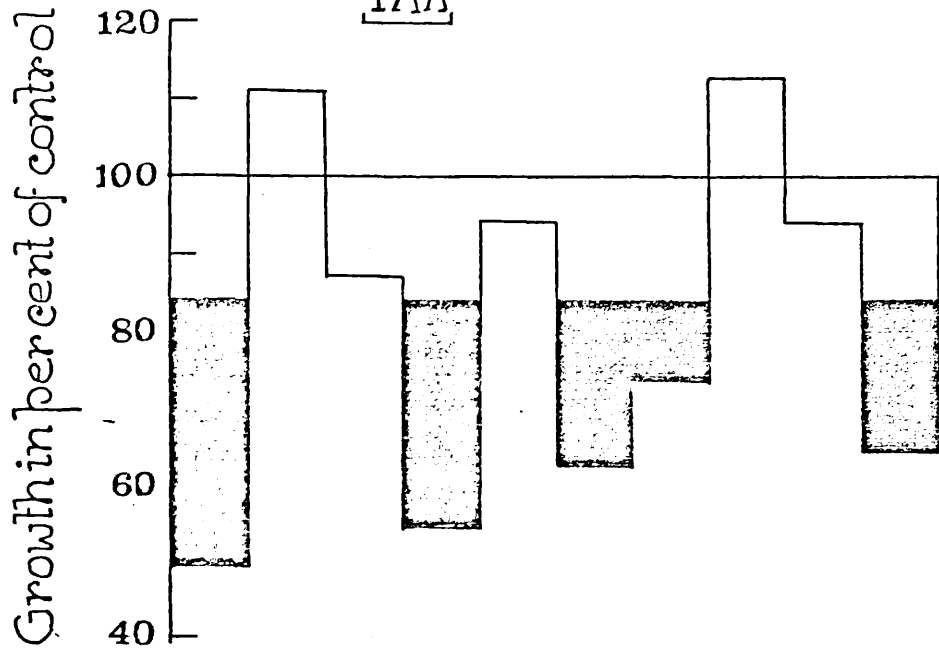
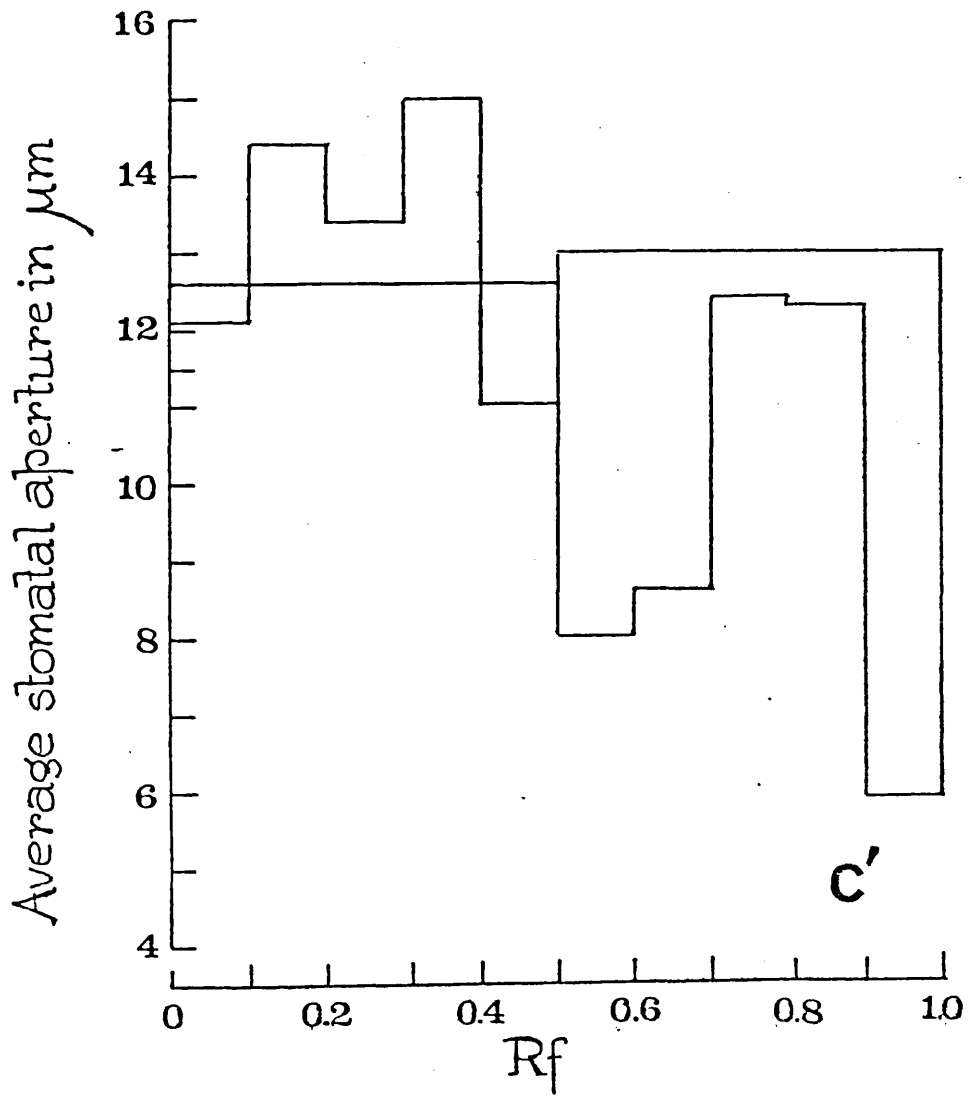


Fig.27 (Contd.)

IAA



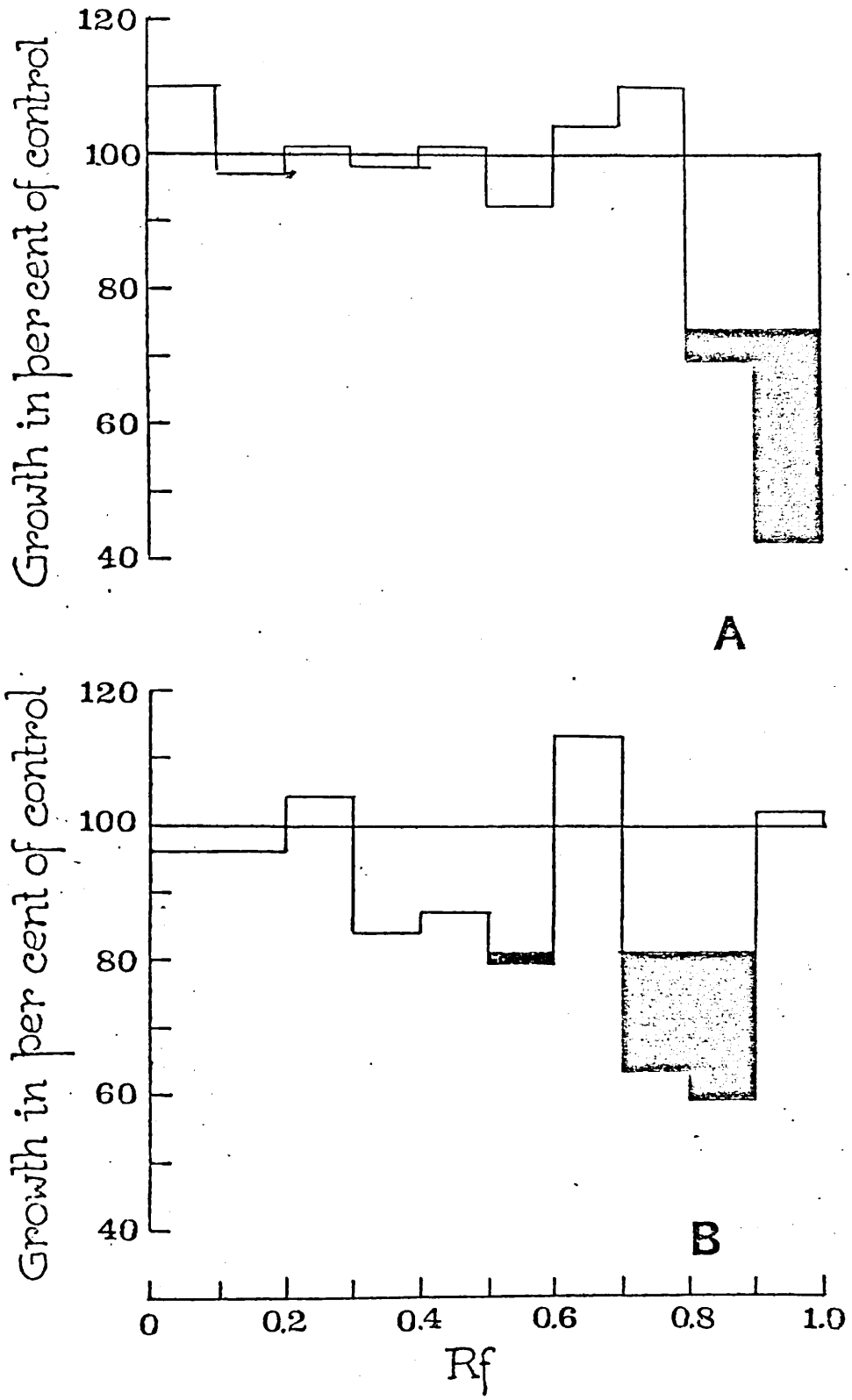
C



C'

Fig. 28 Assay of chromatograms of authentic xanthoxin
(10 μ l of 1%) in different solvents showing
Rf value. A. isopropanol:ammonia:water
solvent, B. isobutanol:methanol:water solvent.

Fig.28



limits of confidence but here too, the presence of the same was indicated by the stomata-closure activity. This was probably due to greater sensitivity of the latter assay.

Of these two inhibitors, having antitranspirant properties, one corresponding to the Rf of the 'cap inhibitor' may very well be ABA itself. The identity of the other substance in the relevant Rf of the 'inhibitor-3' was speculative at this stage.

Xanthoxin, a neutral growth inhibitor and a close relative of ABA had been reported to have an Rf value of 0.85 in solvent I (Taylor, 1967) which was very close to Rf 0.9-1.0 for this unknown compound. This substance has been found in a number of plant species and occurs at concentrations similar to concentrations of ABA (Firn et al., 1972).

So far as the Rf value was concerned authentic xanthoxin* appeared in a similar position on the chromatogram corresponding to the 'inhibitor-3' (Figs. 28 A and 28 B) which was between Rf 0.8-1.0 in both solvents. However, being a neutral inhibitor it was found desirable to analyse the neutral ether fraction of the extract before any further characterization of this active substance, apparently suspected as being xanthoxin, was made.

Analysis of the neutral ether fraction in the methanolic extract of root tips:

Like acid ether fraction, no consistent picture was obtained when the neutral ether fraction of the root tip extract was assayed. The results of six individual assays and the composite diagram derived from them were presented in Fig. 29. As shown from the

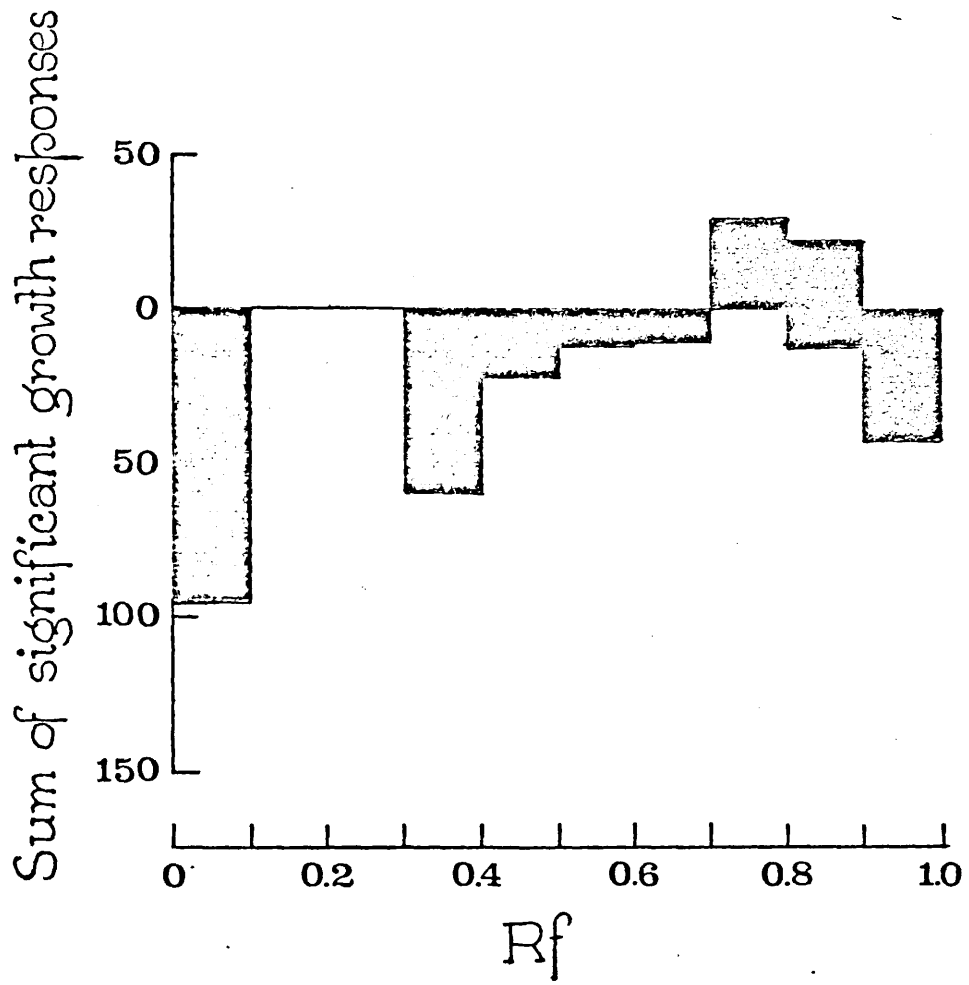
* a generous gift from Dr. H.F. Taylor

Fig. 29 Chromatograms of neutral ether fraction of the extract of the root tips (5 mm). One third of the total extract of a hundred root tips has been assayed (running solvent - isopropanol: ammonia:water).

A. Composite diagram from six separate chromatograms.

B-G Individual chromatograms from each occasion.

Fig. 29



A

Fig.29(Contd.)

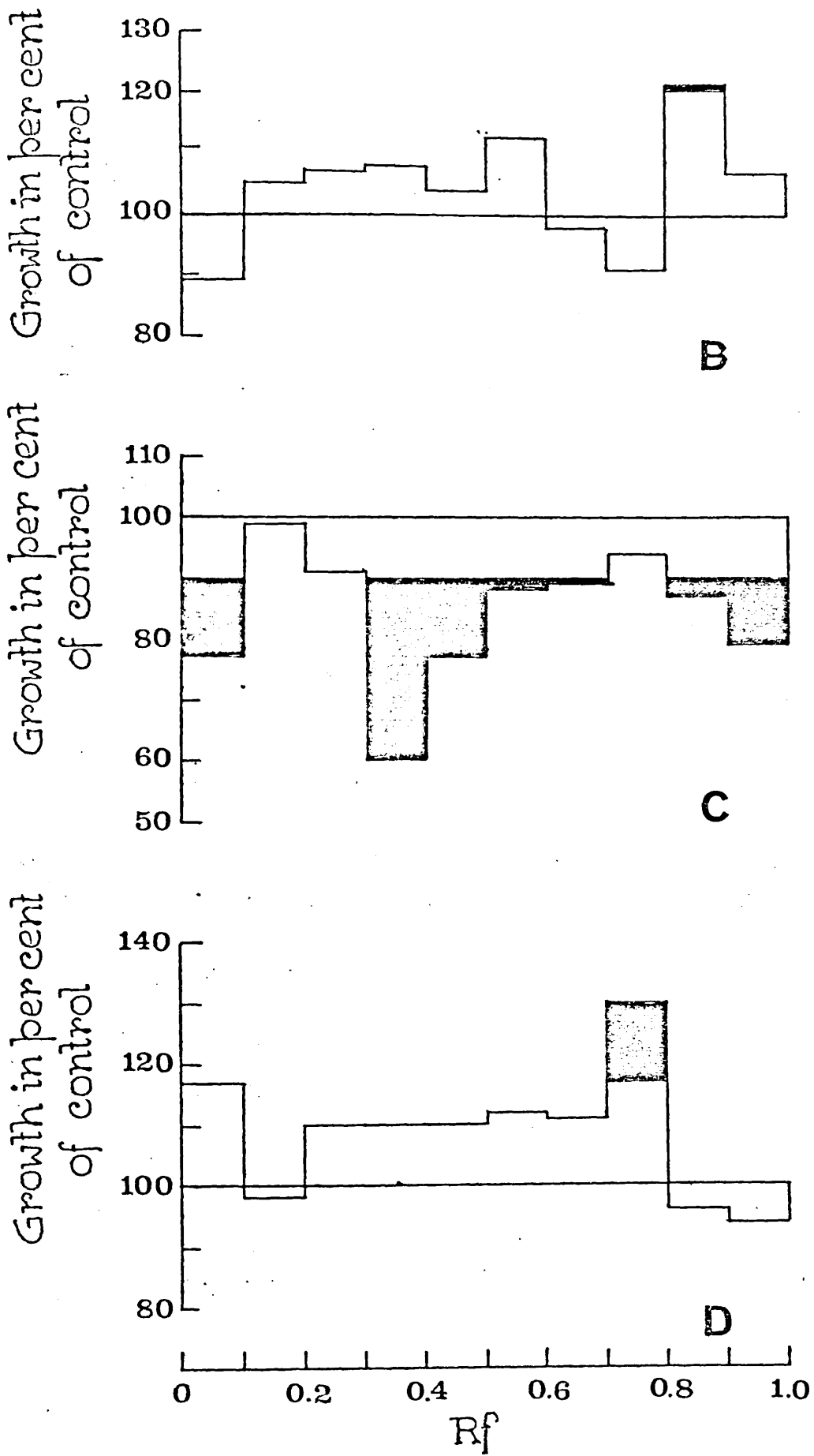


Fig.29 (Contd.)

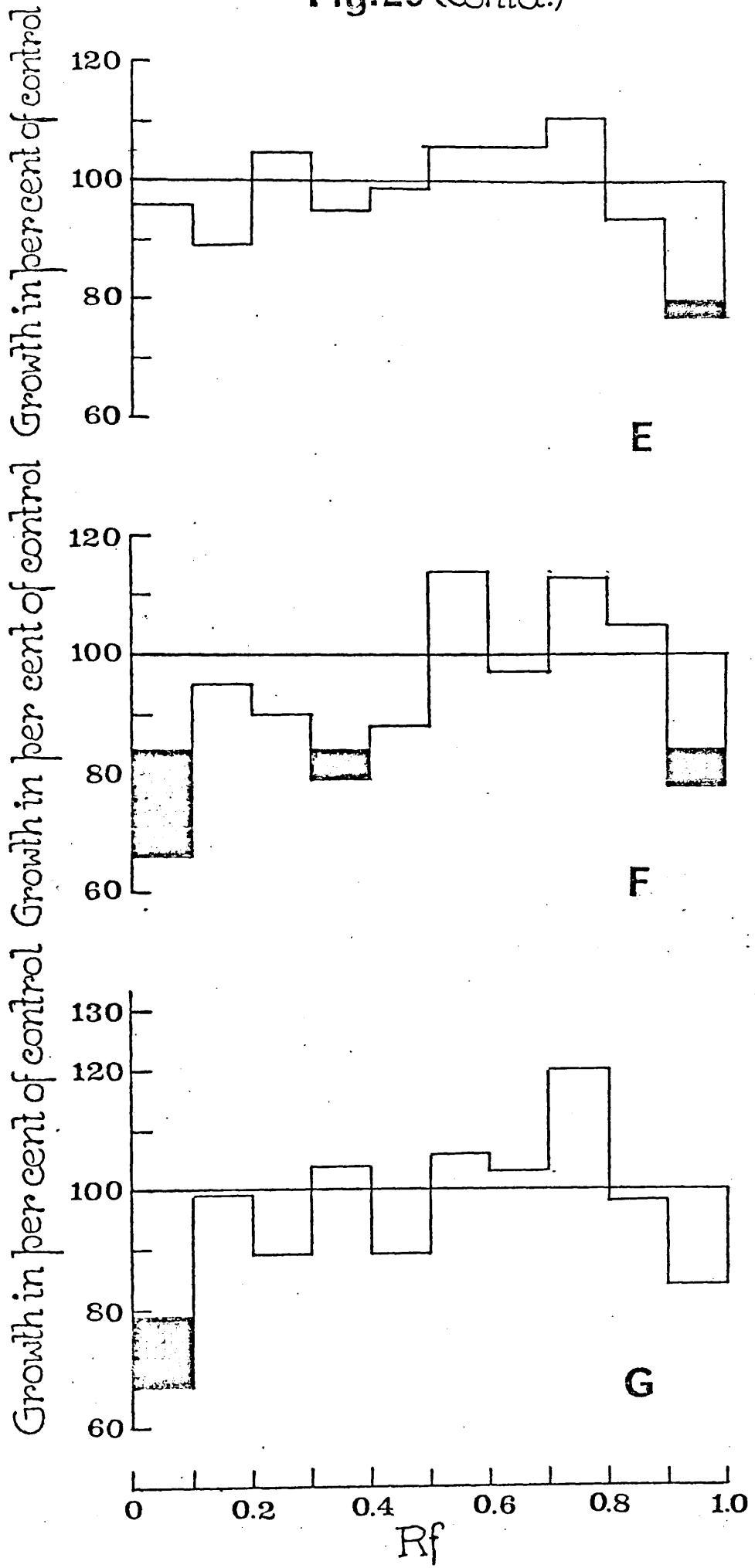
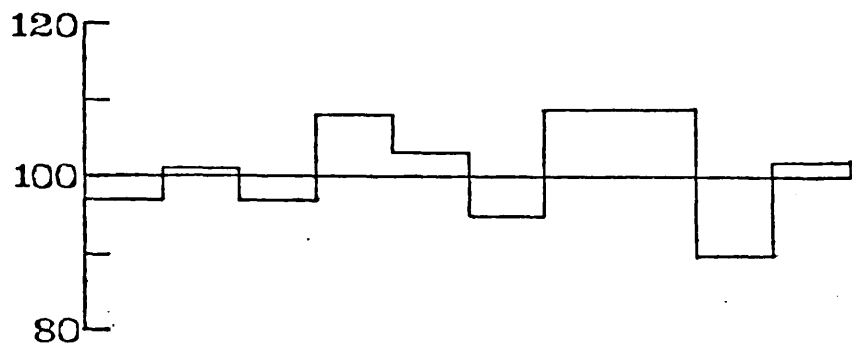


Fig. 30 A and B chromatograms of neutral ether fraction of the extract of a hundred root caps showing the absence of any significant growth active regions. One third of the total extract has been assayed. Running solvent - isopropanol: ammonia:water. Fiducial limits are 19.79 and 6.58 per cent in the case of A and B respectively.

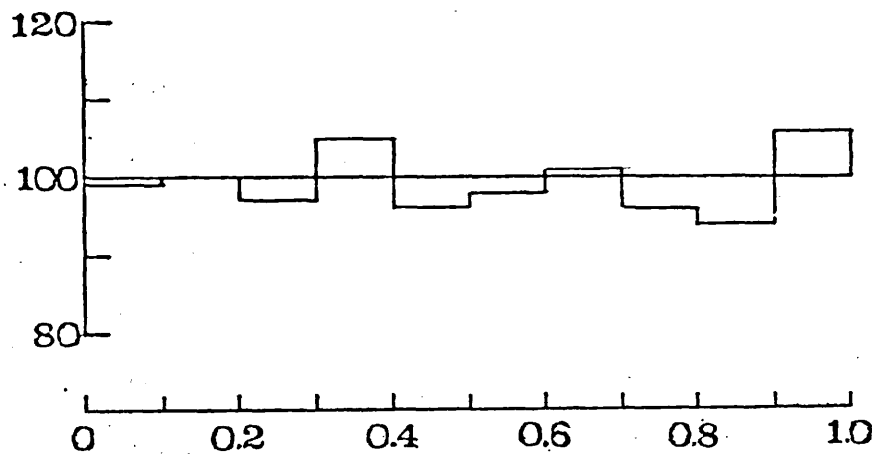
Fig.30

Growth in per cent
of control



A

Growth in per cent
of control



Rf

B

composite diagram, three active zones were present in the chromatogram of the neutral ether fraction. The first active zone, due to an inhibitor, was restricted to the origin of the chromatogram between Rf 0-0.1. This inhibitor appeared in three out of six extracts (Figs. 29 C, 29 F and 29 G). The second active zone fell between Rf 0.3-0.7. This substance also inhibitory to root segment growth, appeared only on two occasions (Figs. 29 C and 29 F). The third active zone appeared more or less regularly (five out of six extracts) in the solvent front between Rfs 0.7-1.0. Here inhibition as well as promotion of root segment growth was obtained in varying amounts. None of these substances were found to be present in the root cap (Figs. 30 A and 30 B).

If xanthoxin is expected to be present in the neutral fraction, its probable position in the chromatogram would be between Rf 0.7-1.0. To find out whether this active zone corresponds with that of the 'inhibitor-3' in the acid ether fraction, a similar stomatal-aperture assay was carried out with the neutral ether fraction. The result presented in Fig. 31 was conclusive. On both occasions distinct peaks of stomata-closure activity were obtained at Rfs 0.7-1.0 corresponding to their inhibitory activity in the root segment assays thus indicating the identity of this neutral inhibitor with that of the 'inhibitor-3'. This substance could be xanthoxin, though the antitranspirant property of xanthoxin, upto the time of writing, was not clearly established.

In one of the stomatal aperture assays (Fig. 31 B) a considerable amount of stomata-closure activity was found in the Rf 0.4-0.5. Also in the corresponding root segment assay a little, but significant inhibition, was found in the same Rf. This might be the identical substance with that of the 'cap inhibitor'. If this happened to be positive then of the three inhibitors in the neutral fraction, two

Fig. 31 Stomatal aperture assay of the chromatograms of neutral ether fraction. Extract of two hundred root tips has been applied on the chromatogram and half of the extract has been assayed by root segment assay while the corresponding other half has been assayed by stomatal aperture assay (running solvent - isopropanol:ammonia:water).

A and B Root segment assays on two occasions.
A' and B' Corresponding stomatal aperture assays two occasions showing the peaks of stomata closure activities on the chromatogram.

Fig.31

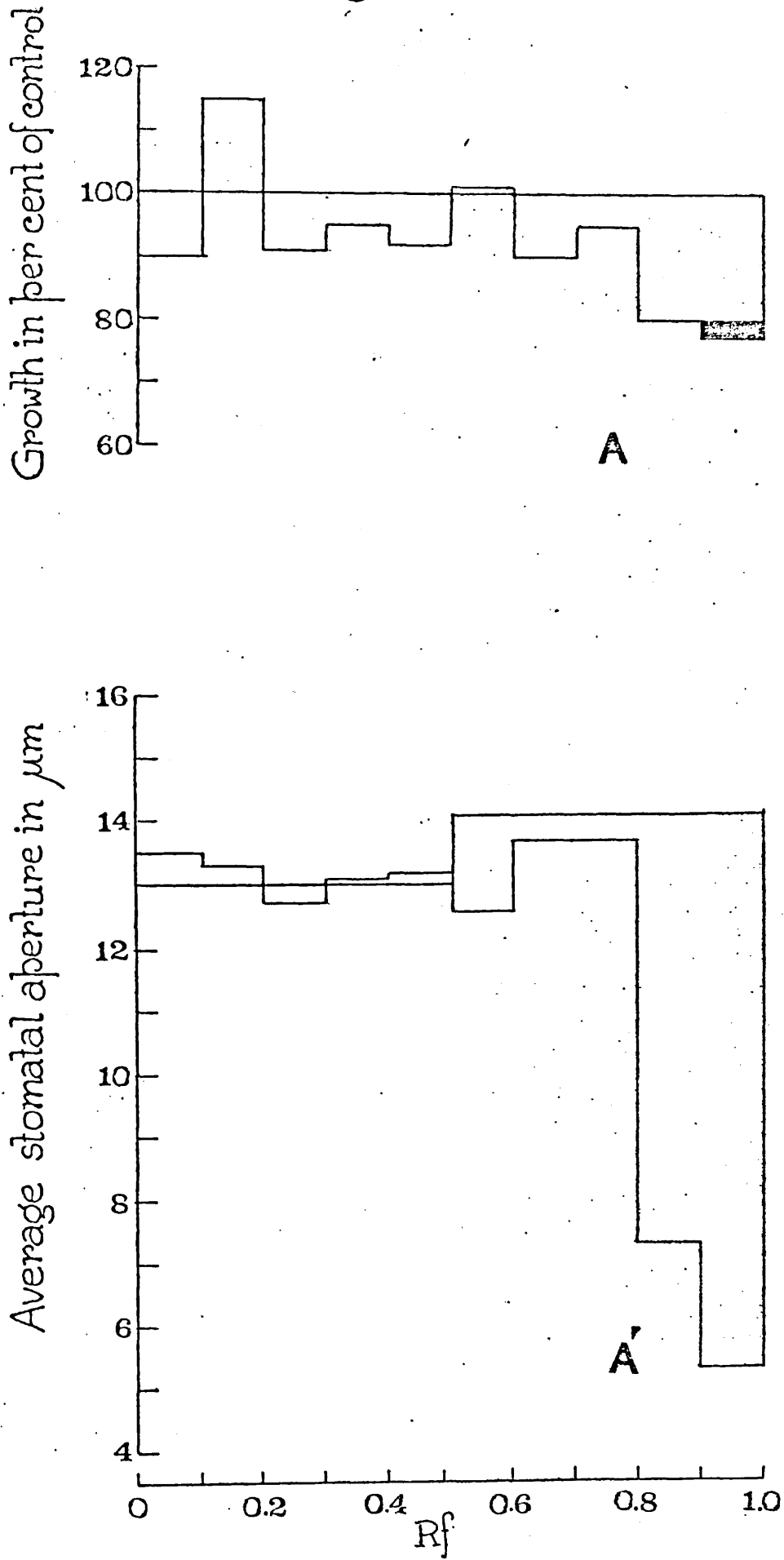
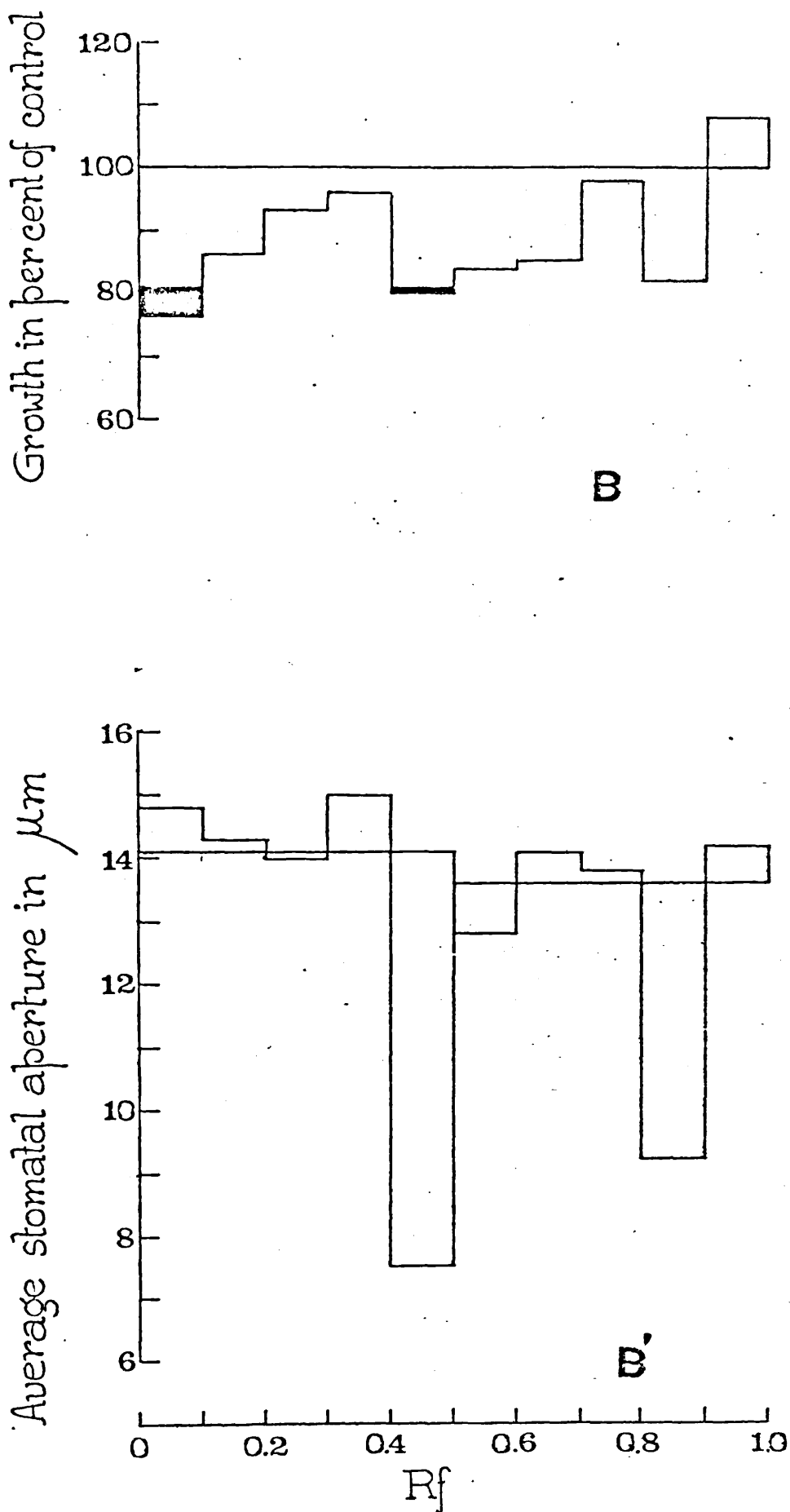


Fig.31 (Contd.)



of them (one between Rf 0.4-0.7 and the other between Rf 0.7-1.0), may be identical with that of the 'cap inhibitor' and the 'inhibitor-3' respectively. Their appearance in both the fractions was possibly due to incomplete separation of these fractions. The occasional inhibitory activity in the origin of the chromatogram might also be due to the presence of the 'meristem inhibitor', which appeared in the neutral fraction owing to similar incomplete partitioning of two ether fractions. However, it is also possible that this inhibition was partly due to an oily substance (shown by a greasy halo around the initial spot) as observed by Thresh (1956). This was not present in the acid extract.

Due to small qualitative differences in the growth substances in the acid and neutral fractions obtained so far and at the same time assuming a possible loss of growth substances during partitioning of these two fractions the final investigation was performed to assay the primary ether fraction (before partitioning) of the extracts of the cap as well as serial 2 mm segments, stretching back proximally from the cap/meristem region as before. This investigation was found to be necessary to give a comprehensive account of all the ether soluble inhibitors present in the root tip. Assay of each root segment, as well as each root cap sample was repeated on three consecutive occasions. As shown below a clear and more or less consistent picture was obtained in each case.

Analysis of the primary ether fraction in the methanotic extract of different root materials:

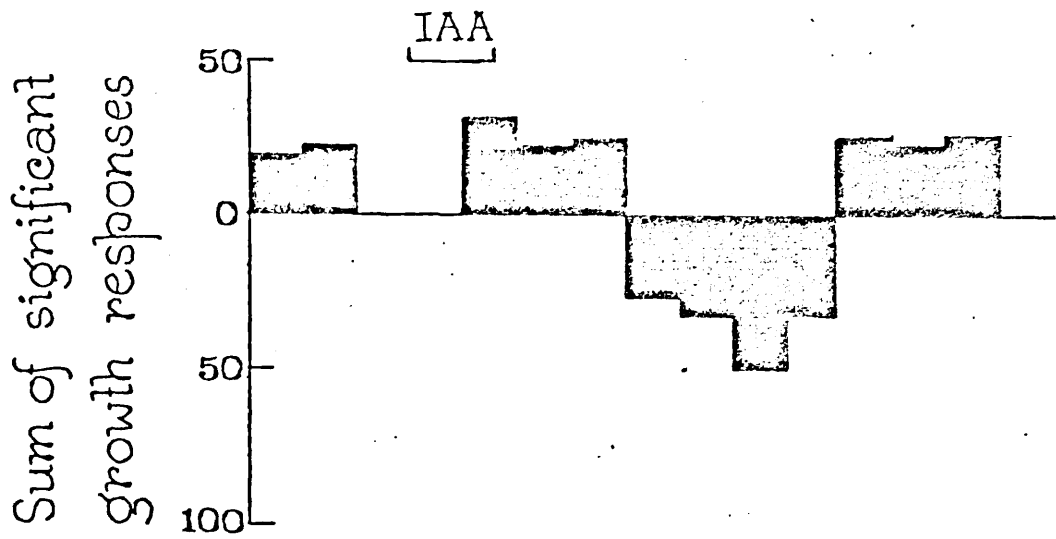
A. Root cap

Fig. 32 represents the composite diagram (A) and a typical chromatogram (B) respectively. On all of the three observations, only the 'cap inhibitor' was found to be present and this was in

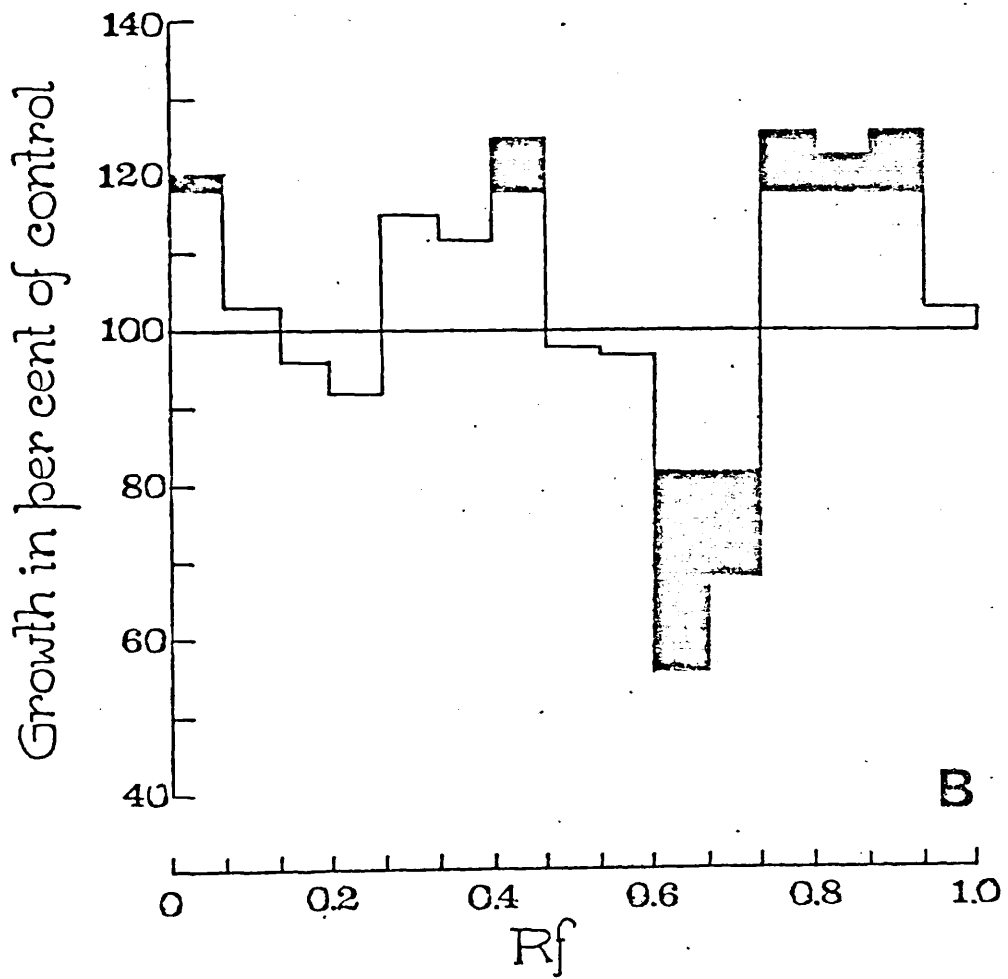
Fig. 32 Chromatograms of the primary ether fraction of
the extract of root caps (100) (running solvent
- isopropanol:ammonia:water). The entire
amount of the extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.32



A



B

accordance with the previous observations. Here, on two occasions stimulation of growth was found in the Rf of the 'meristem inhibitor' and that of IAA. Similar promotion of growth was also observed only on one occasion in the region of the 'inhibitor-3'. Promotion of growth of these inhibitors at low concentrations might possibly be due to contamination of the 'meristematic tissue'.

B. First 2 mm segment

In the first 2 mm segment next to the cap the 'cap inhibitor' was present almost in the same amount as shown in Fig.33. The 'meristem inhibitor' appeared in only one extract whereas IAA appeared on two occasions. Occasionally stimulation as well as inhibition were found to occur in the Rf of the 'inhibitor-3'. Another inhibitor appeared more or less constantly between Rf 0.2-0.3. This substance might be equivalent to the 'inhibitor-4' in solvent III.

C. Second 2 mm segment

Here the 'meristem' and the 'cap inhibitor' were present in all three observations, but the activity of the latter inhibitor was comparatively less than that of the previous segment (Fig. 34). The 'inhibitor-3' also appeared in all the extracts. No activity was found in the Rf of IAA whereas slight inhibition was observed once in the position of the 'inhibitor-4'.

D. Third 2 mm segment

In this segment the activity of the 'meristem inhibitor' was found to be maximal (Fig. 35). This inhibitor was present in considerable amounts. On the other hand, the activity of the 'cap inhibitor' was comparatively low, though this inhibitor was found to be present in all three extracts. IAA and the 'inhibitor-3' appeared sporadically, but the 'inhibitor-4' was totally absent.

- Fig. 33 Chromatograms of the primary ether fraction of the first 2 mm segments (100) next to the root cap (running solvent - isopropanol:ammonia:water). The entire amount of the extract has been assayed.
- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.33

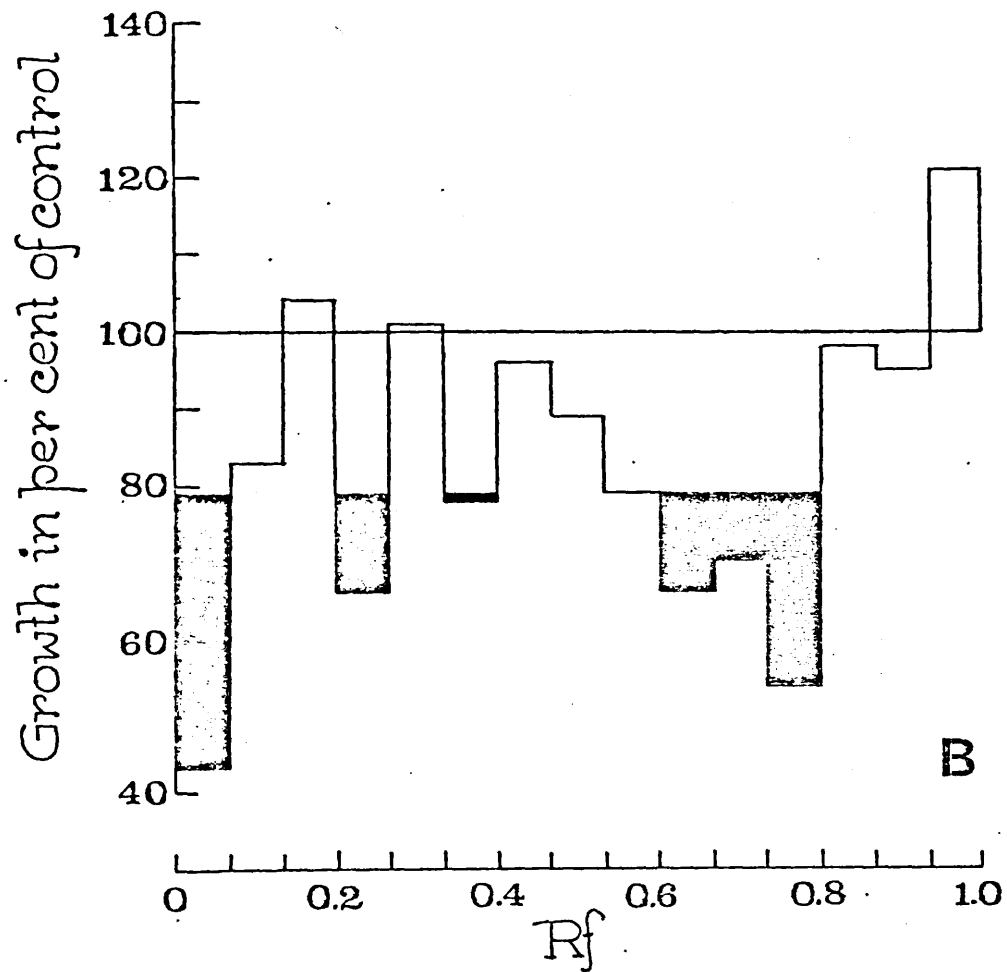
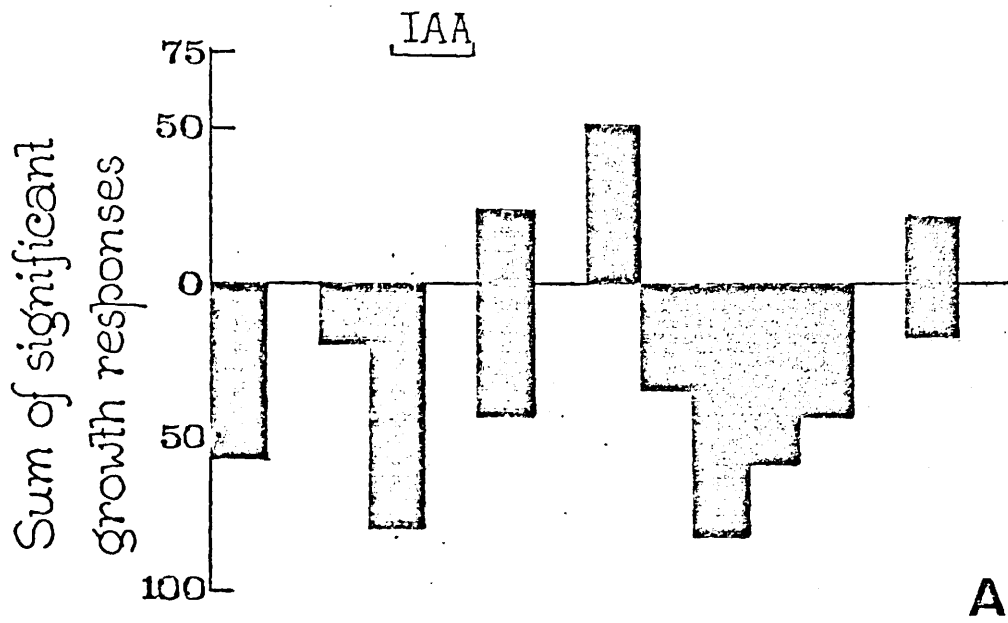


Fig. 34 Chromatograms of the primary ether fraction of the extract of the second 2 mm segments (100) next to the first segment (running solvent - isopropanol:ammonia:water). The entire amount of the extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.34

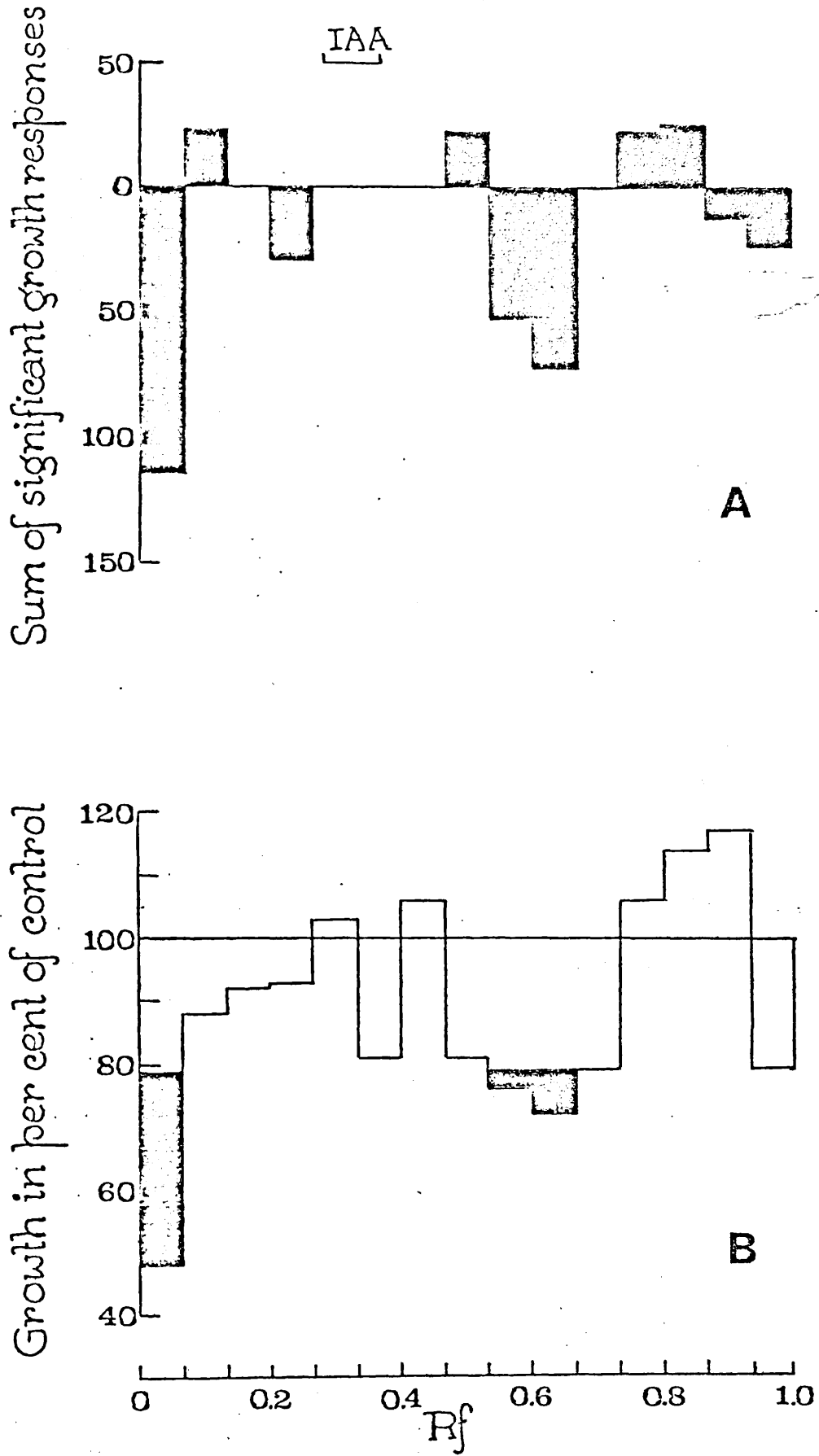


Fig. 35 Chromatograms of the primary ether fraction of the extract of the third 2 mm segments (100) next to the second segment (running solvent - isopropanol:ammonia:water). The entire amount of the extract has been assayed.

- A. Composite diagram from three separate chromatograms.
- B. A typical chromatogram from one of the three occasions.

Fig.35

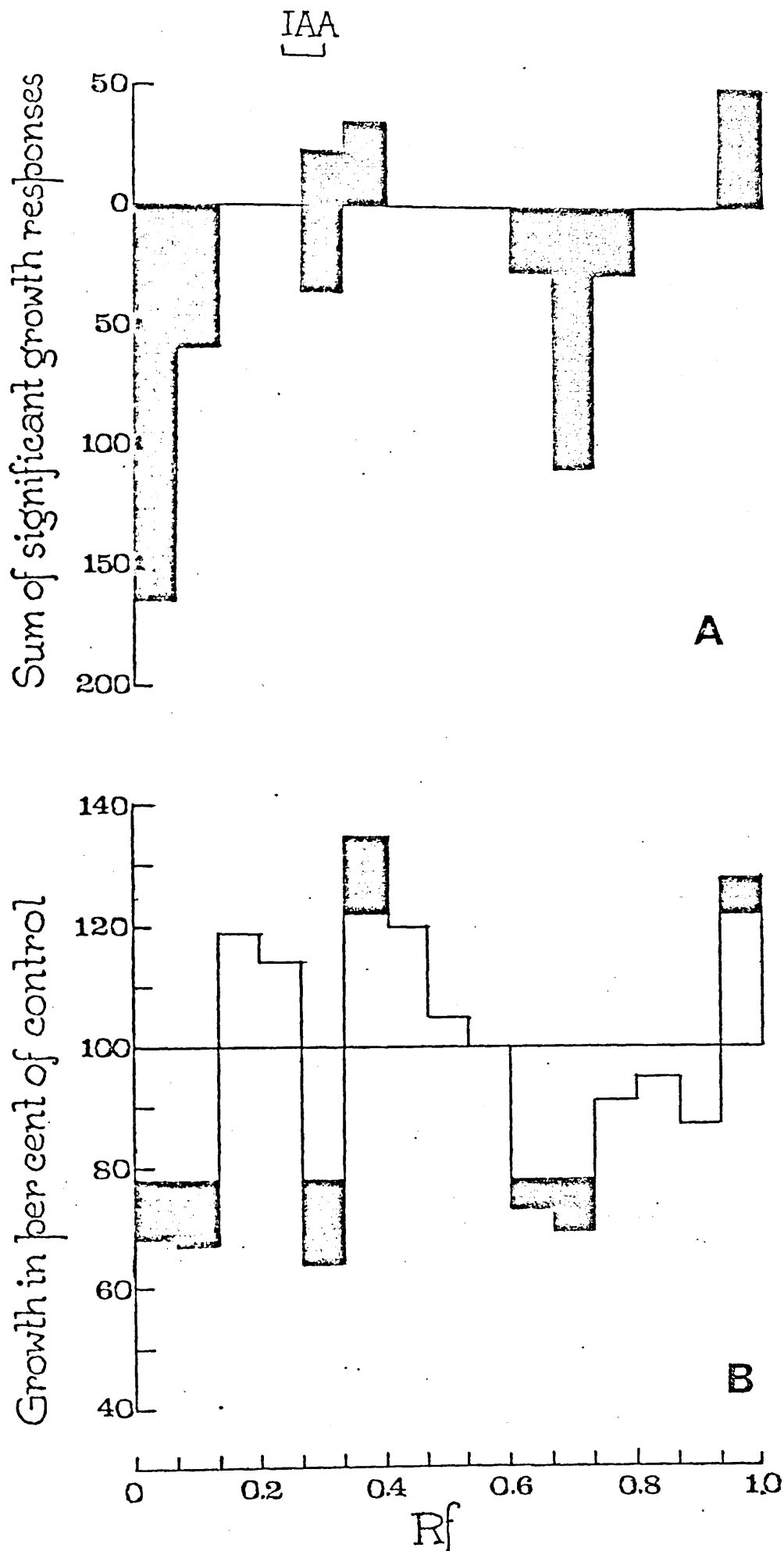
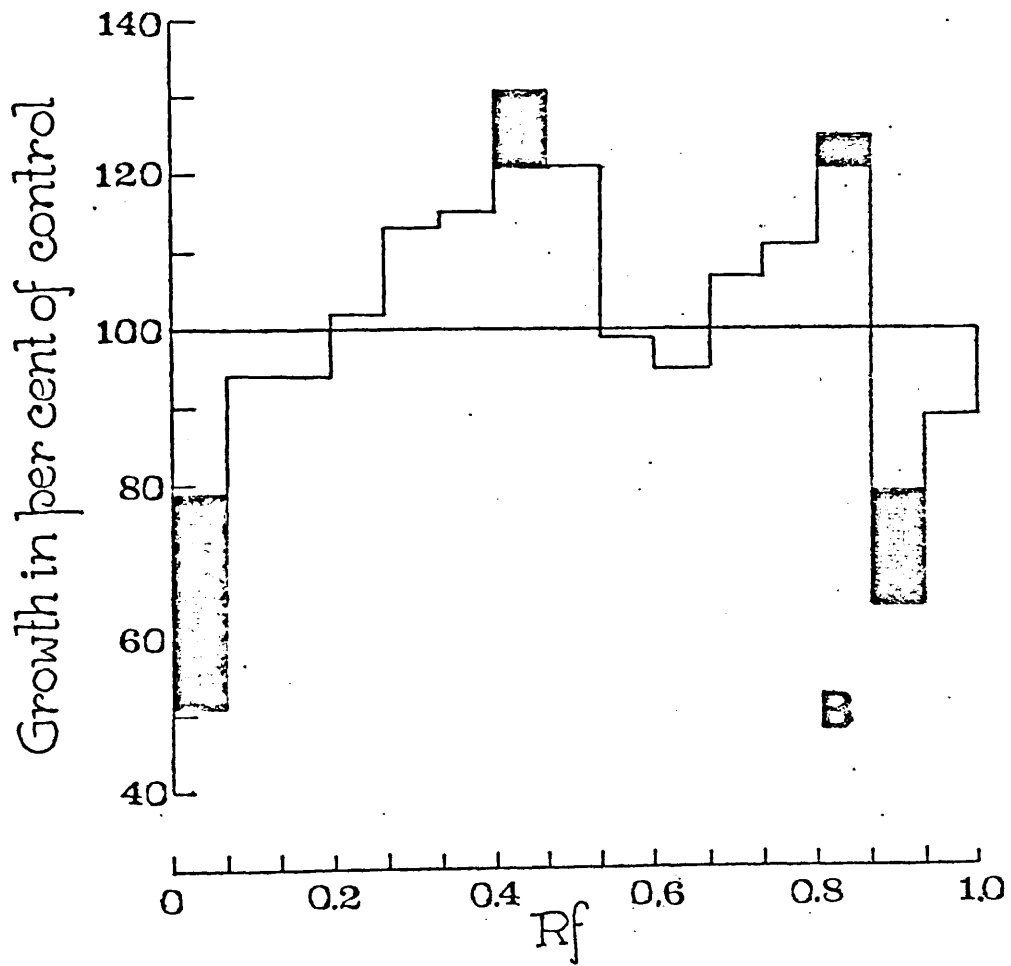
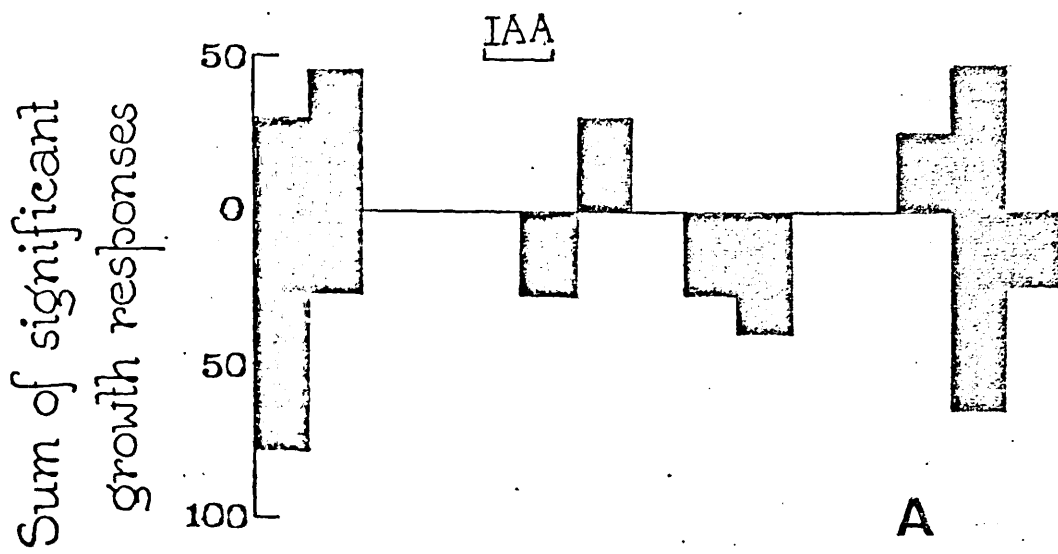


Fig. 36 Chromatograms of the primary ether fraction of the fourth 2 mm segment (100) next to the third segment (running solvent - isopropanol:ammonia: water). The entire amount of the extract has been assayed.

- A. Composite diagram from three separate occasions.
- B. A typical chromatogram from one of the three occasions.

Fig.36



E. Fourth 2 mm segment

In the more matured tissue, traces of the 'cap inhibitor' were obtained occasionally. The 'meristem inhibitor' was found on all three occasions, though its activity was found to be slightly less than that of the previous segment (Fig. 36). Here also IAA appeared sporadically whereas the 'inhibitor-3' appeared consistently and its activity was found to be maximum in all the segments. The 'inhibitor-4' was absent in this segment.

The following facts can be established from the results obtained so far.

(1) The 'cap inhibitor' is the only ether soluble growth active substance present in the root cap of Zea mays. Judging from its position on the chromatogram and its antitranspirant property it may very well be ABA.

(2) The 'meristem inhibitor' is the major inhibitor present in the meristem and in the mature region of the root tip. This substance is presumably not an indole compound as it is negative to any one of the colour reactions for indole. However, eluates of this substance from the paper chromatogram when rechromatographed by the thin layer, gave rise to two separate faint yellow colourations when sprayed with sodium cobaltinitrite (see page 58). One of the coloured zones restricted to the origin of the chromatogram, and the other slightly above indicating it may contain two or more phenolic substances. As no further experiments have been done to characterize this substance its true chemical nature is yet to be resolved.

(3) No positive colour reaction was obtained in the position of IAA however, from its growth activity and the Rf value it is suspected as being IAA. It is absent in the cap but appears sporadically in the whole root tip extracts.

(4) The 'inhibitor-3' is also present in the root tip extract except in the root caps. This substance has similar antitranspirant properties to the 'cap inhibitor'. From its position on the chromatogram it is suspected to be xanthoxin though the antitranspirant property of xanthoxin is not yet established.

(5) Nothing is known about the chemical nature of the 'inhibitor-4' though it is always present in the meristematic region of the root tip. However, its activity is less than that of the other inhibitors, which consistently appeared in the extract.

Results of the assay of root materials after geotropic stimulation:

A. Stimulation in the dark

In the dark, root tips of the vertically grown roots contained predominantly the 'meristem inhibitor' (Fig. 37). The 'cap inhibitor' was apparently absent in three out of four extracts (or, if present its activity was not significant up to the limits of confidence). On only one occasion (Fig. 37 D) a little inhibition was found in the relevant Rf value of the 'cap inhibitor' and on another occasion slight promotion of growth was observed in the same position (Fig. 37 B). The 'meristem inhibitor', on the other hand appeared consistently and its considerable activity was noticed on all the four chromatograms. The other inhibitors appeared sporadically.

After 30 mins of stimulation an increased production of the 'cap inhibitor' was obtained (Fig. 37 A'). This observation was consistent on all occasions. This increased production was presumably due to the gravitational stress as observed earlier by Audus and Lahiri (1961). However, there was no marked difference in the activity of the 'meristem inhibitor' in the stimulated root tips when compared with that of the vertical roots. Here also the other inhibitors appeared inconsistently in the chromatogram.

Fig. 37 Comparison of the chromatograms of the acid ether fraction from vertical and stimulated roots (100 root tips in each case) in strictly identical conditions in the dark.

- A. Composite diagram from four separate chromatograms of the extract of vertical root tips run in isopropanol:ammonia: water solvent.
- B-E Chromatograms of four individual assays of root tips of the above.
- A' Composite diagram from similar four separate chromatograms of the extracts of root tips from the stimulated roots.
- B'-E' Chromatograms of four individual assays of root tips from corresponding stimulated roots.

Fig.37

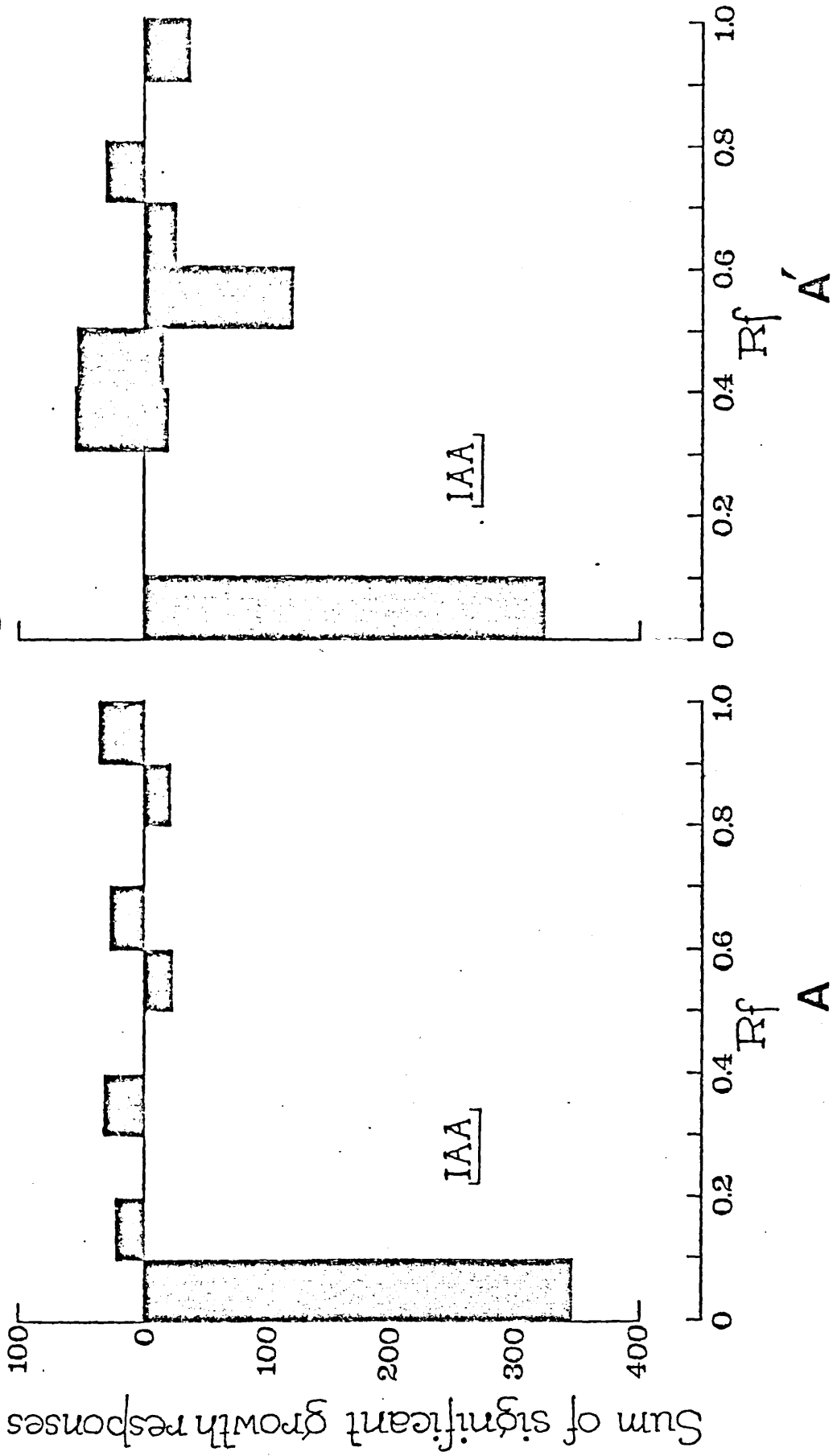


Fig. 37 (Conld.)

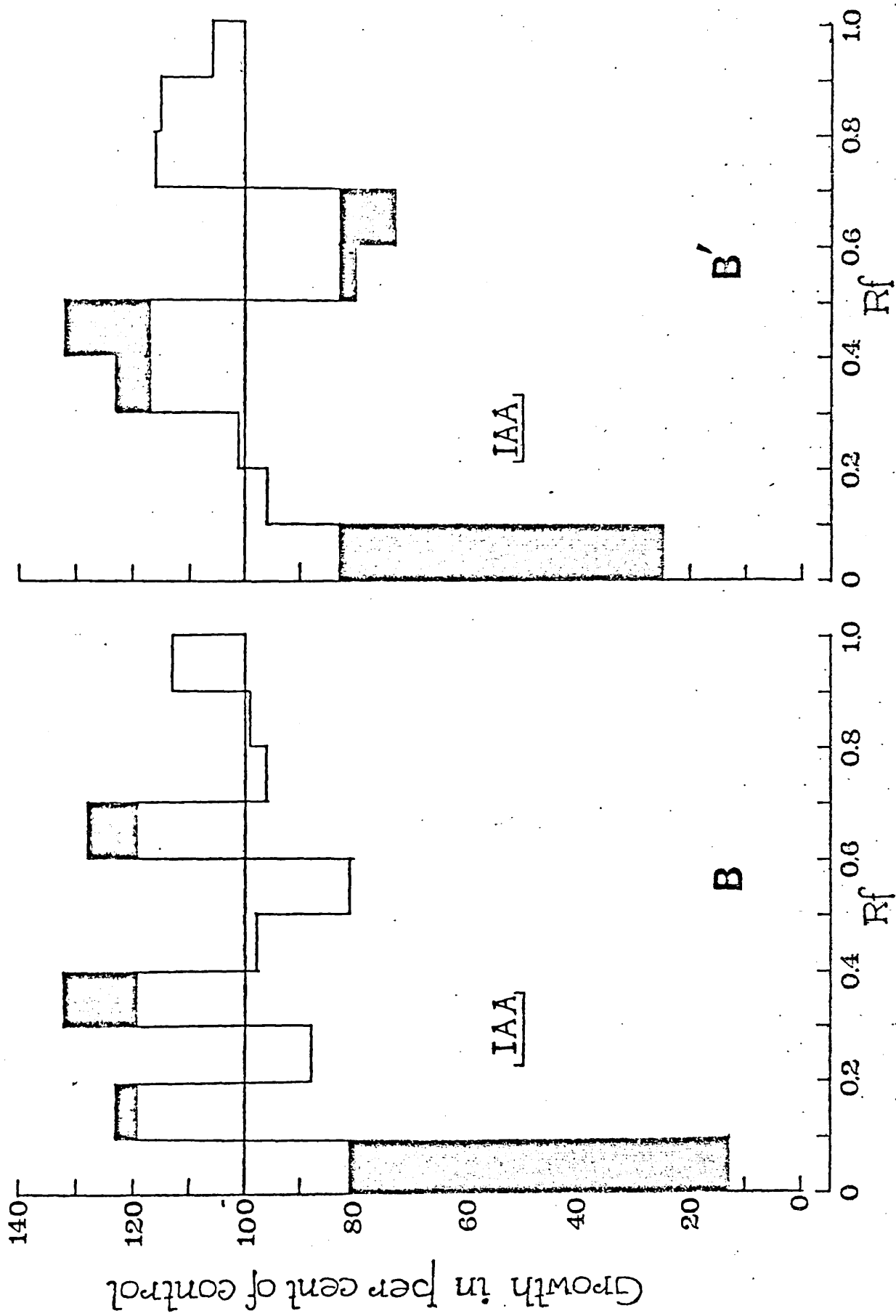


Fig. 37 (Contd.)

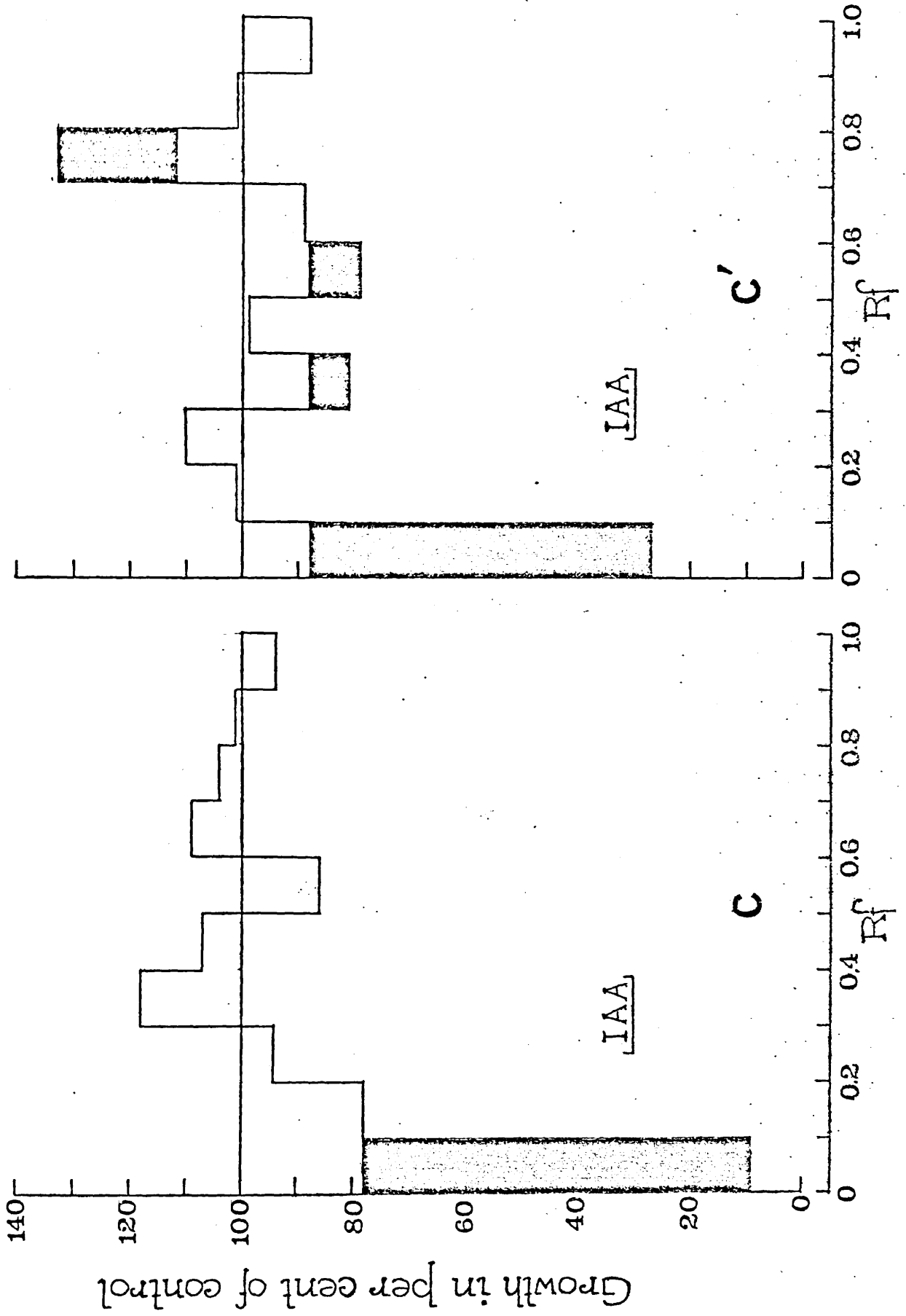


Fig. 37 (Contd.)

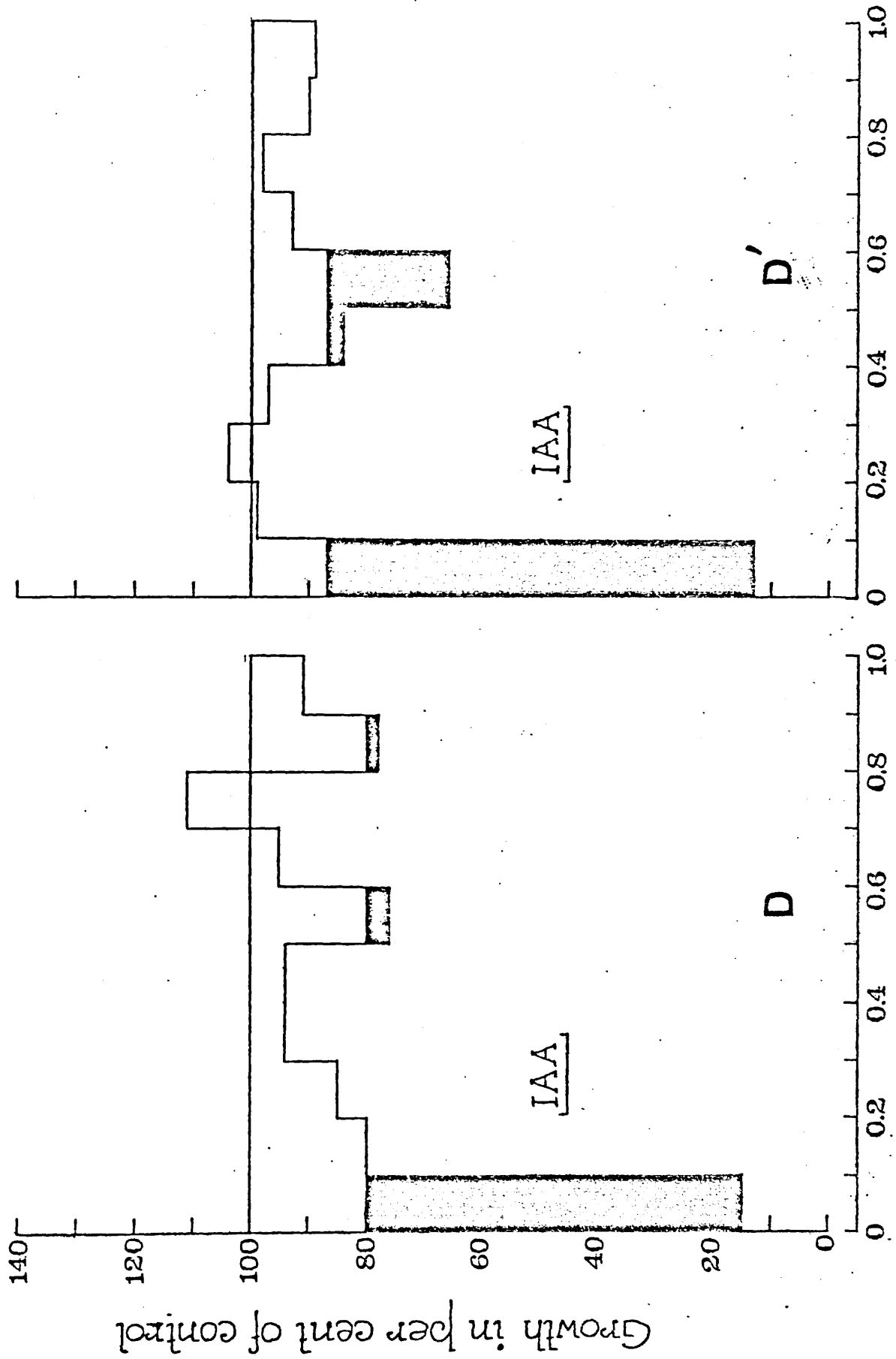
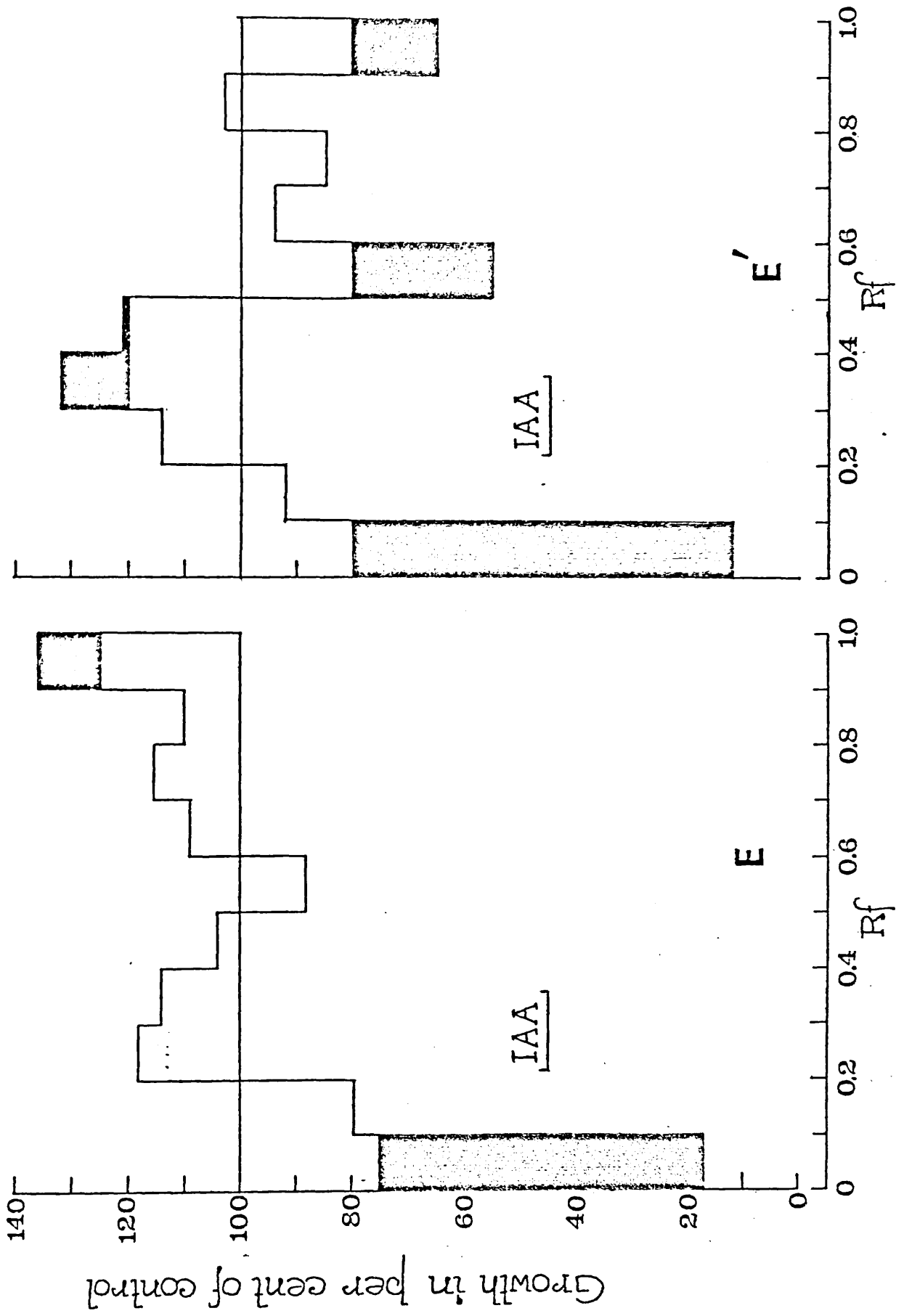


Fig. 37 (contd.)



A greater production of the 'cap inhibitor' in the stimulated root was further evident from the assay of the root caps. The root caps from the vertical roots contained less of this inhibitor when compared with that of the stimulated root caps (Fig. 38). On several occasions the 'cap inhibitor' was apparently absent in the caps of the vertical roots.

In the next experiment extracts of the two equal halves of the 5 mm root tip were assayed to find out any asymmetric distribution of the active substances during geotropic stimulation in the dark. The results of this experiment are presented in Fig. 39.

In stimulated root tips, the activity of the 'cap inhibitor' in the lower half of the root tip was significantly greater on ^{out of five} four occasions, indicating its greater concentration in the lower half than that of the upper half. A similar differential distribution was obtained in the case of 'inhibitor-3' which appeared consistently in these samples of root tips. However, from the assay of the acid ether fraction alone it is difficult to judge the distribution of this inhibitor unless the primary ether fraction of these two halves have been analysed. On one occasion (Fig. 39 F) a significant level of inhibition was observed in the position of IAA in the upper half. In the case of the 'meristem inhibitor' no appreciable difference in the activity was obtained in these two halves of the root tip.

On the other hand, chromatograms of the two halves of the vertical root tip revealed more or less identical patterns of distribution of the inhibitors (Fig. 40).

B. Stimulation in light

When compared with the vertical root tips in the dark, a greater amount of the 'cap inhibitor' was found in the tips of the

Fig. 38 Comparison of the chromatograms of the acid ether fraction of root caps (100) from vertical and stimulated roots in strictly identical conditions in the dark.

A. Composite diagram from four separate chromatograms of the extracts of root caps from vertical root tips run in isopropanol:ammonia:water solvent.

B and C Chromatograms of two of the individual assays of root caps from vertical roots.

A' Composite diagram from similar four separate chromatograms of the extracts of root caps from the stimulated roots.

B' and C' Results of two of the individual assays of root caps from the stimulated roots.

Fig.38

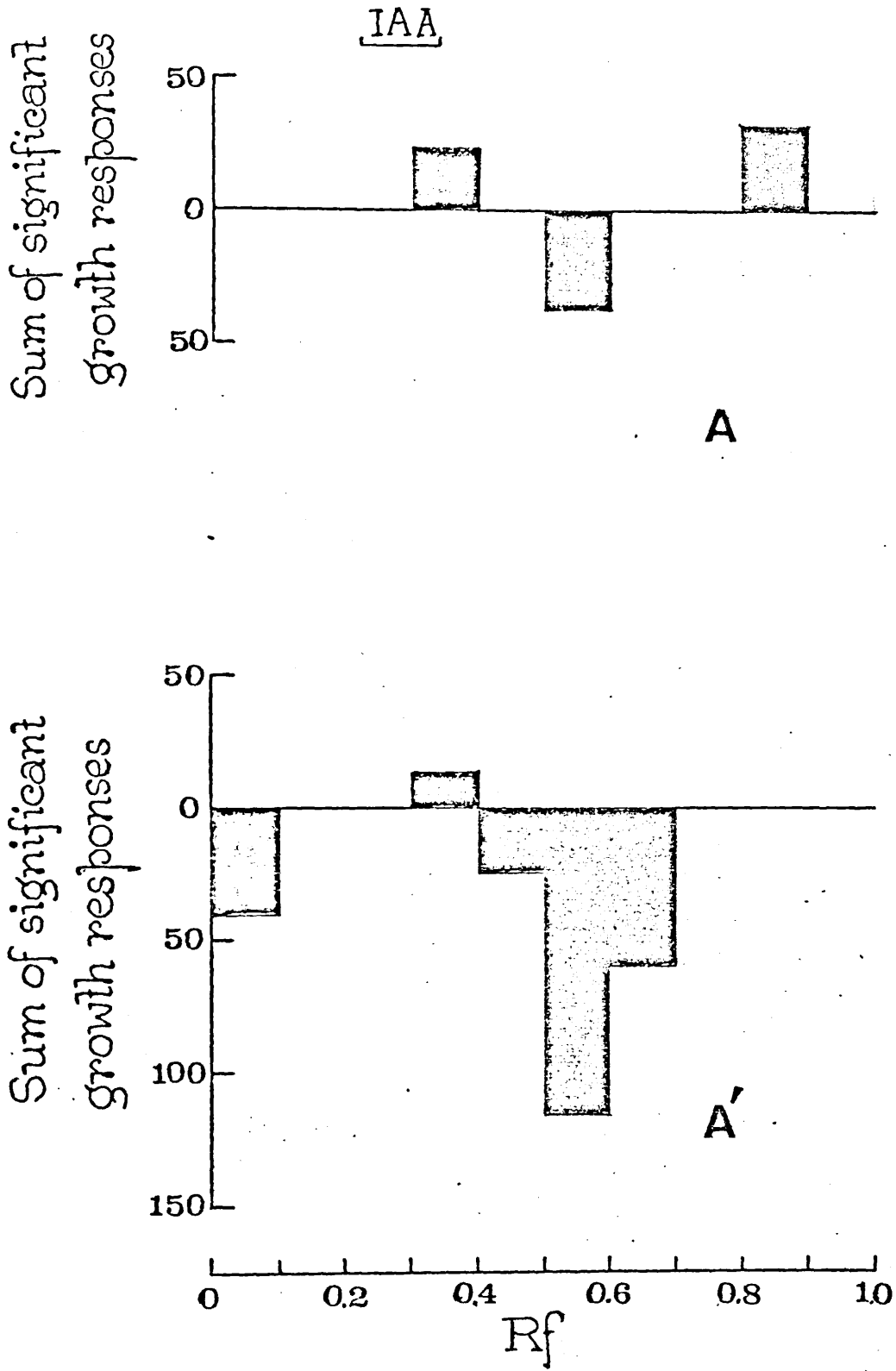


Fig.38 (Contd.)

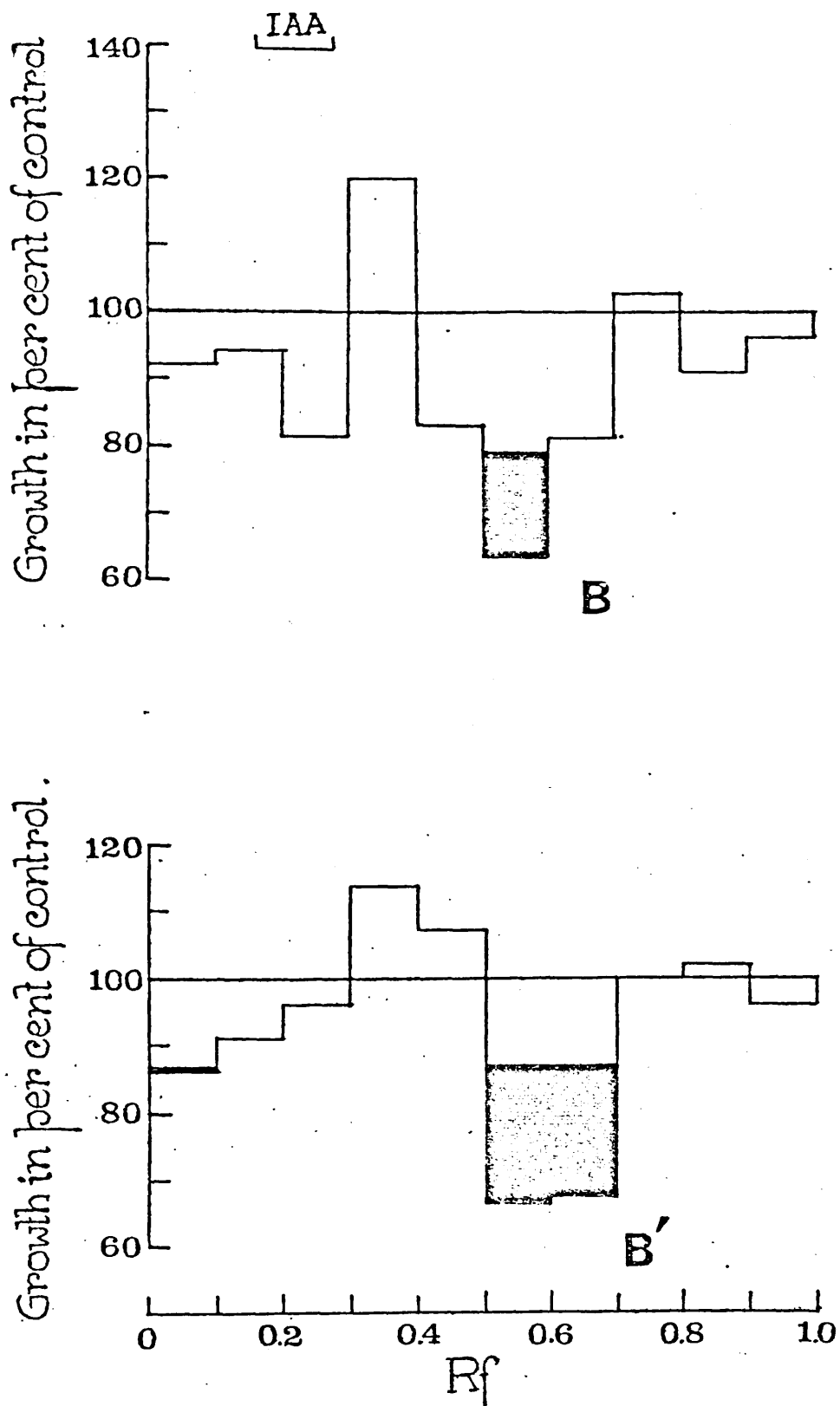


Fig.38(Contd.)

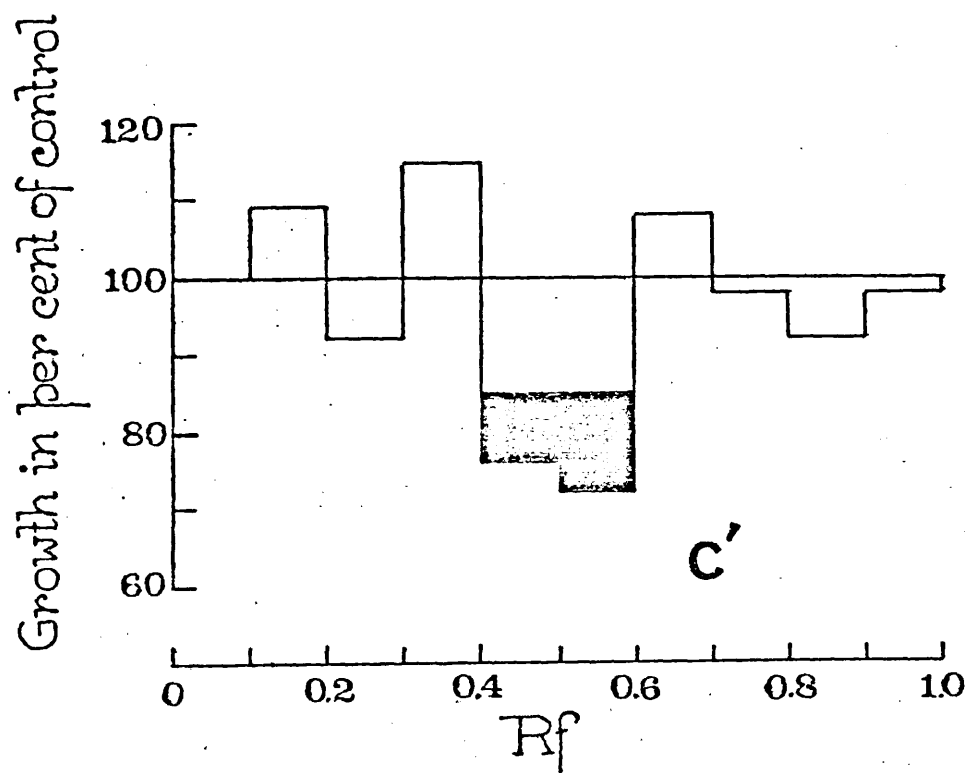
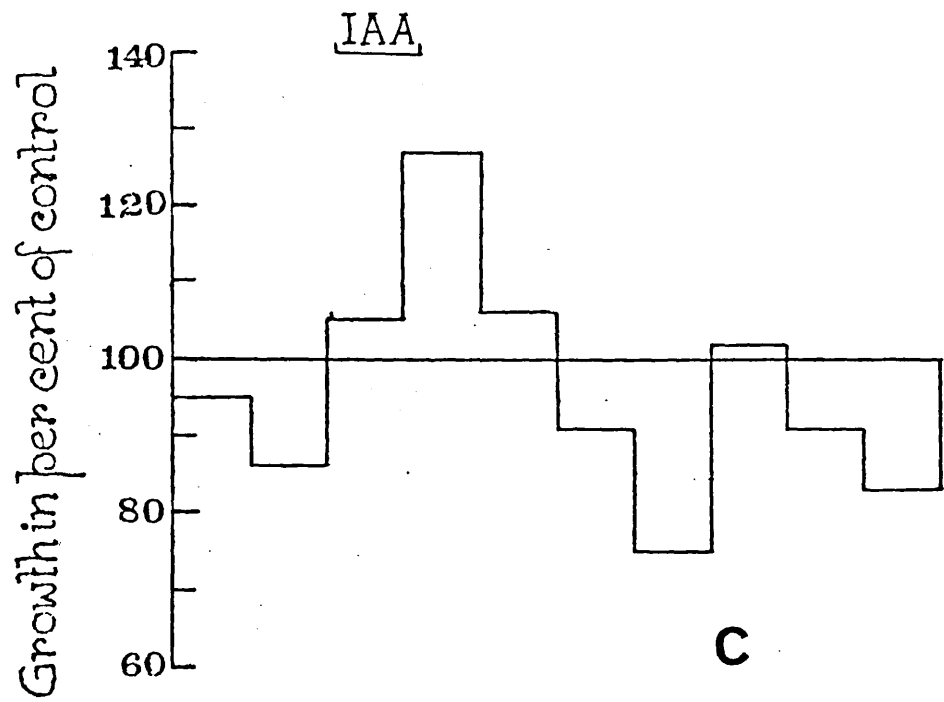


Fig. 39 Comparison of the chromatograms of acid ether fractions of upper and lower halves of root tip from roots stimulated in the dark. On each occasion a hundred root materials have been assayed.

A. Composite diagram from five separate chromatograms run in isopropanol:ammonia: water solvent showing the growth active regions in the upper half of root tips from stimulated roots.

B-F Results of five individual assays of the above.

A' Composite diagram from five similar separate chromatograms of the extract of lower half of root tips from stimulated roots.

B' - F' Chromatograms of corresponding five individual assays of the above.

Fig. 39

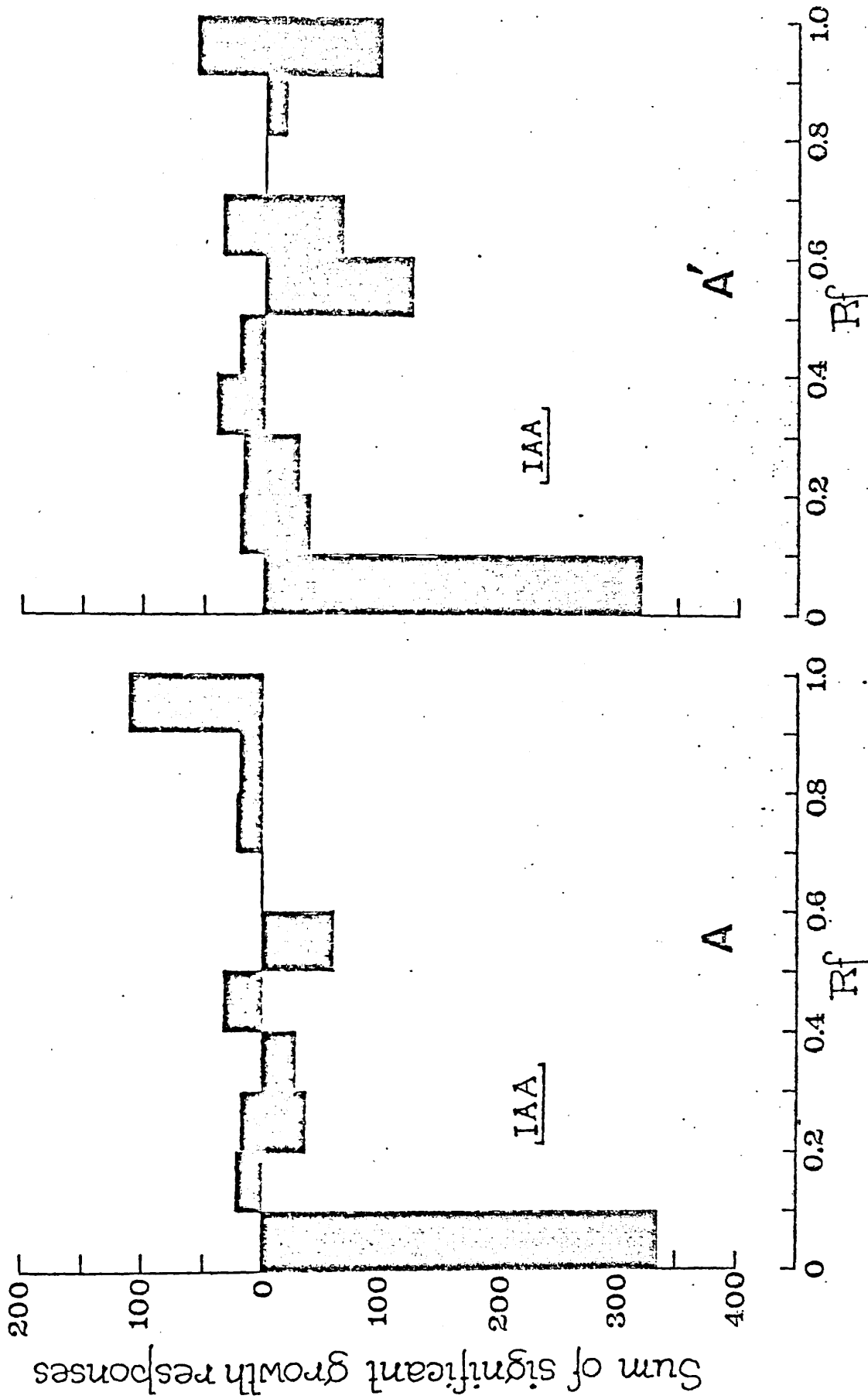


Fig. 39 (Contd.)

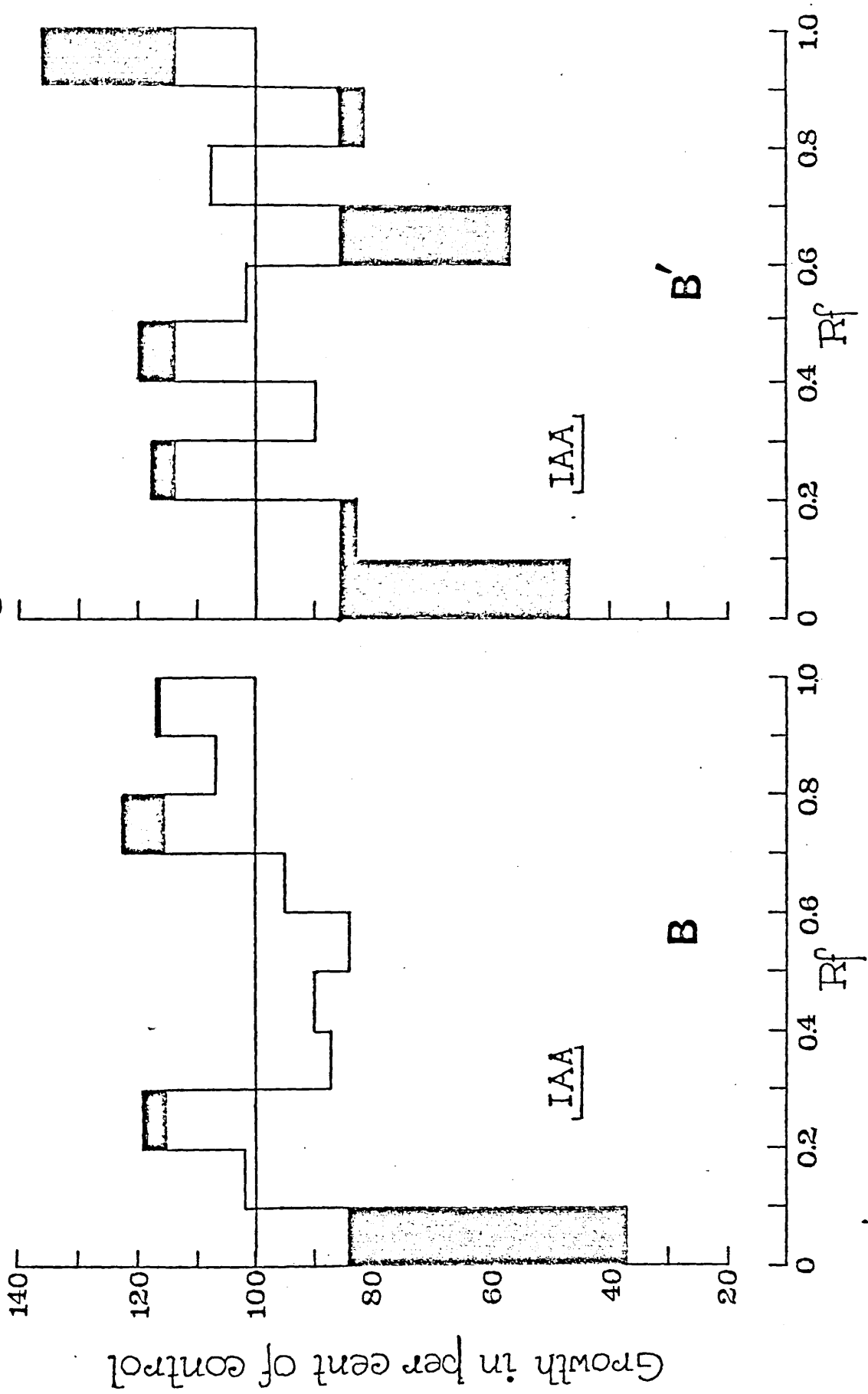


Fig.39(Contd.)

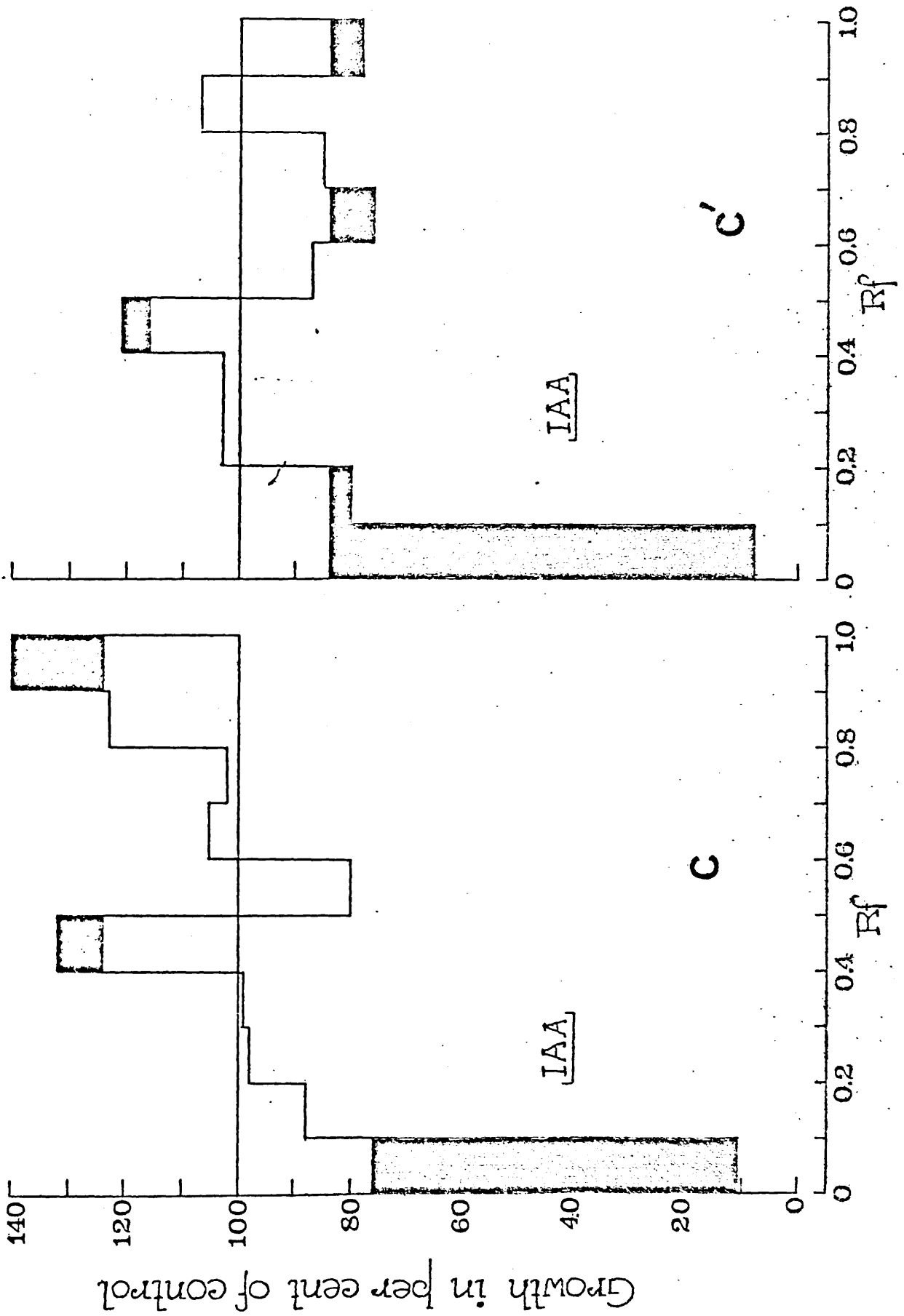


Fig. 39 (Contd.)

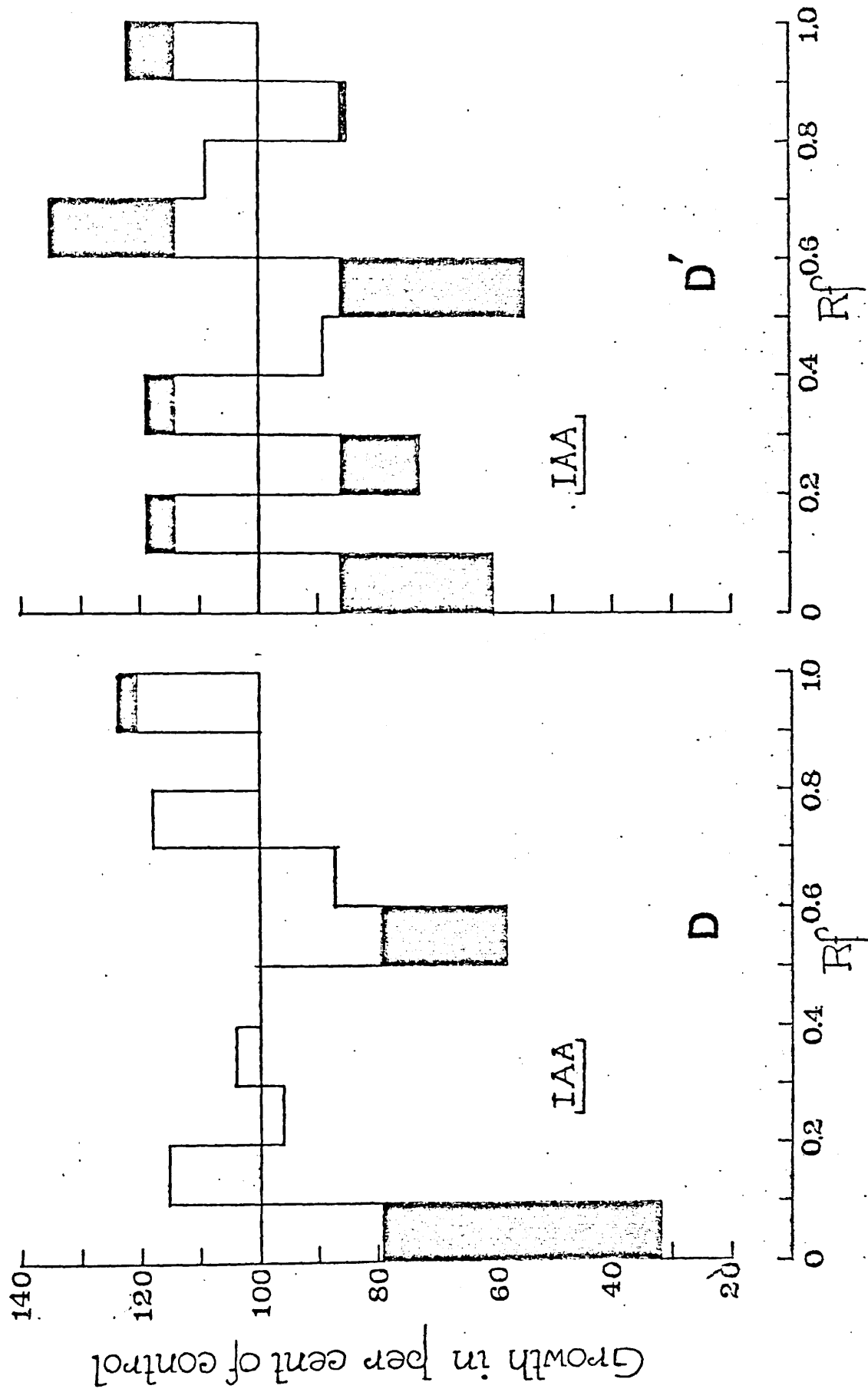


Fig. 39 (Contd.)

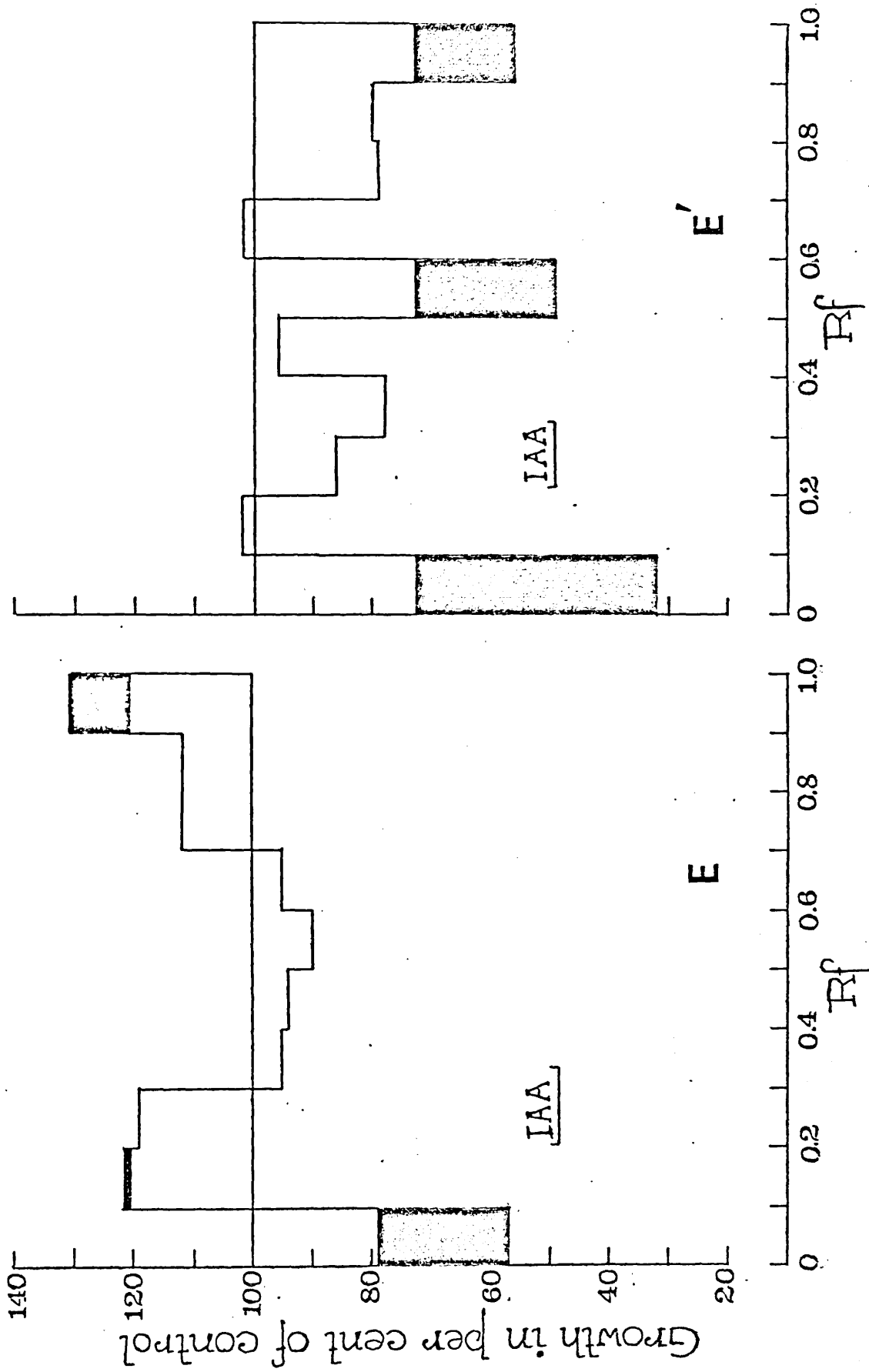


Fig. 39(contd.)

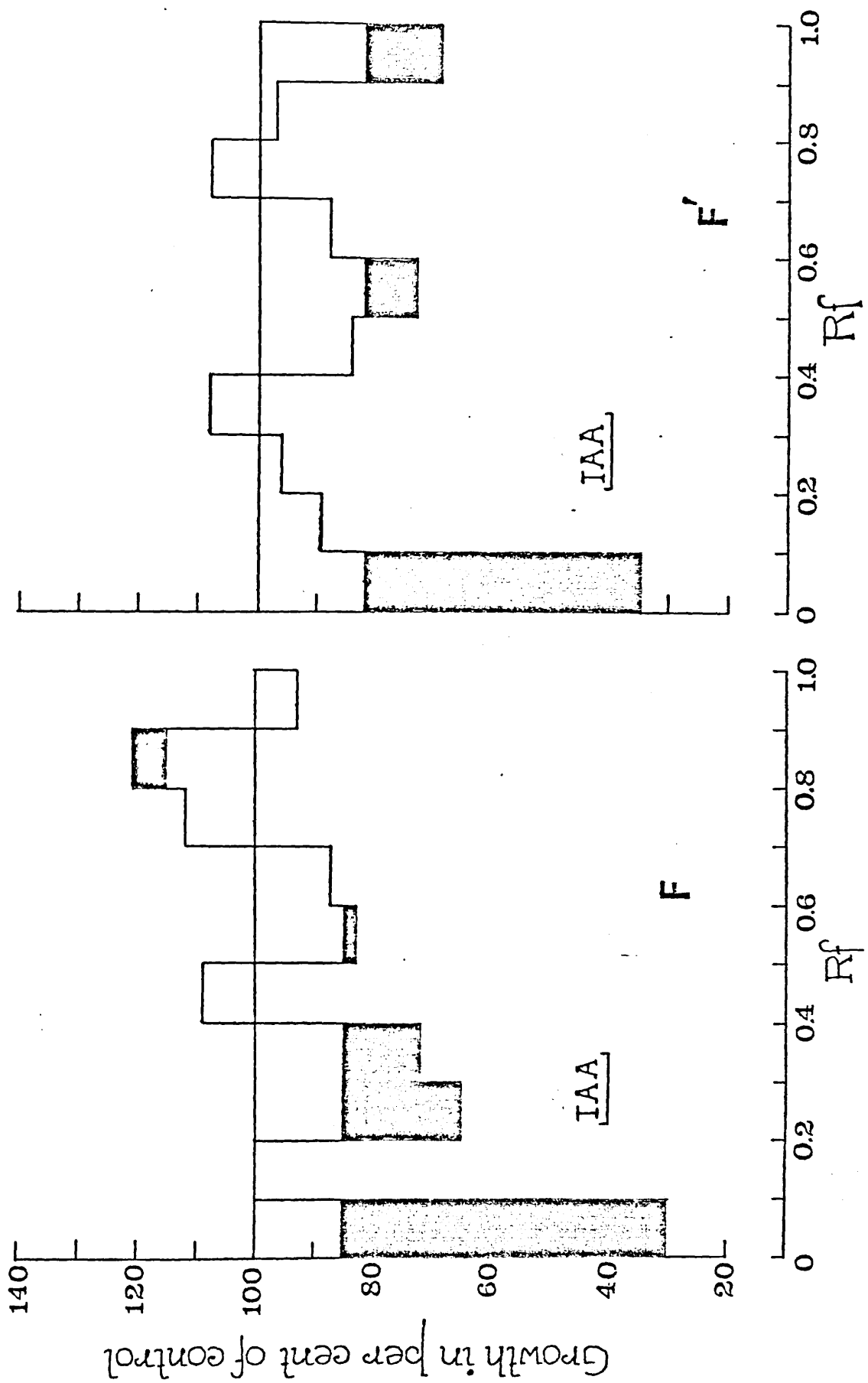
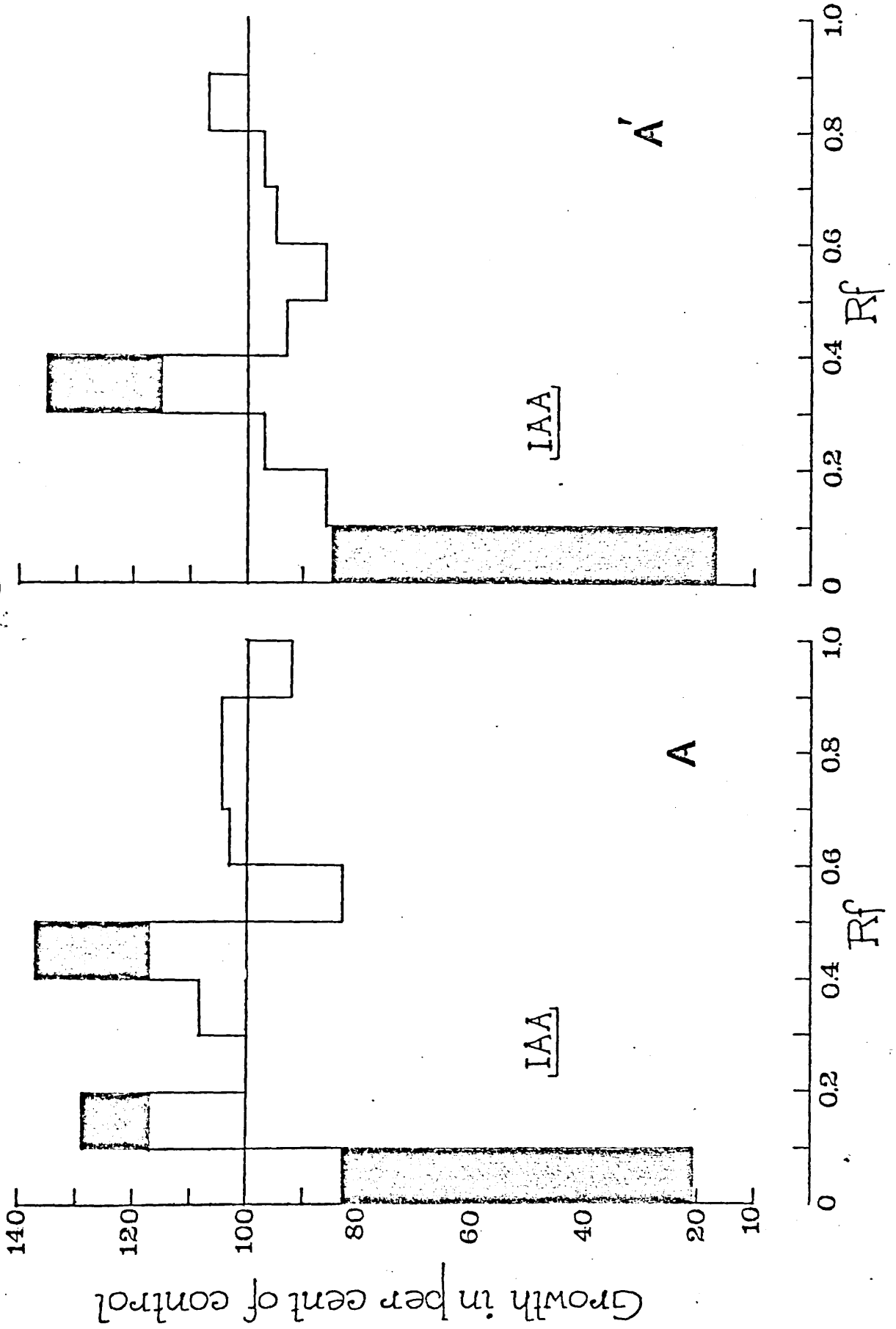


Fig. 40 Comparison of the chromatograms of acid ether fraction of the two equal halves of root tips grown in vertical position. On each occasion the extract of a hundred root materials has been used.

Fig. 40



vertically grown roots in the presence of light (Figs. 41 A to 41 D). This may be due to the effect of light enhancing the production of this inhibitor. A similar comparison with the dark grown vertical root tips showed a slight increase in IAA-like substance in the illuminated root tips. However, no difference in the activity of the 'meristem inhibitor' was observed in vertical roots grown either in the dark or in the presence of light.

When compared with the roots either stimulated in the dark or grown in a vertical position in the presence of light a maximal amount of the cap inhibitor content was found in the root tips stimulated in the presence of light (Figs. 41 A' to 41 D').

On the other hand, no appreciable difference in growth activity was observed in the case of the 'meristem inhibitor' in stimulated and vertical root tips in light. On two occasions a little increase in inhibition was found in the position of IAA in the vertical root tips (Figs. 41 C and 41 D) compared with that of the stimulated tips. There were no traces of 'inhibitor-3' in the illuminated roots (either grown in a vertical position or stimulated) except in one extract of the vertical roots (Fig. 41 C).

Contrary to the previous observation in the presence of light there was a greater accumulation of the 'cap inhibitor' in the upper half of the root tip (Figs. 42 A to 42 F) whereas the lower half contained more of the 'meristem inhibitor' (Figs. 42 A' to 42 F'). This unusual and unequal distribution of these two inhibitors was very consistent on all five occasions. A similar and relatively clear picture was obtained using solvent II (Fig. 43). Moreover,

the composite diagram (Fig. 43) suggests that in the light the redistributions of the two inhibitors due to gravity are "complementary", there being roughly the same degree of asymmetry of the 'meristem inhibitor' towards the lower side as is shown by the 'cap inhibitor' towards the upper side.

Fig. 41 Comparison of the chromatograms of the acid ether fraction of a hundred root tips from vertical and stimulated roots in strictly identical condition in presence of light. On each occasion the entire extract of 100 root samples has been assayed.

- A. Composite diagram from three separate chromatograms of the extract of root tips from the vertical roots run in isopropanol: ammonia:water solvent.
- B-D Individual chromatograms of three individual assays of the above.
- A' Composite diagram from similar three separate chromatograms of the extract of root tips from the stimulated roots.
- B'-D' Results of three individual assays.

Fig.41

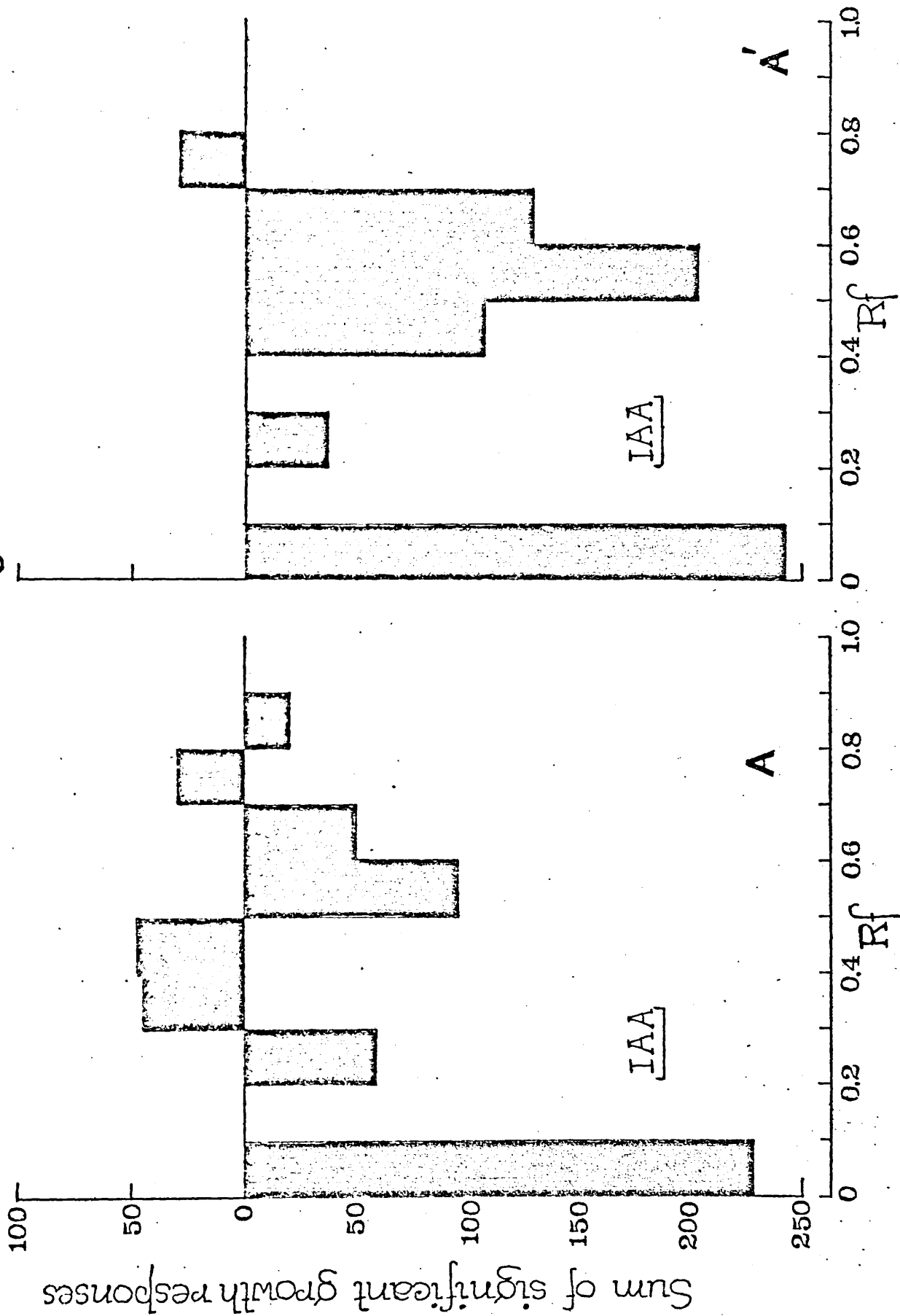


Fig. 41 (contd.)

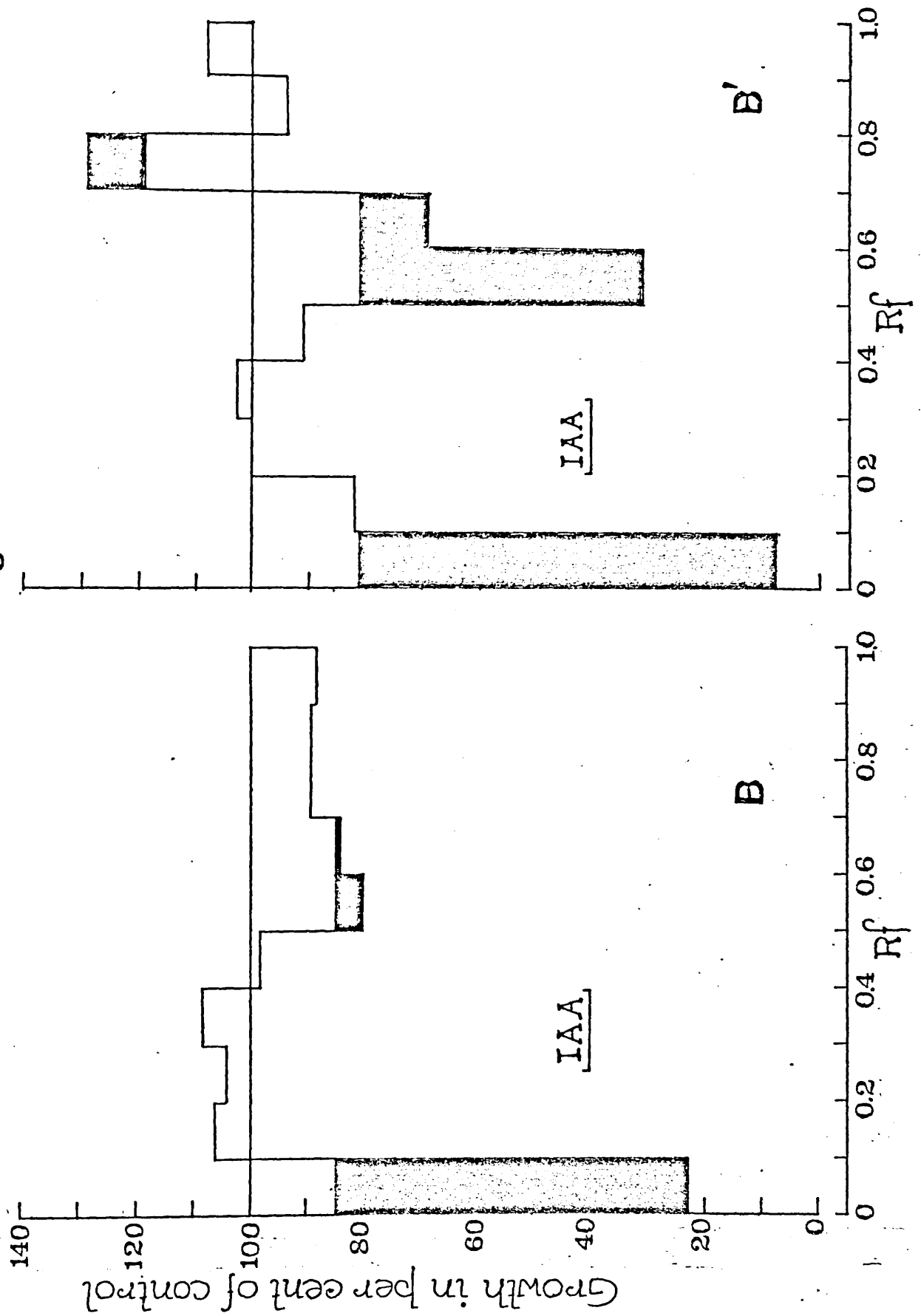


Fig. 41(Contd.)

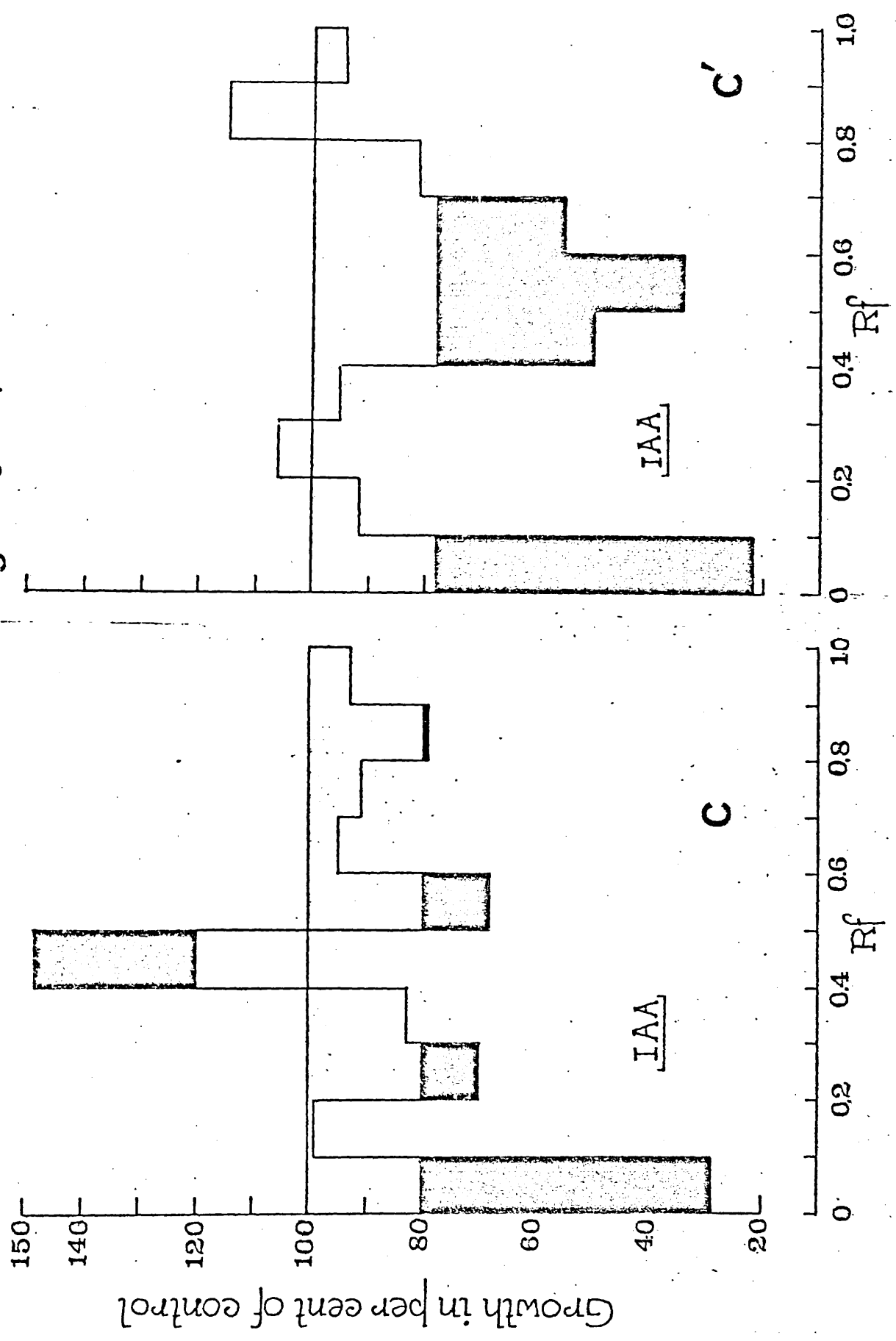


Fig. 41 (contd.)

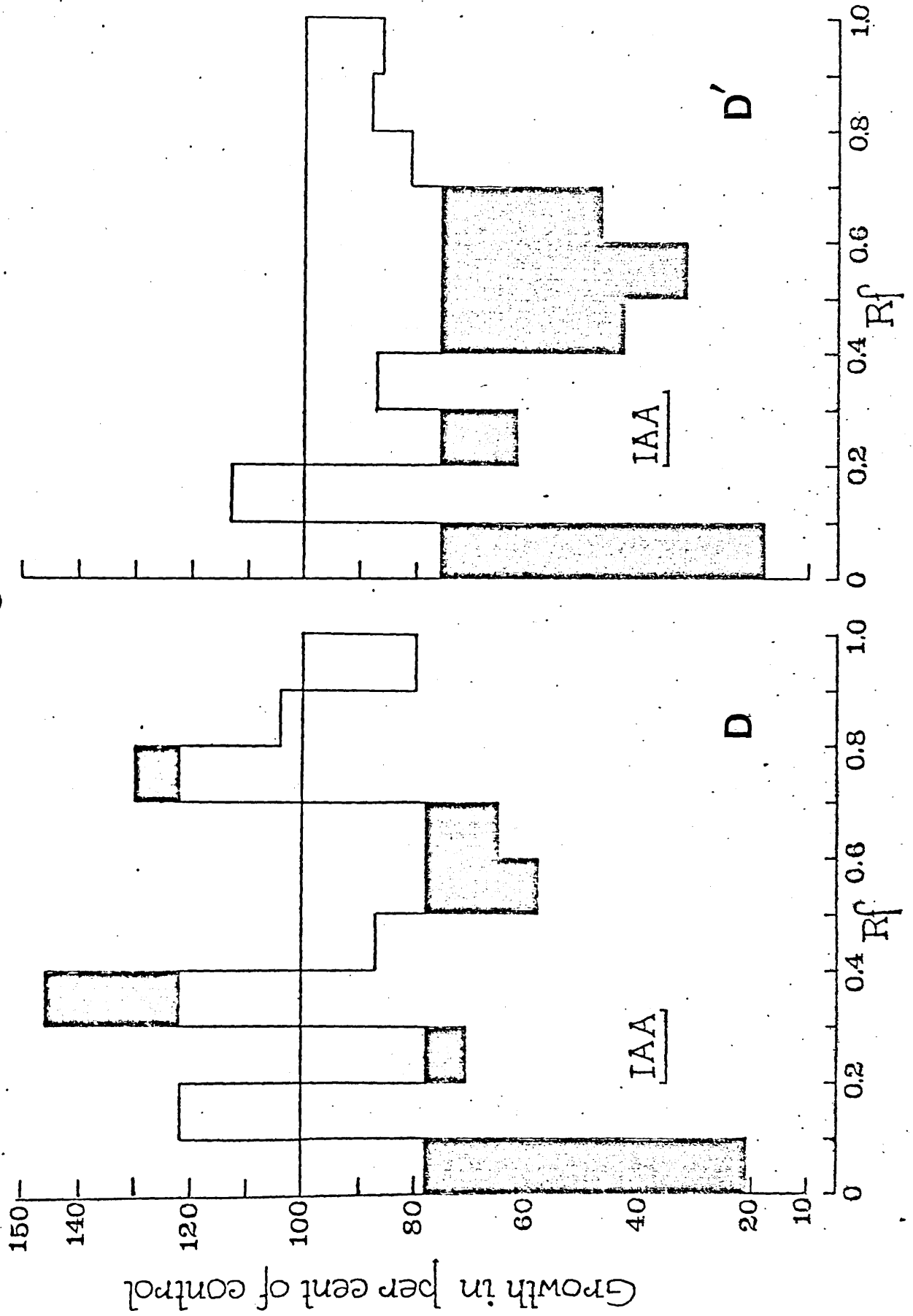


Fig. 42 Comparison of the chromatograms of acid ether fraction of upper and lower halves of root tips from stimulated roots in the presence of light. On each occasion the entire extract of a hundred root materials has been assayed.

A. Composite diagram from five separate chromatograms run in isopropanol:ammonia:water solvent showing the active regions in upper halves of the stimulated roots.

B-F Chromatograms of five individual assays of the above.

A' Composite diagram from similar five separate chromatograms of the extract of lower halves of root tips from stimulated roots.

B' - F' Chromatograms of corresponding five individual assays of the above.

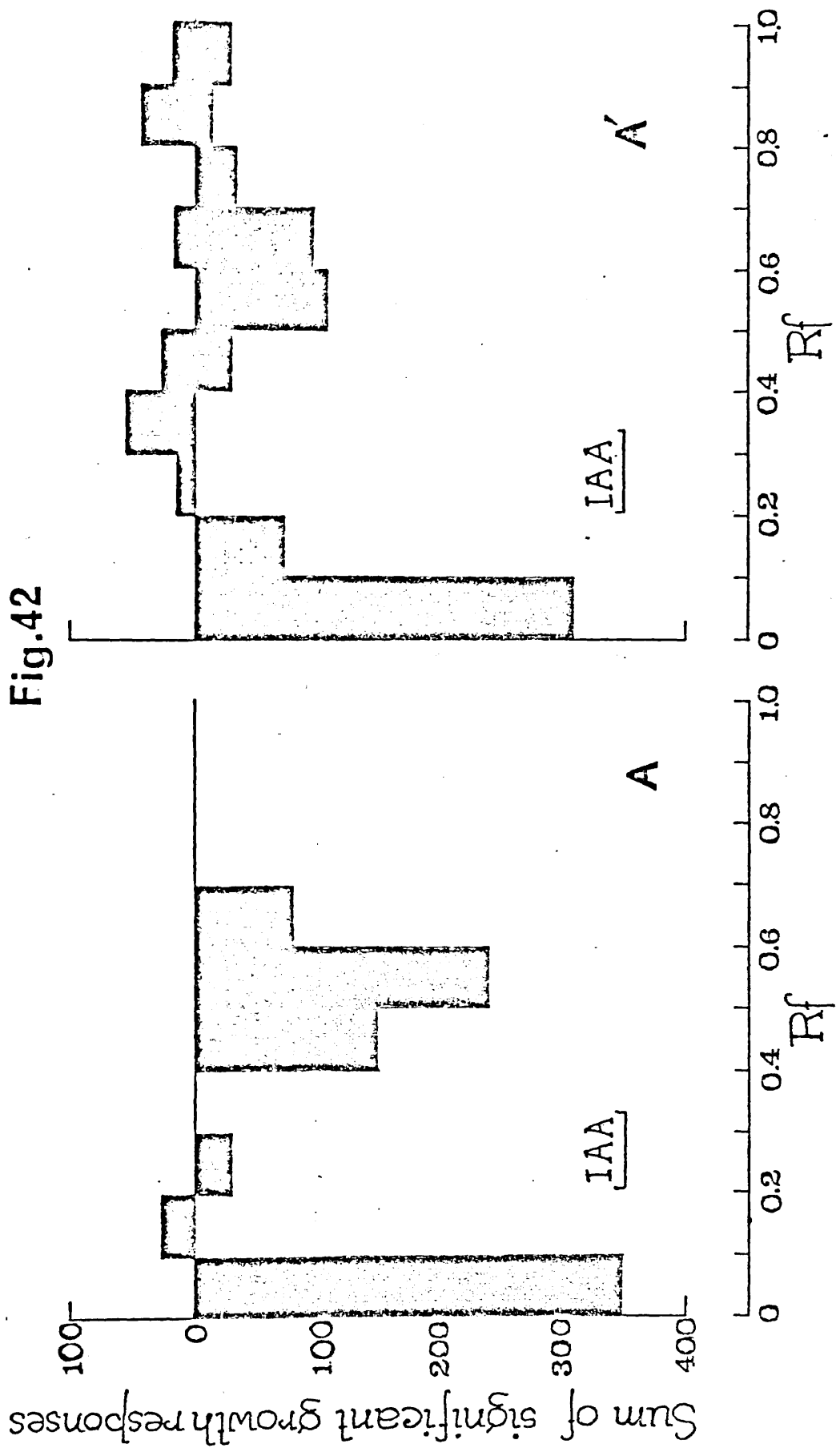


Fig. 42(Contd.)

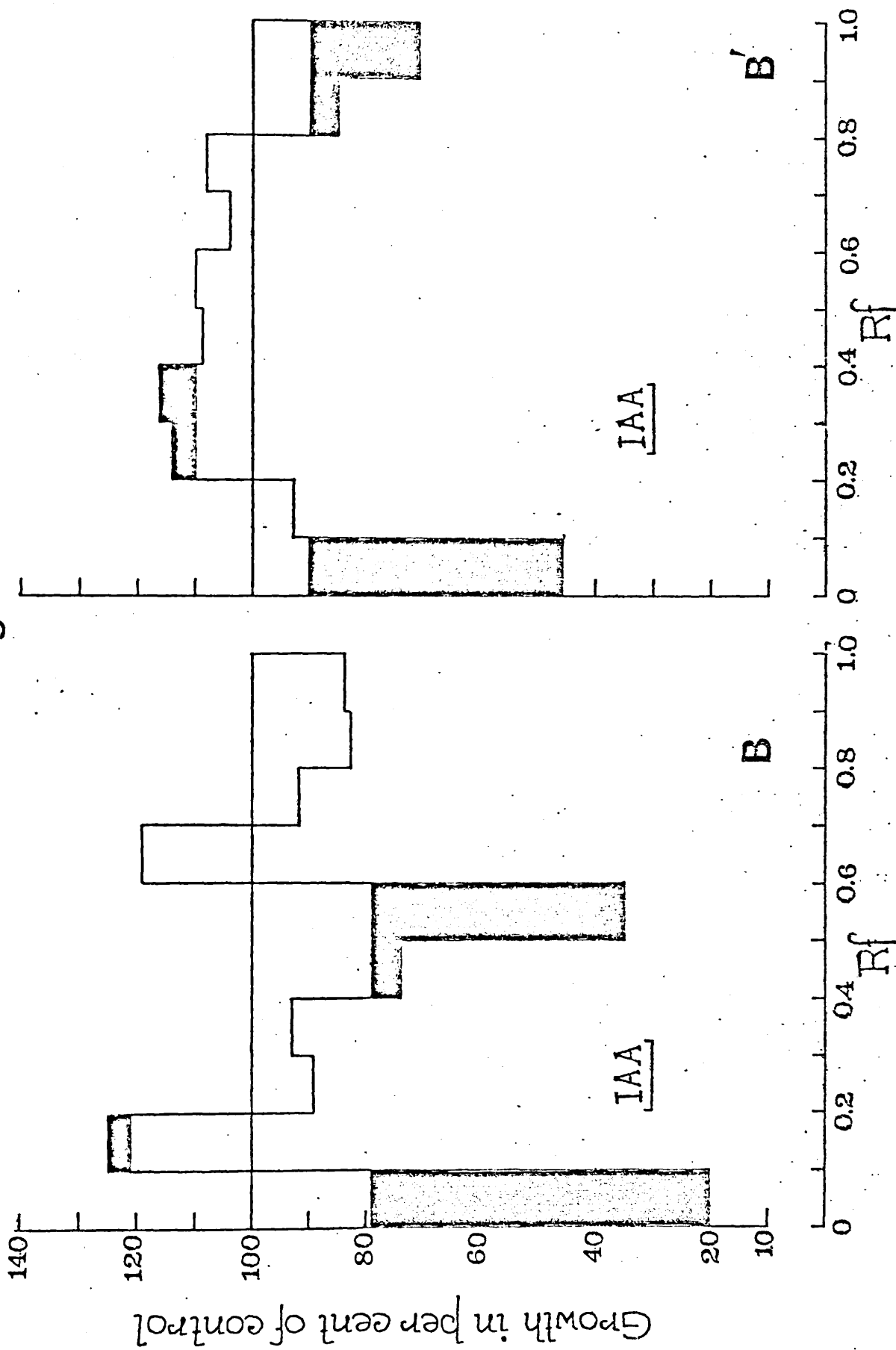


Fig. 42 (Contd.)

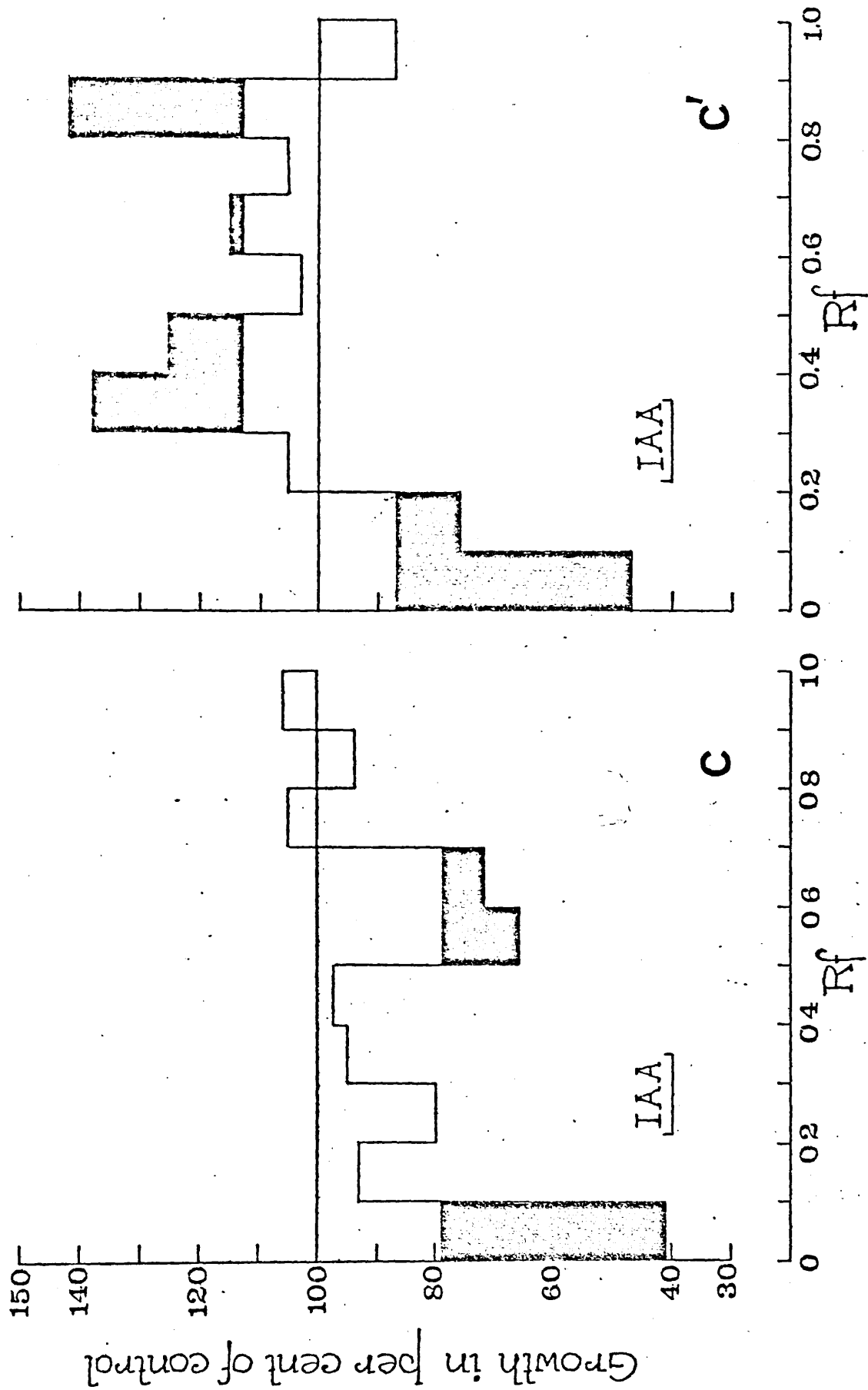


Fig. 42 (Contd.)

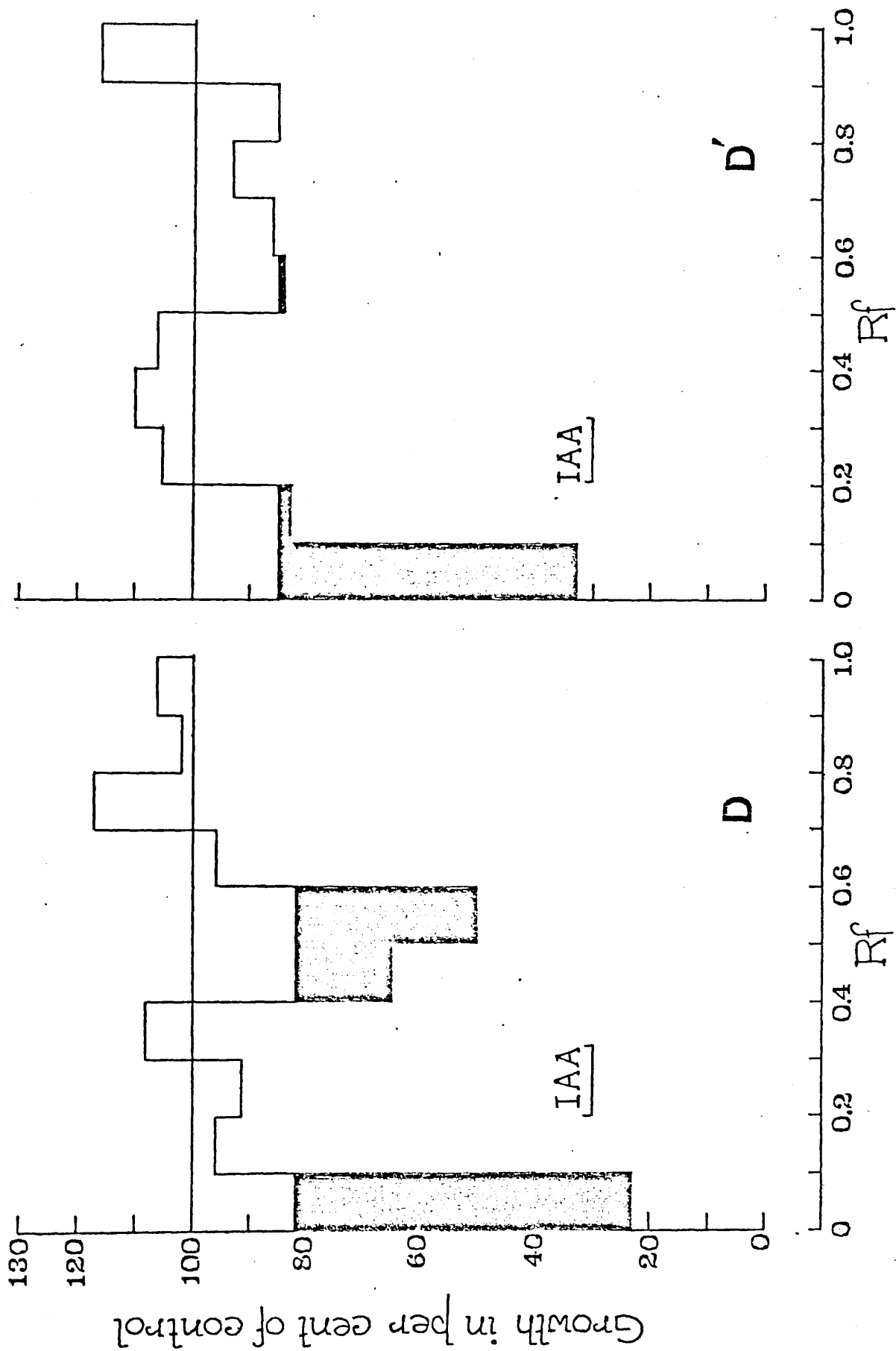


Fig. 42(contd.)

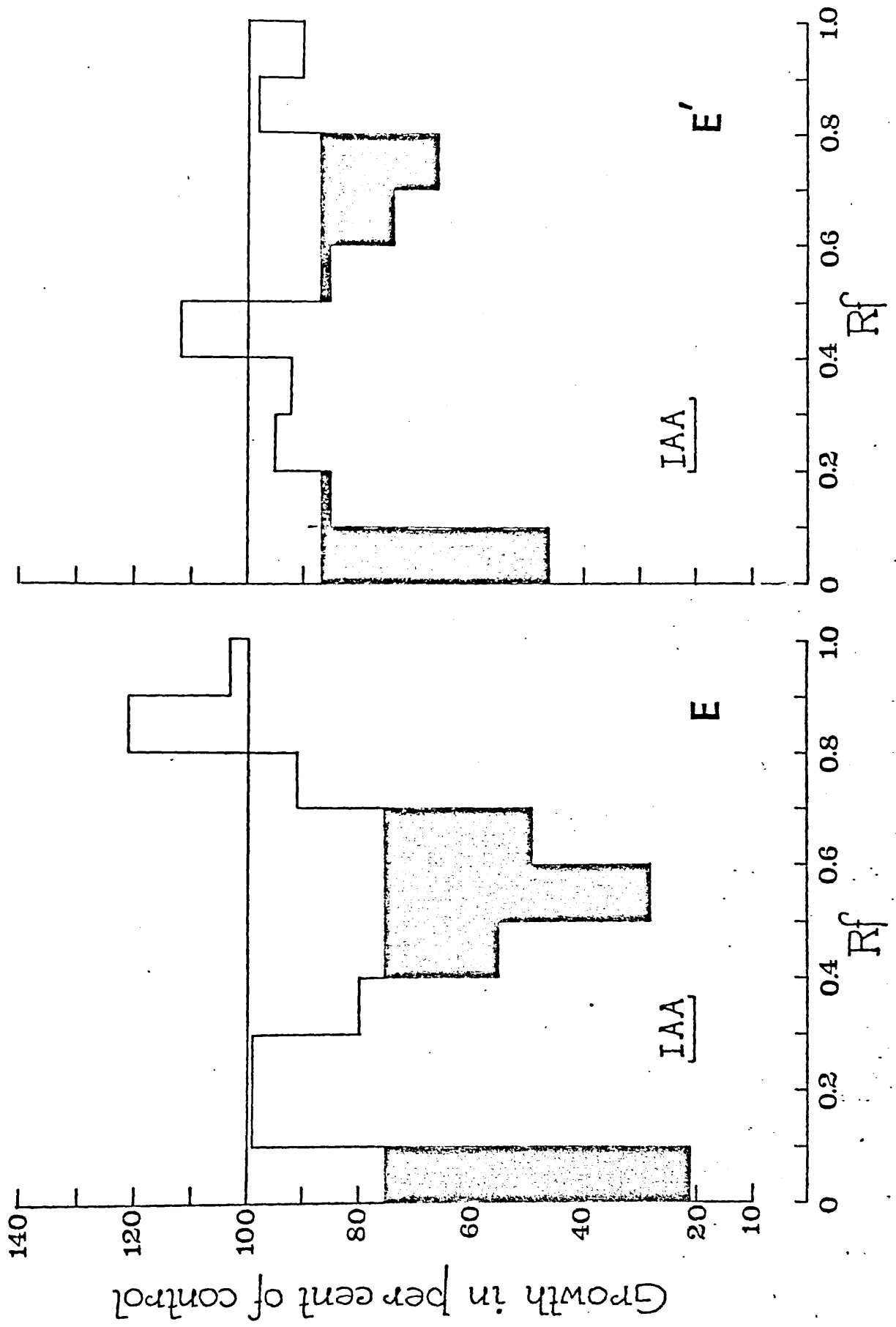


Fig. 42(Contd.)

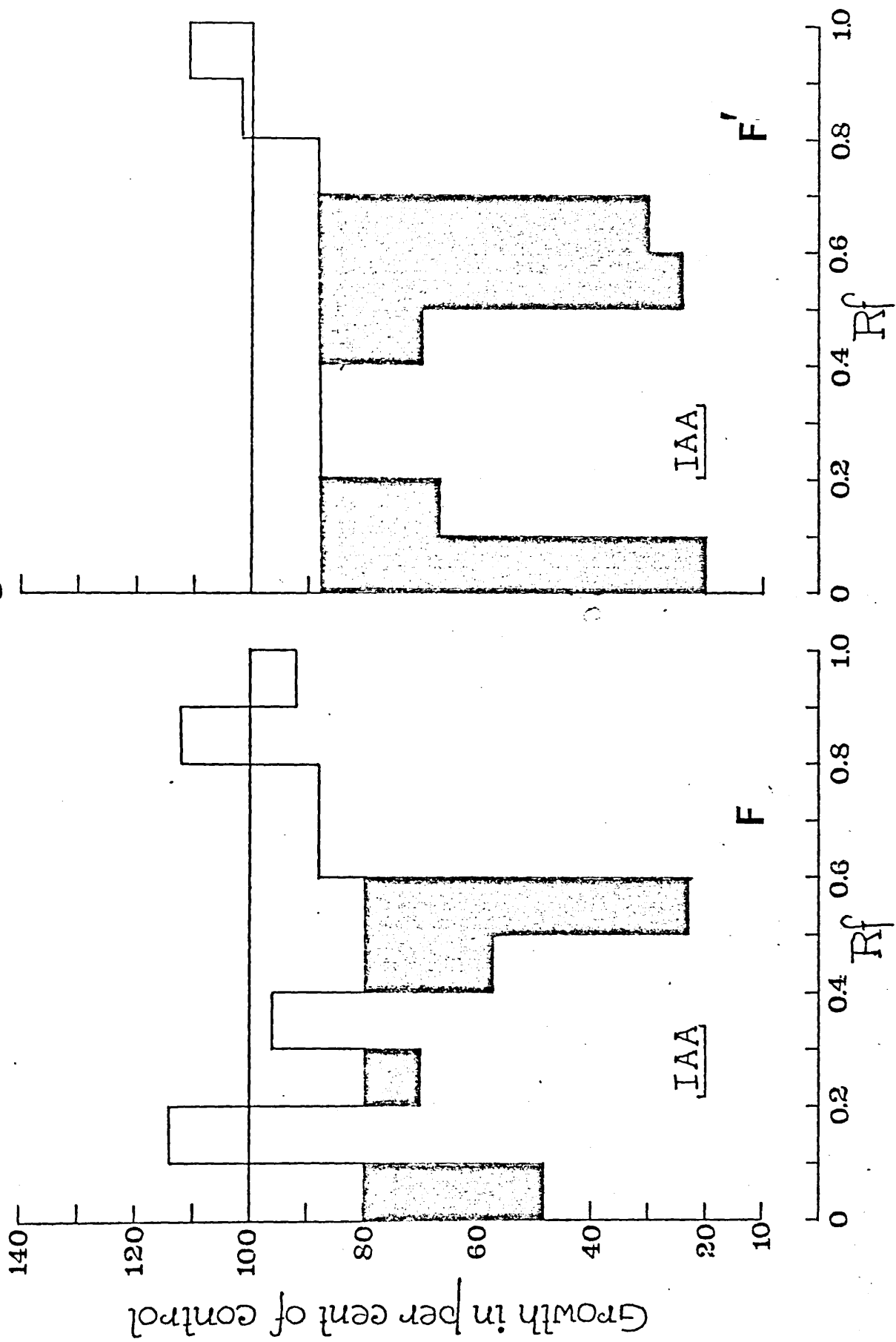


Fig. 43 Comparison of the chromatograms of acid ether fraction of upper and lower halves of root tips from stimulated roots in presence of light. On each occasion the entire extract of a hundred root materials has been assayed.

A. Composite diagram from four separate chromatograms run in isobutanol:methanol:water solvent showing the active regions in upper halves of the stimulated root tips.

B-E Chromatograms of the four individual assays of the above.

A' Composite diagram from similar four separate chromatograms of the extract of lower halves of root tips from stimulated roots.

B' - E' Chromatograms of corresponding four individual assays of the above.

Fig.43

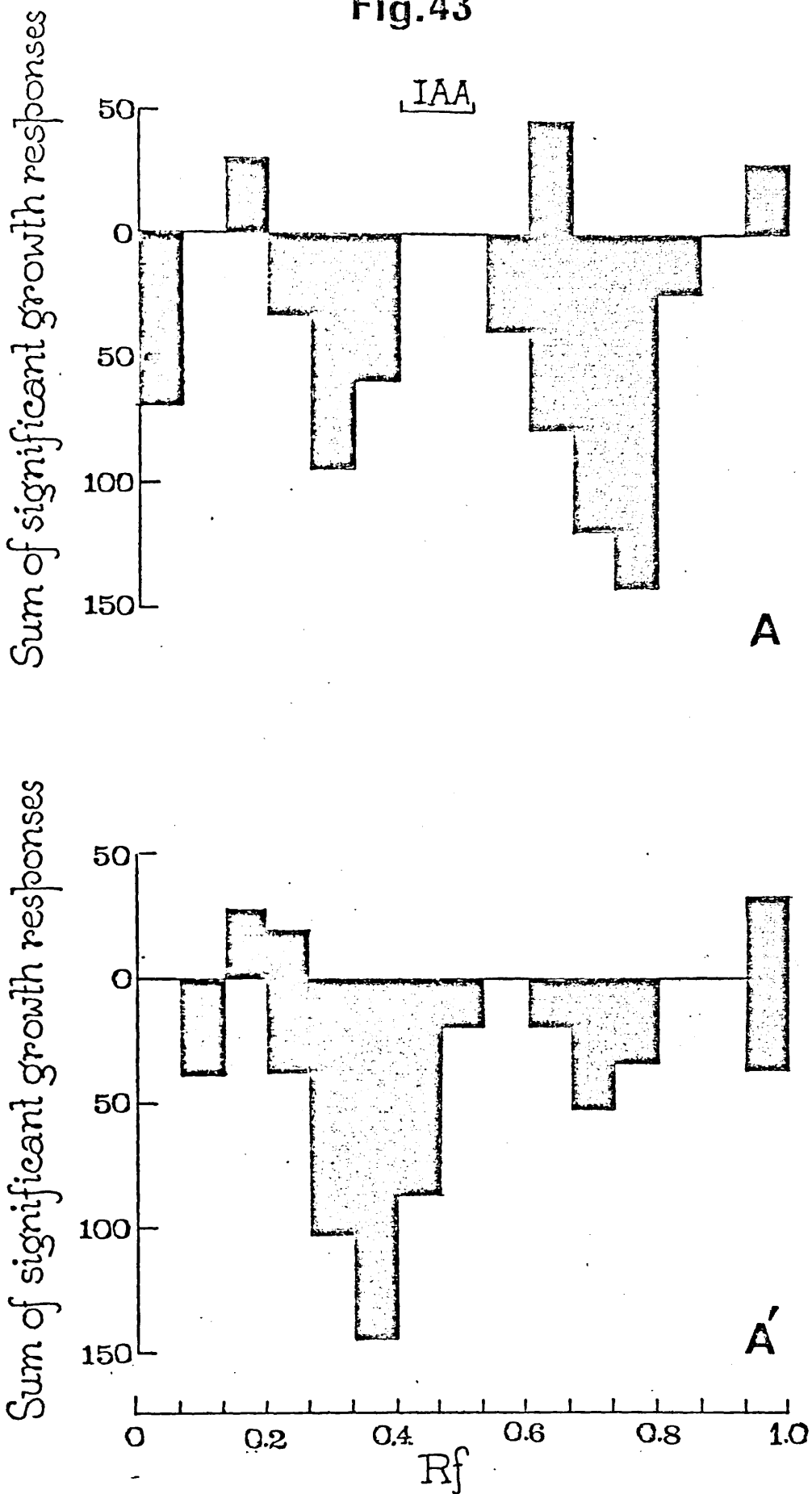


Fig.43(Contd.)

IAA

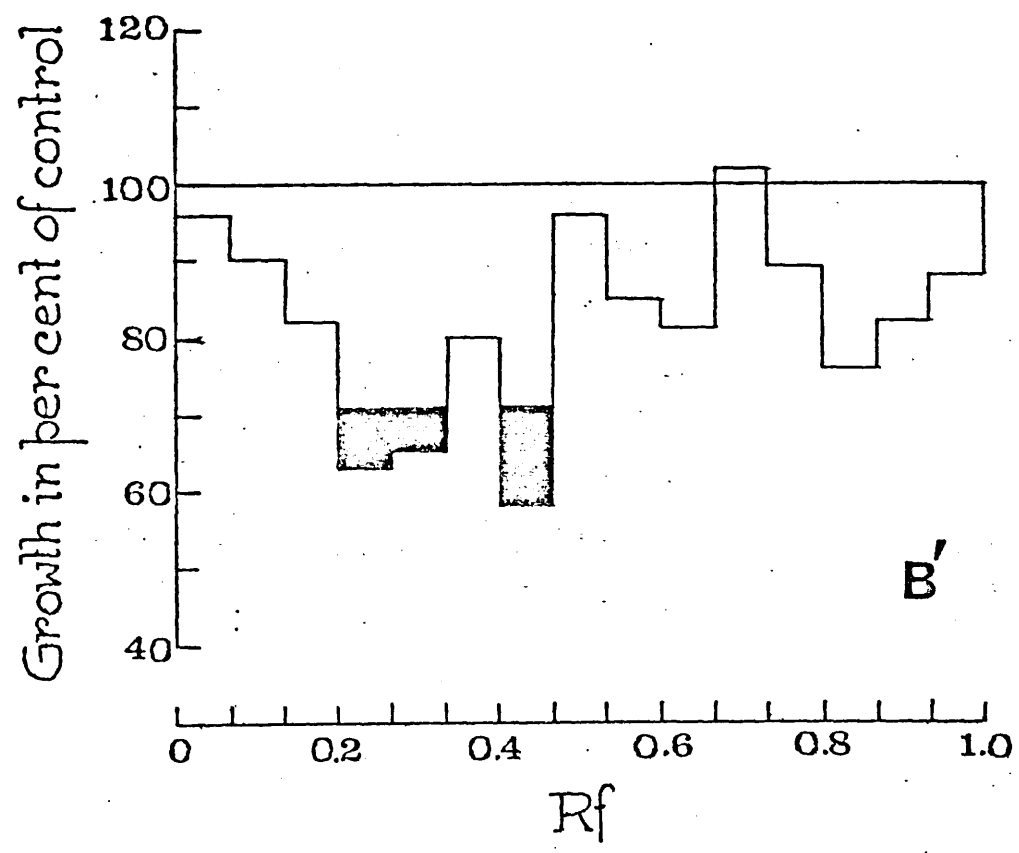
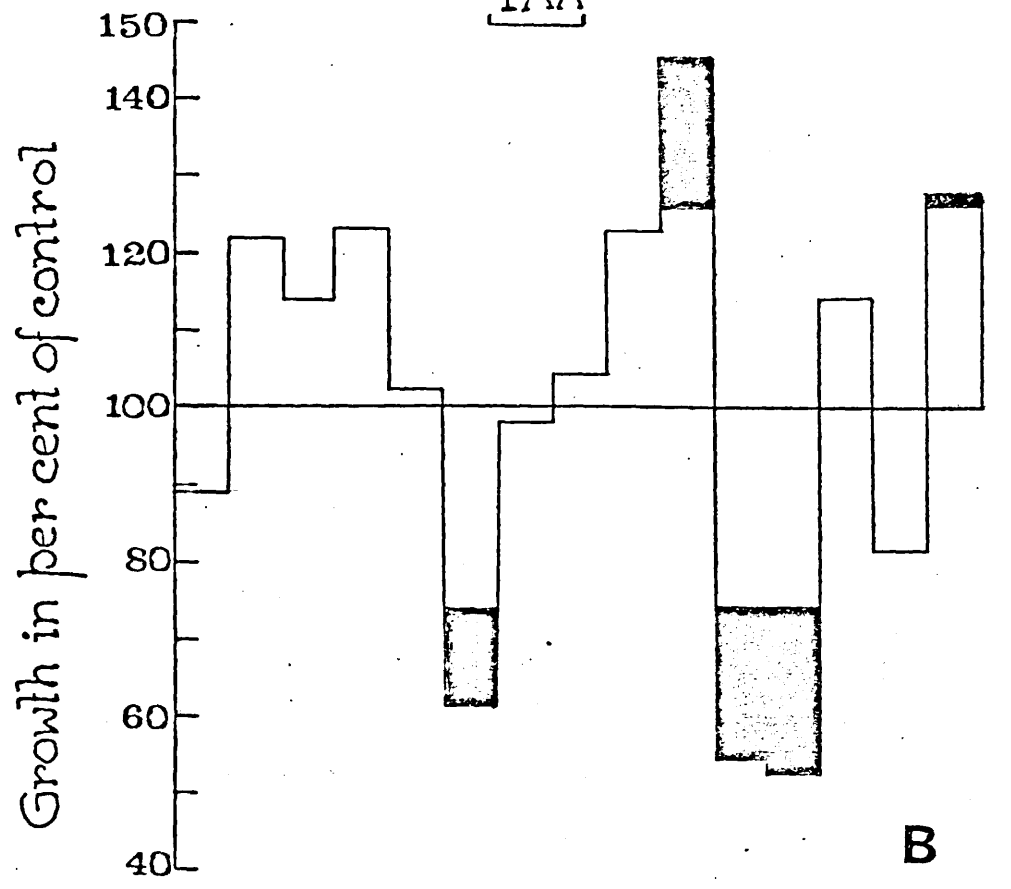
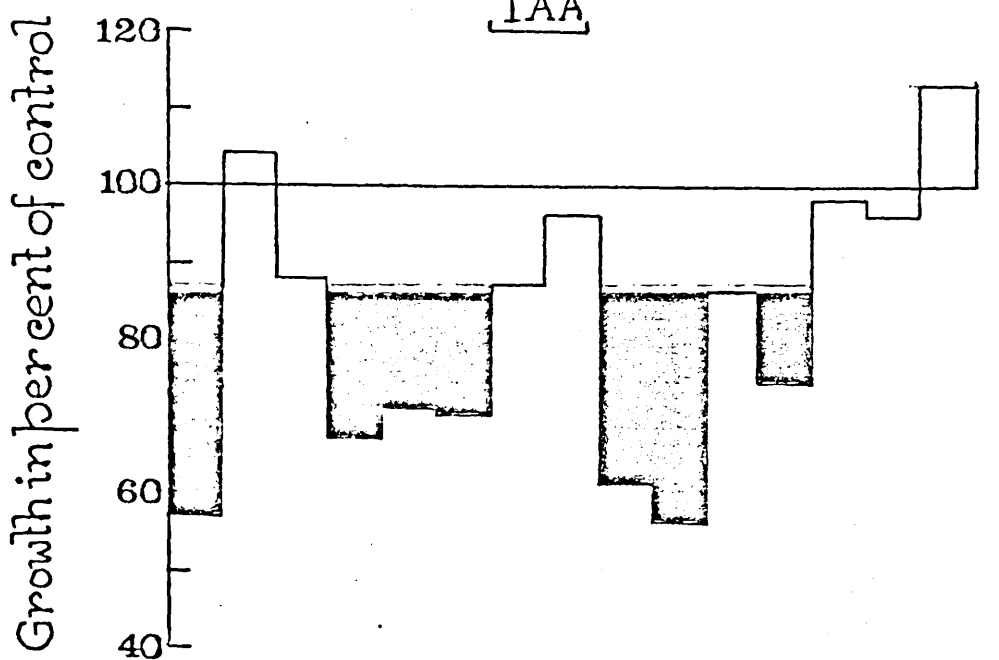
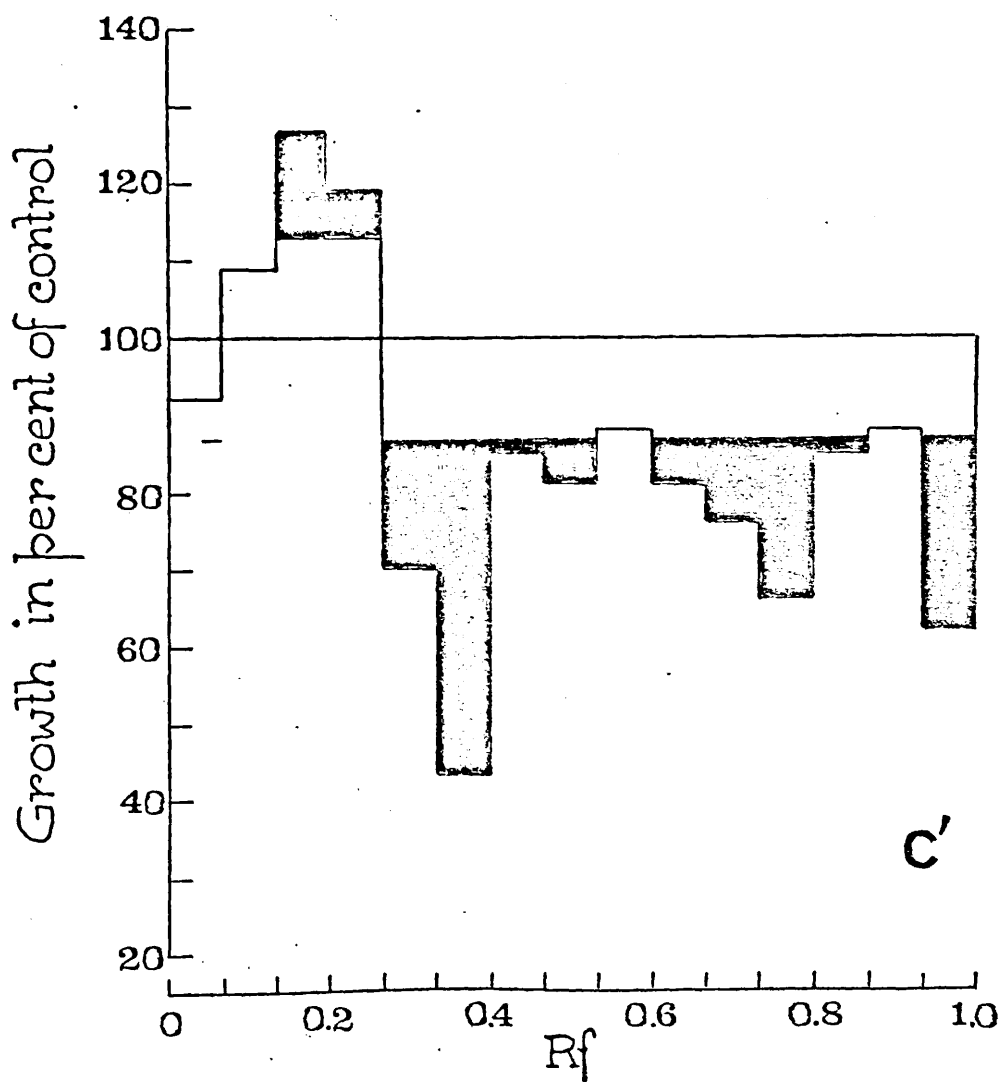


Fig.43(Contd.)

IAA



C



C'

Fig.43(Contd.)

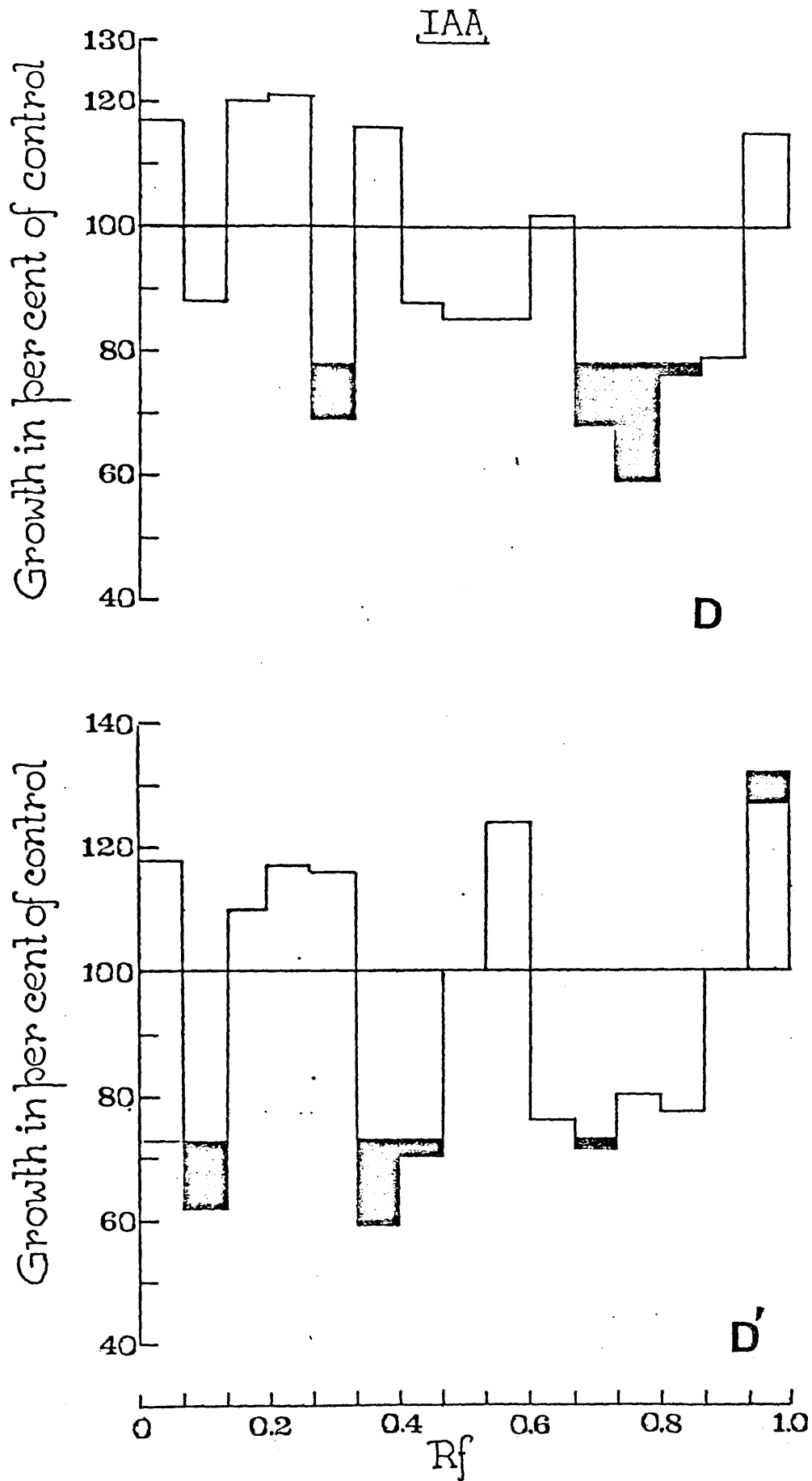
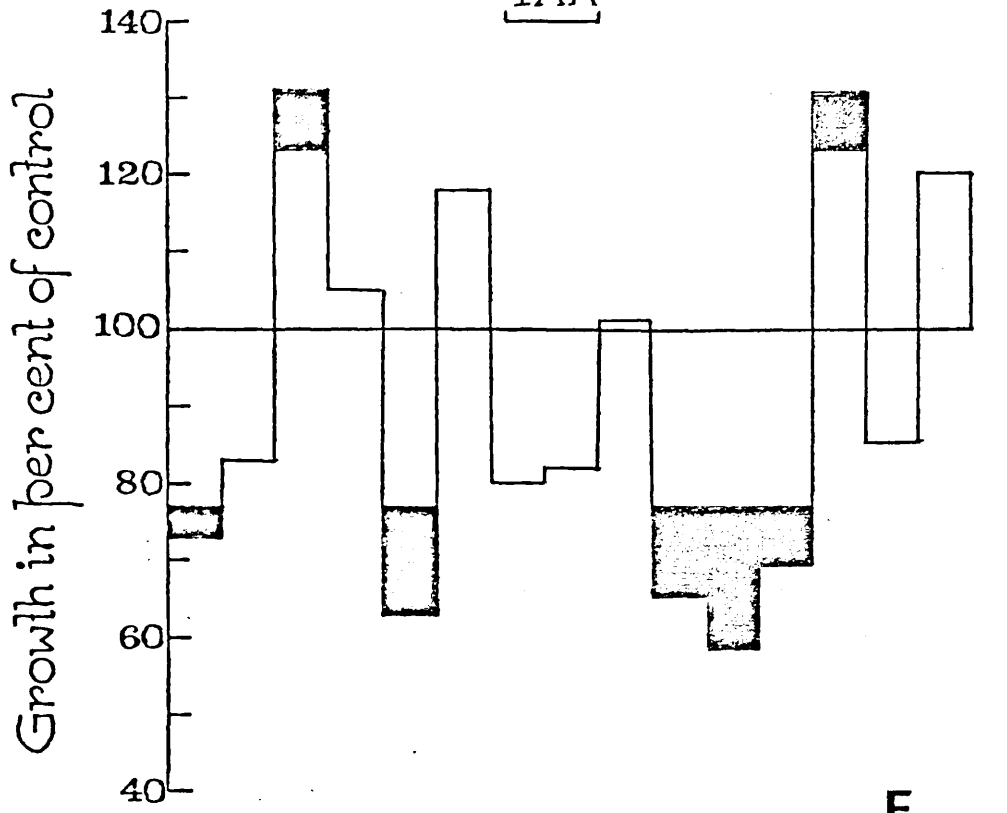
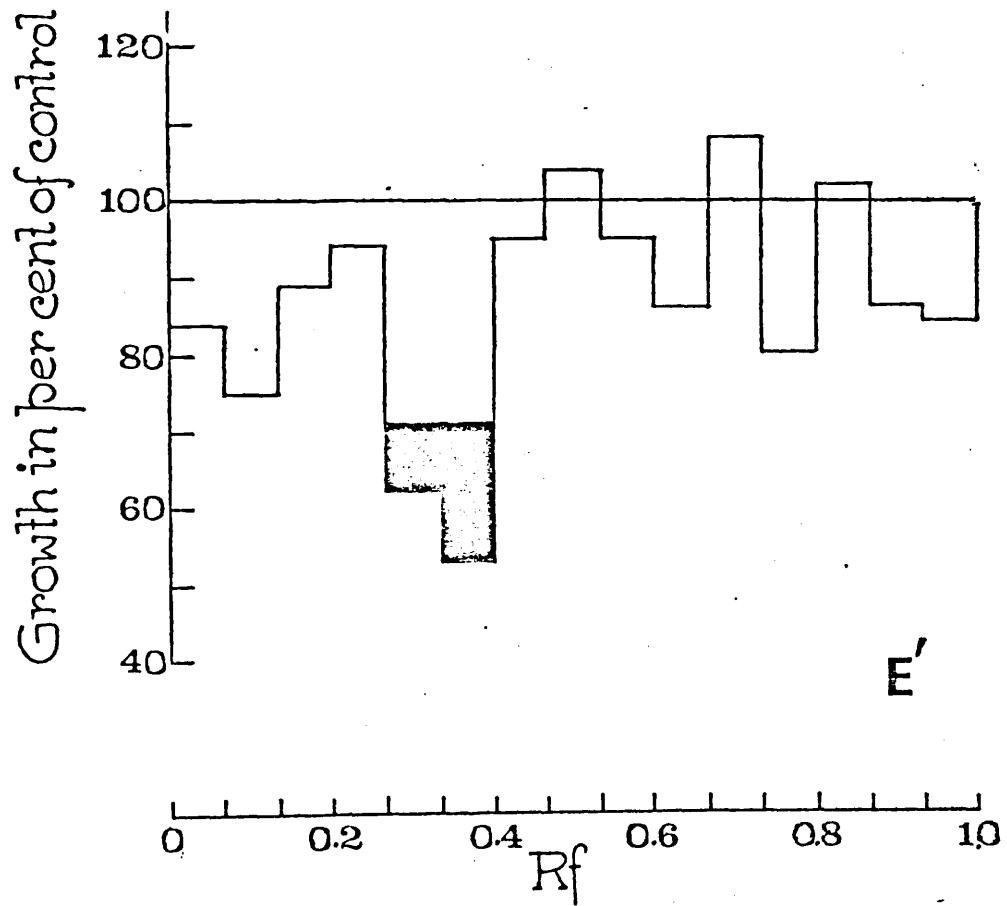


Fig.43(Contd.)

IAA



E



E'

CHAPTER IV

DISCUSSION

Before discussing the results in detail, it is necessary to point out that the name "inhibitor" used in the preceeding chapter (and also in the succeeding pages) represents the active zones on the chromatogram which appear more or less regularly and uniformly between particular Rf values and are chosen tentatively for the ease of description. They are not intended to represent single substances.

The present investigation reveals the presence of two major ether soluble growth inhibitors in the acid fraction of the extracts of the extreme 5 mm tips of young seminal roots of Zea mays. One of these, the 'cap inhibitor', is principally located in the root cap and from its chromatographic properties it resembles inhibitor β , a common growth active substance frequently reported to be present in roots (see the review of Åberg, 1957). This is possibly the same substance 'AI(ii)', obtained in pea roots by Audus and Gunning (1958) and also by Lahiri and Audus (1960) in Vicia faba. From the rechromatography of the eluates of this active substance, using three different solvent systems it appears to be a single substance rather than a complex of inhibitors at the same Rf.

The main active component of inhibitor β has already been established to be ABA. The 'cap inhibitor' has a similar Rf value with the authentic ABA (Fig. 26) and also possesses anti-transpirant activity, a specific and characteristic property of ABA. Thus it is tempting to conclude that the 'cap inhibitor' contains predominantly this substance. However, contrary to the

authentic ABA, the 'cap inhibitor', on the other hand, yields a distinct purple colouration with Salkowski's reagent. This differential reaction towards this chromogenic spray suggests that this zone of inhibition may contain other substances in addition to ABA. Dörffling (1972) in a recent review on ABA mentioned that one should be very careful not to interpret the occurrence and quantitative variation of inhibitor β in terms of ABA, as inhibitor β is nothing else than the unpurified fraction of a one dimensional chromatogram which is inhibitory in some bioassays. For instance, in pea root extracts, inhibitor β consists of several compounds including trans-cinnamic acid and ABA (Tietz, 1971). So, the colour reaction obtained here may be attributed to other substances present in the same Rf with ABA which is not separable by the present method of chromatography. This needs a further critical investigation. Since the work was completed ABA has been identified in the caps of maize roots grown in light by Wilkins and Wain (1974).

The other major inhibitor, or the 'meristem inhibitor' is present all along the root tip except in the root cap and there is a slight indication that its concentration is considerably higher, approximately 5-6 mm away from the apical extremity (Fig. 35). The nature of this substance is unknown and whether or not this inhibiting zone is attributable to one single substance or an inhibitor complex is equally uncertain at this stage. It may contain one or more phenolic substances (see page 136).

The 'inhibitor-3', frequently appeared in the acid and neutral fractions of the extract (but consistently appeared in the primary ether fraction) is also found all along the root tip except in the root cap. Its activity is, however, substantially low compared to the other two inhibitors. Judging from the Rf value

alone this substance is suspected to be xanthoxin (Fig. 28), a neutral inhibitor and a close relative of ABA which is found to occur in several plants at concentrations similar to concentrations of ABA (Firn et al., 1972). This substance also possesses anti-transpirant activity like ABA, though the antitranspirant property of xanthoxin is yet to be established. Firn and Raschke (1973) have observed that xanthoxin supplied via the transpiration stream causes a rapid closure of stomata in wheat, oat, barley and beans. The speed and magnitude of the response are similar to that found following the application of the same concentration of ABA to the same tissue. So, there is a possibility that xanthoxin per se is active, however, the possibility of a rapid and efficient conversion of xanthoxin to ABA in the above tissues cannot be excluded. From the recent study of mass spectrometry by Stanbury (see Kundu and Audus, 1974) xanthoxin is found to be present in the bulk extract of maize roots. This observation is at variance with the findings of Wilkins and Wain (1974) who were unable to detect any xanthoxin in Zea mays roots. On the other hand, they found another compound 6-methoxy-2 benzoxazoline, a compound having certain chromatographic properties similar to xanthoxin from the whole maize roots but not in the cap.

No significant growth activity was obtained in the region of IAA in the chromatogram of the cap extract, indicating its absence in the root cap (Figs. 14 and 32). Its appearance in one of the cap extracts (indicated by the inhibition of growth at the same Rf of authentic IAA : Fig. 14 D) is very likely due to the contamination of the samples of the root caps by the meristematic tissue. In the whole root tips, growth activity in the position of

IAA appeared sporadically. Assuming this substance is IAA, from such activity on chromatogram the IAA content (based on the calibration curve presented in Fig. 9) in the root tip is of the order of 10^{-10} to 10^{-9} M equivalent per 33 root tips. Calculations based on the fresh weight this comes to about 3.3 ng/kg (fresh weight of 100 tips is 0.75 gm) which is substantially low. Recently IAA has been positively identified in maize root by Greenwood et al. (1973) but this IAA is principally located in the stele. They have estimated the IAA content of different regions of maize root using the Avena coleoptile straight growth bioassay which shows that the stele contains about 142 µg/kg, whereas 2-3 mm root tips contain approximately 11 µg/kg of IAA. Rivier and Pilet (1974) using mass fragmentography found about 365.6 ± 16.2 µg/kg fresh weight of IAA present in the root cap of maize. If this figure is taken into account then the presence of IAA must show up in the assay. The reason for such a discrepancy is yet to be resolved.

For a long time the hormonal mechanism behind the geotropism of the root has been based on the Cholodny-Went theory. However, the presence of supra-optimal concentration of IAA in root tips, the basic condition necessary to establish the theory is still controversial. A major objection of this theory came from the results of Audus and Brownbridge (1957) who found a marked overall decrease in growth rates of both upper and lower halves of pea root following geotropic stimulation. They also found that during downward curvature of pea roots, when according to the Cholodny-Went theory the lower half of the tip was supposed to contain auxin at much more supra-optimal levels, the growth rate of both the upper and lower halves of the root is promoted by low concentration of IAA. This observation posed a

serious challenge of the direct participation of IAA in root geotropism. With the above findings, acropetal polarity in the movement of IAA in roots and a greater concentration of IAA in the steler region of root rather than the tip, indicate the participation of a separate inhibitor mediating the geotropism of roots. Decapping experiments with maize roots by Juniper et al. (1966), indirectly but convincingly, proves that the root cap is the possible source of this inhibitor. This observation was subsequently confirmed by Gibbons and Wilkins (1970) and Pilet (1971). They removed one half of the root cap in maize by microsurgery and orientating them either vertically or horizontally found the development of large curvatures towards the remaining half cap, regardless of the orientation of the root with respect to gravity. These findings further suggest that the root cap is the source of at least one growth inhibiting substance responsible for the geotropic response. Another important observation was by Scott and Wilkins (1968) who found that the primary root of maize grown in total darkness did not exhibit a positive geotropic response. Furthermore, the apical region of maize root has been shown to be involved in the response of roots to white light, with removal of root cap completely eliminating the light induced inhibition of root elongation (Wilkins and Wain, 1974). Correlating all the above information, it is logical to conclude that the root cap is the source of a growth inhibiting factor and plays a critical role in geotropic response and/or perception to white light. In the succeeding paragraphs the possible role of the 'cap inhibitor' - which is the only ether soluble inhibitor present in the root cap of maize, has been discussed in connection with the root geotropism. This discussion is based mainly on the assumption^m that the chemical nature of the 'cap inhibitor' is ABA.

When the roots are grown in a strictly vertical position in the dark the cap inhibitor is either absent or present in a substantially low amount (Fig. 38 A). However its production is augmented in illuminated vertical roots (Fig. 41 A). This clearly suggests that the production of this inhibitor is light dependent. The light dependent production of inhibitor β has also been observed by Masuda (1962) and recently a similar production of ABA in the root cap by Wilkins and Wain (1974). The intricate mechanism behind the light-induced ABA synthesis in plants is not clear. Either light intensity directly or resulting water stress due to wilting or both simultaneously account for increasing ABA level in the illuminated tissues (Dörffling, 1972).

Gravity, on the other hand, induces the production of the 'cap inhibitor' even in the dark (Fig. 38 A') and this production is maximal when the roots are stimulated in the presence of light. The idea of gravity induced hormonal production is nothing new. Audus and Brownbridge (1957) suspected de novo production of inhibitor under the stimulus of gravity. Direct evidence of this is from the observation of Audus and Lahiri (1961) who found an 'explosive production' of AP(ii) (IAA-like substance) after 40 minutes of stimulation in Vicia faba root tips. How gravity influences the production of this inhibitor is speculative at this stage. A brief review of some of the current findings is necessary to outline the probable mechanism of gravity induced inhibitor production in the root tips of maize.

Recently there has been more or less conclusive evidence that sedimenting amyloplasts are the organelles directly acted on by gravity and are therefore considered as gravity sensors particularly in the root tip cells (Schachar, 1967; Iversen, 1969; Barlow and Grundwag, 1974). Convincing proof of this came from the work of

Iversen (1969) who found that the roots of Lepidium sativum after incubating in solutions containing $4.3 \times 10^{-5}M$ gibberellic acid and kinetin at $35^{\circ}C$ for 29 hours, thus causing a complete disappearance of starch from the amyloplast of the root cap cells, when geotropically stimulated for 30 minutes and then rotated on a klinostat at $21^{\circ}C$, showed no trace of curvature after 60 minutes of rotation. Control roots similarly pretreated at $35^{\circ}C$ in water had curved to 22° after 60 minutes. However there were no substantial changes in the growth rate of the treated and control seedlings during that period. When the hormone treated seedlings are illuminated, formation of starch in the amyloplasts took place after 20-24 hours and the geotropic responsiveness was restored at the same time. Barlow (1974) observed that the removal of the cap from the root tip of Zea immediately stimulates the development of amyloplasts in the quiescent centre and remaining apices and the gravity sensitivity reappeared well before the regeneration of the new cap which is not complete until after 3-4 days. This finding further suggests that the amyloplasts are the distinct organelles related to the geosensitivity of roots.

The sedimenting amyloplasts in the next reaction sequence somehow or other exert a mechanical pressure on some other cell component or cell surface thus causing the release of the inhibitor. This cell component may be the endoplasmic reticulum as suggested by Sievers and Volkmanns (1972) or as suggested by Audus (1971) that the amyloplasts directly exert a mechanical pressure on the membranes along the tangential longitudinal walls of the cap cells thus releasing the inhibitor. There is another possibility suggested by Barlow (1974) that the sedimenting amyloplasts themselves may be the source of this inhibitor as the geotropic

response in Zea mays is recovered before a new cap starts to regenerate. Whether the ABA-like cap inhibitor is produced in the amyloplasts is not known but cell-free preparations of the chloroplasts are capable of ABA synthesis from mevalonic acid as has already been demonstrated by Milborrow (1974) in Avocado fruits.

On the whole, the gravitational stress as well as illumination, increase the amount of the 'cap inhibitor' to a maximal level to mediate the geotropic response in horizontal roots. In the dark, synthesis of ABA is hindered and the amount of the inhibitor does not reach that level. This may be one of the possible reasons for the absence of curvature of the stimulated roots of maize in the dark.

Pioneering work of Hawker (1932) clearly indicated that the lower half of the tip of the stimulated root of Zea mays contained more inhibitor than that of the upper half of the tip. Recent microsurgical experiments by Shaw and Wilkins (1973) and Pilet (1973) indicate that the positive geotropic responses of the primary roots of Zea mays and Fisum sativum seedlings depend upon at least one inhibiting factor which arises in the root cap and which moves basipetally through the apex into the extending zone. The downward curvature caused by restricting the supply of this inhibitor to the upper half of the growing zone of a horizontal root is greater than the upward curvature caused by restricting the supply of inhibitor to the lower half, regardless of whether the restriction is achieved by the insertion of a unilateral barrier (mica foil) into the upper or lower half of the root just behind the cap, or by the removal of either the upper or lower half of the root cap. These findings suggest that the gradient

in the concentration of an inhibitor or its effectiveness is established in the growing zone of geotropically stimulated roots of Zea mays. However, these observations are difficult to correlate with my findings for the following reasons.

In the first place, I have shown the inhibitor content of the lower half of the root tip to be greater than that of the upper half and thus establishing a concentration gradient between the two halves of the tip, only when the roots are stimulated in the dark (Fig. 39). But once again, it must be mentioned that the curvature does not take place in the dark particularly in Zea mays roots.

Secondly, in light after half an hour of stimulation an unusual distribution of the inhibitors takes place. Here the 'cap inhibitor' moves into the upper half whereas the 'meristem inhibitor' is mainly restricted to the lower half of the horizontal root tip (Figs. 42 and 43). This movement of the cap inhibitor towards the upper half is definitely light dependent. A similar distribution of 'AP(ii)' in the upper half of the stimulated roots of Vicia faba has also been reported by Lahiri (1959).

All the above observations are quite contrary to the classical hormonal redistribution theory of root geotropism. However, the present findings and the observations of Shaw and Wilkins (1973) and Pilet (1973) can be correlated in several ways. One explanation

could be that the 'cap inhibitor' is not directly involved in producing the curvature. This inhibitor after reaching the upper half is converted to another growth factor (an inhibitor), which is then transported laterally downwards into the extending zone thus increasing its concentration in the lower half resulting in curvature. The positive curvature in a horizontal root is attributable, at least in part, to a downward lateral transport of inhibitor from the upper to the lower half of the Zea mays roots has also been suspected by Shaw and Wilkins (1973). The latter inhibitor in that case must show up in the assay, and ^{as} the 'meristem inhibitor' is the only inhibitor present in the extending zone in a reasonable concentration for such a response; it is tempting to believe that the meristem inhibitor as a whole (or, one of its components) is the product of such a conversion. There is not much variation in the content of the 'meristem inhibitor' in the vertical as well as stimulated roots either in the dark or in the presence of light. However, its greater concentration in the lower half (in a reciprocal amount of the 'cap inhibitor' it moved to the upper half (Fig. 43)) further supports this contention. In the dark grown vertical roots, the absence of the 'cap inhibitor' but the presence of the 'meristem inhibitor' can be interpreted as a total conversion of the cap inhibitor already present in the tissue before placing them in the dark. However, why the cap inhibitor moves to the upper half in light is difficult to explain at this stage and needs further experimentation.

Another possibility postulated by Shaw and Wilkins (1973) is that the root apex may be a source of factors which interact with the regulators from the cap to effect an inhibition of cell elongation in the growing zone. Thus the cap inhibitor instead of being itself converted to the meristem inhibitor activates the

the meristem inhibitor present in the growing zone. This seems improbable as the 'meristem inhibitor' itself is an inhibitor per se.

All the above explanations, needless to say are extremely speculative. The role of the inhibitor-3 can only be guessed at this stage. Taylor and Burden (1973) demonstrated very high incorporation levels of 2- $[^{14}\text{C}]$ -cis, trans-xanthoxin into ABA in the shoot segments of tomato and dwarf beans. From this observation they suggested that plants can synthesize ABA either from a sesquiterpenoid precursor (mevalonic acid) or via the oxidation of a xanthophyll depending upon such factors as plant species, physiological age of the tissue, and environmental conditions. Such conversion may take place in the root tips of maize. However, this possibility is based on the assumption that the inhibitor-3 is xanthoxin. In that case xanthoxin has some indirect role in root geotropism. Recent studies by Wilkins et al. (1974) have shown that a 10 μM solution of xanthoxin can inhibit root growth of wheat seedlings by up to 83% in the dark but is less effective in light. This means that in the presence of xanthoxin there is no significant effect of light on growth. Implications of the above observations are far reaching in the context of the present investigation unless the chemical nature of the inhibitor-3 is fully identified.

In summing up, the present investigation does not reveal the complete chemical nature of the inhibitors present in the root tips of Zea mays. Until their chemical identifications are complete the actual hormonal mechanism behind the root geotropism cannot be

fully envisaged. However, the present observation, once again, clearly indicates that the geotropism of root is not merely the redistribution of auxin in the root tip under the gravitational stress as postulated by the classical Cholodny-Went theory. Further investigations on this problem are being actively pursued in several laboratories. Whatever the outcome of these investigations the results of the present experiments have thrown further doubt on the participation of IAA in the geotropic response of root.

SUMMARY

1. A micro-assay based on the growth inhibition of root segments of Zea mays roots has been designed to investigate the root growth inhibiting substances present in the root tips of Zea mays. This micro-assay is sensitive to 50 pg of IAA.

2. The endogenous inhibitors in roots have been studied using paper chromatographic methods and using three different solvent systems.

3. The chromatogram of the acid ether fraction of the root tip extract reveals the presence of two major growth inhibiting substances. One of them is principally located in the cap and the other is present in the meristem and absent in the cap.

4. The cap inhibitor appears in the relevant position of abscisic acid on the chromatogram and possesses the stomata-closure property evidenced from the antitranspirant assay. This observation suggests that the 'cap inhibitor' contains predominantly this substance.

5. Analyses of the extracts of successive 2 mm segments stretching back proximally from the cap/meristem junction as well as the diffusion studies indicate that the 'cap inhibitor' cannot move far beyond the meristem.

6. The 'meristem inhibitor' on the other hand, is present all along the meristem. The nature of this substance is not known. This substance may be a complex of inhibitors in the same Rf and possibly contains two phenolic substances.

7. There is no indication of the presence of IAA in the root cap. However its presence in the meristem is sometimes indicated by small inhibitions (or stimulations) at the characteristic Rf of IAA. Judging from its activity the amount of IAA present in the root tips seems to be of the order of 3.0 ng/kg fresh weight.

8. Another inhibitor, apart from the major inhibitors is also present consistently in the meristem but is not detected in the cap. From its position on the chromatogram it resembles xanthoxin. This substance also possesses the antitranspirant property like the 'cap inhibitor'.

9. When the roots are grown in strictly vertical position in the dark, the cap inhibitor is found to be absent; but it is present in the roots grown vertically in the presence of light. There is not much variation in the content of the 'meristem inhibitor' under either of these conditions.

10. When the roots are stimulated in the presence of light a maximum production of the 'cap inhibitor' is found, which is contrary to the low cap inhibitor content of similar tips stimulated in the dark.

11. In the dark, a greater concentration of both the inhibitors are found to be in the lower halves of the stimulated tips. In the presence of light, on the other hand the 'cap inhibitor' moves to the upper half of the stimulated tip whereas the concentration of the meristem inhibitor is found to be greater in the lower half.

BIBLIOGRAPHY

(An asterisk before a reference means that it was taken from a review, book or another paper and not consulted in the original.)

- ° Åberg, B. (1952). On the effects of weak auxins and anti-auxins upon root growth. *Physiol. Plantarum*, 5: 305-319.
- ° Åberg, B. (1957). Auxin relations in roots. *Ann. Rev. Pl. Physiol.*, 8: 153-180.
- *Audus, L. J. (1959). In: 'Proceedings of the symposium on plant growth regulators', pp.9-22. Poland : Torun.
- Audus, L. J. (1971). Linkage between detection and the mechanisms establishing differential growth factor concentrations. In: "Gravity and the organism" (eds. Gordon, S. A. and Cohen, M. J.) The University of Chicago Press, pp. 137-150.
- Audus, L. J. and Brownbridge, M. E. (1957) Studies on the geotropism of roots. I. Growth rate distribution during response and the effects of applied auxins. *J. Exptl. Bot.*, 8 : 105-124.
- Audus, L. J. and Das, N. (1955) The interactions of auxins and antiauxins in the stimulation of root growth. *J. Exptl. Bot.*, 6: 328-347.
- Audus, L. J. and Garrard, A. (1953) Studies on the growth and respiration of roots. I. The effects of stimulatory and inhibitory concentrations of β -indolylacetic acid on root sections of *Pisum sativum*. *J. Exptl. Bot.*, 4: 330-348.
- Audus, L. J. and Gunning, B. E. S. (1958) Growth substances in the roots of *Pisum sativum*. *Physiol. Plantarum*, 11: 685-697.
- Audus, L. J. and Lahiri, A. (1961) Studies on the geotropism of roots. III. Effects of geotropic stimulation on growth substance concentrations in *Vicia faba* root tips. *J. Exptl. Bot.*, 12: 75-84.
- Audus, L. J. and Thresh, R. F. (1953) A method of plant growth substances assay for use in partition chromatography. *Physiol. Plantarum*, 6: 451-465.

- Barlow, P. W. (1974) Recovery of geotropism after removal of the root cap. *J. Exptl. Bot.*, 25: 1137-1146.
- Barlow, P. W. and Grundwag, M. (1974) The development of amyloplasts in cells of the quiescent centre of Zea roots in response to removal of the root cap. *Z. PflPhysiol.*, 73: 56-64.
- Bennet-Clark, T. A. and Kefford, N. P. (1953) Chromatography of the growth substances in plant extracts. *Nature*, 171: 645-647.
- Bennet-Clark, T. A., Tambiah, M. S. and Kefford, N. P. (1952) Estimation of plant growth substances by partition chromatography. *Nature*, 169: 452-453.
- Bennet-Clark, T. A., Younis, A. F. and Esnault, R. (1959) Geotropic behaviour of roots. *J. Exptl. Bot.*, 10: 69-86.
- Bhatia, I. S., Bajaj, K. L., Verma, A. K. and Joginder Singh (1971) A general spray reagent for phenolic compounds on thin-layer plates. *J. Chromatogr.*, 62: 471-472.
- Bonner, J. and Koepfli, J. B. (1939) The inhibition of root growth by auxins. *Amer. J. Bot.*, 26: 557-566.
- Bowen, M. R., Wilkins, M. B., Cane, A. R. and McCorquodale, I. (1972) Auxin transport in roots VIII. The distribution of radioactivity in the tissues of Zea root segments. *Planta (Berl.)*, 105: 273-292.
- *Boysen-Jensen, P. (1933) Die Bedeutung des Wuchsstoffes für das Wachstum der Wurzeln von *Vicia Faba*. *Planta*, 20: 688-698.
- Bridges, I. G., Hillman, J. R. and Wilkins, M. B. (1973) Identification and localization of auxin in primary roots of Zea mays by mass spectrometry. *Planta (Berl.)*, 115: 189-192.

- Burnett, D., Audus, L. J. and Zinsmeister, H. D. (1965) Growth substances in the roots of Vicia faba. II. Phytochemistry, 4: 891-904.
- *Bünning, E. (1928) Zur physiologie des Wachstums und der Reizbewegungen der Wurzeln. *Planta*, 5: 635-659.
- *Cholodny, N. (1926) Beiträge zur Analyse der geotropischen Reaktion. *Jahrb. Wiss. Botan.*, 65: 447-459.
- *Cholodny, N. (1931) Zur Physiologie des pflanzlichen Wuchshormons. *Planta*, 14: 207-216.
- Darwin, C. (1880) In: "The power of movement in plants". John Murray, London.
- Dörffling, K. (1972) Recent advances in ABA research. In: "Hormonal regulation in plant growth and development" (eds. Kaldeway, H. and Vardar, Y.) Verlag Chemie, Weinheim, pp. 281-295.
- Dullaart, J. (1967) Quantitative estimation of indoleacetic acid and indolecarboxylic acid in root nodules and roots of Lupinus luteus. *Acta. Bot. Neerl.*, 16: 222-230.
- Dullaart, J. (1970) The auxin content of root nodules and roots of Alnus glutinosa (L.) Vill. *J. Exptl. Bot.*, 69: 975-984.
- Elliott, M. C. (1971) α -N-malonyl-D-tryptophan in seedling wheat roots. *New Phytol.*, 70: 1005-1015.
- Firn, R. D., Burden, R. S. and Taylor, H. F. (1972) The detection and estimation of the growth inhibitor xanthoxin in plants. *Planta (Berl.)*, 102: 115-126.
- Firn, R. D. and Raschke, K. (1973) The effect of xanthoxin on stomatal aperture. In: Annual report MSU/AEC plant research Lab. Michigan State University, pp.158.

- Gibbons, G. S. B. and Wilkins, M. B. (1970) Growth inhibitor production by root caps in relation to geotropic responses. *Nature*, 226: 558-559.
- Greenwood, M. S., Hillman, J. R., Shaw, S. and Wilkins, M. B. (1973) Localization and identification of auxin in roots of Zea mays. *Planta (Berl.)*, 109: 369-374.
- Hawker, L. E. (1932) Experiments on the perception of gravity by roots. *New Phytol.*, 31: 321-328.
- Housley, S. and Bentley, J. A. (1956) Studies in plant growth hormones. IV. Chromatography of hormones and hormone precursors in cabbage. *J. Exptl. Bot.*, 7: 219-238.
- Iversen, T. H. (1969) Elimination of geotropic responsiveness in roots of cress (Lepidium sativum) by removal of statolith starch. *Physiol. Plantarum*, 22: 1251-1262.
- Jenkins, P. A. and Shepherd, K. R. (1972) Identification of abscisic acid in young stems of Pinus radiata D. Don. *New Phytol.*, 71: 501-511.
- Juniper, B. E., Groves, B., Landau-Schacher, B. and Audus, L. J. (1966) Root cap and the perception of gravity. *Nature*, 209: 93-94.
- Keeble, F., Nelson, M. G. and Snow, R. (1929) The integration of plant behaviour. I. Separate geotropic stimulation of tip and stump in roots. *Proc. Roy. Soc. (London)*, series B, 105: 493-498.
- Keeble, F., Nelson, M. G. and Snow, R. (1931) The integration of plant behaviour. III. The effect of gravity on growth of roots. *Proc. Roy. Soc. (London)*, ser. B., 108: 360-365.
- Kefford, N. P. (1955) The growth substances separated from plant extracts by chromatography. I. *J. Exptl. Bot.*, 6: 129-151.

- Konings, H. (1967) On the mechanism of the transverse distribution of auxin in geotropically exposed pea roots. Acta Bot. Neerl., 16: 161-176.
- Konings, H. (1968) The significance of root cap for geotropism. Acta. Bot. Neerl., 17: 203-211.
- *Kögl, F., Haggen-Smit, A. J. and Erxleben, H. (1934) Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Gras-Koleoptilen. Zeit. Physiol. Chemie., 228: 104-112.
- Kundu, K. K. and Audus, L. J. (1974) Root-growth inhibitors from root tips of Zea mays L. Planta (Berl.), 117: 183-186.
- Lahiri, A. N. (1959) "Studies in the hormone relations of root growth". Ph. D. Thesis, University of London.
- Lahiri, A. N. and Audus, L. J. (1960) Growth substances in the roots of Vicia faba. J. Exptl. Bot., 11: 341-350.
- Lane, R. H. (1936) The inhibition of roots by growth hormone. Amer. J. Bot., 23: 532-535.
- Larsen, P. (1961) Biological determination of natural auxins. In: "Encyclopaedia of plant physiology". (ed. Rhuland, W.) Vol. 14, pp. 521-582. Springer-Verlag.
- Lexander, K. (1953) Growth regulating substances in roots of wheat. Physiol. Plantarum, 6: 406-411.
- Marmer, D. (1937) Growth of wheat seedlings in solutions containing chemical growth substances. Amer. J. Bot., 24: 139-145.
- Masuda, Y. (1962) Effects of light on a growth inhibitor in wheat roots. Physiol. Plantarum, 15: 780-789.
- Milborrow, B. V. (1967) The identification of (+)-Abscisin II ((+)-Dormin) in plants and measurement of its concentration. Planta (Berl.), 76: 93-113.

- Milborrow, B. V. (1974) Biosynthesis of abscisic acid by a cell-free system. *Phytochemistry*, 13: 131-136.
- *Nielsen, N. (1930) Untersuchungen über einen neuen Wachstumsregulierenden Stoff: Rhizopin. *Jahrb. Wiss. Bot.*, 73: 125-191.
- *Ochs, G. (1954) Zur Wirkungsweise des Wuchsstoffes auf das Streckenwachstum der Wurzel. *Planta*, 44: 412-436.
- Ogunkanmi, A. B., Tucker, D. J. and Mansfield, T. A. (1973) An improved bio-assay for abscisic acid and other anti-transpirants. *New Phytol.*, 72: 277-282.
- Pilet, P. E. (1964) Auxin transport in roots (*Lens culinaris*) *Nature*, 204: 561-562.
- Pilet, P. E. (1971) Root cap and georeaction. *Nature*, 233: 115-116.
- Pilet, P. E. (1972) Root cap and root growth. *Planta (Berl.)* 106: 169-171.
- *Pilet, P. E. (1972) Geotropisme et georeaction racinaires. *Physiol. Veg.*, 10: 347-361.
- Pilet, P. E. (1973) Growth inhibitor from the root cap of *Zea mays*. *Planta (Berl.)*, 111: 275-278.
- *Pohl, R. (1951) Die Wirkung von β -Indolacetaldehyd auf das Wachstum der Kressewurzeln. *Ber. Deut. Botan. Ges.* 64: 132-135.
- Rivier, L. and Pilet, P. E. (1974) Indolyl-3-acetic acid in cap and apex of maize roots : Identification and quantification by mass fragmentography. *Planta (Berl.)* 120: 107-112.
- Robinson, P. M. and Wareing, P. F. (1964) Chemical nature and biological properties of the inhibitor varying with photoperiod in Sycamore (*Acer pseudoplatanus*) *Physiol. Plantarum*, 17: 314-323.
- Rufelt, H. (1957) Influence of growth substances on the geotropic reactions of wheat roots. *Physiol. Plantarum*. 10: 500-520.

- Schachar, B. (1967) "The root cap and its significance in geoperception". Ph.D. Thesis, University of London.
- Scott, T. K. and Wilkins, M. B. (1968) Auxin transport in roots. II. Polar flux of IAA in Zea roots. Planta (Berl.), 83: 323-334.
- Shaw, S. and Wilkins, M. B. (1973) The source and lateral transport of growth inhibitors in geotropically stimulated roots of Zea mays and Pisum sativum. Planta (Berl.), 109: 11-26.
- Sievers, A. and Volkmann, D. (1972) Verursacht differentieller Druck der Amyloplasten auf ein Komplexes Endomembransystem die Geoperzeption in Wurzeln? Planta (Berl.), 102: 160-172.
- Stowe, B. and Thimann, K. V. (1954) The paper chromatography of indole compounds and some indole containing auxins of plant tissues. Arch. Biochem. and Biophys. 51: 499-516.
- Taylor, H. F. (1969) "Studies on the regulation of plant growth with chemicals" Ph.D. Thesis, University of London.
- Taylor, H. F. and Burden, R. S. (1973) Preparation and metabolism of 2-(¹⁴C)-cis, trans-xanthoxin. J. Exptl. Bot. 24: 873-880.
- *Terpstra, W. (1953) "Extraction and identification of growth substances". Doctoral thesis, University of Utrecht.
- Thimann, K. V. (1936) Auxins and the growth of roots. Amer. J. Bot., 23: 561-569.
- Thresh, R. F. (1956) "Studies on the effects of the applications of synthetic growth substances, on the natural hormone content of plants". Ph.D. Thesis, University of London.
- *Tietz, A. (1971) Die Wirkung von Abscisinsäure und Gibberellinsäure auf Wachstum und Hormongehalt des Erbsenkeim lings unter besonderer Berücksichtigung des Wurzelsystems. Diss., Hamburg.

- Torrey, J. G. (1959) A chemical inhibitor of auxin induced lateral root initiation in roots of Pisum. *Physiol. Plantarum*, 12: 873-887.
- Tucker, D. J. and Mansfield, T. A. (1971) A simple bio-assay for detecting antitranspirant activity of naturally occurring compounds such as abscisic acid. *Planta (Berl.)*, 98: 157-160.
- Vardar, Y. and Tözün, B. (1958) Role played by decapitation in growth and differentiation of Lens culinaris roots. *Amer. J. Bot.*, 45: 714-718.
- Walker, D. R., Hendershott, C. H. and Snedecor, G. W. (1958) A statistical evaluation of a growth substance bioassay method using extracts of dormant peach buds. *Pl. Physiol.* 33: 162-166.
- Willmer, C. M. and Mansfield, T. A. (1969) A critical examination of the use of detached epidermis in studies of stomatal physiology. *New Phytol.*, 68: 363-375.
- Wilkins, H., Larqué-Saavedra, A. and Wain, R.L. (1974) Studies on plant growth regulating substances. XXXVII. The effects of light and hormone inhibitors on plant root growth. *Ann. Appl. Biol.*, 78: 169-177.
- Wilkins, H., Larqué-Saavedra, A. and Wain, R. L. (1974) Control of Zea root elongation by light and the action of 3,5-di-iodo-4 hydroxy-benzoic acid. *Nature*, 248: 449-450.
- Wilkins, H. and Wain, R. L. (1974) The root cap and control of root elongation in Zea mays L. seedlings exposed to white light. *Planta (Berl.)*, 121: 1-8.
- Wilkins, M. B. (1971) Hormone movement in geotropism. In: "Gravity and the organism" (eds. Gordon, S. A. and Cohen, M. J.) The University of Chicago Press, pp.107-126.
- Wilkins, M. B. and Scott, T. K. (1968) Auxin transport in roots. III. Dependence of the polar flux of IAA in Zea roots upon metabolism. *Planta (Berl.)*, 83: 335-346.

Yeomans, L. M. and Audus, L. J. (1964) Auxin transport in roots
(Vicia faba), Nature, 204: 559-561.

Younis, A. F. (1954) Experiments on the growth and geotropism of
roots. J. Exptl. Bot., 5: 357-372.

APPENDIX

Table 1

A. Percent increase in length of the root segments after 24 hours of incubation in different volumes of sucrose. Each figure represents the mean segment growth from five replications on each occasion.

Volume of sucrose in ml.	0.03	0.05	0.07	0.10	0.15
Occasion 1	136.00	185.50	178.00	169.50	145.00
Occasion 2	151.00	176.00	159.00	188.50	140.00
Occasion 3	131.50	171.00	168.00	140.50	124.00
Occasion 4	146.50	157.00	158.00	147.00	140.50
Mean	141.25	172.37	165.75	161.37	137.37

B. Table of the analysis of variance of the growth data presented in 'A'. The significant values at 5% probability level are marked with '*'.

Source of variance	Degrees of freedom	Total sum of squares	Mean square variance	Variance ratio (F)
Occasion	3	6.3666	2.1222	2.1813*
Volume	4	22.9089	5.7272	5.8872*
Occasion X volume	12	9.1745	0.7645	0.7657
Sample variance	80	15.9637	0.1995	0.2050
Residual	200	194.5901	0.9729	
Total	299	249.0038		

Table 2

A. Percent increase in length of the root segments in optimal volume of sucrose at different time intervals. Each figure represents the mean segment growth from five replications on each occasion.

Time in hours	3	6	12	24	48
Occasion 1	49.50	84.00	108.00	166.50	158.50
Occasion 2	53.00	100.50	127.00	177.50	178.50
Occasion 3	38.50	75.50	117.00	151.00	155.00
Occasion 4	44.50	88.00	124.50	161.50	163.00
Mean	46.37	87.00	119.12	164.12	163.75

B. Table of the analysis of variance of the growth data presented in 'A'. The significant values at 5% probability level are marked with '*'.

Source of variance	Degree of freedom	Total sum of squares	Mean square variance	Variance ratio (F)
Occasion	3	5.8586	1.9528	6.7014*
Hour	4	245.0581	61.2645	210.2419*
Occasion X Hour	12	1.7358	0.0014	0.0048
Sample variance	80	25.3213	0.3165	1.0861
Residual	200	58.2976	0.2914	
Total	299	336.2714		

Table 3

A. Growth of the root segments in different concentrations of IAA (growth expressed as % of control). Each figure represents the mean segment growth from five replications on each occasion.

Log concentrations in molar	SET I					SET II		
	-4	-5	-6	-7	-8	-9	-10	-11
Occasion 1	42.26	54.29	74.57	81.09	89.12	85.26	100.70	98.24
Occasion 2	39.20	47.84	70.14	79.13	96.08	102.49	99.61	86.18
Occasion 3	41.57	52.05	71.16	80.14	88.97	106.04	102.28	106.90
Occasion 4	44.83	61.20	75.80	86.12	94.18	107.82	104.56	105.09
Mean	41.96	53.84	72.91	81.62	92.08	93.38	103.98	103.92

B. Table of the analysis of variance of the growth data presented in 'A'. The significant values at 5% probability level are marked with '*'. *

Source of variance	Degree of freedom	Total sum of squares	Mean square variance	Variance ratio (F)
Occasion	3	I 3.0549	1.0183	4.4525*
		II 2.9016	0.9672	3.5624*
Concentration	4	I 97.5388	24.3847	106.6230*
		II 5.6541	1.4135	5.2062*
Occasion X concentration	12	I 0.6183	0.0515	0.2252
		II 2.3457	0.1954	0.7197
Sample variance	80	I 16.0241	0.2003	0.8785
		II 21.5532	0.2694	0.9922
Residual	200	I 45.7542	0.2287	
		II 54.3170	0.2715	
Total	299	I 162.9885		
		II 86.7716		

Table 4

A. Growth of root segments in different concentrations of \pm ABA (growth expressed as % of control). Each figure represents the mean segment growth from five replications on each occasion.

Log concentrations in molar	-3	-4	-5	-6	-7	-8
Occasion 1	43.10	50.34	85.17	100.34	114.13	105.17
Occasion 2	50.92	63.56	92.19	107.80	124.90	115.60
Occasion 3	47.38	58.12	87.32	90.08	121.76	95.59
Mean	47.13	57.34	88.27	99.40	120.26	105.45

B. Table of the analysis of variance of the growth data presented in table 'A'. The significant values at 5% probability level are marked with '*'.

Source of variance	Degrees of freedom	Total sum of squares	Mean square variance	Variance ratio (F)
Occasion	2	26.6146	13.3073	28.2173*
Concentration	6	177.1694	29.5282	62.6128*
Occasion X concentration	12	5.2285	0.4357	0.9238
Sample variance	84	23.1415	0.2754	0.5839
Residual	210	99.0398	0.4716	
Total	314	331.1938		

PUBLISHED PAPERS

1. Kundu, K. K. and Audus, L. J. (1974) Root growth inhibitors from root tips of Zea mays L. Planta (Berl.), 117: 183-186.

2. Kundu, K. K. and Audus, L. J. (1974) Root growth inhibitors from root cap and root meristem of Zea mays L. J. Exptl. Bot., 25: 479-489.