SOME PHYSIOLOGICAL ASPECTS OF THE AUXIN TRANSPORT INHIBITOR, ACPI-55, ON THE ROOTS OF *ZEA MAYS* L.

by

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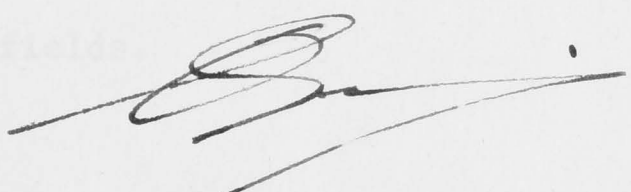
Except where it is acknowledged to have been performed by others, all the work described in this thesis was performed by me.



(A.E. Geissler)

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SUMMARY

A method was developed to test chemicals for geotropic activity in *Zea mays* roots as well as for fixation of these roots in specific orientations.

It was shown with the aid of the former method that the auxin transport inhibitor, ACPI-55, abolished the positive geotropic response in *Zea mays* roots. By statistical analyses it was determined that ACPI-55 treated roots were ageotropic rather than negatively geotropic. Curvature development was not due to a phototropic reaction and it appeared to be associated with some "intrinsic factor" within the seed or root. Evidence was produced that ACPI-55 itself, has no auxin-type growth activity.

Evidence from histological studies on the root cap of *Zea mays*, showed that the ageotropic response of ACPI-55 treated roots was not due to interference of the compound with the size, number and movement of amyloplasts. These studies did not reveal any apparent phytotoxicity effects of ACPI-55 (at a concentration of 10^{-6}M) on the root cap and neighbouring tissues. In addition, herbicide data showed that ACPI-55 at moderate to high concentrations was not phytotoxic on the above-ground parts of *Zea mays* plants.

The response of *Zea mays* roots to ACPI-55 treatment, both as regards growth inhibition and curvature development was linked to the properties of ACPI-55 as an auxin transport inhibitor. Some evidence was produced that ACPI-55 acted independently of light induced root cap inhibitors.

I. INTRODUCTION

A chemical was discovered in our laboratory which was not only a powerful inhibitor of auxin transport, but also the most active compound then known to abolish the geotropic response in roots (Brown *et al.* 1972). As the chemical, 1-(2'-carboxyphenyl)-3-phenylpropane-1,3-dione, was jointly developed by the Divisions of Applied Chemistry and Plant Industry (CSIRO), it was given the code name ACPI-55. The activity of ACPI-55 and its derivatives was published by Brown *et al.* (1973) while the activity of related compounds has been reported recently (Huppatz *et al.* in press). In this thesis experiments were initiated to study the effect of ACPI-55 in *Zea mays* roots. It was hoped that by these investigations new insights could be gained into the process of geotropism.

There is strong evidence that geoperception occurs in the root cap (Juniper *et al.* 1966) and that the geotropic response occurs in the extension zone (Cholodny 1924; Navez 1933; Pilet and Nougarède 1974). The overall process of geotropism is poorly understood but it could be regarded as a chain of reactions which are causally linked in sequence.

Perception of gravity	→	Transformation of physical into biochemical information	→	Hormone transport	→	Geotropic response

It is generally believed that the initial physical action of the gravity stimulus on the plant involves an interaction between the mass of the earth and the masses of some constituents of the plant (Larsen 1971). Evidence for this statement comes from experiments in which gravity was substituted by centrifugal forces (Knight 1806). Under these conditions, roots curved in the direction of the centrifugal force while shoots

curved away from it. Most hypotheses concerning the perception of gravity in plants propose that certain particles, termed statoliths, are responsible for geoperception. These statoliths are solid inclusions in plant cells which are free to move under the influence of gravity. Observations of organelle distribution in root cap cells of *Vicia faba* favour the theory that amyloplasts (i.e. starch grains that sediment under the influence of gravity) are the statoliths which are responsible for geoperception rather than mitochondria on Golgi bodies (Griffith and Audus 1964). The main strength of the starch (amyloplast) statolith theory (Haberlandt 1900; Nemec 1900) is that cells which possess amyloplasts are almost invariably found in geotropic sensitive organs of plants. They are thus characteristic of root cap cells and coleoptile tips. Haberlandt (1900) and Nemec (1900) reported observations that showed a high correlation between the occurrence and movement of amyloplasts on the one hand and the presence of geotropic sensitivity in tissues on the other. Iversen (1969) found that removal of statolith starch in roots of *Lepidium sativum* led to loss of geotropic responsiveness. The theory could however be criticised on the ground that geotropic responses have been observed to occur apparently in the absence of amyloplasts. Examples are the naturally starch free aerial roots of *Laelia anceps* (Tisher 1905) and the perianth of *Clivia nobilis* (Linsbauer 1907). In addition, it has been claimed by Pickard and Thimann (1966) that artificially starch free wheat coleoptiles retained their capacity to respond to gravity. These contradictory findings could indicate that there are different mechanisms of geoperception in different organs and in different species. However, the experimental results of Iversen (1969) strongly support the view that at least in some roots, gravity is perceived by amyloplasts. These amyloplasts rest on the distal walls of the cells when roots grow

downwards. Angular displacement of the root from the vertical to the horizontal plane will cause sedimentation of the amyloplasts to the lateral walls. According to the starch statolith theory, mechanical pressure on the membranes lining these lateral walls will initiate a chain of reactions culminating in geotropic curvature (Haberlandt 1928).

The mechanism(s) involved in the transformation of physical into biochemical information is at present unknown.

Audus and Brownbridge (1957) showed that the positive geotropic response of horizontal roots was caused by a greater decrease of elongation in the lower half than in the upper half of the roots, suggesting that curvature development is due to a redistribution of a growth inhibiting substance. The hypothesis that hormones or inhibitors are involved in the process of geotropism was proposed long ago. Darwin (1880) emphasized that tropism was a kind of correlation phenomenon and on root geotropism he concludes: "that it is the tip alone which is acted on and that this part transmits some influence to the adjoining parts causing them to curve downwards". It was not until 1928 that Went obtained the substance from coleoptile tips that was responsible for both the correlative nature of the tropistic response in *Avena* coleoptiles as well as the control of growth rates. In 1931 this substance was given the name Auxin (Gr. Auxein = to increase) by Kögl and Haagen-Smit. In 1935 Thimann isolated and purified the active compound from *Rhizopus* cultures and identified it as indole-3-acetic acid. Auxins were linked to tropism in the now classical Cholodny-Went theory (Cholodny 1926; Went 1926). The theory postulates that the normal symmetrical flow of auxin from the tip to the extension zone would be disturbed when roots are displaced from the vertical to the horizontal plane. This would result in lateral migration of the hormone to the lower side of the root. Auxin levels would be optimal for growth in the upper half and supraoptimal for growth in the lower half of the root.

Because auxin is inhibitory for growth at high concentrations, a positive curvature develops.

Early support for the Cholodny-Went theory was provided by Hawker (1932) who showed that a greater positive curvature could be induced in root stumps of *Vicia faba* by the diffusates from the lower halves of root tips placed horizontally, than from the upper halves. Boysen-Jensen (1933), Thimann (1936) and Van Raalte (1937) showed that the substance which diffused out of the root tips of *Vicia faba* and *Avena* into agar, was indeed an auxin, as measured by the *Avena* curvature test. Because evidence has now been provided for the presence of indolyl-3-acetic acid in *Zea mays* roots (Bridges *et al.* 1973; Elliott and Greenwood 1974) and root caps (Rivier and Pilet 1974), it is quite possible that this hormone is involved in geotropism and growth. Previously it was suggested that the root cap of maize produces or releases at least one growth regulator which reduces root elongation (Gibbons and Wilkins 1970; Pilet 1972). Basipetal transport from root cap to extension zone of this inhibitor (Shaw and Wilkins 1973; Pilet 1973) could explain the georeaction of roots (Pilet 1971; Shaw and Wilkins 1973; Wilkins *et al.* 1973). The role of auxin transport in geotropic responses has been investigated with the aid of synthetic plant growth regulators which inhibit movement of auxin. For instance, synthetic plant growth regulators such as 2,3,5-triiodobenzoic acid (TIBA), 1-naphthylphthalamic acid (NPA) and 1-(2'-carboxyphenyl)-3-phenylpropane-1,3-dione (ACPI-55) are not only highly effective inhibitors of auxin transport (Keitt and Baker 1966; McCready 1968; Brown *et al.* 1972), but they also abolish the normal positive geotropic response of roots (Fuente and Leopold 1968; Jones *et al.* 1954; Brown *et al.* 1972).

Because of its extensive use in studies related to root geotropism, *Zea mays* was chosen as test species. A method was developed, whereby the response of healthy roots to chemical treatment could be analysed accurately

(Chapter II). Geotropism and growth of roots treated with ACPI-55 was determined quantitatively (Chapter III). A histological study on the root cap and neighbouring tissues of *Zea mays* roots was carried out (Chapter IV). This work was followed by a general discussion (Chapter V).

II. METHODS

A method was developed to test chemicals for geotropic activity and root growth effects. The hybrid maize variety PX-52 (purchased from A. Yates & Co. Pty. Ltd.) was chosen from several other varieties because a high degree of germination was obtained and the primary roots were straight. The method described below was chosen from several other methods which were investigated because it produced seedlings with a healthy primary root which could be treated accurately.

1. *Germination, Growth and Treatment*

a. General procedure

A diagrammatic presentation of the procedure is given in Figure 1. Although graded seeds were used which had been treated commercially against fungus and insect attack (with a Lindane-Malathion-Tetramethyl-Thiuram Disulphide mixture), some further selection was carried out to ensure a high degree of synchronized germination and uniform growth. Small and damaged seeds were discarded (selection of seeds : A in Fig. 1). Surface sterilization was done with a 4% sodium hypochlorite solution for 45 seconds to provide a sterile environment for germination and growth (sterilization : B). The seeds were rinsed for four minutes in running distilled water to wash off the sodium hypochlorite (washing : C). The seeds were subsequently soaked in distilled water for five hours to facilitate imbibition. Significantly shorter periods of soaking resulted in unsynchronized germination. In many studies corn seeds are soaked for 6-8 hours (e.g. Scott and Wilkins 1968), but in the present study longer periods of soaking did not increase germination (imbibition :D). After imbibition, seeds were rinsed with running distilled water for 1-2 minutes to wash off any residual chemical which might have been present on the seed coat, or possible germination inhibitors which might have been released from the seeds (washing : E). The seeds were then transferred to test tubes (3 cm diameter; 18 cm long) which contained one piece of

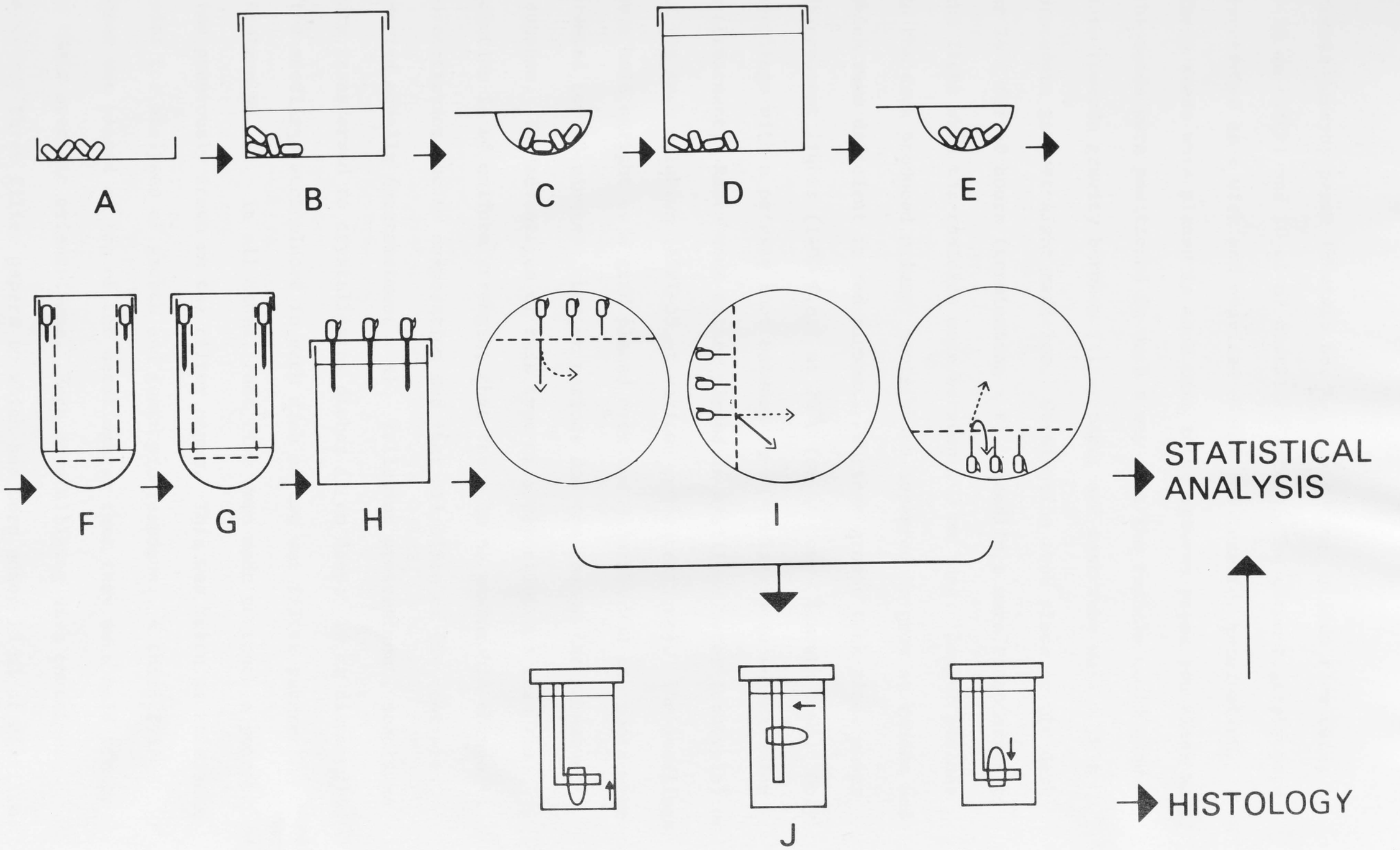


FIGURE 1 DIAGRAMMATIC PRESENTATION OF PROCEDURE

chromatography paper (Whatman No. 1, 10 x 16 cm; medium flow rate; 0.16 mm thick) and 10 ml of distilled water. The chromatography paper functioned as a wick and provided enough moisture for germination. Three seeds were placed in each test tube, between paper and glass wall. The seeds were positioned in such a way that the radicle could grow down towards gravity between filter paper and test tube wall. This procedure gave straight radicles. Germination took place in the dark at 24°C for 48 hours (germination : F). Seedlings were transferred to the light when the radicles were between 1-3 mm long. Longer periods in the dark produced primary roots which appeared to grow at random and which were deficient in red pigment. Further growth took place under fluorescent lights (1400 f.c.) at 24°C for 24 hours (growth : G). Only seedlings with a primary root between 1½-2½ cm long were selected for pretreatment. Roots were either placed in distilled water (controls) or in the test solution (ACPI-55 at various concentrations). The seedlings were held upright by a grid placed over the solution and the roots were treated for 30 minutes. Longer periods did not change the subsequent response. The advantages of this type of application are that the solution is of uniform strength, that there is no measurable change in concentration due to evaporation and that all sides of the root are treated equally (pretreatment : H). Following pretreatment, seedlings were transferred to crystallizing dishes (3 cm deep; 16 cm diameter). Five seedlings were placed in each dish on two wet filter papers (Whatman No. 1). In all cases, root tips were made to touch a pencil line previously drawn on the filter paper. This was taken as reference point for analyses of growth and geotropic response. A third filter paper was placed on top of the seedlings so that they were held firmly in their specific orientations. Each crystallizing dish contained therefore three filter papers to which had been added 18 ml of distilled water or 18 ml of ACPI-55 in solution. Practically no excess liquid

was present in the crystallizing dishes and evaporation was prevented by a "Gladwrap" (transparent plastic) cover over each dish. The dishes were placed on their sides under fluorescent light (1400 f.c.), and turned in such a way that roots pointed either vertically down (0^{o}), horizontally (90^{o}) or vertically up (180^{o}). The temperature within the crystallizing dishes was kept constant by an efficient air circulation system from fans situated in the constant temperature room. This kept condensation of water on the Gladwrap plastic to a minimum thus preventing a rise in the concentration of the test solution within the crystallizing dishes. The solid arrows in Figure 1 show the approximate directions in which most control roots grew, while the dotted arrows show directional growth of a large number of treated roots at a concentration of 10^{-6}M. However, fairly large standard errors were obtained on these curvatures and the indicated directions are therefore only approximate (curvature development and growth : I). After the growth period roots were analysed statistically or fixed in acrolein (see Chapter 4) for further histological studies (fixation : J).

b. Dark experiments

In order to investigate root growth and behaviour of roots grown in the dark, the same procedure was followed as described above for light grown roots, except that the plant material was kept in complete darkness (at a constant temperature of 24^{o}C). During treatment and subsequent transfer of seedlings to the crystallizing dishes, green safety lights were used.

c. Light-dark experiments

An extension of the above experiment was done to determine the influence of light grown shoots on the geotropic response of dark grown roots. Treatment of roots and transfer to crystallizing dishes, was done under green safety lights. The experiment was set up in such a

way that the seed and the growing shoot were positioned outside the crystallizing dish while the root could grow inside the dish in the usual manner. This was possible as holes had been made in the walls of the dishes. The roots were kept in darkness as the crystallizing dishes were sealed with specially made black linen covers. The seeds and shoots were positioned under fluorescent lights. Proper air circulation took place throughout the duration of the experiment. The temperature in an extra control dish was checked from time to time. After 24 hours of growth, the black covers were removed and the geotropic response of the roots observed. The roots were then allowed to grow for an additional six hours under fluorescent light.

2. *Statistical Analysis*

a. Root growth

After 24 hours of growth, roots were transferred from the crystallizing dishes to graph paper and growth measurements (in millimetres) were taken immediately. The Student's t test was applied to determine the extent of the differences between two populations.

b. Curvature development

After 24 hours of growth, roots were transferred from the crystallizing dishes to graph paper and curvature measurements (in degrees) were taken. In all instances, the plumb-line was taken as 0^{o} and the deviations from this line were given in degrees. Before transfer of the seedlings from the crystallizing dishes to graph paper, the orientation of the roots was checked to see if any displacement had occurred during the growth period. As the roots were always placed alongside a pencil line, any deviation from the original position could be checked visually. Nearly all roots developed root hairs very quickly thus providing adequate support. Those seedlings which had moved were discarded. The statistical procedure was discussed above.

3. *Investigation into the Possible Effect of Sodium Hypochlorite and the Lindane-Malathion-Tetramethyl Thiuram Disulphide Mixture on Root Geotropism and Root Growth*

Sobieszczanski (1965) found with wheat and lettuce test species that fungi as well as bacteria could be stimulatory, inhibitory or neutral for growth. Rovira (1965) concludes that the mechanisms by which the microflora affect higher plants is not established but that the production of metal chelating compounds and growth-regulating substances could be important. From the literature it appears that little attention has been given to the occurrence of exocellular hydrolytic enzymes associated with the roots of higher plants. It could be that these enzymes solubilize macromolecular constituents of organic origin, thus permitting uptake and utilization by the plant of the resultant low molecular weight organic compounds. The work of Chang and Bandurski (1964) supports this concept by the demonstration that several exocellular hydrolytic enzymes probably of bacterial origin, were associated with the roots of corn seedlings. For this reason it was considered important to sterilize seeds with sodium hypochlorite and to use seeds which had been treated commercially with a Lindane-Malathion-Tetramethyl Thiuram Disulphide mixture which contains an anti-fungal component (i.e. TMTD). The possibility could however not be excluded that these chemicals would influence root geotropism and growth. Two experiments were therefore carried out to ascertain that the effects observed with ACPI-55 treated roots were not due to any intrinsic activity of the above mentioned compounds.

In the first experiment, seeds which had not been treated commercially with the Lindane-Malathion-TMTD mixture were either sterilized with a solution of 4% sodium hypochlorite or they were used without prior sterilization. Subsequent curvature development and growth of roots were statistically analysed. Because of a possible interaction of ACPI-55

TABLE 1. *ZEA MAYS*. CURVATURE DEVELOPMENT AND GROWTH.
STERILIZED (S) AND UNSTERILIZED (U) SEEDS.
ROOTS PLACED HORIZONTALLY. TREATMENT: ACPI-55

Treatment	Experiment	Curvature			Growth		
		$\bar{x}$	S.D.	t	$\bar{x}$	S.D.	t
Control	S	35	15		28.1	4.6	
				N.S.			N.S.
	U	41	19	0.69	30.7	3.5	1.40
10^{-4}M	S	170	75		17.3	4.3	
				N.S.			N.S.
	U	217	77	1.69	16.0	2.6	0.81
10^{-5}M	S	183	38		9.9	1.9	
				N.S.			N.S.
	U	153	46	1.55	9.6	2.9	0.28
10^{-6}M	S	130	61		16.2	4.9	
				N.S.			N.S.
	U	133	27	0.12	15.8	3.4	0.21
10^{-7}M	S	49	5		30.4	4.5	
				N.S.			N.S.
	U	45	16	0.77	31.6	5.5	0.53

Analyses: Curvature = Degrees

Growth = MM

N = 10

$\bar{x}$ = Mean; S.D. = Standard deviation

t = Student's t

N.S. = Not significant

TABLE 2. *ZEA MAYS*. CURVATURE DEVELOPMENT AND GROWTH. SEEDS TREATED WITH LINDANE-MALATHION-TMTD MIXTURE (T) OR UNTREATED (U). ROOTS PLACED HORIZONTALLY. TREATMENT: ACPI-55

Treatment	Experiment	Curvature			Growth		
		$\bar{x}$	S.D.	t	$\bar{x}$	S.D.	t
Control	T	32	17		28.2	6.1	
				N.S.			N.S.
	U	36	16	1.95	28.1	4.6	0.18
10^{-4}M	T	175	35		16.7	2.0	
				N.S.			N.S.
	U	170	75	0.30	17.3	4.3	0.39
10^{-5}M	T	139	49		11.2	2.3	
				N.S.			N.S.
	U	169	38	1.52	9.9	1.9	0.65
10^{-6}M	T	104	42		19.9	4.3	
				N.S.			N.S.
	U	130	61	1.01	16.2	4.9	1.76
10^{-7}M	T	52	9		29.8	5.2	
				N.S.			N.S.
	U	49	5	0.67	30.4	4.5	0.72

Analyses: Curvature = Degrees

Growth = MM

N = 10

$\bar{x}$ = Mean; S.D. = Standard deviation

t = Student's t

N.S. = Not significant

with residual sodium hypochlorite, ACPI-55 treatment was included in this experiment. Differences of curvature development and growth between sterilized and unsterilized seeds were not significant ($P > 0.05$) for both control and ACPI-55 treated roots (Table 1).

In the second experiment statistical analyses were carried out on curvature development and root growth of seeds which had been treated with the Lindane-Malathion-TMTD mixture and seeds which had not been treated. Surface sterilization was carried out on both samples. Differences in curvature development and growth between treated and untreated seeds were not significant ($P > 0.05$; Table 2).

4. *Summary*

The method which was developed for the *in vivo* testing of the effect of chemicals on geotropism and root growth in *Zea mays* can be used in future studies. Several important experimental requirements are met by this method: (i) seedlings produce a healthy root system; (ii) reproducible results are obtained; (iii) the method is simple; (iv) roots are amenable to accurate chemical treatment; (v) the response of the roots to chemical treatment can be measured accurately; (vi) histological studies on the roots are possible.

Sterilization of seeds with sodium hypochlorite and the use of seeds treated with the Lindane-Malathion-TMTD mixture provided an environment in which roots could grow free from infection by fungi. These chemicals did not affect curvature development and root growth in either control or ACPI-55 treated roots.

III. THE EFFECT OF ACPI-55 ON ROOT GEOTROPISM AND GROWTH IN *ZEA MAYS* L.

1. Introduction

Previous investigations (Brown *et al.* 1972) demonstrated that ACPI-55 abolished the normal geotropic response in roots of cress (*Lepidium sativum*) and ryegrass (*Lolium rigidum*) grown on agar. However, no quantitative results were published on root curvature and root growth in these species.

The effect of ACPI-55 on various aspects of root curvature and root growth has now been analysed quantitatively using *Zea mays* as test species.

2. Methods

The method which was used has been fully described in Chapter II.

3. Results

(a) *Curvature Development and Growth of Roots Orientated at 0^o, 90^o and 180^o with Respect to Gravity*

The activity of ACPI-55 on roots which were placed vertically down (0^o) is shown in Figure 2. At high concentrations most roots curved away from their original orientation (Appendix 1). The difference between zero response (i.e. no curvature development) of control roots and curvature development of treated roots (10^{-4}M - 3.3×10^{-7}M) was highly significant ($P < 0.001$; Table 3). The difference in growth between control and treated roots (10^{-4}M - 10^{-6}M) was also highly significant ($P < 0.001$; Table 4).

The activity of ACPI-55 on roots which had been placed horizontally (90^o) is shown in Figure 3. Roots treated at high to moderate concentrations (10^{-4}M - 10^{-6}M) curved upwards (if the embryo was placed uppermost; see further results), while control roots and roots treated at low concentrations, curved downwards (Appendix 2). The difference in curvature development between control roots and treated roots (10^{-4}M -

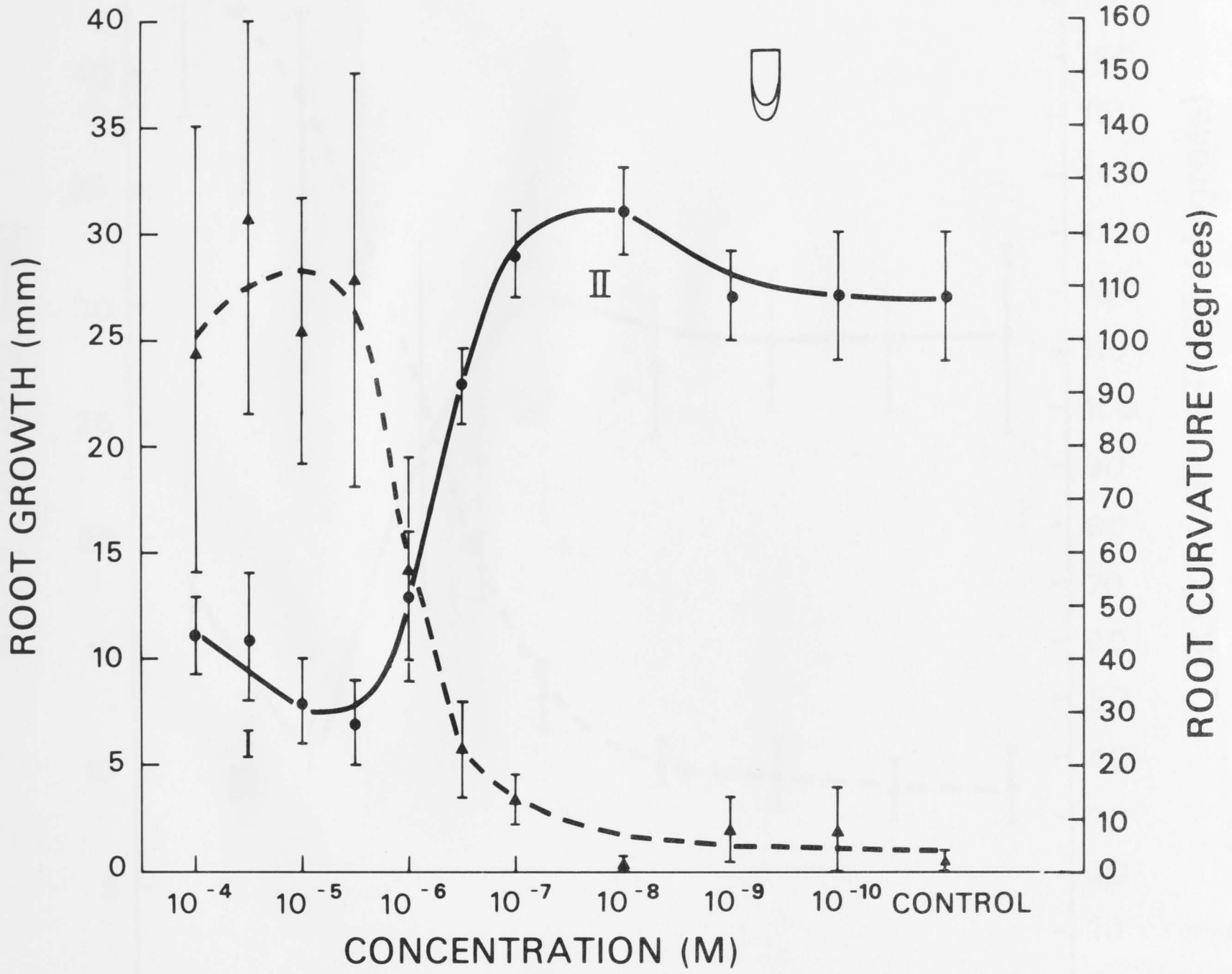


FIGURE 2 *ZEA MAYS.* TREATMENT ACPI-55
ROOT CURVATURE ▲--▲
ROOT GROWTH ●—●

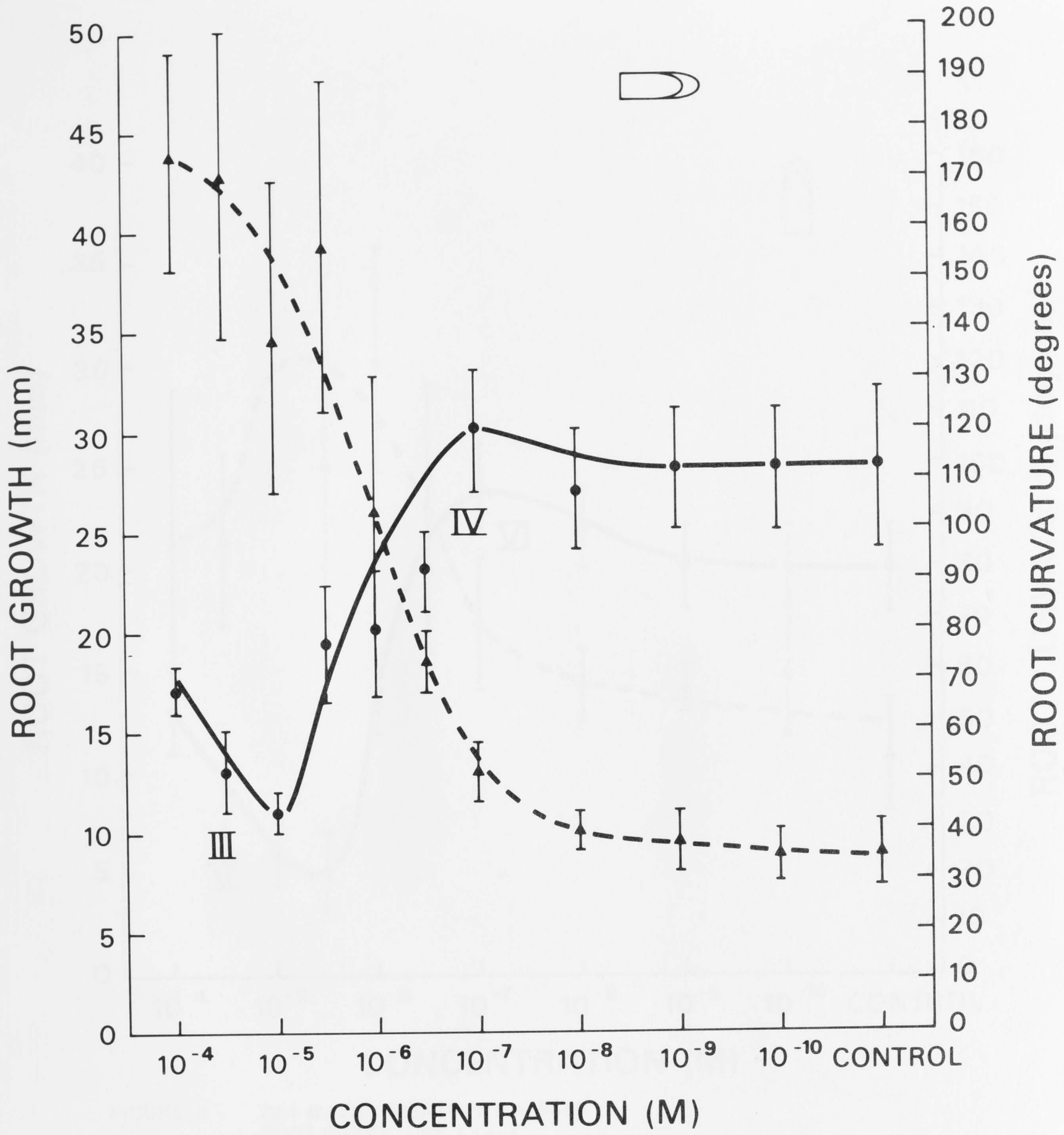


FIGURE 3 *ZEA MAYS.* TREATMENT ACPI-55
ROOT CURVATURE ▲- - -▲
ROOT GROWTH ●——●

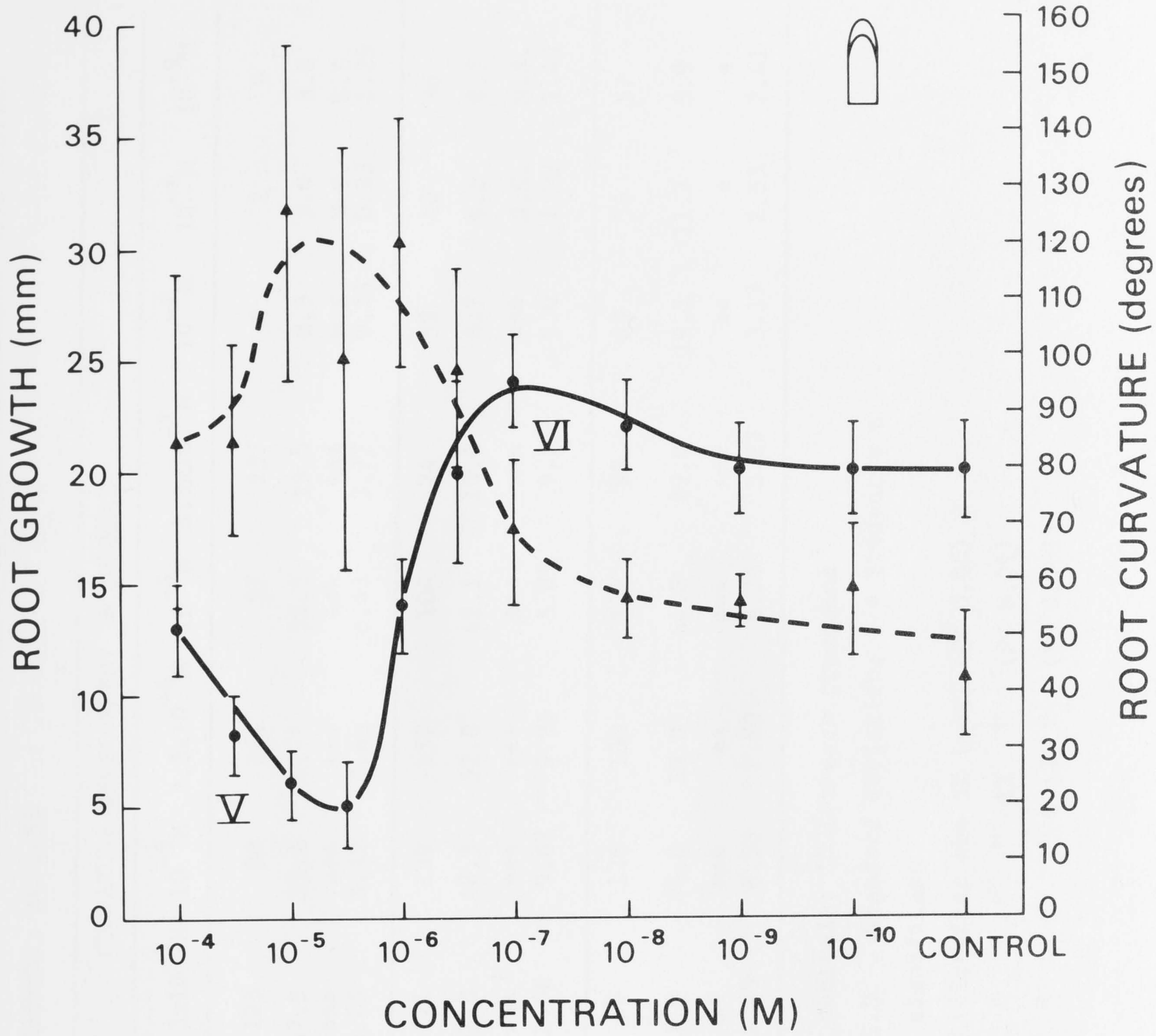


FIGURE 4 *ZEA MAYS.* TREATMENT ACPI-55
ROOT CURVATURE ▲- - -▲
ROOT GROWTH ●——●

TABLE 3. *ZEA MAYS*. CURVATURE DEVELOPMENT OF ROOTS. ROOTS POSITIONED IN THREE ORIENTATIONS WITH RESPECT TO GRAVITY. TREATMENT: ACPI-55

Experiment	Analyses	CURVATURE DEVELOPMENT IN DEGREES										
		Control	10^{-4}M	3.3x10^{-5}M	10^{-5}M	3.3x10^{-6}M	10^{-6}M	3.3x10^{-7}M	10^{-7}M	10^{-8}M	10^{-9}M	10^{-10}M
Roots 0^{o}	$\bar{x}$	3	98	123	99	111	57	23	4	2	9	8
	S.D.	4.2	67.8	57.5	55.4	61.2	33.5	13.5	8.5	3.0	8.8	13.8
	t		*** 6.11	*** 8.85	*** 8.32	*** 7.60	*** 6.43	*** 3.77	N.S. 0.24	N.S. 0.33	N.S. 1.29	N.S. 0.88
Roots 90^{o}	$\bar{x}$	32	175	171	139	157	104	74	52	40	38	35
	S.D.	17.9	35.3	51.0	48.5	52.8	42.1	10.0	8.7	6.5	8.7	7.3
	t		*** 16.38	*** 11.66	*** 9.62	*** 9.95	*** 5.85	*** 9.52	*** 5.0	N.S. 1.92	N.S. 1.43	N.S. 0.83
Roots 180^{o}	$\bar{x}$	43	86	86	126	100	121	98	69	58	57	59
	S.D.	17.8	47.2	41.9	48.0	59.3	34.8	29.0	19.2	11.7	5.9	18.5
	t		** 2.67	** 2.98	*** 8.53	** 2.89	*** 6.25	*** 5.25	** 3.17	* 2.53	* 2.41	* 2.09

N = 20 for controls; 10 for each treatment

$\bar{x}$ = Mean; S.D. = Standard deviation; t = Student's t

N.S. = Not significant

* = Significant at the 5% level ($P < 0.05$)

** = " " " 1% " ($P < 0.01$)

*** = " " " 0.1% " ($P < 0.001$)

TABLE 4. *ZEA MAYS*. GROWTH RESPONSE OF ROOTS. ROOTS AT 3 ORIENTATIONS WITH RESPECT TO GRAVITY.
TREATMENT : ACPI-55

Experiment	Analyses	GROWTH RESPONSE IN MM									
		Control	10^{-4}M	$3.3x10^{-5}$M	10^{-5}M	$3.3x10^{-6}$M	10^{-6}M	$3.3x10^{-7}$M	10^{-7}M	10^{-8}M	10^{-9}M	10^{-10}M
Roots - 0^{o}	$\bar{x}$	26.6	10.5	10.7	8.4	7.0	12.9	23.7	28.8	30.6	26.5	26.9
	S.D.	3.1	2.8	4.8	2.5	2.9	4.2	2.4	3.7	3.2	3.5	4.8
	t		*** 11.79	*** 9.85	*** 12.04	*** 14.24	*** 8.99	* 2.20	N.S. 1.45	** 2.78	N.S. 0.10	N.S. 0.15
Roots - 90^{o}	$\bar{x}$	28.2	16.7	12.8	11.2	19.0	19.9	22.9	29.8	27.2	27.9	28.1
	S.D.	6.1	2.0	3.5	2.3	4.7	4.3	3.5	5.2	5.2	4.7	5.6
	t		*** 6.78	*** 8.48	*** 9.91	*** 3.81	*** 4.79	** 2.90	N.S. 0.83	N.S. 0.48	N.S. 0.13	N.S. 0.02
Roots - 180^{o}	$\bar{x}$	20.4	12.6	8.3	5.7	5.1	13.7	19.7	24.1	21.5	19.9	20.0
	S.D.	4.4	3.0	3.6	3.1	3.0	3.0	5.9	3.5	3.2	3.7	4.1
	t		*** 4.62	*** 6.28	*** 8.65	*** 9.16	*** 3.89	N.S. 0.30	* 2.09	N.S. 0.64	N.S. 0.27	N.S. 0.80

N = 20 for controls; 10 for each treatment

$\bar{x}$ = Mean; S.D. = Standard deviation; t = Student's t

N.S. = Not significant

* = Significant at the 5% level ($P < 0.05$)

** = " " " 1% " ($P < 0.01$)

*** = " " " 0.1% " ($P < 0.001$)

10^{-7}M) was highly significant ($P < 0.001$; Table 3) as was the difference in root growth between control and treated (10^{-4}M - 10^{-6}M) roots (Table 4).

The activity of ACPI-55 on roots which had been placed vertically up (180^{o}) is shown in Figure 4. Control and treated roots turned away from the plumb-line (Appendix 3). The difference in curvature development between control and treated roots was significant (Table 3), while the difference in growth between control and treated roots (10^{-4}M - 10^{-6}M) was highly significant ($P < 0.001$; Table 4).

The results show that there is a positive correlation between curvature development and root growth inhibition.

(b) *Growth Response to High and Low Concentrations of ACPI-55*

The differences in growth between roots treated at 10^{-4}M and those treated at lower concentrations (3.3×10^{-6}M and 10^{-5}M) were highly significant ($P < 0.01$; Table 5).

The differences in growth between control roots and roots treated at 10^{-8}M was significant at the 1% level ($P = 0.01$; Table 5) when roots were placed vertically down (0^{o}). In the two other orientations (90^{o} and 180^{o}) there was a tendency for growth stimulation at 10^{-7}M (Table 5).

It can be concluded that similar growth curves were obtained regardless of the orientation in which the roots had been placed.

(c) *Curvature Deviations from the Longitudinal Axes of Treated Roots Placed Vertically Down (0^{o}), Horizontally (90^{o}) and Vertically up (180^{o})*

Mean curvature deviations from the longitudinal axes (0^{o}, 90^{o}, 180^{o}) in which treated roots (10^{-4}M - 3.3×10^{-6}M) were placed are shown in Table 6. The differences between these deviations were not significant (Table 6).

It can thus be concluded that curvature development in ACPI-55 treated roots (10^{-4}M - 3.3×10^{-6}M) was independent of the gravitational force.

TABLE 5. *ZEA MAYS*. ROOT GROWTH AT HIGH AND LOW CONCENTRATIONS OF ACPI-55. ROOTS PLACED IN THREE ORIENTATIONS (0^{o}, 90^{o}, 180^{o}) WITH RESPECT TO GRAVITY

Orientation	Treatment	Analyses		
		$\bar{x}$	S.D.	t
0^{o}	10^{-4}M	10.5	2.8	
	$3.3x10^{-6}$M	7.0	2.9	2.96**
90^{o}	10^{-4}M	16.7	2.0	
	10^{-5}M	11.2	2.3	5.73***
180^{o}	10^{-4}M	12.6	3.0	
	10^{-5}M	5.7	3.1	5.00***
0^{o}	Control	26.6	3.1	
	10^{-8}M	30.6	3.2	2.78**
90^{o}	Control	27.7	6.1	
	10^{-7}M	29.8	5.2	N.S. 0.83
180^{o}	Control	20.4	4.4	
	10^{-7}M	24.1	3.5	2.09*

Analysis = MM

N = 20 for control; 10 for treatment

$\bar{x}$ = Mean; S.D. = Standard deviation; t = Student's t

N.S. = Not significant

* = Significant at the 5% level ($P<0.05$)

** = " " " 1% " ($P<0.01$)

*** = " " " 0.1% " ($P<0.001$)

TABLE 6. *ZEA MAYS*. COMPARISON BETWEEN AMOUNTS OF DEVIATION IN THREE ORIENTATIONS (0^{o}, 90^{o}, 180^{o}). ROOTS TREATED WITH ACPI-55

Concentration	Orientation	Mean curvature deviation (degrees)	t Values	
			0^{o}	90^{o}
10^{-4}M	0^{o}	99		
	90^{o}	85	0.51; N.S.	
	180^{o}	94	0.21; N.S.	0.47; N.S.
$3.3x10^{-5}$M	0^{o}	123		
	90^{o}	81	1.72; N.S.	
	180^{o}	94	1.44; N.S.	0.18; N.S.
10^{-5}M	0^{o}	99		
	90^{o}	49	2.01; N.S.	
	180^{o}	54	1.88; N.S.	0.02; N.S.
$3.3x10^{-6}$M	0^{o}	111		
	90^{o}	67	1.72; N.S.	
	180^{o}	80	1.15; N.S.	0.60; N.S.

N = 10

N.S. = Not significant

(d) *Direction of Curvature*

In many roots, curvature developed towards the side of the seed from which the shoot emerged (Appendices 1,2,3). The possible influence of the shoot on curvature development was therefore investigated. Removal of the entire shoot in horizontally placed roots (Appendix 4) was found to have no effect on curvature development either in control or treated (10^{-6}M) roots (Table 7). Furthermore, the differences in curvature development between control and treated (10^{-6}M) roots were highly significant ($P < 0.001$; Table 7), irrespective of whether the shoot had been excised or not.

The shoot therefore has no apparent influence on curvature development in roots of *Zea mays*.

In previous experiments carried out with horizontally placed roots, seedlings had been orientated in the crystallizing dishes with the embryo uppermost (Appendix 2). In this orientation, treated roots (for example at 10^{-6}M) curved upwards. In order to determine whether treated roots (10^{-6}M) would always curve upwards, regardless of the orientation of the embryo, seeds were placed in the crystallizing dishes with the embryo lowermost (Appendix 5). The difference in curvature development between control roots with the embryo uppermost and control roots with the embryo lowermost was not significant (Table 7). However, the difference in curvature development between treated roots with the embryo uppermost and treated roots with the embryo lowermost was highly significant ($P < 0.001$; Table 7). The difference in curvature development between control and treated roots with the embryo lowermost was not significant (Table 7).

These results indicate that curvature in treated roots (10^{-6}M) is preferentially towards the embryo side of the seed rather than towards the endosperm side.

TABLE 7. *ZEA MAYS*. CURVATURE DEVELOPMENT. ROOTS TREATED WITH ACPI-55. ALL ROOTS PLACED HORIZONTALLY

Treatment	Concentration	Analyses		
		$\bar{x}$	S.D.	t
Shoots not excised	Control	24	6	
Shoots excised	Control	29	12	N.S. 0.50
Shoots not excised	10^{-6}M	154	36	
Shoots excised	10^{-6}M	128	34	N.S. 1.47
Shoots not excised	Control	24	6	
Shoots not excised	10^{-6}M	154	36	*** 11.23
Shoots excised	Control	29	12	
Shoots excised	10^{-6}M	128	34	*** 5.85
Embryo uppermost	Control	24	6	
Embryo lowermost	Control	26	14	N.S. 0.40
Embryo uppermost	10^{-6}M	154	36	
Embryo lowermost	10^{-6}M	41	19	*** 8.74
Embryo uppermost	Control	24	6	
Embryo uppermost	10^{-6}M	154	36	*** 11.23
Embryo lowermost	Control	26	14	
Embryo lowermost	10^{-6}M	41	19	N.S. 1.71

Analysis = Degrees

N = 10

$\bar{x}$ = Mean; S.D. = Standard deviation; t = Student's t

N.S. = Not significant

*** = Significant at the 0.1% level ($P<0.001$)

TABLE 8. *ZEA MAYS*. CURVATURE DEVELOPMENT AND GROWTH OF DARK GROWN ROOTS. ROOTS TREATED WITH ACPI-55. ALL ROOTS PLACED HORIZONTALLY

Experiments	Analyses	Control	Concentration (M)	
			10^{-6}	10^{-7}
Curvature	$\bar{x}$	88	162	88
	S.D.	8	63	22
	t		*** 3.62	N.S. 0.07
Growth	$\bar{x}$	33.6	26.2	28.7
	S.D.	6.2	3.9	6.2
	t		** 3.08	N.S. 1.76

Analyses : Curvature development = Degrees; Growth = MM

N = 10

$\bar{x}$ = Mean; S.D. = Standard deviation; t = Student's t

N.S. = Not significant

** = Significant at the 1% level ($P<0.01$)

*** = " " " 0.1% " ($P<0.001$)

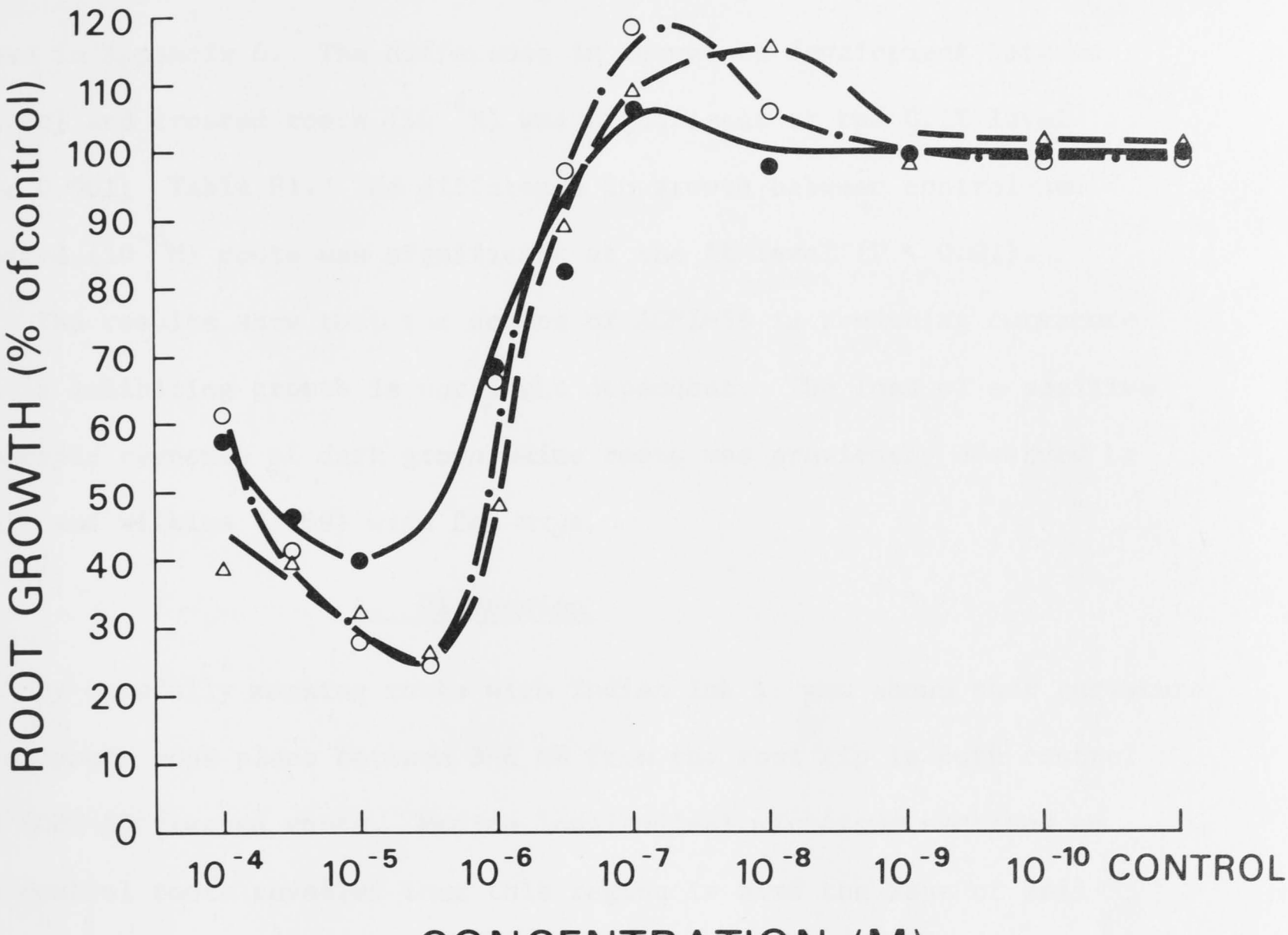


FIGURE 5 *ZEA MAYS.* TREATMENT ACPI-55

●—● HORIZONTAL

○– –○ VERTICALLY DOWN

△–·–△ INVERTED

(e) *Curvature Development and Growth of Control and ACPI-55 Treated Roots Grown in the Dark*

Curvature development of dark grown roots placed horizontally is shown in Appendix 6. The difference in curvature development between control and treated roots (10^{-6}M) was significant at the 0.1% level ($P < 0.001$; Table 8). The difference in growth between control and treated (10^{-6}M) roots was significant at the 1% level ($P < 0.01$).

The results show that the action of ACPI-55 in producing curvature and in inhibiting growth is not light dependent. The loss of a positive geotropic response of dark grown maize roots was previously observed by Scott and Wilkins (1969) with *Zea mays*.

4. Discussion

By carefully marking roots with Indian ink it was shown that curvature development took place between 3-6 MM from the root tip in both control and ACPI-55 treated roots. Median longitudinal microtome sections on the control roots revealed that this region is also the zone of cell elongation. If ACPI-55 inhibits root growth by inhibiting cell elongation, then its effect on curvature development and growth might be expected to be in the same region, i.e. in the extension zone. This could explain the positive correlation between curvature development (Table 3) and root growth inhibition (Table 4). Curvature development in horizontally placed roots treated with ACPI-55 could have been due to a differential in auxin levels between the lower and upper halves of the roots while growth inhibition could have been due to an overall decrease in auxin content in the extension zone. Alternatively growth inhibition could have been due to a reduction in cell divisions in the meristem of the root tip as a result of a lower auxin content in this region. Because IAA is known to stimulate cell elongation and cell division (see Review by Scott 1972), any interference in the supply of IAA to the extension zone and/or meristematic region by ACPI-55 could inhibit growth. From

the histological studies (see Chapter IV) it seems unlikely that curvature development was caused by different rates of cell division on the two sides of horizontally placed roots, treated with ACPI-55. Audus and Brownbridge (1957) have shown that the positive geotropic response in horizontally placed pea roots was caused by a greater decrease in elongation in the lower halves than in the upper halves of these roots. Whether the reverse takes place in treated roots which curve upwards (Fig. 3; Appendix 2) has still to be verified.

Of particular interest were the growth response curves (Fig. 5). At the highest concentrations (10^{-4}M) growth was less inhibited than at 3.3 x 10^{-6}M (for roots orientated vertically down, Fig. 2, Region I) or at 10^{-5}M (for roots which were orientated in the horizontal plane, Fig. 3, Region III and roots which were orientated vertically up, Fig. 4, Region V; Table 5). The trend of increased growth inhibition with a decrease in concentration in these regions was significant (negative regression coefficients were obtained in these three instances). The mode of action of ACPI-55 is unknown but it could be that the molecules block the passage of a growth substance, possibly IAA, passing through the plasma membranes. When the concentration of ACPI-55 is raised too high (10^{-4}M), the compound could bring about changes in membrane permeability due to "phytotoxicity". The flow of the growth substance may in fact increase at this concentration, thus stimulating growth. Electron-microscopy studies on membranes could perhaps indicate whether membrane structure is altered at high (10^{-3}M, 10^{-4}M) concentrations.

At 10^{-8}M (for roots orientated vertically down, Fig. 2, Region II) and at 10^{-7}M (for roots which were orientated in the horizontal plane Fig. 3, Region IV, and roots which were orientated vertically up Fig. 4, Region VI) root growth was stimulated over growth of the control roots. The trend of decreased growth from the optimum (at 10^{-7}M and 10^{-8}M) to control growth was significant for Regions II and VI (negative regression

coefficients were obtained in these two instances), while it was not significant for Region IV. According to the Cholodny-Went theory, auxin levels in roots are supraoptimal for growth. This means that growth is inhibited slightly. ACPI-55 as an auxin transport inhibitor could lower the endogenous auxin levels slightly at 10^{-7}M and 10^{-8}M. As a result auxin levels could become optimal for growth thus stimulating root growth over that of control roots. However the evidence presented for this action is at present not conclusive (Table 5). Only by testing a large number of roots per concentration could supporting evidence be produced for or against the Cholodny-Went theory. Audus and Brownbridge (1957) were able to increase as well as decrease both linear growth and the positive geotropic response in pea roots by adding different concentrations of IAA. Konings (1969) showed that IAA applied to the base of intact pea roots actually enhanced the curvature at the tip. These results would indicate that auxin levels are suboptimal for growth. In this event, the increase in growth due to applications of ACPI-55 at 10^{-7}M and 10^{-8}M cannot be explained except by proposing that the compound in itself stimulates growth at these concentrations. There is now evidence that this is not the case (see Chapter 5).

Curvature deviations from the longitudinal axes of treated roots (10^{-4}M - 3.3 x 10^{-6}M) placed in the three orientations (0^{o}, 90^{o}, 180^{o}) were analysed to determine whether curvature development was a geotropic response or whether the response was independent of the gravitational force. The results show (Table 6) that there were no significant differences in the curvature deviations between roots placed in the three orientations. Treated roots could therefore be classified as being ageotropic, i.e. they are not responding to the gravitational force. Because treated roots generally curve in the same direction, the response could not be classified as being random.

In many roots curvature developed towards the embryo side of the seed, rather than to the endosperm side. This is clearly shown for control roots which had been orientated upwards (180^{o}; Appendix 3) and for treated roots (at active concentrations) placed in all three orientations (Appendices 1, 2, 3). Curvature development in horizontally placed treated roots, was always towards the embryo side of the seed, whether the seeds were placed in the crystallizing dishes with the embryo uppermost or lowermost (Table 7; Appendix 5). The difference in curvature development (10^{-6}M) between seeds which had been placed with the embryo uppermost and those which had been placed with the embryo lowermost was highly significant ($P < 0.001$). These results clearly show that there is some intrinsic factor in these maize roots which influences curvature preferentially in one direction. This factor is present in both control and treated roots. It is not derived from the shoot, because no significant differences were observed in curvature development between seedlings with shoots and those of which the shoots had been excised (Table 7; Appendix 4). These results also support the statement that treated roots could be classified as being ageotropic, because roots of seeds placed with the embryo uppermost curved away from gravity while roots of seeds placed with the embryo lowermost curved towards gravity.

It was observed that control roots did not exhibit a positive geotropic response in darkness (Appendix 6; see also Scott and Wilkins 1969). When horizontally placed seedlings were positioned in the crystallizing dishes in such a way that the seeds with developing shoots grew under light conditions (1400 f.c.) while roots grew in darkness, no positive geotropic response was observed. Within one hour after light had been applied to these dark grown roots, positive curvature development became noticable. These experiments established that light is an important factor in the positive geotropic response of control roots. Other experiments showed that the downward curvature of horizontally

placed control roots was not a negative phototropic response (lights were always placed above the growing seedlings) since these roots curved towards the light source when they were illuminated from below. The response in ACPI-55 treated roots (at active concentrations), placed horizontally, was not a positive phototropic response since a curvature developed away from the light source when roots were illuminated from below. In actual fact the effect of ACPI-55 on curvature development was not light dependent at all, because curvature developed even in the dark (Table 8; Appendix 6).

Light had also a pronounced effect on root growth. Root growth of horizontally placed control seedlings which were grown under light conditions was inhibited by 16% as compared with dark grown control roots. Reduction in growth with light grown seedlings of *Zea mays* var. LG-11 was reported by Wilkins *et al.* (1974). These authors also showed that removal of the root cap completely released the root from this inhibitory effect of light on cell elongation. Wilkins and Wain (1974) have shown that abscisic acid and other unidentified growth inhibitors were present in the root caps of light grown roots, but they were not detected in dark grown *Zea mays* roots. The interesting observation was made that ACPI-55 treated roots (10^{-6}M) placed horizontally were inhibited in the light as well as in the dark. While growth of treated roots (10^{-6}M) in the dark was inhibited by 22% (as compared with dark grown control roots; Table 8), growth of treated (10^{-6}M) roots under light conditions was inhibited by 30% (as compared with light grown control roots; Table 4). Thus the light induced inhibitors reduced growth by 16%, while ACPI-55 reduced growth by 22%. When these two inhibitory factors (light induced inhibitors and ACPI-55) act at the same time (as was the case with treated roots grown under light) and if their action is independent of one another, it is expected that reduction in growth would be approximately 38%. In actual fact growth

inhibition of treated roots grown in the light (10^{-6}M, compared with growth of control roots grown in the dark), was close to this figure, i.e. 40%.

Because ACPI-55 inhibited growth even in dark grown roots in which no root cap inhibitor was detected, it is suggested that the compound acts independently of any endogenous inhibitors. This view is supported by the demonstration that the effect of ACPI-55 and any root cap inhibitors was additive rather than synergistic. Inhibition of growth by ACPI-55 in dark grown roots was probably due to its effect on auxin transport inhibition, which could lower the auxin levels in the growing tissues.

5. Summary

1. There is some evidence that curvature development in ACPI-55 treated roots takes place in the extension zone. However, it is not yet established whether root growth inhibition also occurs in this region. The positive correlation between these two parameters could possibly be explained in terms of auxin transport inhibition (both acropetal and lateral : see Chapter V) by ACPI-55.

2. Growth stimulation at low concentrations of ACPI-55 could be due to a shift in endogenous auxin levels from supraoptimal to optimal.

3. Curvature development in ACPI-55 treated roots was found to be independent of gravity. These roots were classified as being ageotropic.

4. Curvature development in most control and treated roots was in the direction of the embryo side of the seed. The factors responsible for this phenomenon are at present unknown.

5. Light is essential for the positive geotropic response in control roots. The positive geotropic response occurs only when the

root is illuminated. The shoot and seed have no apparent influence on this process.

6. The action of ACPI-55 on curvature development and growth is not dependent on light.

7. There is some evidence that ACPI-55 acts independently of any endogenous root cap inhibitor.

IV. HISTOLOGICAL STUDY ON THE ROOT CAP OF *ZEA MAYS*

1. Introduction

Juniper *et al.* (1966) demonstrated that removal of the root cap of *Zea mays* completely abolished geoperception. There is now strong evidence that amyloplasts are involved in the initial reactions which lead to the geotropic response (Wilkins 1969). In the previous Chapter it was shown that ACPI-55 treated roots were ageotropic and this could have been due to interference of the compound with the perception mechanism situated in the root cap or with the root cap itself. For this reason a histological study was made on the root cap as well as on the size, number and movement of amyloplasts in the statocytes of both control and treated roots. In addition, the effect of ACPI-55 on the root cap cells and on the cells of the quiescent centre was determined.

In general the histological methods as outlined by Feder and O'Brien (1968) were followed. However, the procedure had to be adapted to suit *Zea mays* roots. In addition a method was developed for fixing root tips in different orientations. A detailed description of the method used follows.

2. Methods

In the studies listed below, all roots were originally placed in the horizontal (90^{o}) orientation. Histological studies were carried out on roots (orientations of roots with respect to gravity are given in degrees; 0^{o} = direction of gravity) to determine the following:

(i) *Position of amyloplasts.* Roots were grown in the light for 24 hours and the final orientations were:-

- Control: $\simeq 30^{o}$
- Treated: 90^{o}

(ii) *Position of amyloplasts.* Roots were grown in the dark for 24 hours and the final orientations were:-

- Control: 90^{o}
- Treated: 180^{o}

(iii) *Movement of amyloplasts.* Roots were grown in the light for 24 hours and they were then reorientated to the vertical plane (0^{o}; vertically down)

- Control: $\simeq 30^{o} \rightarrow 0^{o}$
- Treated: $90^{o} \rightarrow 0^{o}$

(iv) *Movement of amyloplasts.* Roots were grown in the light for 24 hours and they were then reorientated as under (iii); followed by reorientation in the inverted position (180^{o}; vertically up)

- Control: $0^{o} \rightarrow 180^{o}$
- Treated: $0^{o} \rightarrow 180^{o}$

With this root material the following histological method was used.

(a) *Preparation of plant material prior to fixation*

The reorientation of roots was carried out by turning the crystallizing dishes in which the roots grew to 0^{o} or 180^{o}. The duration of each reorientation was one hour. Treatment was done with ACPI-55 at 10^{-6}M. The procedure was as follows: After the growth or reorientation periods, root tips were excised, put on razor blades (see below), and immediately lowered in cold fixative (Acrolein, kept on ice). The root tips were excised at an angle so that the direction of gravity could be identified at a later stage from the microtome sections. All manipulations of the material were carried out in the fume cupboard because of the toxicity of the fixative used. The transfer time of the root tips from the crystallizing dishes to the razor blades was between 5-10 seconds. Dark grown roots were kept in darkness as long as possible before transfer and received between 10-40 seconds of

diffuse light before fixation commenced. During transfer, root tips were kept in the specific orientations in which they grew, before excision. A method was developed whereby rootstips could be fixed, in the exact orientations in which they grew originally. Fixation of roots could not be done in the crystallizing dishes because of the toxicity of Acrolein. The equipment which was developed for fixation of roots (as well as for dehydration and infiltration) is shown in Figure 1 (J). The small glass vials which were used could hold 24 ml of solution. Slits had been made in special lids of perspex to hold cut razor blades. In some instances razor blades were spot welded at right angles. All blades had one sharp edge and root tips were slit on these sharp edges as indicated by the arrows in the Figure.

(b) *Fixation*

Fixation was carried out with a 10% solution of Acrolein for 24 hours. The main advantage of this fixative is that it penetrates and fixes rapidly (Feder and O'Brien 1968). This was of particular importance as the root tissue was relatively large (between 4-7 mm long) and fixation had to be carried out quickly to arrest the amyloplasts in their specific locations within the statocytes. The main disadvantage of Acrolein is its toxicity. Being a tear gas it could not be used as a direct application to the roots in the crystallizing dishes.

(c) *Dehydration*

During the process of washing and dehydration, roots were kept on razor blades. The procedure was as follows: The Acrolein was carefully decanted with the aid of a Pasteur pipette and in the fume cupboard. The root tissue was subsequently rinsed with cold distilled water. Following this, dehydration was done with 2-methoxyethanol, 100% ethanol, n-propanol and finally n-butanol. Two changes were made of

each solution in 24 hours. All solutions were kept cold. Extreme care was taken that no ice crystals or water would accidentally contaminate the solutions. Low temperatures were used because of the strengthening effect of cold on most macromolecular materials. This gives more resistance to mechanical distortion.

(d) *Infiltration*

After the last change of the dehydration process, the vials containing the root material were brought to room temperature first, before opening since atmospheric moisture could condense on the cold surface of the liquid. For infiltration glycol methacrylate (GMA, a plastic) was used. The GMA mixture (referred to as GMA from now on) was made up as follows:

Glycol methacrylate : 94.87%

2,2'-Azobis[2-methylpropionitrile] : 0.13% (initiator)

Polethylene glycol 400 : 5% (plasticizer)

Care was taken during the preparation that all glassware was dry. The GMA was stirred for at least one hour to dissolve all the initiator as any solid residue could make microtome sections inferior due to "stress marks". Stirring was done in the dark and without heat to prevent polymerization. The first two changes were made with a GMA-n-butanol mixture (50:50). This was followed by infiltration of the specimens with a solution of GMA for at least a week. During this period, the solution was changed three times.

(e) *Embedding*

For embedding pure GMA was used. A thin layer of GMA (1-3 mm) was placed in plastic dishes (microsize weighing trays - $1\frac{5}{8}$" square), and put under u.v. light in a specially constructed perspex box for polymerization. After the GMA had hardened some fresh solution was added followed by the specimen. Further polymerization was carried

out as described above. The advantage of this procedure is that the specimen is covered on all sides by the GMA. This gives strong support to the tissue which is important when microtome sections are cut. Polymerization was done under a flow of high purity nitrogen as oxygen inhibits this process. The u.v. lamp (Philips 15W) was situated 30 cm above the specimen. U.V. light entered the box through a gladwrap (clear plastic) window as perspex could filter out the u.v. rays. Three factors effect the hardness of the GMA blocks and the speed at which polymerization takes place: (i) the distance between the u.v. source and the specimen (the further away the u.v. lamp is placed, the longer it takes for the polymerization process to be completed); (ii) the plasticizer (if the GMA blocks are too hard or brittle, more polyethylene glycol 400 must be added); (iii) the polymerization initiator (if the blocks are too soft, the concentration of 2,2'-azobis[2-methylpropionitrile] may have to be altered). The best results were obtained with the preparation of GMA as discussed in the previous section on infiltration and under the conditions mentioned above. The main advantage of GMA and most other plastics is their strength and hardness, which means good support for the tissue elements. Most waxes are relatively soft and lack tensile strength. This means that hard and tough tissue elements are easily torn or displaced by the knife edge. With GMA moreover, sections of 1μ or less can be obtained with relative ease while most wax sections are in the range of 5-20μ. The disadvantage is that trimming of the specimen blocks and sectioning are generally slower with plastic than with wax. GMA sections have to be handled individually, while wax sections can be obtained in a ribbon.

(f) *Sectioning*

Before sectioning, blocks were trimmed in which the plant material was embedded and these were mounted on polymerized GMA cylinders. These cylinders were made with the aid of gelatine capsules (self locking caps). The blocks were fastened with a cyanoacrylate adhesive ('Loctite superbonder') to the GMA cylinders. Much care was taken with the orientation of the root tips during the processes of embedding, trimming and mounting so that median sections could be obtained and the original direction of gravity could be identified from the sections. Sectioning was done on a Reichert microtome with the aid of glass knives. The sections (0.5, 1 and 2 micron thick) were put individually in drops of sterilized distilled water on microscope slides and were fastened with low heat from a hot plate.

(g) *Staining*

(i) *Periodic acid - Schiff (PAS) stain.* The PAS stain was used to show the amyloplasts. Starch and some complex polysaccharides are stained red. The PAS stain is preceded by an "aldehyde blockage". If this blockage is not performed a general background staining will occur of many tissue elements besides starch and polysaccharides. Completeness of the blockage was tested by transfer of a treated slide (in each batch), directly to the Schiff's reagent (thus by passing the periodic acid step), followed by the usual metabisulfite and water rinses. If the blockage is complete, no background staining should be noticeable. The blockage was performed with a saturated solution of 2,4-dinitrophenylhydrazine (DNPH) in 15% acetic acid in water, for 10 minutes at room temperature. This step was followed by a thorough rinse with running tap water for 30 minutes and a 1% periodic acid

treatment for 10 minutes. The sections were rinsed again thoroughly in running tap water for 10 minutes and this was followed by the Schiff's reagent treatment lasting 30 minutes. After the Schiff's reagent treatment, slides were quickly transferred to three successive baths (2 minutes each) of 0.5% sodium metabisulfite (Lilie 1965) and a final rinse for 2 minutes in distilled water.

(ii) *Toluidine blue*. Most sections were counterstained for 3 minutes with a 0.05% solution of toluidine blue. DNA stains blue.

(h) *Mounting*

Mounting was done with fast hardening "Eukitt" and No. 1 cover slips (22 mm diameter).

(i) *Microscopy*

All microscope examinations were done on a Zeiss microscope. Photographs were taken through a Leitz microscope using Kodak Panatomic-X (32 ASA) films.

(j) *Analyses*

Counts and measurements were made on 19 median sections of 4 light grown control roots and on 15 median sections of 4 light grown treated roots. No statistical analysis was carried out on dark grown roots, but several control and treated root caps were examined and photographed. The following measurements and counts were taken from the central core statocytes of the median sections: amyloplast size, amyloplast number, nucleus and nucleolus size and cell size of central core statocytes.

(k) *Treatment*

Roots were treated with a 10^{-6}M solution of ACPI-55 (Na salt). The compound dissolves readily in water and no solvent was therefore used.

3. Results

(A) *Key to Photograph Labels*

C.C. = Central core

P.C. = Peripheral cells

ST. = Statocytes

D.ST. = Developing statocytes

SL.C. = Sloughed off cells

C.B. = Cap boundary

C.M. = Cap meristem

Q.C. = Quiescent centre

AM. = Amyloplasts

N = Nucleus

NC = Nucleolus

G + Arrow = Direction of gravitational stimulus

R + Arrow = Direction in which roots grew or were orientated

Approximate magnifications of photographs are given in the text.

(B) *Roots Grown in the Light*

Figure 6. Amyloplast position of control (A) and treated (B) roots in their final orientation after 24 hours of growth (x 200).

The amyloplasts of the central core statocytes of control and treated roots rest on the transverse and longitudinal walls of the cells. The amyloplasts of the peripheral cells of the root cap were often distributed at random.

Figure 7. (i) Amyloplast movement of control (A) and treated (B) roots which were, after 24 hours of growth, reorientated for one hour in the vertical position (0^{o}; vertically down). (ii) Amyloplast movement of control (C) and treated (D) roots which were reorientated, after 24 hours of growth, first for one hour vertically down (0^{o}), followed by one hour vertically up (180^{o}; x 180).

Figure 8. High magnification of control root (A : x 400 and C : x 1600) and treated root (B : 400 and D : x 1600) which had been reorientated as mentioned under Figure 7 (i).

Figure 9. High magnification of control root (A : x 400 and C : x 1600) and treated root (B : x 400 and D : x 1600) which had been reorientated as mentioned under Figure 7 (ii).

Figure 10. Root cap meristem and growing cells of a control root (A : x 1700) which grew vertically down (0^{o}) and a treated root (B : x 1700) which grew horizontally (90^{o}). Roots were originally placed horizontally.

Figure 11. Quiescent centre of a control (A) and a treated (B) root. Control root grew vertically down (0^{o}), treated root grew horizontally (90^{o}; x 1800). Roots were originally placed horizontally.

Table 9. The differences in amyloplast size and number between control and treated roots were not significant ($P > 0.05$).

The differences in the size of the nuclei and nucleoli between control and treated roots were not significant ($P > 0.05$).

The difference in cell size between control and treated roots was also not significant ($P > 0.05$).

Figure 12. The dimensions of the root caps of control and treated roots are shown in histogram form. All measurements were taken from median sections. Very little difference was observed between the root cap sizes of control and treated roots.

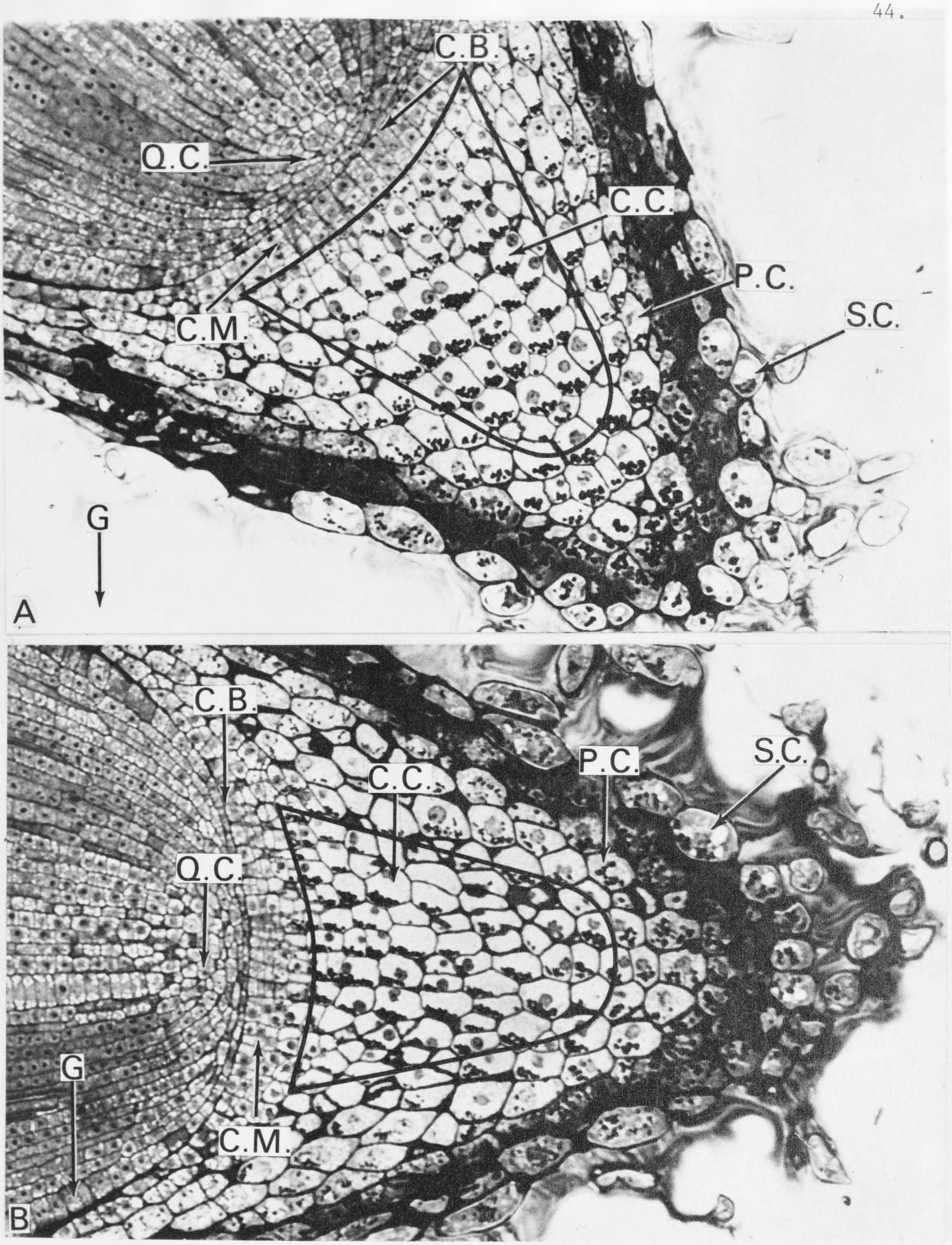


Figure 6

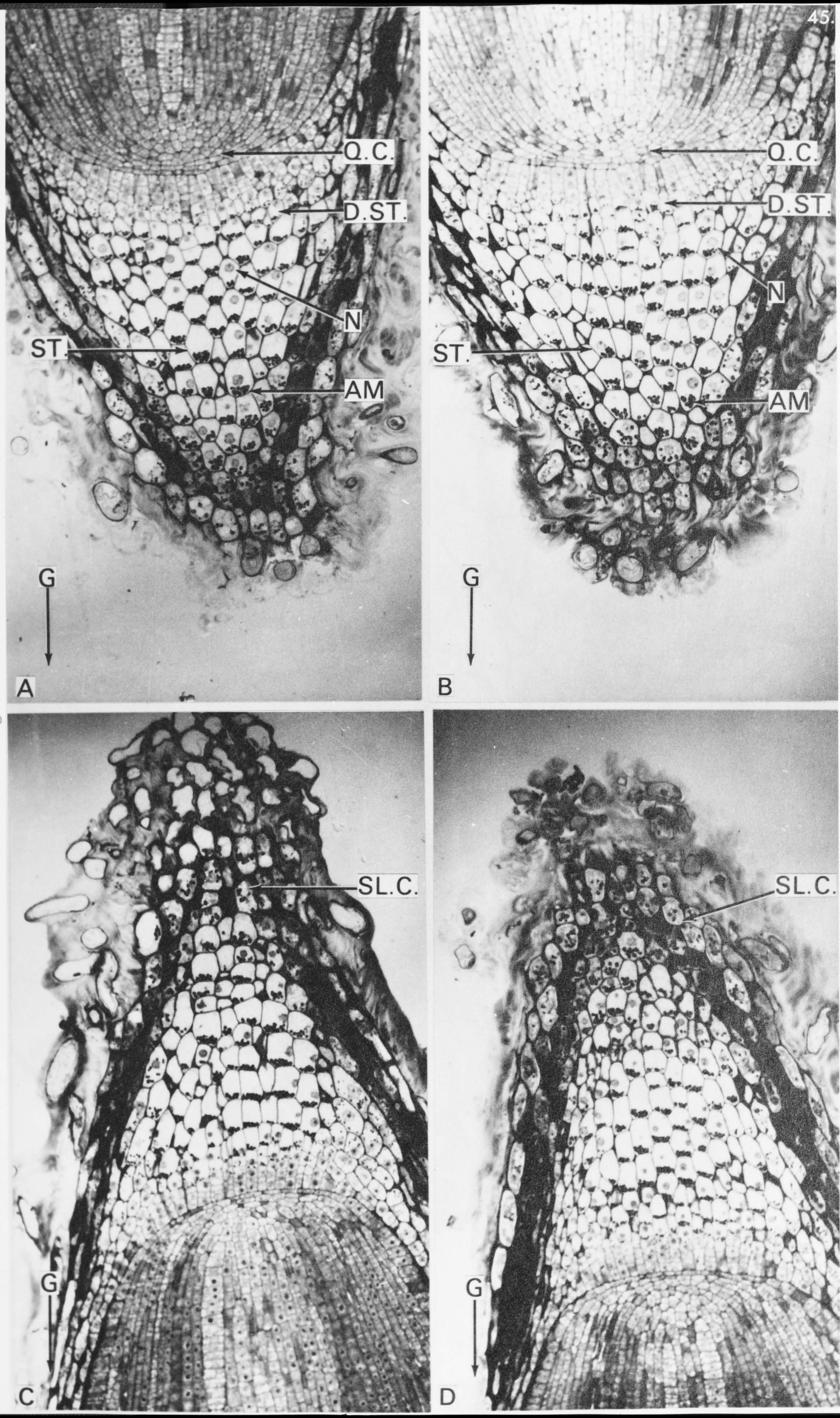


Figure 7

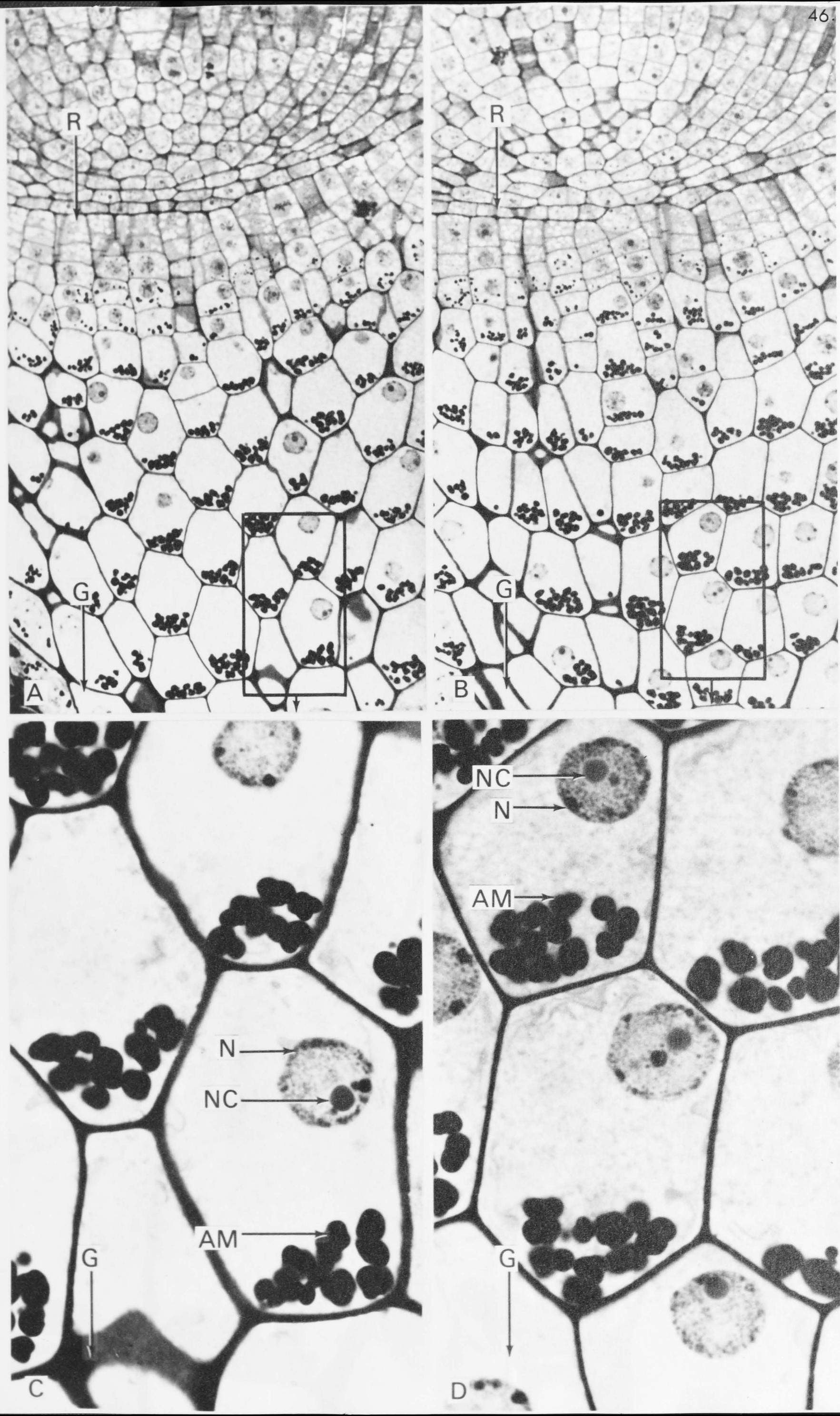
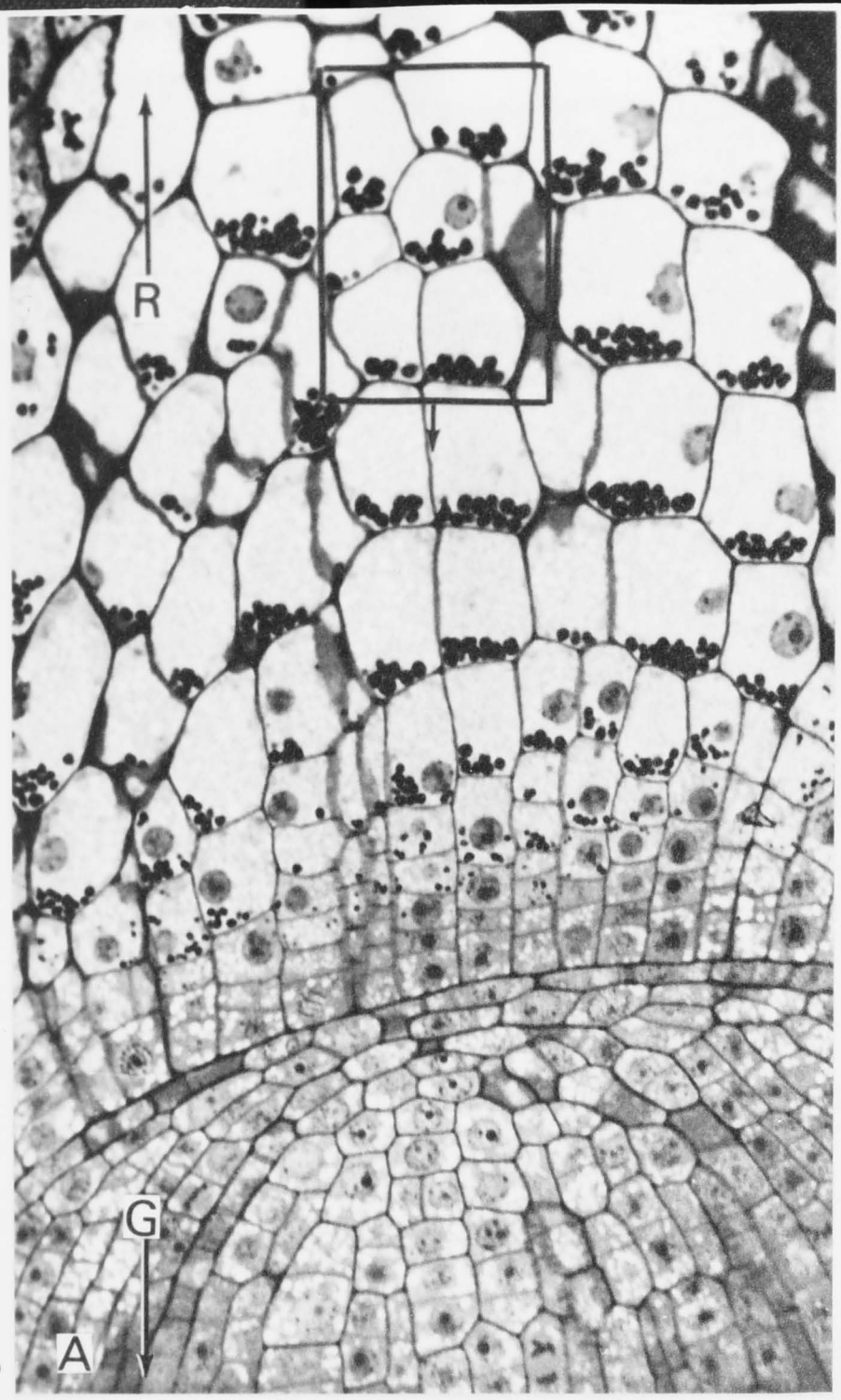
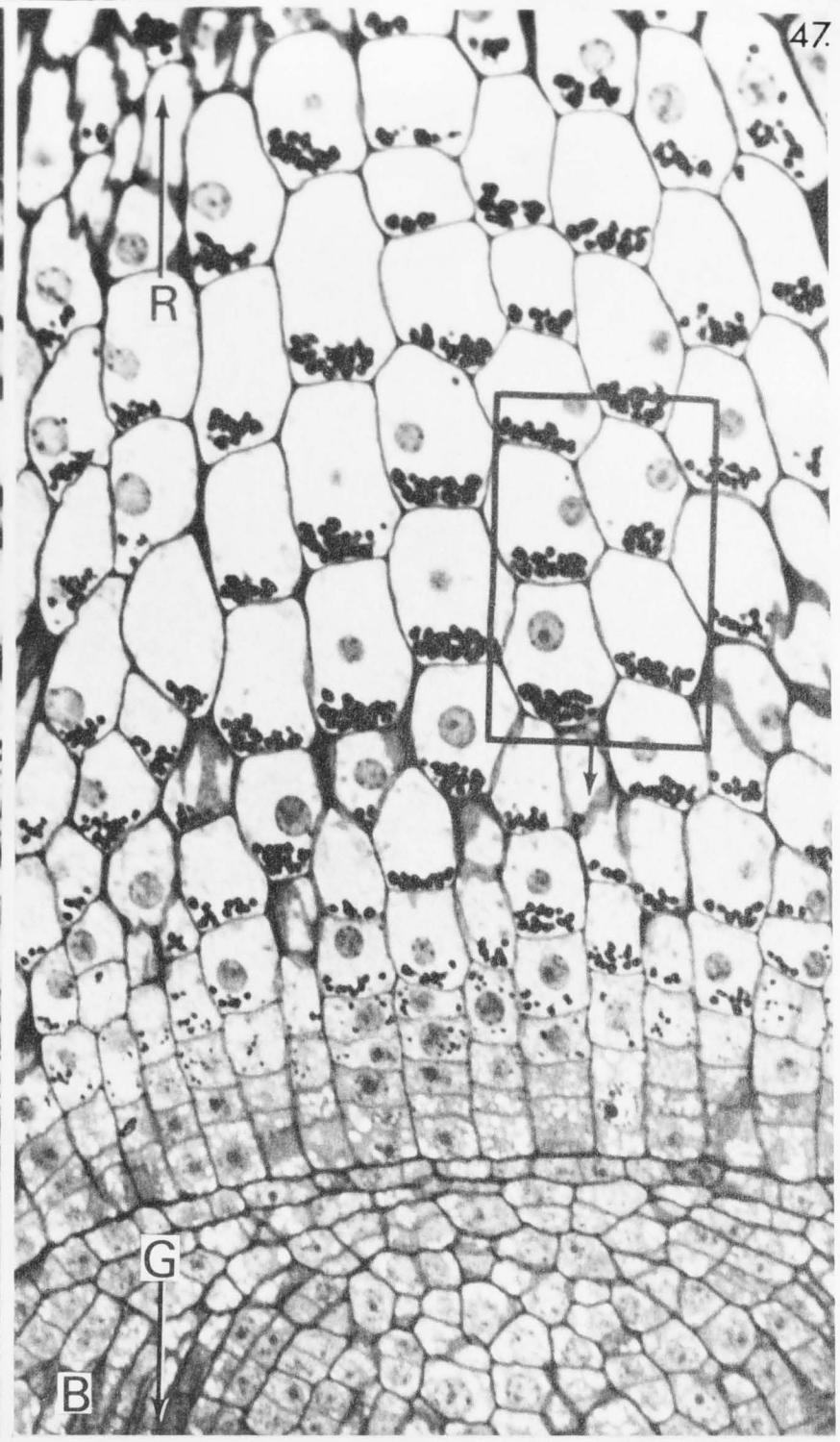
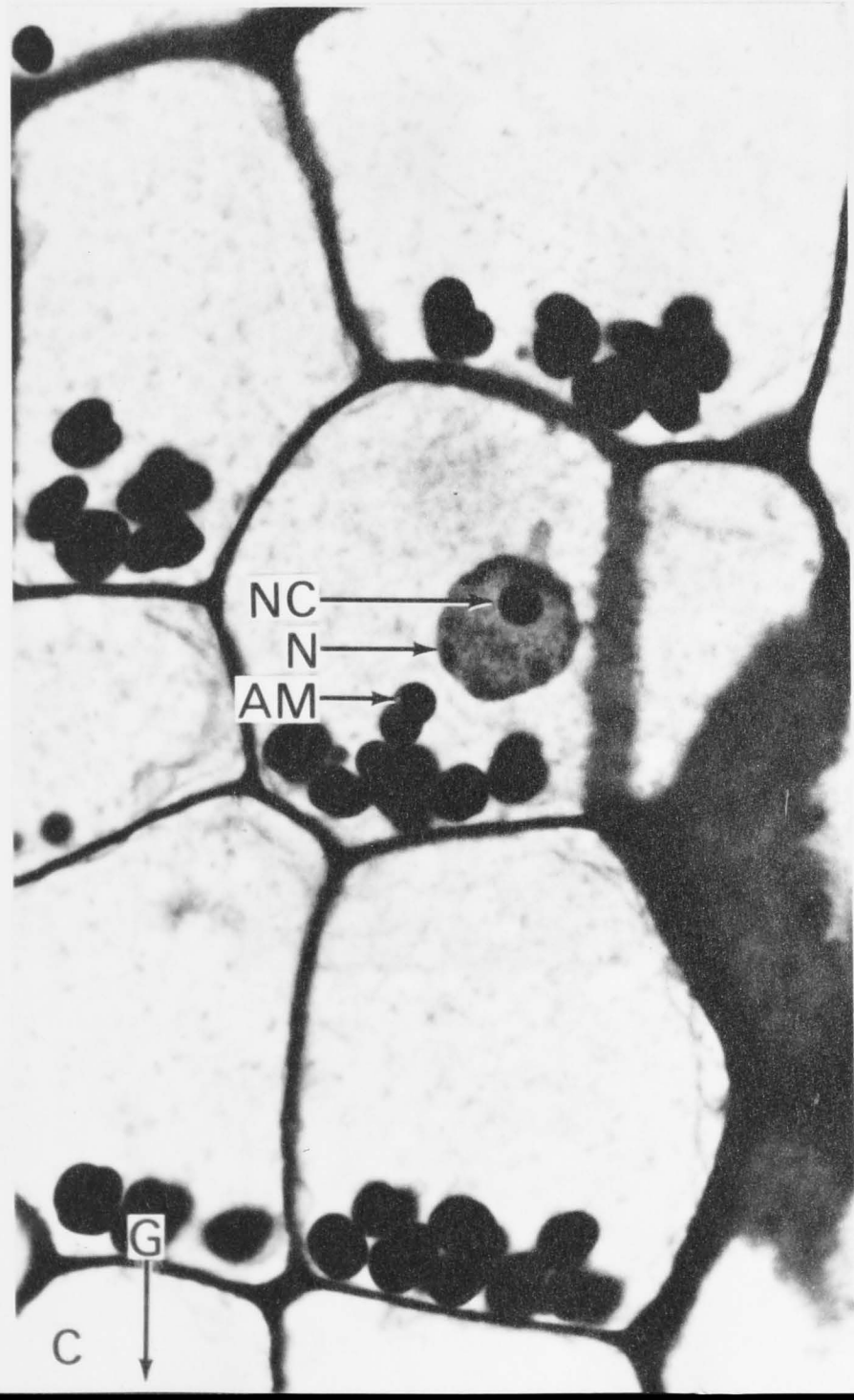


Figure 8







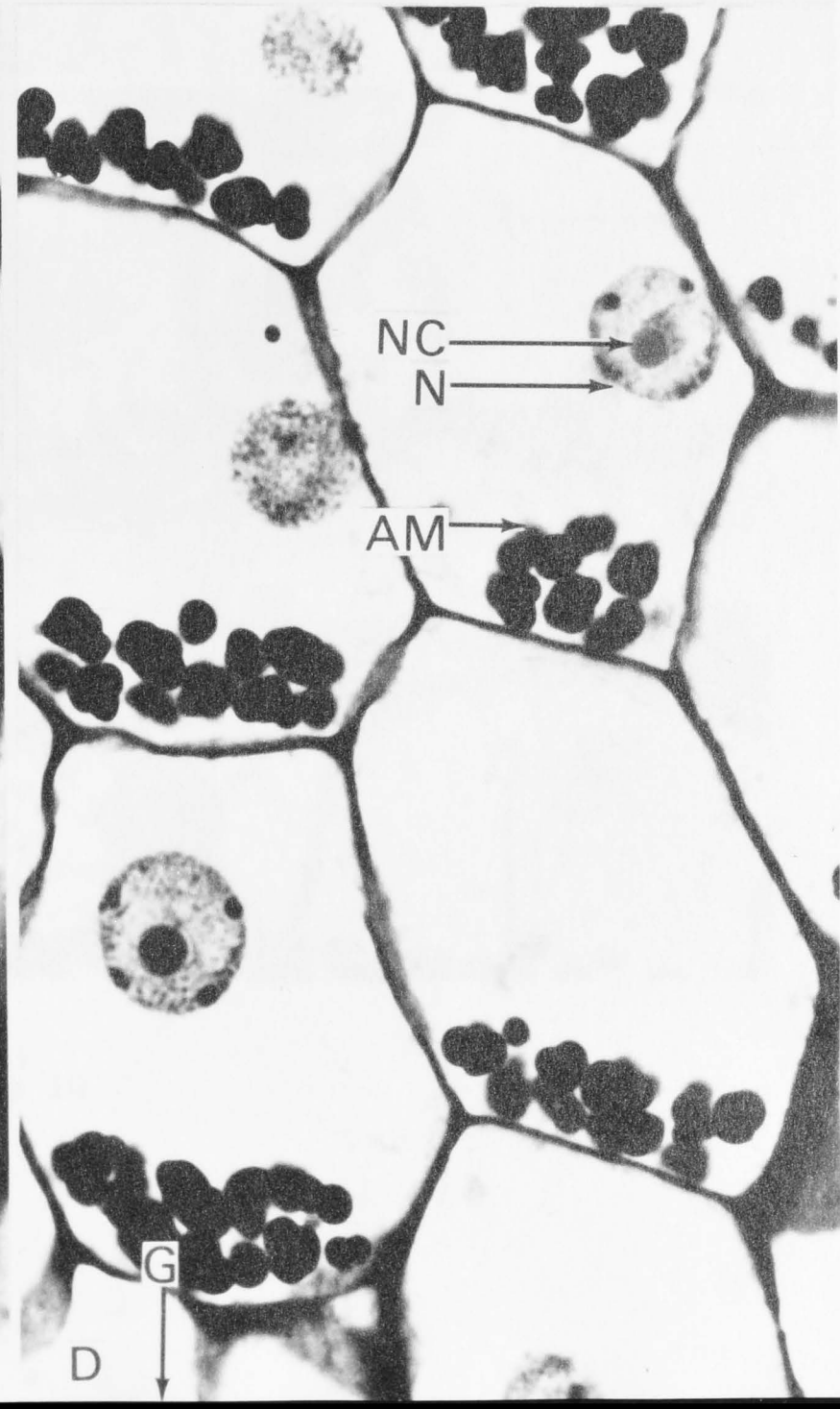


Figure 9

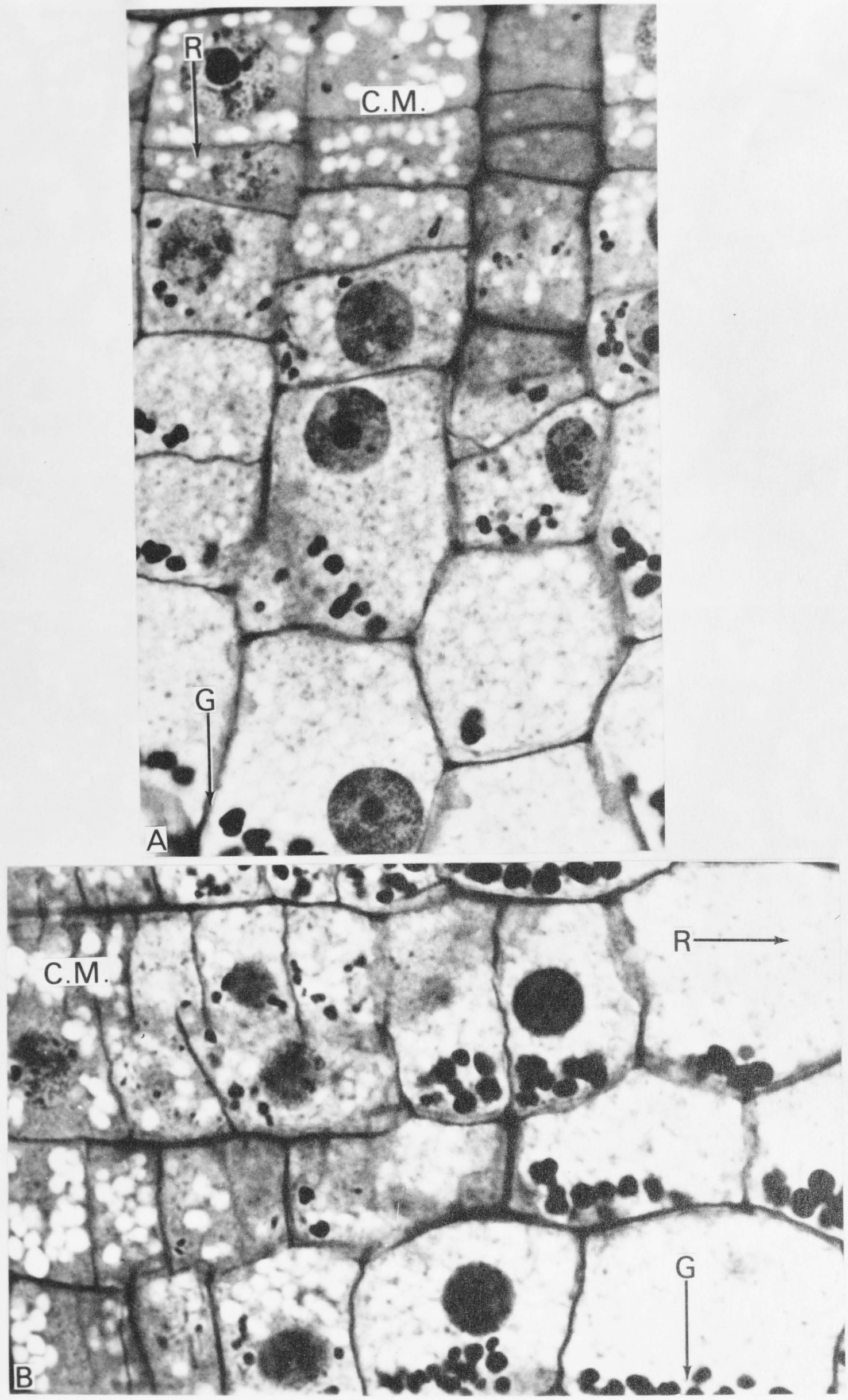


Figure 10

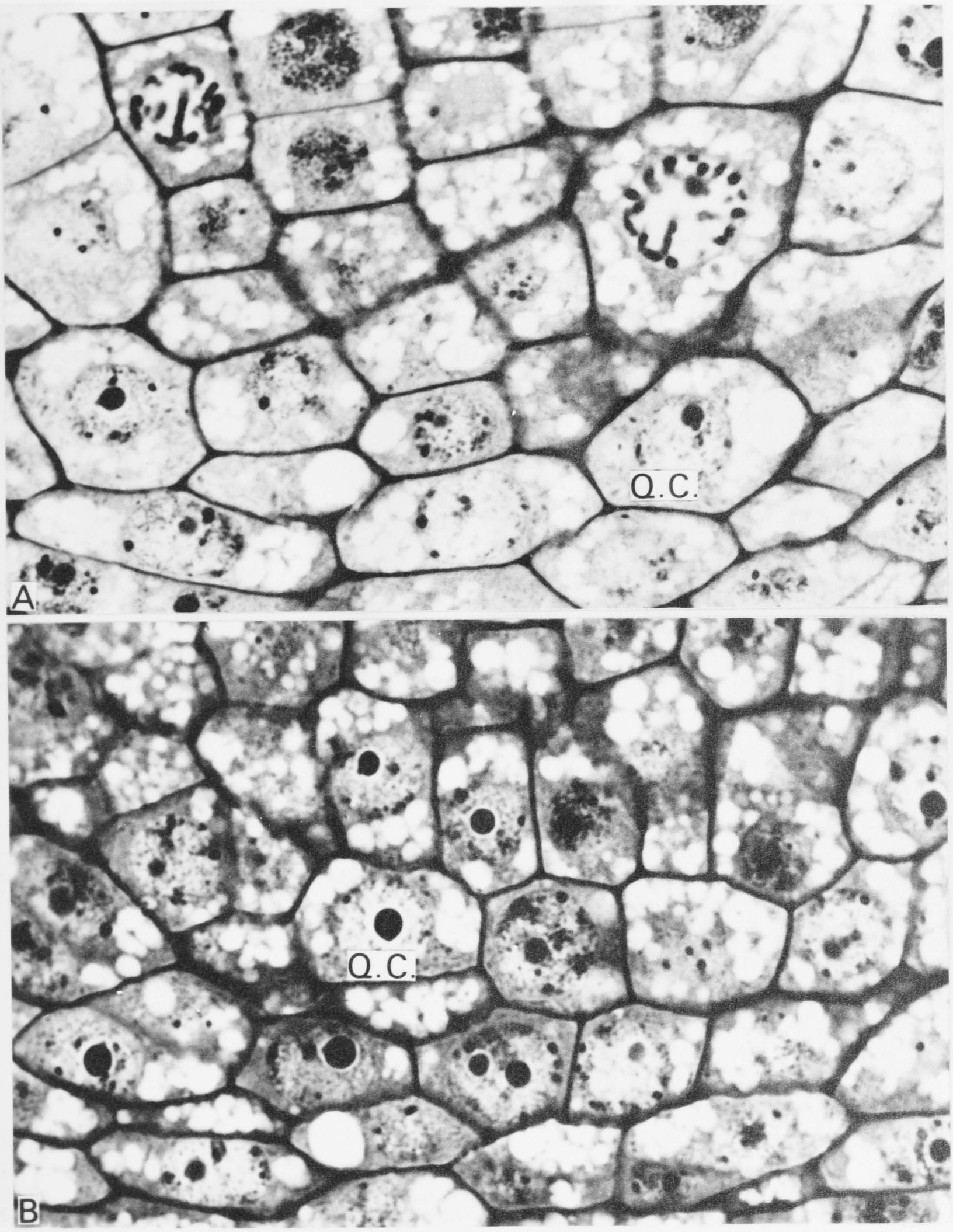


Figure 11

TABLE 9. *ZEA MAYS*. ANALYSES OF ROOT CAP ORGANELLES AND SIZE OF ROOT CAP CELLS

Type of Analyses	Control	Treated	Student's t
Mean amyloplast size (μ^2)	10.53	10.18	1.53 N.S.
N	140	112	
Mean number of amyloplasts per cell area	6.14	6.27	0.05 N.S.
N	355	297	
Mean size of nucleus (μ^2)	86.08	81.16	1.29 N.S.
N	19	15	
Mean size of nucleolus (μ^2)	5.73	5.48	1.71 N.S.
N	19	15	
Mean cell size (μ^2)	607	587	1.01 N.S.
N	355	297	

N.S. Not significant ($P>0.05$)

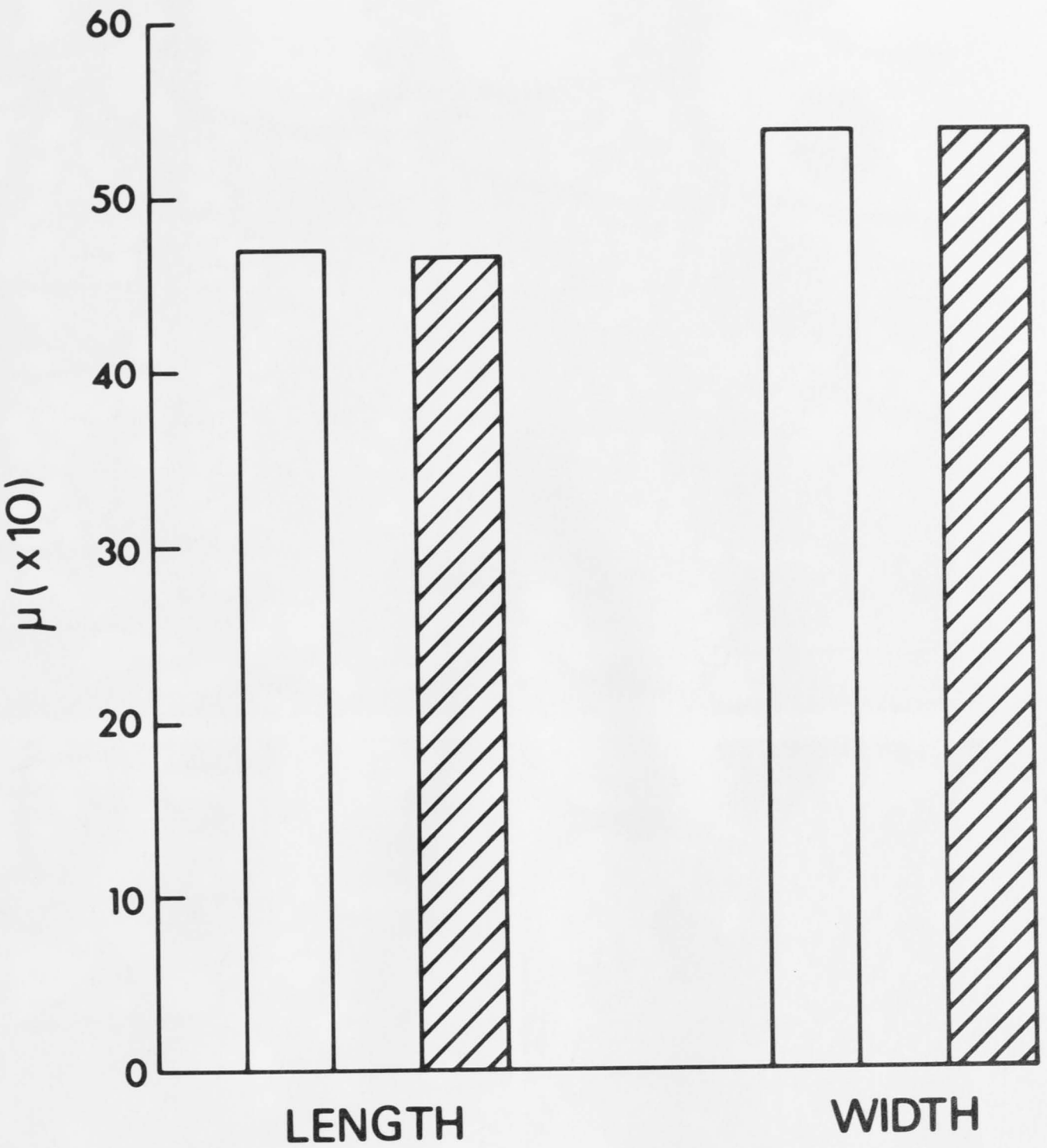


Fig. 12. *Zea mays*. Dimensions of root cap

Length: Straight line from root cap boundary to tip of root cap

Width: Straight line across root cap boundary

N = 4

☐ Control ▨ ACPI-55, 10^{-6}M

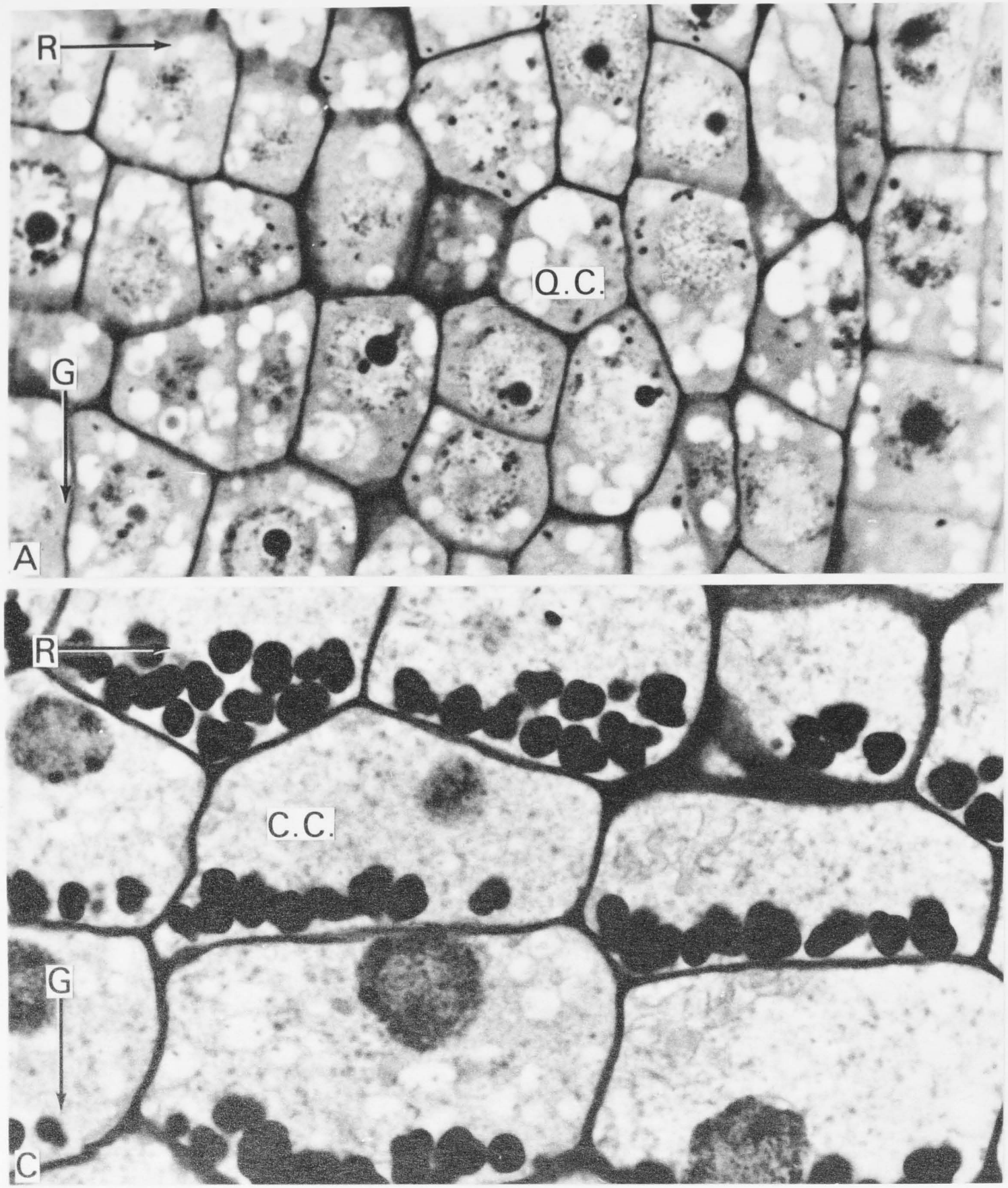
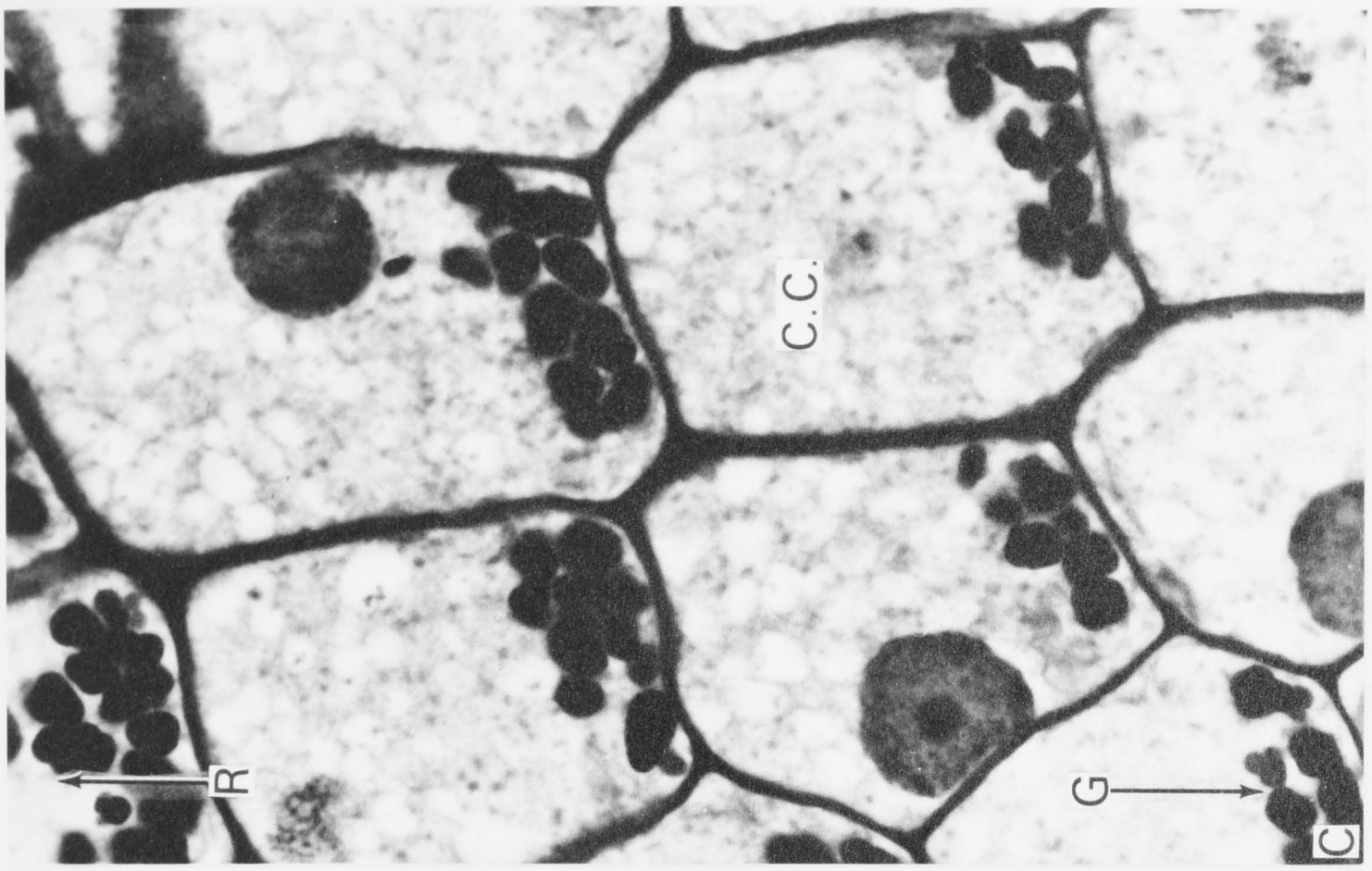
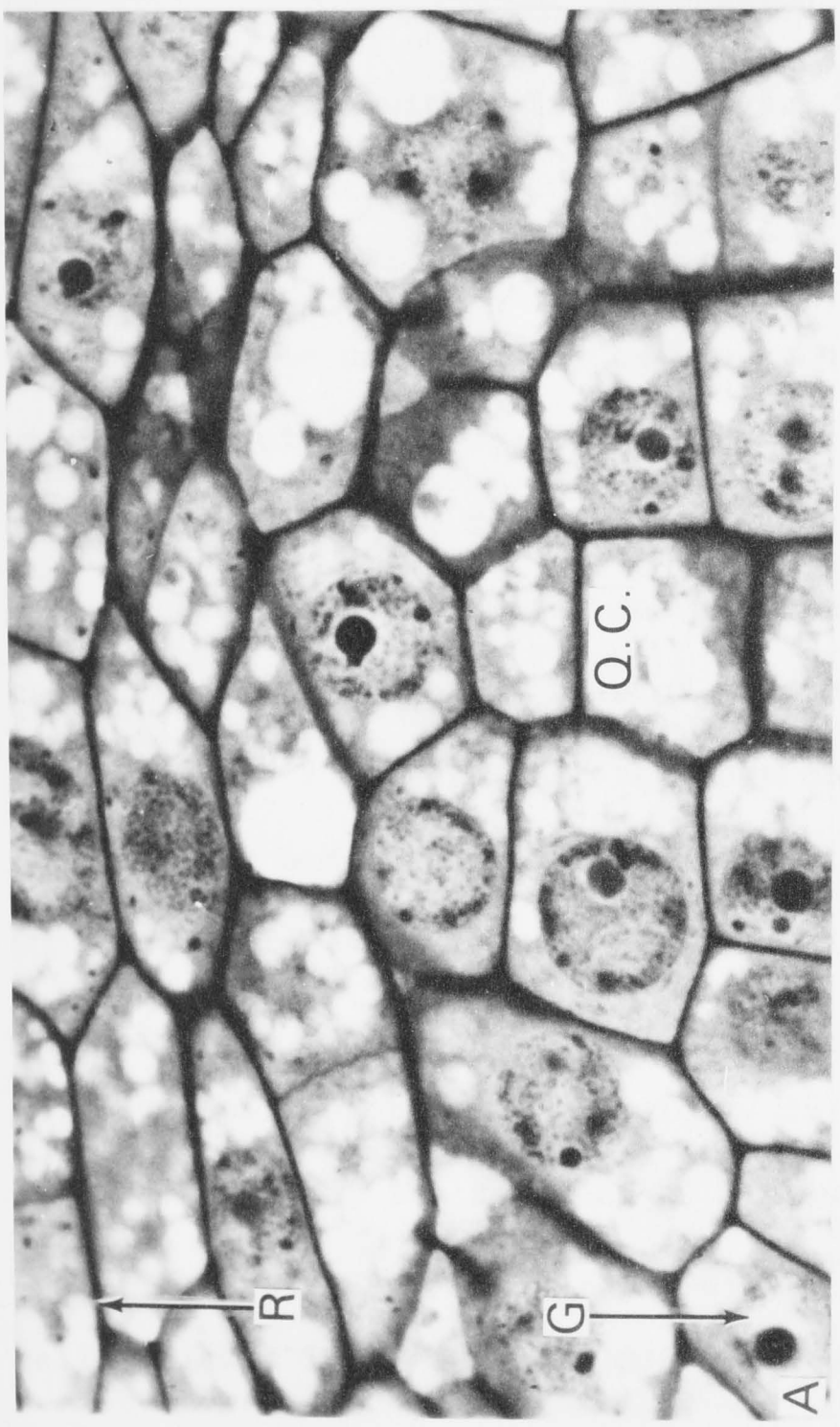


Figure 13





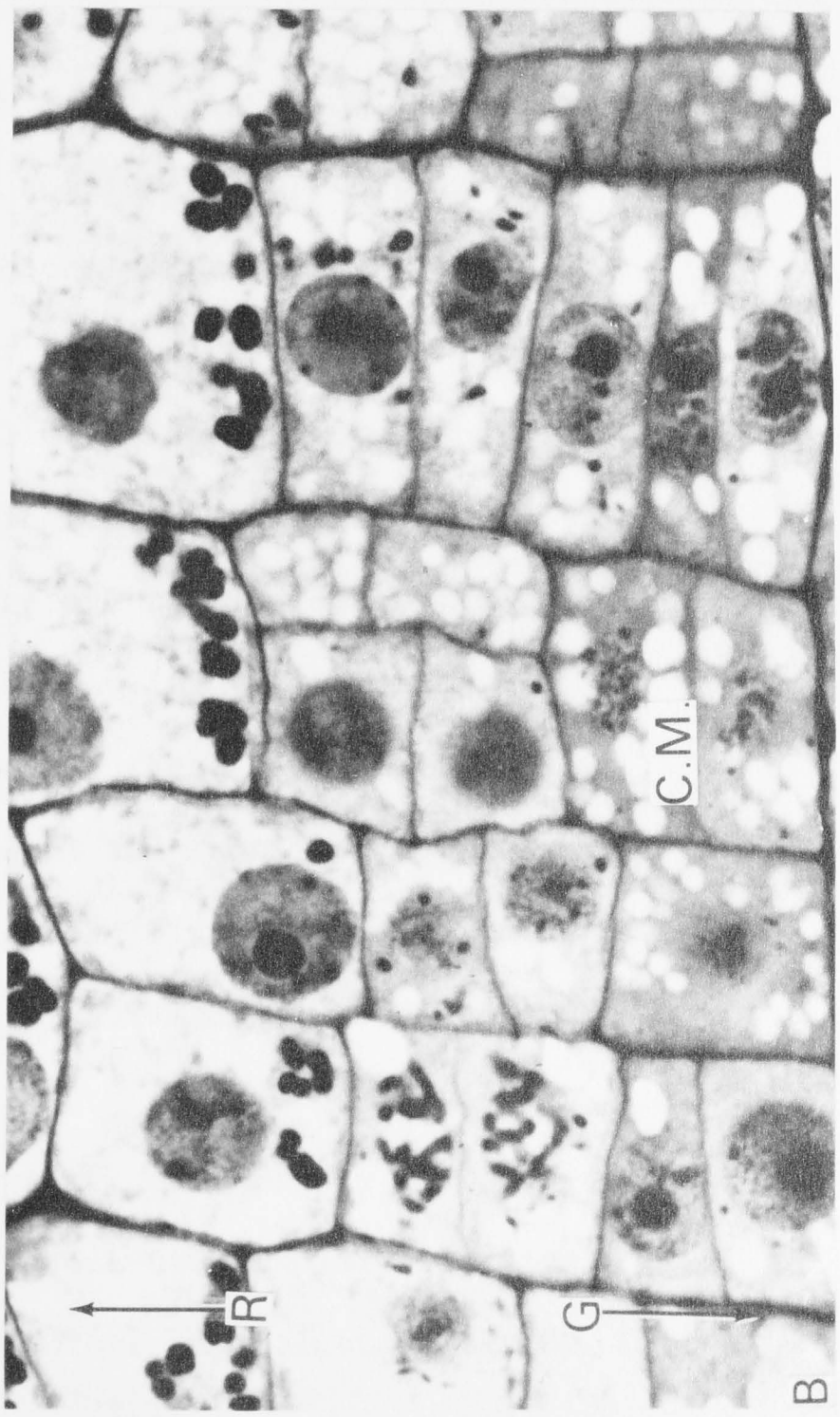


Figure 14

(C) *Roots Grown in the Dark*

Qualitative information on a dark grown control root with respect to the cells of the quiescent centre, cap meristem and size, number and position of amyloplasts is presented in Figure 13. The root was fixed in Acrolein after 24 hours of growth in the horizontal plane (90^{o}; x 1650).

The same qualitative information on a dark grown treated root is shown in Figure 14. The root was fixed in Acrolein after 24 hours of growth vertically upwards (180^{o}; x 1650).

4. Discussion

It was shown with maize (*Zea mays*) and pea (*Pisum sativum*) roots, that removal of the root cap (decapping) abolishes geoperception (Juniper *et al.* 1966; Konings 1967; Gibbons and Wilkins 1970). The presence of the cap boundary between root cap and root proper (Fig. 6) which is rarely, if ever, crossed by cell divisions (Juniper and French 1970; Fig. 8 and 9), facilitates decapping without causing damage to the roots. The experimental results therefore provide overwhelming evidence for a regulatory role of the root cap in geoperception. Figures 6 and 7 suggest that the root caps of these roots are normal (see Juniper and French 1970 for comparison) and that there are no apparent differences between control (Fig. 6A, Fig. 7A and C) and treated (Fig. 6B, Fig. 7B and D) roots. Measurements on median sections of root caps support this view (Fig. 12).

The cells of the root cap of *Zea mays* are produced by the cap meristem (Fig. 6, 8, 9, 10), a region of about 6600 rapidly dividing cells (Clowes 1971) which do not possess mature amyloplasts (Fig. 8, 9, 10). These cells are probably not responsible for geoperception because re-establishment of the meristem which takes place after decapping is not sufficient to restore the ability to perceive the gravitational stimulus (Schachar 1967). This view is supported by the

work of Cercek (1970) who showed that barley (*Hordeum vulgare*) roots which possessed a cap meristem failed to respond to gravity when all the differentiated cells of the cap had been cut. No apparent differences were observed between the cap meristems of control (Fig. 10A) and treated (Fig. 10B) roots.

Surrounding the whole cap is a layer of cells which are termed the peripheral cap cells (Fig. 6). The Figure shows that these cells possess only small amyloplasts which do not often sediment under the influence of gravity. Schachar (1967) showed that decapped roots soon recovered the ability to perceive gravity even in the absence of the peripheral cells. It is therefore likely that these cells are not involved in the perception of gravity but they could have a protective function for the root cap as a whole. The presence of peripheral cells was observed in both control (Fig. 6A) and treated (Fig. 6B) roots.

If the gravitational stimulus is perceived by plant cells as a result of the displacement of some intracellular particle of a density different from that of the cytoplasm, then an appreciable sedimentation must take place during the presentation time (i.e. the minimum exposure to the gravitational stimulus necessary to induce a just detectable response). Attempts have therefore been made to calculate the theoretical rate of sedimentation of particles of various sizes and densities in the cytoplasm, using Stokes Law, which governs such movement in a viscous medium. Audus (1962) has calculated that only a starch grain is large and dense enough to traverse half a cell width of a bean root tip cell (10μ) in an average presentation time of three minutes. If however, smaller distances are involved then a body like the mitochondrion could cover these smaller distances in a reasonable short time. Because these calculations do not rule out the possibility that particles of

mitochondrion dimensions could be statoliths, Griffiths and Audus (1964) made direct observations of organelle distributions in root cap cells of *Vicia faba* by electron microscopy and analysed statistically the changes induced by displacement of growing roots. They found that both Golgi bodies and mitochondria showed little change in distribution except as an indirect result of amyloplast sedimentation. There was no evidence that these particles were affected by gravity over the stimulation period of two hours. Further support for the starch (amyloplast) statolith theory comes from Iversen (1969) who found no geotropic responsiveness in starch free, but still elongating roots of cress (*Lepidium sativum*) rotated on a klinostat (to eliminate the unilateral action of the gravitational force) after horizontal stimulation. These studies are supporting evidence for the theory proposed by Nemec (1900, 1902) that the central cap cells (Figs. 6, 8, 9) are responsible for geoperception on the grounds that only these cells contain movable amyloplasts. The results of experiments which were carried out to study amyloplast movement supports the theory proposed by Nemec (Fig. 7). The data also shows that amyloplasts of control (Fig. 7A; Fig. 8A and C) and ACPI-55 treated roots (Fig. 7B; Fig. 8B and D) sediment under the influence of the gravitational stimulus. In addition, the experimental results show that amyloplasts of control (Fig. 7C; Fig. 9A and C) and treated roots (Fig. 7D; Fig. 9B and D) could traverse the entire cell length within one hour. This means that ACPI-55 treatment does not block amyloplasts in specific locations within the statocytes and that they are free to move under the influence of gravity. Statistical analyses on amyloplast size and number in the central core statocytes revealed no significant differences between control

and treated roots (P>0.05); Table 9) and this would indicate that ACPI-55 does not interfere with the synthesis and growth of amyloplasts.

Because no significant differences were obtained in the size of nuclei and nucleoli between control and treated roots (P>0.05; Table 9), this could indicate that ACPI-55 has little, if any, effect on the synthesis of nuclear and nucleolar material. Observations made on the quiescent centre supports this view (Fig. 11). These cells, which rarely divide (Clowes 1956) are small and the space which is taken up by the nuclei is relatively large. No obvious differences were observed between the nuclei of the control and those of the treated roots.

The difference in the size of the statocytes between control and treated roots was not significant (P>0.05; Table 9) and this suggests that ACPI-55 has no apparent effect on the growth of these particular cells.

The histological data obtained from dark grown roots was of particular interest because horizontally orientated control roots did not respond to the gravitational stimulus (see Chapter III; Appendix 6), although the statocytes appear to have the full complement of amyloplasts (Fig. 13C). The lack of response in dark grown *Zea mays* roots is obviously not due to a reduction in size or number of amyloplasts. The quiescent centre (Fig. 13A) and the cap meristem (Fig. 13B) appears to be normal. The same observation was made for dark grown treated roots (Fig. 14A and B) which grew vertically upwards. The amyloplasts of the statocytes had sedimented under the influence of gravity to the transverse walls (Fig. 14C).

5. Summary

Histolgical studies on the cells of the root caps of *Zea mays* revealed no structural differences between control and treated roots. It was demonstrated that amyloplasts could move freely inside the

statocytes. The ageotropic response of ACPI-55 treated roots was therefore not due to a reduction in amyloplast size and/or number or to interference with their movement within the statocytes.

Microscopic examination revealed no obvious differences in cell structure, amyloplast size, number and movement between dark and light grown control roots. The abolishment of a positive geotropic response in dark grown control roots can therefore not be explained in terms of an absence or reduction in amyloplast size and number. No structural differences were observed between dark and light grown roots which had been treated with ACPI-55.

V. GENERAL DISCUSSION

Although ACPI-55 could act on any of the chain of events that lead to the geotropic response in *Zea mays* roots, only two aspects were examined. The aspects studied involved auxin transport and the part played by the root cap in regulating the geotropic response. The ageotropic response in ACPI-55 treated roots could be explained if either system is interfered with.

During the course of this thesis work it became clear that ACPI-55 acts on root growth. Root growth was inhibited at those concentrations at which the compound abolishes the geotropic response. Moreover, it was determined by growing *Zea mays* roots in aerated water for 7 days, that lateral root formation was also inhibited at these concentrations (Appendix 7). It could be argued that phytotoxicity could cause all these effects. Herbicide tests showed that ACPI-55 at moderate to high concentrations had no apparent phytotoxicity effects on the above ground parts of maize plants (Appendix 8). In addition, histological studies showed that there were no significant differences between control and treated roots (at a concentration of 10^{-6}M) as regards the size of the root caps, central core statocytes, nuclei and nucleoli. It seems therefore that the effects observed at moderate concentrations of ACPI-55 were due to an interference with the *rate* of some endogenous process rather than the *destruction* of such a process. Strong evidence is presented in the literature that auxin is involved in root growth (by cell division and cell elongation) and secondary root formation (Scott 1972; MacLeod 1966). It is also possible that IAA is involved in root geotropism (Konings 1967). The problem is clearly to determine how ACPI-55 acts on auxin transport.

At present it is not established whether growth inhibition by ACPI-55 is caused by inhibition of cell elongation (in the extension

zone) or by a reduced rate of cell division (in the root tip meristem) or by both these processes. Changes in rates of cell division in the root tip meristem of ACPI-55 treated roots was not investigated in this study.

It has been shown by time lapse photography (Audus and Brownbridge 1957) that growth inhibition in the extension zone of control roots is part of the geotropic response. The action of ACPI-55 as an auxin transport inhibitor (Brown *et al.* 1972) could change IAA levels in the root, thereby interfering with growth and perhaps curvature development.

That IAA concentrations effect growth is an established fact (Scott 1972), but it is not yet known whether the geotropic response is also due to IAA. Two different theories which have been postulated are the following:

(i) The Cholodny-Went (Cholodny 1926; Went 1926) hypothesis proposes that the positive geotropic response is due to a differential in auxin concentration between upper and lower halves of horizontal roots, (with inhibitory concentrations for growth in the lower halves) causing curvature towards gravity.

(ii) Pilet (1975) suggested that auxin plays only a secondary role, i.e. it stimulates the action of growth inhibitors such as ABA. The accumulation of ABA in the lower half of the horizontal root would lead to curvature development towards gravity.

It has been shown by Brown *et al.* (1972) that ACPI-55 is a highly active auxin transport inhibitor and in the present study evidence was produced that the application of the compound interferes drammatically with the geotropic response in *Zea mays* roots. The characteristics of IAA movement in root tissues are less well established than in shoot tissues. Wilkins and Scott (1968) produced conclusive evidence for a

marked acropetal flux of labelled IAA through segments of *Zea mays* roots. Basipetal transport occurred to only a small degree. Shaw and Wilkins (1974) produced further evidence for a predominant acropetal flux of radioactive IAA which was more efficient in the stele than in the cortex. Basipetal movement occurred to only a slight extent and with equal efficiency in both stelar and cortical tissues. Cane and Wilkins (1970) also observed that movement of IAA was polarized acropetally and that acropetal as well as basipetal transport was more pronounced near the root apex than in the more basal regions of the root. If these studies with radioactive IAA are any indication of the movement of endogenous auxin it appears that there is a preferential movement of IAA towards the root cap. Brown *et al.* (1972) showed that ACPI-55 acts on active auxin transport in *bean petioles* (*Phaseolus vulgaris*). In following up experiments, Geissler and Katekar (1974, unpublished data - Appendix 9A), have confirmed that the active basipetal movement was inhibited by ACPI-55 and they have also found that acropetal movement (non active movement : McCready 1968) in *bean petioles* was not inhibited (Appendix 9B).

Because of the above findings it is proposed here that ACPI-55 acts on the polarized *acropetal* movement in *Zea mays roots*, which could therefore inhibit growth.

Besides acropetal and basipetal movement, IAA is also transported laterally in roots (Konings 1967).

Juniper *et al.* (1966) showed that the root cap is responsible for geoptropism. Recently IAA was detected in root caps of *Zea mays* (Rivier and Pilet 1974) and Konings (1967) showed that labelled IAA when applied to the apex of horizontal pea roots is transversely distributed. Removal of the root cap prevents this unequal distribution of labelled IAA (Konings 1967). The root cap appears therefore

to control both, the asymmetric distribution of labelled IAA and geotropism. It is known that IAA inhibits growth at high concentrations and stimulates it at low concentrations (Scott 1972). The unequal distribution of IAA between upper and lower halves of horizontal roots could result in growth inhibition in lower halves, thereby producing a downward curvature of the root. Interference with the lateral distribution of IAA, by ACPI-55, could account for the ageotropic response in *Zea mays* roots. Future studies on auxin transport (acropetal, basipetal as well as lateral transport) inhibition by ACPI-55 in *Zea mays roots* could shed some light on IAA movement in the root and its role, if any, in the geotropic response.

As well as these documented systems which are thought to determine curvature, an additional observation was made in this study. The direction of curvature development was dependent on some "intrinsic factor" within the root or seed in both control and treated roots. Curvature was nearly always towards the embryo side of the seed rather than towards the endosperm side (Appendices 1, 2, 3). The difference between control and treated roots was that the gravitational force could "override" this "intrinsic factor" in control roots. This was regardless of the orientation of the roots. Curvature development preferentially in the direction of the embryo side of the seed could be explained in terms of a differential sensitivity to IAA between the two halves of the root or there could be a higher level of enzymatic destruction of IAA on one side. That ACPI-55 itself would stimulate growth preferentially in one side of the root, thus influencing curvature development, is unlikely. The use of the pea stem section method and the split pea stem method to detect auxin type growth activity (see Agricultural Handbook No.336, 1968, United States Department of Agriculture) did not reveal any growth promoting activity of ACPI-55 (Appendices 10A and 10B). Alternatively, there could be a preferential

flow of IAA along one side of the root, thus producing curvature in the direction of the embryo. In horizontally placed control roots, active lateral transport of IAA could accumulate the auxin on the lower side of the root, thus producing geotropic response. In horizontally placed treated roots however, this lateral transport would be inhibited and IAA would therefore accumulate on the "embryo side" of the root, thus producing curvature in this direction. Future studies with labelled IAA and the use of mica barriers as used by Pilet (1975) in his studies on ABA movement, could shed some light on this hypothesis.

Some evidence has been put forward in the literature that there are other growth inhibitors present in the root cap besides IAA. Wilkins and Wain (1974) have shown that abscisic acid and other unidentified growth inhibitors were present in the root caps of light grown roots, but they were not detected in dark grown roots. They found that light induced inhibition of the elongation of primary roots of *Zea mays* seedlings is dependent upon perception of light by the root cap. Because ACPI-55 inhibited root growth in the dark as well as in the light, it seems that the compound does not act on the above mentioned root cap inhibitors. Furthermore, it was found that the effect of growth inhibition by ACPI-55 and by the light induced root cap inhibitors was additive rather than synergistic. This finding could be supporting evidence for the suggestion that ACPI-55 acts independently of the root cap inhibitors mentioned by Wilkins and Wain (1974).

It has been suggested that the movement of amyloplasts in the statocytes of the root cap initiates lateral transport of auxin (see Review by Wilkins 1969). The effect of ACPI-55 on the root cap was therefore analysed. The detailed histological study showed that ACPI-55 had no effect on the size, number and movement of amyloplasts.

If the amyloplasts had been absent or if the movement of amyloplasts had been blocked, this could have explained the ageotropic response of ACPI-55 treated roots. It would also have been indirect evidence in support of the stalolith theory as proposed by Haberlandt (1900) and Nemec (1900).

The above findings strongly suggest that the ageotropic response in ACPI-55 treated roots was not due to interference of the compound with the perception mechanism or that it was due to any apparent phytotoxicity. It is proposed that root growth inhibition is caused by inhibition of acropetal (active) transport of IAA in the root proper. Curvature development in horizontally placed treated roots could have been due to inhibition of lateral transport of IAA either in the root cap or in the root proper. It is further suggested that curvature development in both control and treated roots is linked to an intrinsic factor which influences curvature in a particular direction.

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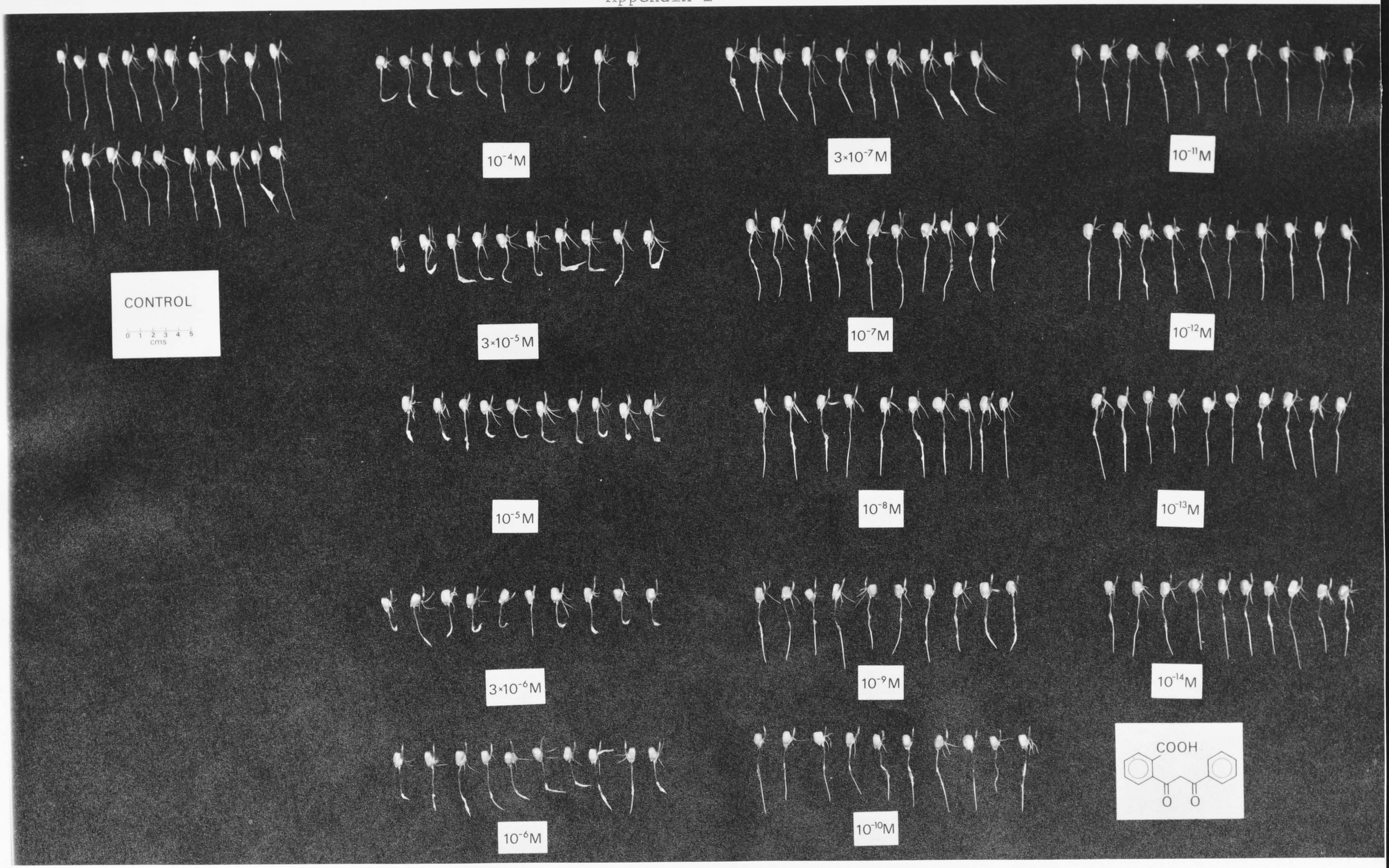
Appendix 1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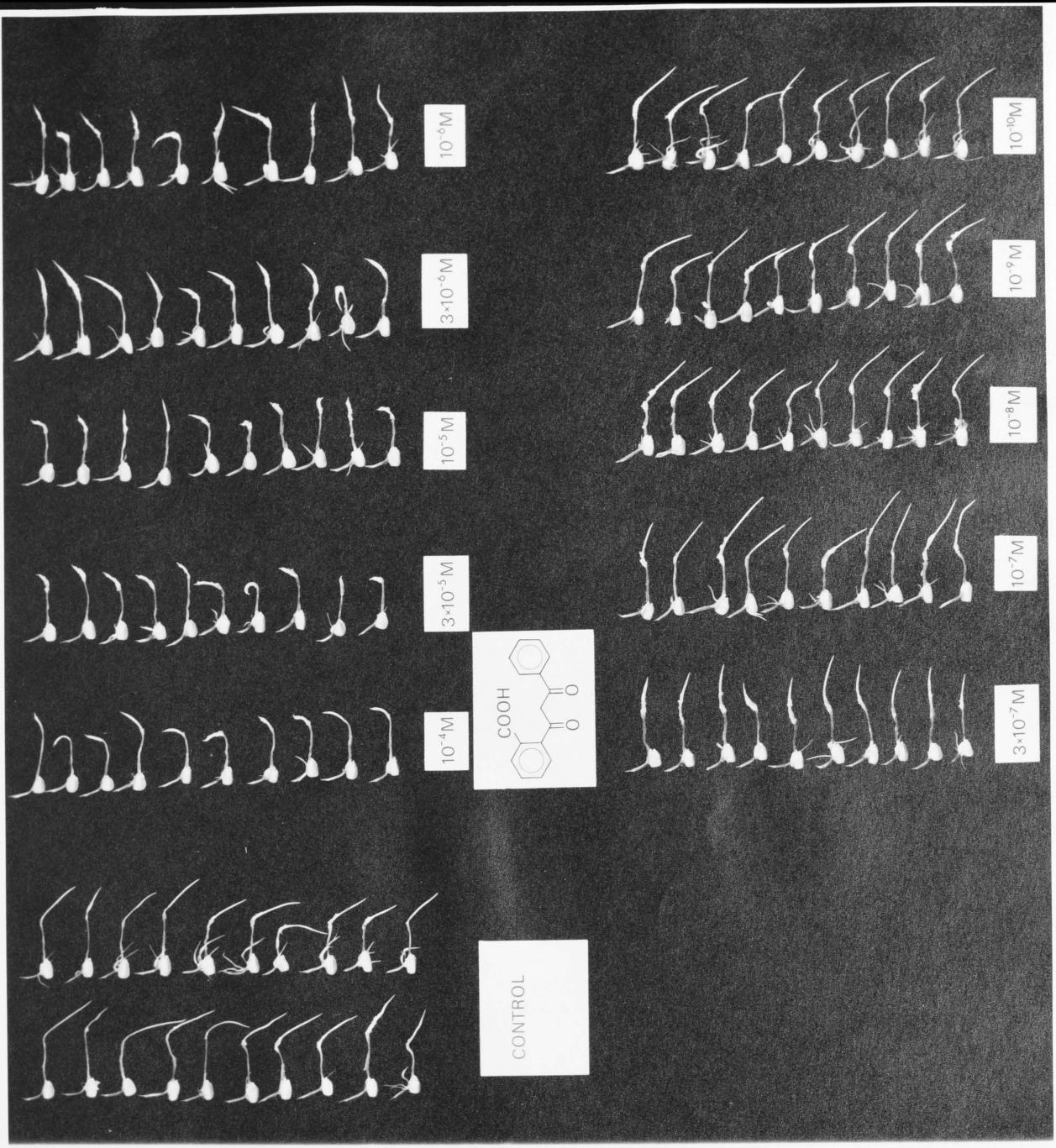
O O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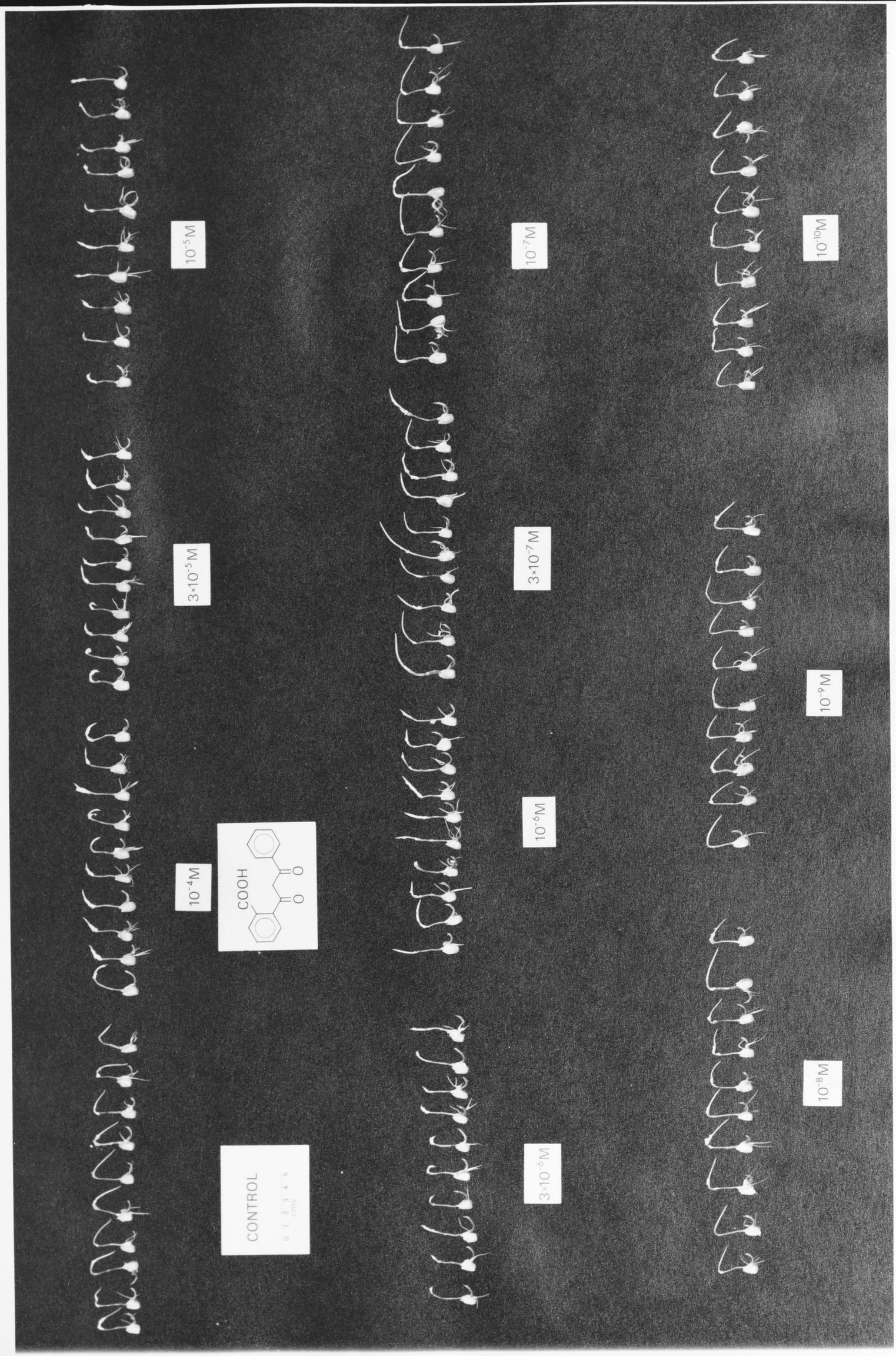
Appendix 2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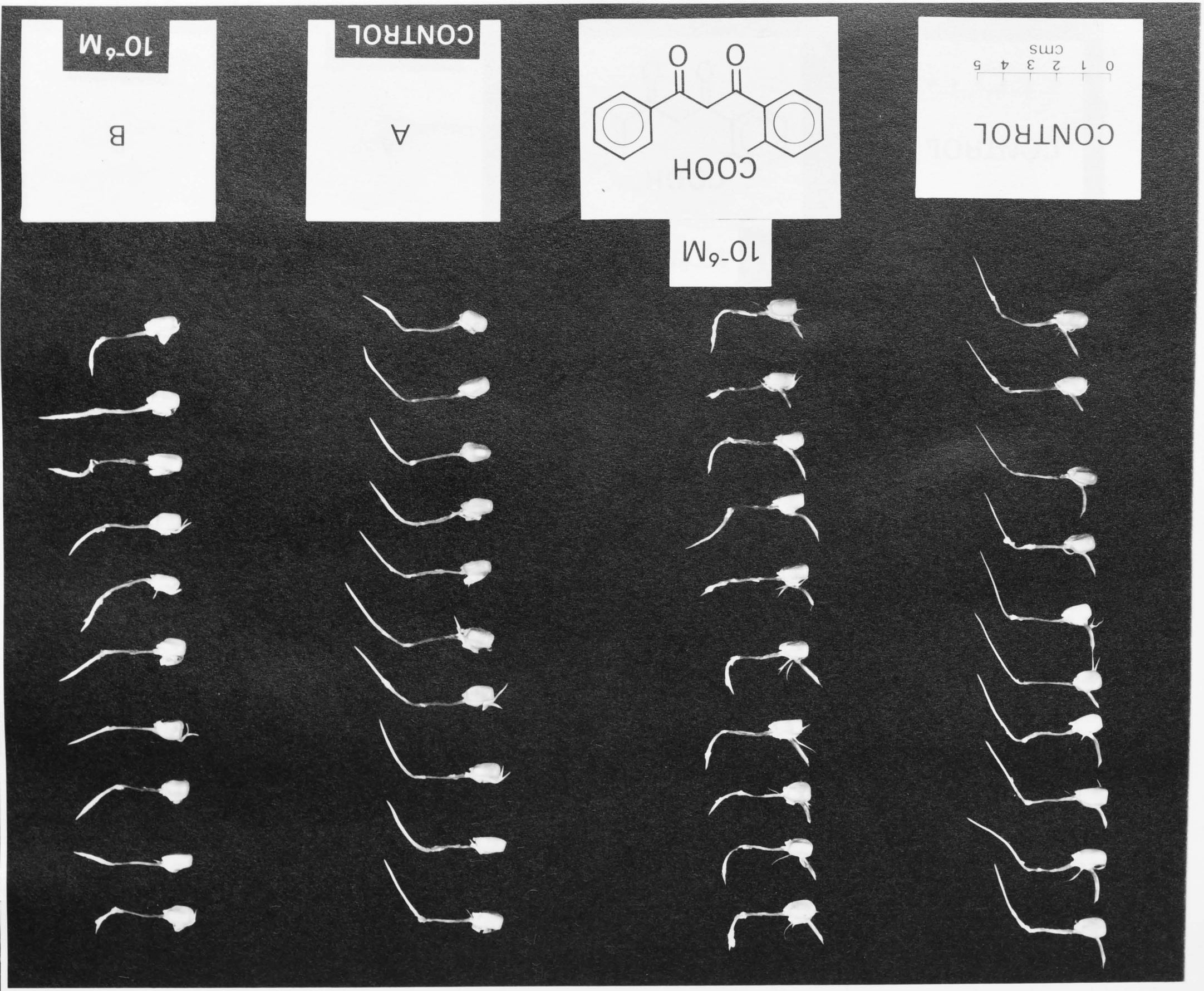
Appendix 3



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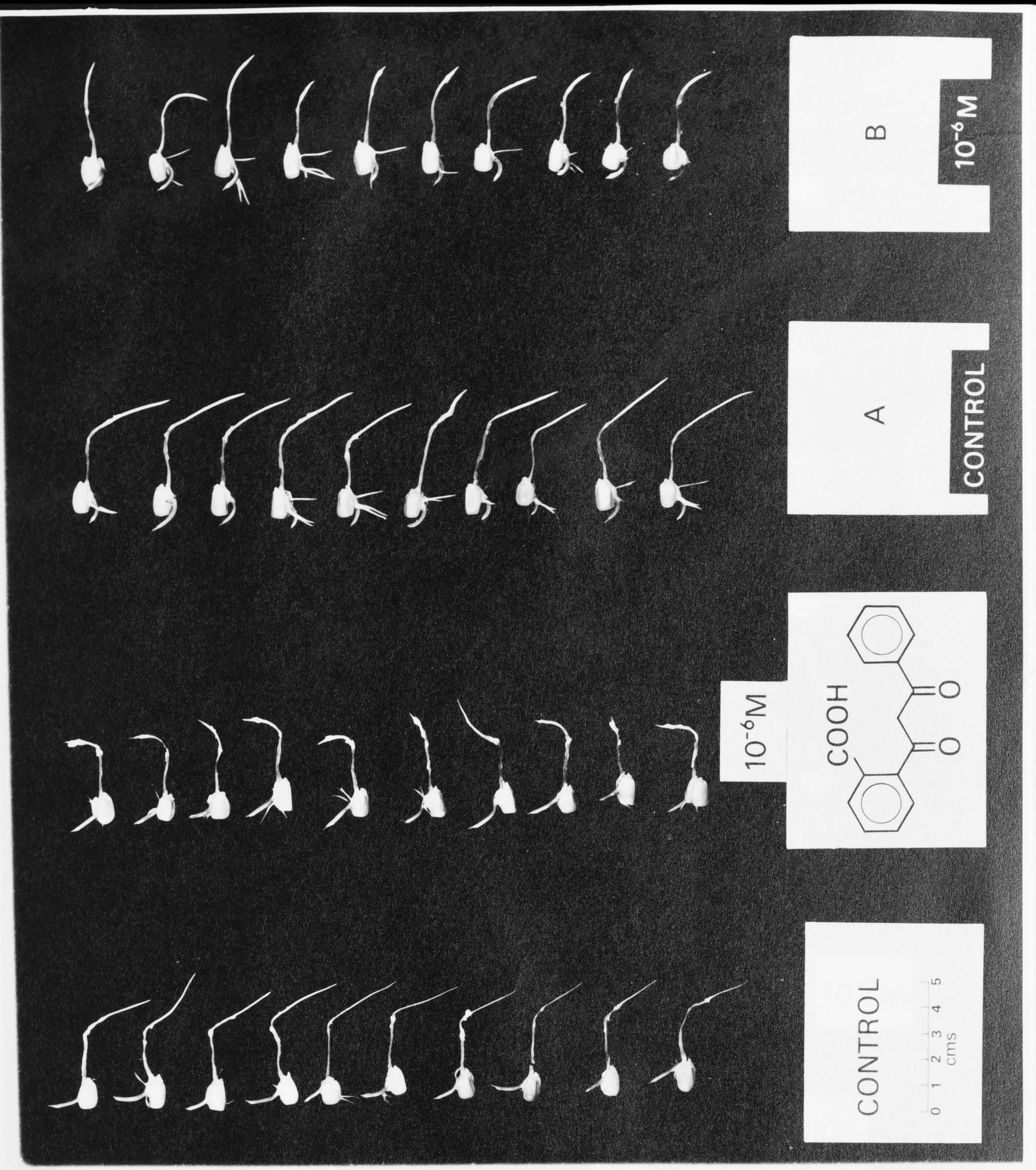

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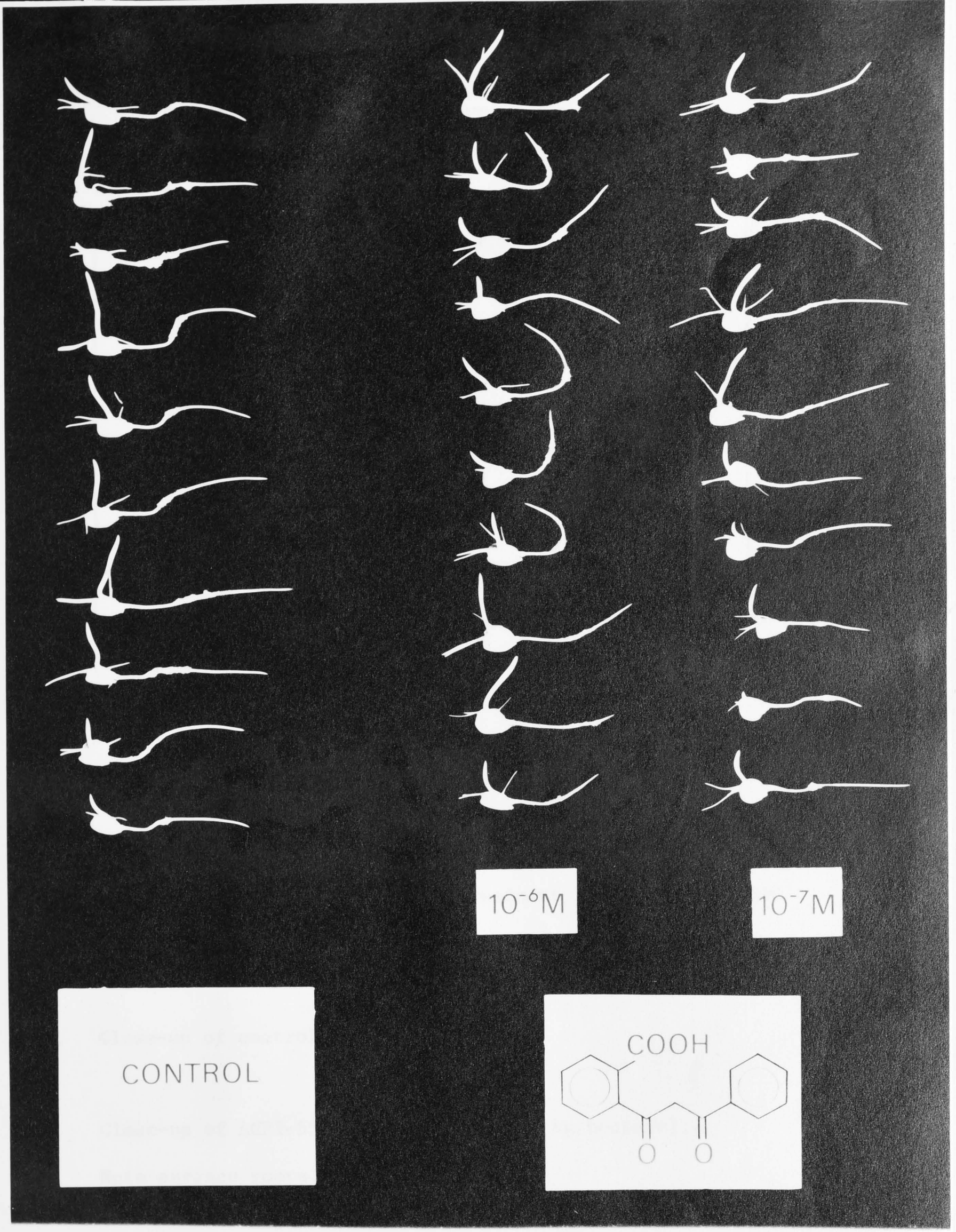


Appendix 6

Appendix 7 - *ZEA MAYS*. SECONDARY ROOT FORMATION IN CONTROL AND ACPI-55 TREATED ROOTS GROWN IN AERATED WATER FOR 7 DAYS. TREATMENT: ACPI-55

Treatment	$\bar{x}$ number of secondary roots
Control	37.3
10^{-8}M	41.6
10^{-7}M	24.6
10^{-6}M	14.2
10^{-5}M	3.0
10^{-4}M	0.0
10^{-3}M	0.0

N = 10

Appendix 8 - PRE-EMERGENCE HERBICIDE TESTS OF ACPI-55 ON *ZEA MAYS* VAR. PX-52

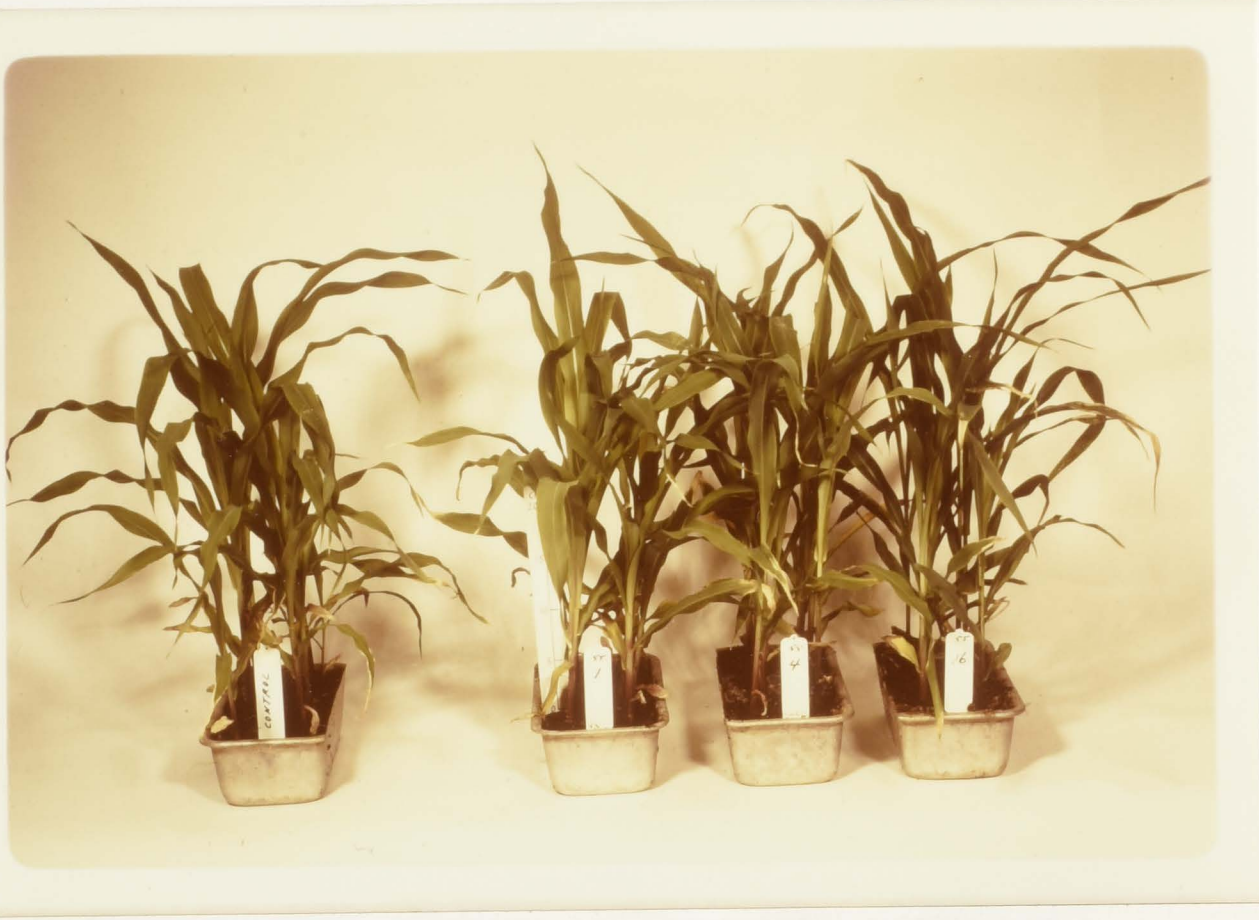
A. Three weeks old maize plants. Treatment 1, 4, 16 kilograms per hectare.

B. Close-up of control plants.

C. Close-up of ACPI-55 treated plants (16 kg/hectare). Note surface roots.

Photographs (see following page)

A



B

C

Appendix 9A - PERCENTAGE RADIOACTIVITY TRANSPORTED BASIPETALLY THROUGH *PHASEOLUS VULGARIS* PETIOLES (PERCENTAGE OF CONTROL)

Compound	Molar concentration in receiver block					
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
ACPI-55[a]	2	4	20	32	52	62
ACPI-55[b]	6	3	15	59	84	82
TIBA[a]	5	5	30	70	100	96
NPA[a]	<5	<5	< 5	30	75	82

[a]Results presented by Brown *et al.* (1972)

[b]Results Geissler and Katekar (1974)

N = 60

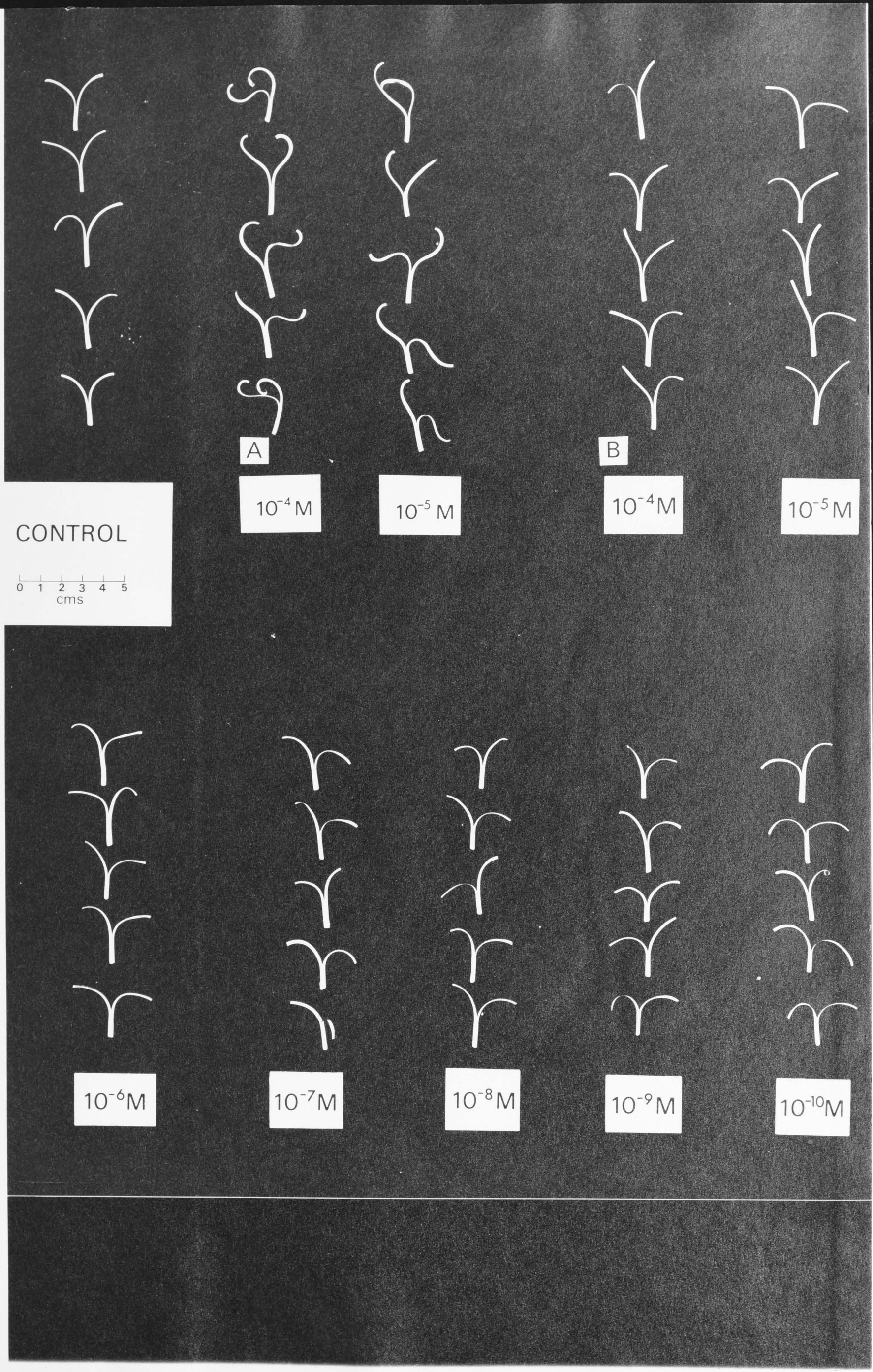
Appendix 9B - PERCENTAGE RADIOACTIVITY TRANSPORTED BASIPETALLY AND ACROPETALLY THROUGH *PHASEOLUS VULGARIS* PETIOLES. TREATMENT: 10^{-5}M ACPI-55. AS A PERCENTAGE OF BASIPETAL TRANSPORT (=100)

	Transport	IAA-2-C^{14} transported
Control	Basipetal	100
	Acropetal	5
ACPI-55	Basipetal	7
	Acropetal	7

Appendix 10A - PEA STEM SECTION METHOD FOR DETECTING AUXIN TYPE ACTIVITY. INCREASE IN GROWTH : PERCENTAGE OF WATER CONTROL (= 100)

Treatment	Concentration	Percentage
ACPI-55	10^{-4}M	100
	10^{-5}M	102
	10^{-6}M	103
	10^{-7}M	99
	10^{-8}M	100
	10^{-9}M	99
	10^{-10}M	101
IAA	10^{-4}M	134
	10^{-5}M	129

N = 12



Appendix 10B - Split pea stem section method for detecting auxin type activity. Curvature development outwards : no auxin activity; curvature development inwards : auxin activity. (A) IAA; (B) ACPI-55