SOME CHARACTERISTICS OF A GROWTH INHIBITING MATERIAL IN THE ALCOHOL-SOLUBLE-EXTRACT FROM AVOCADO SEED COATS

By

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1959

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1961

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ACKNOWLEDGEMENTS

During the course of this study the writer has had much help and guidance from several members of the faculty of Oklahoma State University. He wishes to express grateful appreciation to his major professor, Dr. Samuel C. Wiggans, for his patient assistance and suggestions, not only during the course of the investigation and preparation of this manuscript but also throughout his academic career. Sincere thanks also is due to Dr. David G. White for his guidance and counsel. Indebtedness is acknowledged to Professor George V. Odell for his assistance and help in making certain of the chemical analyses.

Acknowledgement is made to Dr. Walter Reuther of the Riverside Experiment Station, University of California, who provided some of the avocados used in this study.

Dedicated

to the memory of

my father, Pastor P. C. Kochu Koshy

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Concluded)

CHAPTER I

INTRODUCTION

The concept of chemical regulation of plant growth was first suggested by Julius Sachs nearly one hundred years ago. It was 1926, however, before definite proof of the hormonal action in plants took place, when Went (40), working with oat seedlings, demonstrated the presence of a growth substance in the coleoptile which he called auxin. Since that time many workers have reported the presence of different growth factors which regulate the physiological processes in plants. These growth factors may either stimulate or inhibit plant growth and may be found to occur naturally in all parts of the plant.

The ontogeny of the plant is not a continuous process of growth. Interposed with periods of rapid cell division and enlargement are periods when plant growth may be partially or completely inhibited. Both the rapid growth and inhibitory growth periods can be regulated by chemical growth factors present within the plant or which may be applied externally. Certain of these growth factors have been shown to regulate growth in many species of plants, including germination, seedling growth, flower production, fruit set, blossom drop, etc.

The present study was concerned with a naturally occurring plant growth inhibiting substance or substances present in the avocado seed coat. It has been reported (4) that avocado seeds will germinate more rapidly if the seedcoat is removed before placing the seed in the

germination media. Several different assay methods to determine and demonstrate the presence and relative activity of this inhibitory substance(s) were tested. These included a seedling growth test, a split pea bioassay, lettuce and tomato seed germination tests, and certain chromatographic separations.

CHAPTER II

LITERATURE REVIEW

Growth substances have been defined as "organic compounds which, at low concentrations, promote, inhibit, or qualitatively modify growth" (30). They occur widely in nature and have been found to play important roles in regulating the initiation and growth of various plant parts. Plant growth substances have been classified as auxins, anti-auxins, or growth inhibitors, according to their biological effect on plant growth.

The plant growth substances which retard growth were called "negative catalysts" or "antienzymes" by Czapek, a Czech botanist. Since that time investigators working with these materials have identified a number of the naturally occurring inhibitors in plant tissue. Stewart (33) isolated an inhibitor from the leaves and cotyledons of radish (Raphanus sativus) which released auxin(s) on hydrolysis with alkalies. Funke and Soding (12) found a similar inhibitor in the seedlings of corn (Zea mays) and oats (Avena sativa) and in potato tubers (Solanum tuberosum). Linser (23) using column chromatographic techniques, isolated two distinct auxins, one of which was 3-indoleacetic acid, plus a growth inhibitor from the leaves of brussels sprouts (Brassica oleracea gemmifera). He also found two distinct growth inhibiting components in the leaves of elder (Sambucus nigra).

Growth inhibitors are known to play important roles in seed germination. Kockemann (14) called germination inhibitors "blastocholines." He

showed that very low concentrations of these materials were capable of inhibiting seed germination and that enough of these materials are present in the seed of most species to prevent germination while the seed is still associated with the mother plant. Moewus and Schader (27) found blastocholines which inhibited seed germination in the fruit of 33 genera. These included apple (Pyrus malus), pear (Pyrus communis), oranges (Citrus sinensis), tomato (Lycopersicon esculentum), fig (Ficus carica), cabbage (Brassica cleracea capitata), clover (Trifolium sp), lettuce (Lactuca sp), coffee (Coffea arabica), etc.

Evenari (8) said that plant growth inhibitors "are equally as important in the ontogeny of plants as are growth hormones and other stimulating substances." He showed that certain growth inhibiting substances, when excreted, may inhibit the development of other species. He also stated that "all physiological reactions are governed by a set of 'positive' as well as 'negative' catalysts whose function it is to delay or inhibit reactions."

Cox, et al. (5), using embryo culture techniques found that the water soluble fraction of an alcoholic extract of seedcoats of dormant cabbage (Brassica oleracea capitata) contained a germination inhibitor. They also showed that soaking dry seeds in concentrated sulfuric acid for one hour and then washing them thoroughly in tap water for several hours permitted satisfactory seed germination.

Hendershott and Walker (13) working with peach (Prunus Persica) flower buds, found an inhibitor which they called "naringenin." This material was present in high concentrations in August, but by October it had decreased markedly in content. In December and January it again was found in large amounts. However, about two weeks before bloom, and

coinciding with a greatly increased growth of buds and twigs, it had disappeared entirely.

Randolph and Cox (31) found an inhibiting substance present in the endosperm of mature iris (Iris sp) seeds which prevented growth of the embryo while in contact with the endosperm. They suggested that in general, inhibiting substances are highly stable compounds, relatively insoluble in water, and readily diffusible away from the region of the embryo.

Most inhibitors are non-specific in their activity. For example, Froeschel (9, 10, 11) reported that inhibitors present in red clover (Trifolium pratense) and beets (Beta vulgaris) inhibited the germination of seeds of some 28 species. Other workers (8, 16, 17) found that the inhibitor present in tomato juice can effectively inhibit the germination of tomato (Lycopersicon esculentum), wheat (Triticum vulgare), oats (Avena sativa), corn (Zea mays), etc. They also showed that the sensitivity of seed to different concentrations of the inhibitor(s) varies with different species. It has been reported (7, 20, 23, 24) that the inhibiting effect of tomato juice was due solely to its osmotic pressure. Oppenheimer and Evenari (29), however, showed that there was little or no relationship between the inhibitory effect of tomato juice and its osmotic pressure. They showed that extremely low concentrations of tomato juice effectively inhibited the germination of many seeds.

Several workers (18, 34, 36, 37), reported that pH has little or no effect on the action of plant growth inhibitors on seed germination.

Recent studies have indicated a clear relationship between plant growth inhibitors and auxins, both in chemical structure and in their physiological effect on plants. It has been suggested by Tegethoff (35)

that some plant growth inhibitors may be the result of a combination of an auxin with another inhibitor. Funke and Soding (12) and Larsen (20), indicated that these auxin inhibitor complexes have many of the characteristics of auxin precursors and are resistant to both alkali and hydrogen peroxide.

The majority of known plant growth inhibitors are simple organic compounds and include some of the unsaturated lactones. Coumarin and transcinnamic acid, both widely distributed in plants, are highly effective as germination inhibitors (2). It has been suggested that transcinnamic acid acts as a "blastocholine" in the green algae, Chlamydomonas (28).

Amygdalin and other cyanophoric glucosides are found in many seeds, especially those of plants belonging to the Prunaceae and Pomaceae families. Laibach and Keil (19) suggested that the hydrolysis of amygdalin to benzaldehyde, hydrogen cyanide, and beta glucose is one of the most important processes in the activity of natural inhibitors. Sigmund (32) showed that hydrogen cyanide (HCN) is set free from amygdalin and that the CN group has an inhibiting effect on the growth and germination. Barton (3) reported that seed germination is very much delayed or completely inhibited as long as it is kept in an atmosphere containing HCN. She found that seeds were able to retain their germinating capacity, even after six days in HCN, thus enabling them to germinate when they were again placed in an atmosphere free of HCN. Ullman (38) suggested that HCN in seeds acts as an inhibitor to the germination of other seeds. He showed that almond seeds, containing 1 to 3 percent amygdalin, when kept in contact with wheat completely inhibited its germination.

Stout and Tolman (28) reported the enzymatic release of ammonia

from nitrogenous compounds in plants sometimes caused inhibitory effects. The liberation of ethylene from the fruit of apples and pears and from other plant parts also has been shown to exhibit an inhibitory effect on plant growth (6, 14).

Growth inhibiting extracts have been isolated from oils found in many plants of the mustard family (8). These oils, containing mostly allyl-isothyocyanate and betaphenethyl-isothyocyanate, are present in almost all plant parts of the Cruciferae. They are particularly abundant in the Brassica and Sinapis (38).

Aldehydes sometimes act as plant growth inhibitors (8). Benzaldehyde formed during the hydrolysis of amygdalin is a strong inhibitor. Acetaldehyde is an inhibiting substance found in immature pea seeds. Salicylaldehyde, a very strong inhibitor, is found in lemon grass oil.

Germination inhibitors of many plants in the Umbelliferae belong to a class of compounds called phthalides. Moewus and Schader (26) found that the most active of these compounds they isolated was n-butylidene-hexahydro-phthalide.

Essential oils also may be effective as germination inhibitors. They have been isolated from orange and lemon skins, clove flowers, peppermint, rosemary and eucalyptus leaves, fennel fruits etc. (2).

Weintraub and Price (39) reported that certain resinous emanations from wood may inhibit seed germination.

Organic acids also may act as growth inhibiting agents. Malic and citric acids in apple, and caffeic and ferulic acids in tomato, have been found to exhibit inhibiting effects (2).

In general, the biological functions of the plant growth inhibitors thus far reported seems to be to prevent premature germination, to

extend the germination period, and to suppress the germination and growth of other species. No literature was found in which an extract from the seed coat had been applied to a seedling of the same or different species.

CHAPTER III

MATERIALS AND METHODS

In the investigations reported herein a naturally occurring plant growth inhibitor(s) found in the seedcoats of avocado (Persea gratissima), was studied. Avocado fruits, of the Hass variety, were procured from two sources. The first group, obtained directly from California,[1] arrived about the middle of May. They were placed in a 40° F. cold storage until they could be processed since they were quite soft upon arrival. The second group, also from California, were purchased in early October at the Stillwater Fruit Market. All the avocados in this group were hard and, therefore, were left on the laboratory bench for several days to soften before being used.

Each large single seed was removed easily from the soft mushy flesh with a knife. It was then washed thoroughly under tap water and the remaining bits of flesh were picked off by hand. Two hundred seedcoats were ruptured, peeled away from the seed, and placed in erlenmeyer flasks containing a total of 800 cc. of a 95% ethyl alcohol extracting solution. The flasks were placed in a 40° F. refrigerator for seven days at which time the seedcoats were removed from the extracting solution and macerated with fine sand in a mortar. They were then replaced in the extracting solution in the refrigerator for two more days. The extract

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[1] Supplied through the courtesy of Dr. Walter Reuther, Head of the Department of Horticulture, University of California, Riverside.

was centrifuged and the clear supernatent poured off into shallow dishes. The supernatent was allowed to evaporate to dryness at room temperature (70° F.). The yellow residue was then redissolved in a total quantity of 125 ml of distilled water. The dissolved extract was diluted 1:10 and 1:100 with distilled water for assay of its activity in comparison with 3-indoleacetic acid and adenine sulfate each of which have often been used as standards to determine the biological activity of various growth materials.

Four assay procedures were used: (A) Avocado seedling growth, (B) Slit pea bioassay, (C) Seed germination, and (D) Chromatographic separation.

A. Avocado seedling growth:

Avocado seeds, from which the seedcoats had been removed, were planted in the Oklahoma State University greenhouse on June 1, 1960. They were planted in 9 inch pots containing a mixture of 1 part sand, 1 part peat moss, 1 part manure, and 1 part soil. The pots were placed on a greenhouse groundbed maintained at a minimum temperature of 65° F. at night and 70° F. during the day. When the seedlings were approximately seven weeks old (10 weeks after planting) 72 uniform plants were selected for treatment and moved to a bench in a second greenhouse which was maintained at a minimum temperature of 50° F. at night and 60° F. during the day.

All seedling plants were given one of the following nine treatments: (a) check (water control), (b) avocado seedcoat extract (undiluted), (c) avocado seedcoat extract, diluted 1:10, (d) avocado seedcoat extract, diluted 1:100, (e) 10 ppm 3-indoleacetic acid, (f) 100 ppm 3-indoleacetic acid, (g) 1000 ppm 3-indoleacetic acid, (h) 10 ppm adenine sulfate, and

(i) 100 ppm adenine sulfate. The treatments were applied to the plants by one of two methods: (a) From 0.3 to 0.5 ml of these materials were injected with a hypodermic syringe into the stem about 1.5 to 2.0 cm below the growing tip; (b) A small cotton swab, wetted by dipping it into one of the previously mentioned materials, was securely wrapped around the stem just below the growing tip. The same treatments were applied to each seedling twice, the first time 10 weeks after planting (the same day in which the plants were moved to the 60° F. day temperature room), and the second time 60 days later. The experimental design was a randomized block with 4 replications.

The following measurements were made at varying times following treatments, length of stem from pot level to growing tip, number of leaves 1/2 inch or more in width, and diameter of the stem at pot level.

B. Slit pea bioassay

The standard slit pea stem bioassay (22, 41) was used to determine the relative activity of the alcohol soluble extract from avocado seedcoats, in comparison with that of 3-indoleacetic acid and adenine sulfate.

Uniform Alaska pea seeds were germinated in darkness in moist sand flats at 70° F. On the eighth day the seedlings were illuminated with a red light for four hours. On the ninth day, when the stems above the first leaf node were from 1/4 to 1/2 inch long they were decapitated. A longitudinal cut of about 3 cm. was made with a sharp blade splitting the first internodal section of the stem into equal halves. The stems were then cut off about 1/2 inch below the slit end and the slit sections placed in distilled water for exactly one hour before being transferred

to petri-dishes containing one of the nine solutions described in the avocado seedling growth test. After 24 hours they were removed, blue printed, and the degrees of curvatures were measured.

C. Seed germination tests:

Tomato and lettuce seeds were germinated in petri-dishes on filter paper moistened with one of the nine solutions described in the avocado seedling growth test. The germination temperature was approximately 70° F. The percentage germination was recorded each day for each treatment, for 10 days.

D. Chromatographic separation:

A paper chromatographic separation of the avocado seedcoat extract was attempted. The extract was first concentrated in a rotary evaporator, then suspended in methanol and placed on the chromatographic paper for separation. A solvent system of normal butanol, ammonia, and water in the ratio 10:1:1 (which is used in the separation of indoleacetic acid, indolepropionic acid, indolebutyric acid, and certain amino acids) was utilized in this separation. A color test with 4-dimethylaminocinnamaldehyde was used for the detection of certain compounds present in the extract.

CHAPTER IV

EXPERIMENTAL RESULTS

A. Avocado seedling growth:

The effect of the three solutions, avocado seedcoat extract (ASCE), 3-indoleacetic acid (IAA), and adenine sulfate (AS), on avocado seedling growth varied with the concentration used and the method of application. In Figure 1 are shown the effects of ASCE, IAA, and AS, either injected into the stem just below the growing point or applied to the growing point with cotton swabs dipped in water solutions, on the increase in stem length of seven-week old avocado seedlings 60 days and 92 days after the start of treatment. There was little difference between the two methods of application. In some treatments there was a slightly greater stem length increase when the solution was injected into the plant with a hypodermic syringe while in others there was a greater stem length increase when the solution was applied with a cotton swab. The full strength ASCE markedly inhibited stem elongation. However, diluting the ASCE to 1:10 and 1:100 with water lessened the inhibitory effect. Concentrations of 10 and 100 ppm IAA slightly increased stem elongation over that of the control, while 1000 ppm IAA resulted in a marked inhibition of growth. The 1000 ppm IAA treatment also caused considerable injury to the plant, as indicated by the production of weak stems and small twisted leaves (Figure 2). In one plant the injury was so severe that the terminal bud was destroyed, causing the production of numerous weak secondary branches. Both of the AS treatments, 10 and 100 ppm, markedly

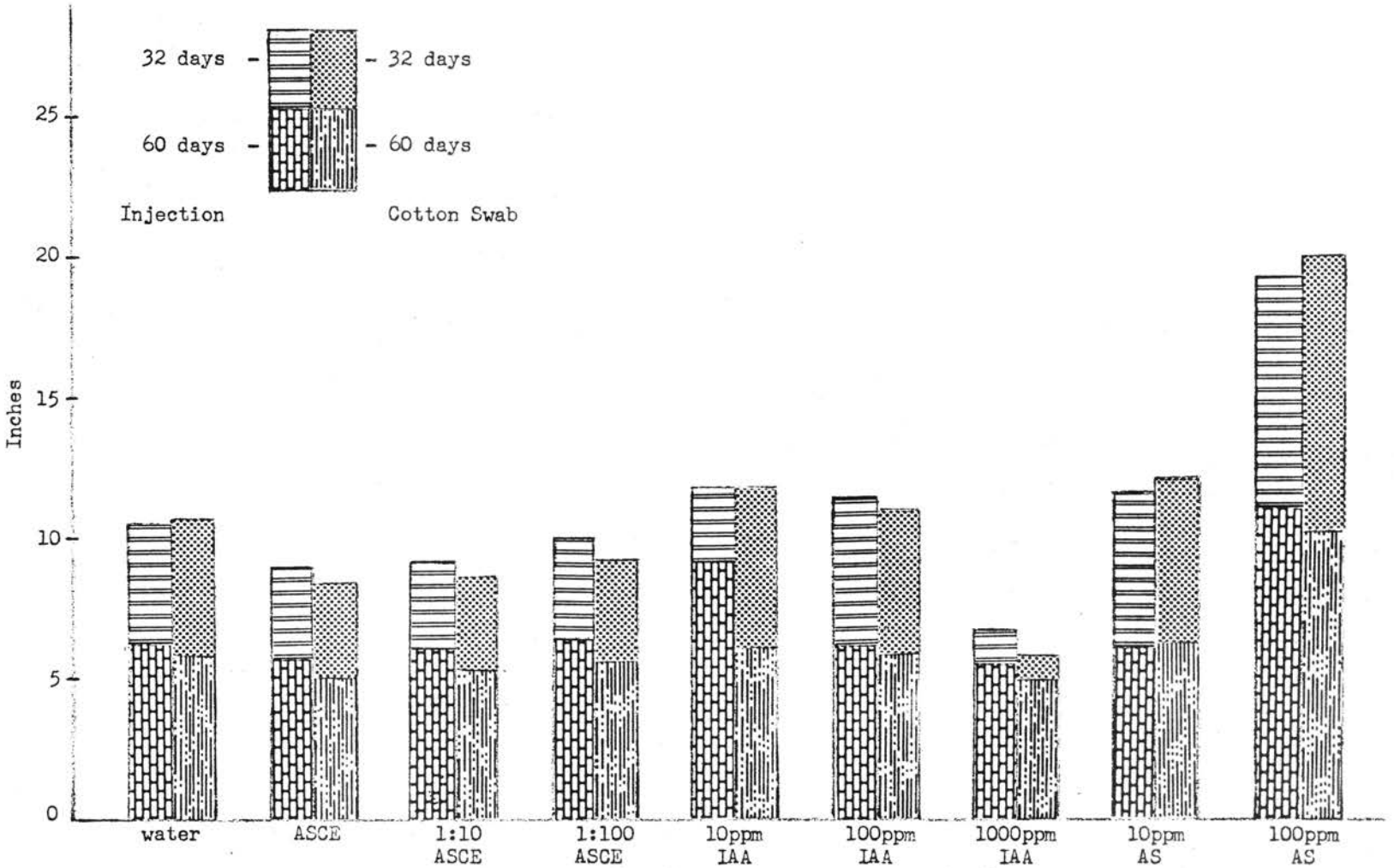

Figure 1. Effect of avocado seedcoat extract (ASCE), 3-indoleacetic acid (IAA), and adenine sulfate (AS) on stem elongation of 7-week old avocado seedlings. The solutions were injected into the stems or applied with cotton swabs to the growing point. Two treatments were applied to each plant, one at 0 days and the second 60 days later. Each bar represents an average of four plants.

Figure 2. Avocado seedling plants treated twice with 3-indoleacetic acid, applied with cotton swabs, once at the 7-week stage and again 60 days later. Picture taken 92 days after first treatment.

increased the stem elongation over that of the control. The growth rate of plants treated with 100 ppm AS was nearly double that of the control.

Since the overall growth with the injection and cotton swab methods of application was so similar (Figure 1) for each treatment they were combined in the growth curves shown in Figures 3, 5, and 6.

The growth rate of ASCE treated plants are shown in Figure 3. In comparison with the control plants, which maintained a uniform rate of growth, the ASCE treatments caused a slight slow down in the growth rate. This was most pronounced in the full strength and the 1:10 dilution of ASCE. About three weeks following treatment a more or less constant rate of seedling growth was resumed, which paralleled that of the control. When the plants were treated again after 60 days, a marked decrease in the growth rate took place. After about three weeks the constant rate of growth again was resumed. ASCE treated plants are shown in Figure 4.

Figure 5 represents the growth rates of plants treated with varying concentrations of IAA. Both 10 and 100 ppm IAA caused an overall growth rate which exceeded that of the control. When the IAA concentration was raised to 1000 ppm, however, plant growth was severely retarded.

Both the 10, and 100 ppm AS treatments were effective in promoting more rapid stem elongation as shown in Figure 6. The 10 ppm AS treatment resulted in a slightly increased rate of stem elongation for about a month following treatment. Then there was a slight decrease in growth paralleling that of the control. After the second treatment the growth rate was increased markedly especially with the 100 ppm AS, which produced almost double the growth of that of the control after 92 days. Avocado seedlings treated with AS are shown in Figure 7.

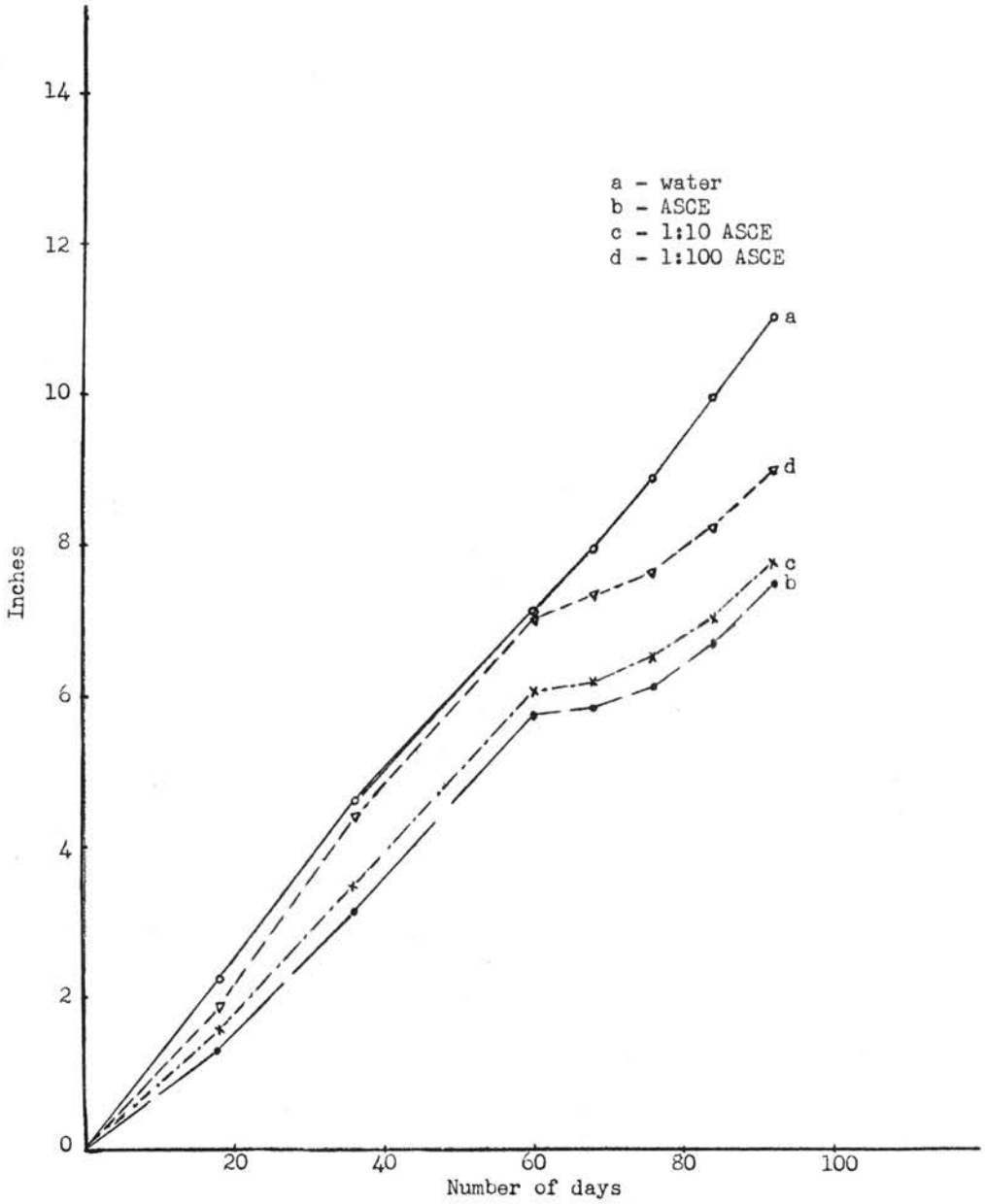

Figure 3. The growth rate of 7-week old avocado seedlings treated with avccado seedcoat extract (ASCE) at 0 days and 60 days. Each curve represents an average of 8 plants.

Figure 4. Avocado seedling plants treated twice with avocado seedcoat extract applied with cotton swabs, once at the 7-week stage and again 60 days later. Picture taken 92 days after first treatment.

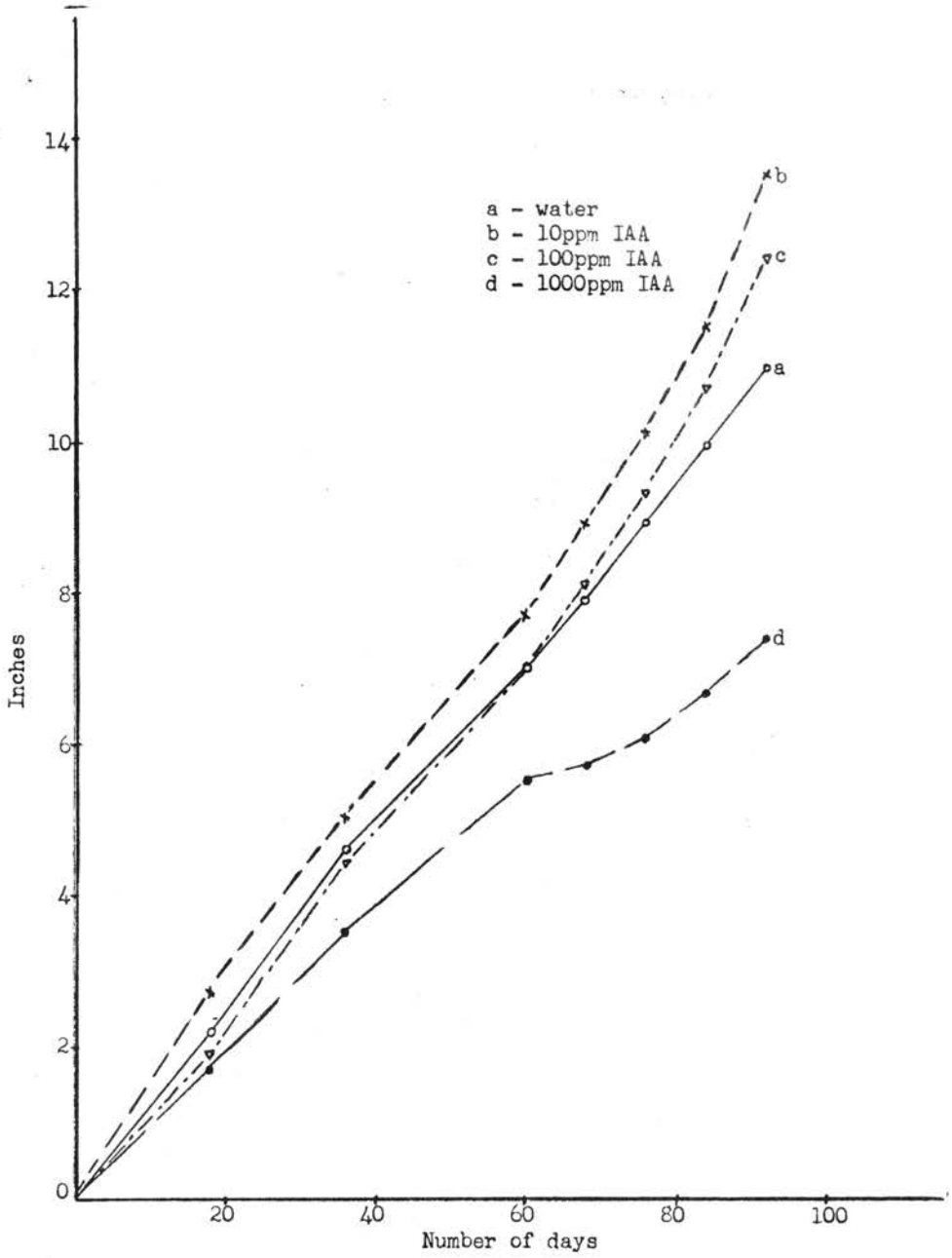

Figure 5. The growth rate of 7-week old avocado seedlings treated with 3-indoleacetic acid (IAA) at 0 days and 60 days. Each curve represents an average of 8 plants.

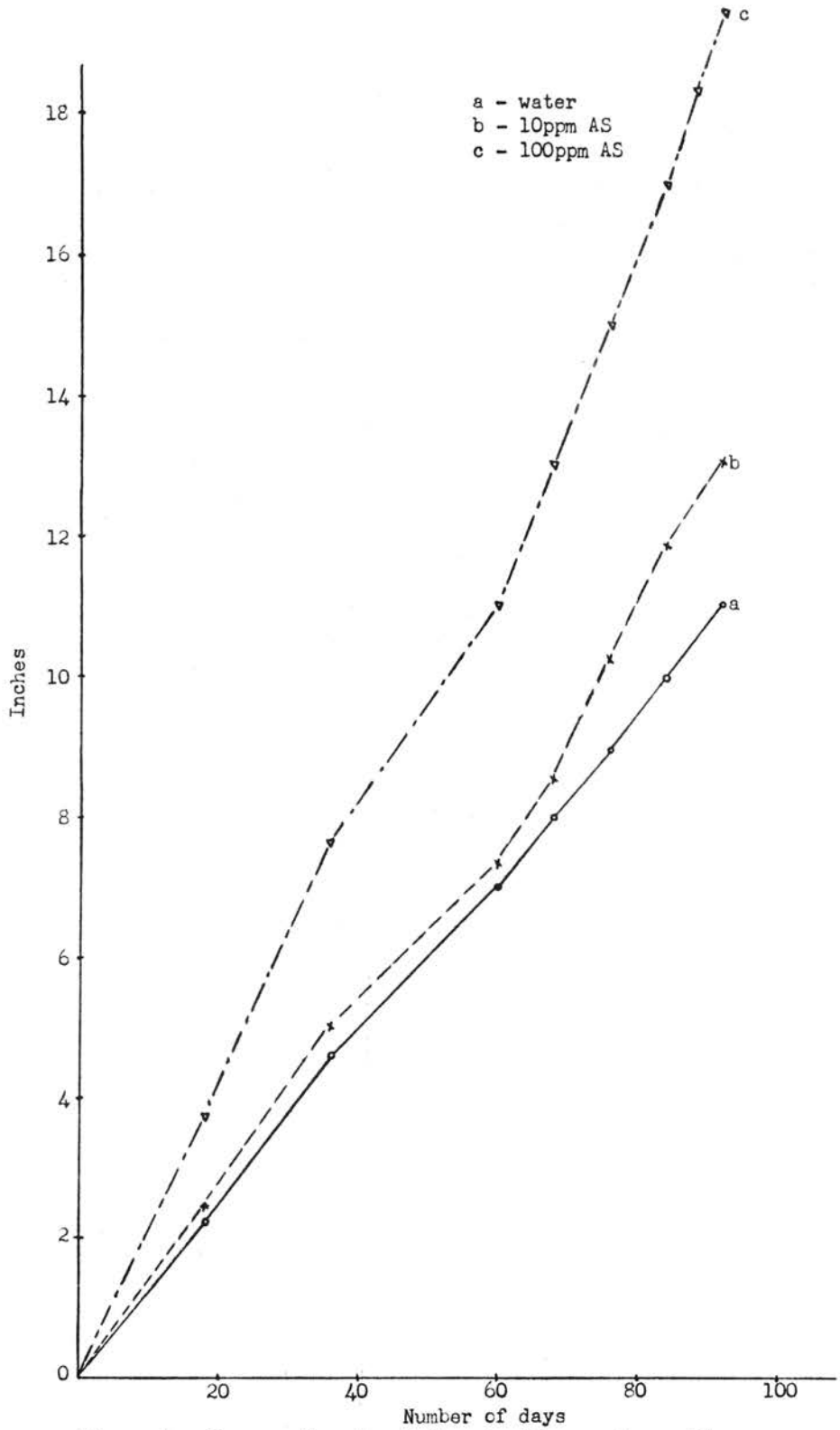

Figure 6. The growth rate of 7-week old avocado seedlings treated with adenine sulfate (AS) at 0 days and 60 days. Each curve represents an average of 8 plants.

Figure 7. Avocado seedling plants treated twice with adenine sulfate, applied with cotton swabs, once at the 7-week stage and again 60 days later. Picture taken 92 days after first treatment.

The effect of ASCE, IAA, and AS on the increase in leaf number of seven week old avocado seedling after 60 and 92 days is shown in Figure 8. In general, there tended to be a slightly larger number of leaves produced with the cotton swab method of application. The ASCE treatments resulted in only a slight inhibitory effect on leaf production. The leaves of the ASCE treated plants were green and appeared to be as healthy as those of the control (Figure 4). The 10 ppm IAA treated plants produced an average of three more leaves per plant than did the control. Plants treated with 1000 ppm IAA, particularly those in which IAA was injected into the plant, were markedly reduced in leaf number, and showed severe formative effects (Figure 2). Plants treated with 10 and 100 ppm AS produced an average of 3 and 13 more leaves per plant, respectively, than did the control plants. These leaves also were dark green and showed no sign of injury (Figure 7).

Since the injection and cotton swab method of application resulted in approximately the same total number of leaves for the ASCE, IAA, and AS treatments they also were combined. Growth curves for the ASCE, IAA, and AS, in comparison with the control, are shown in Figures 9, 10, and 11, respectively.

Figure 9 shows that ASCE treated plants produced a relatively uniform increase in number of leaves. However, for about 3 to 4 weeks following each treatment the leaf number increase was slightly inhibited in comparison with that of the control.

In Figure 10 the overall increase in leaf number of IAA treated plants is shown. Both the 10 and 100 ppm IAA treated plants increased in leaf number slightly faster than did the control. However, the

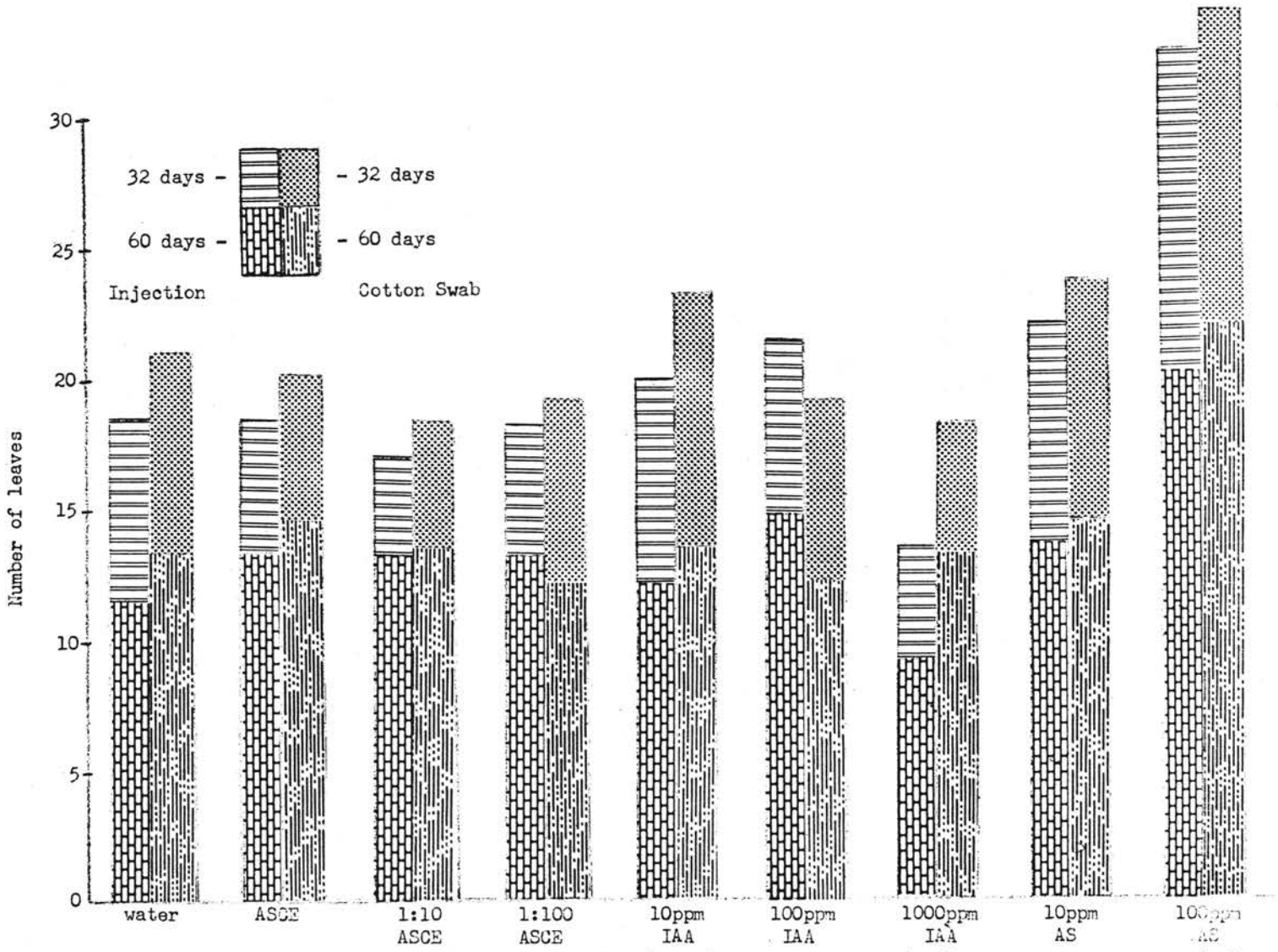

Figure 8. Effect of avocado seedcoat extract (ASCE), 3-indoleacetic acid (IAA), and adenine sulfate (AS) on the increase in number of leaves on 7-week old avocado seedlings. The treatments were injected into the stems or applied with cotton swabs to the growing point. Treatments were applied on each plant two times, one at 0 days and the second 60 days later. Each bar represents an average of four plants.

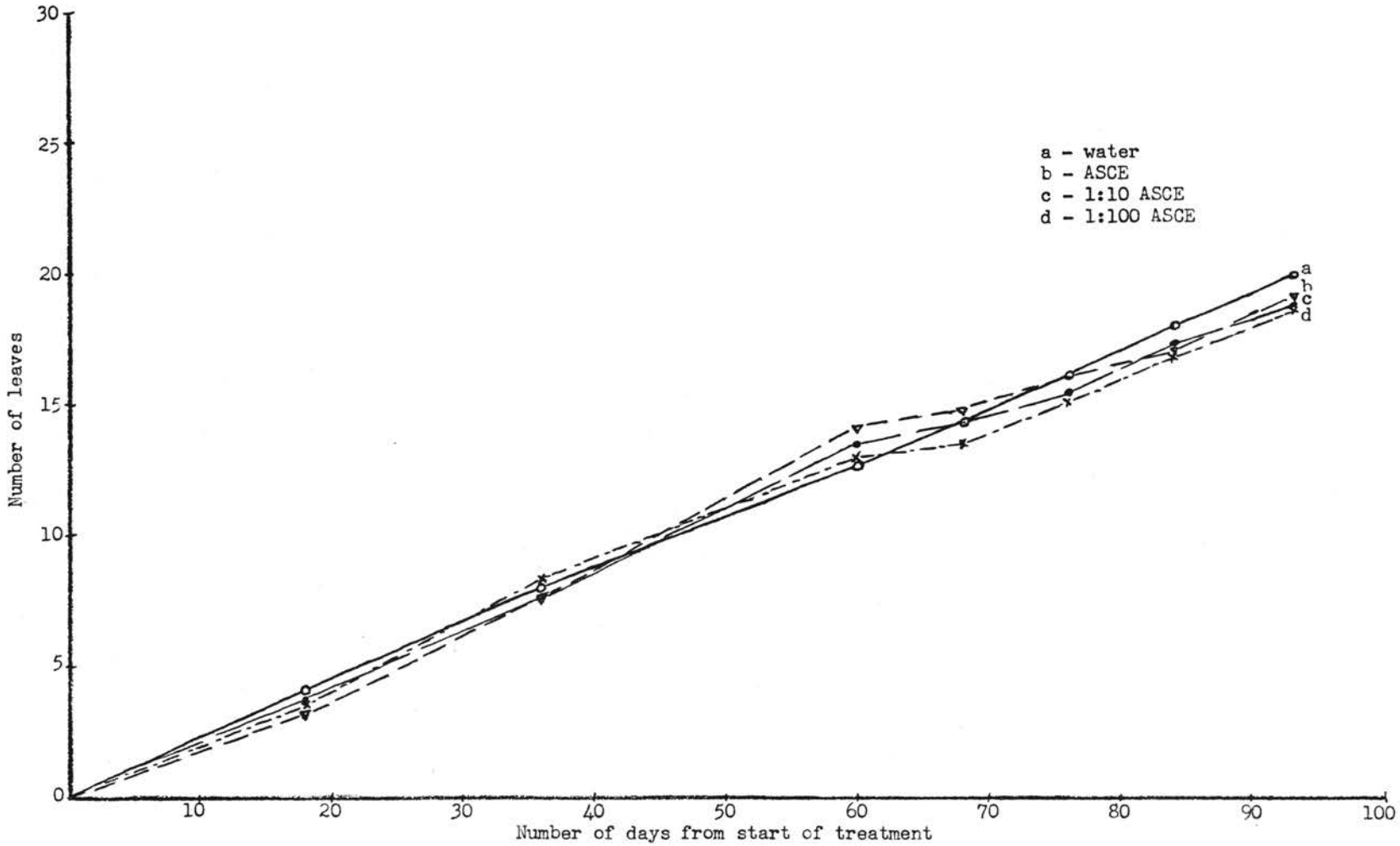

Figure 9. The rate of increase of leaves of 7-week old avocado seedlings treated with avocado seedcoat extract (ASCE) at 0 days and 60 days. Each curve represents an average of 8 plants.

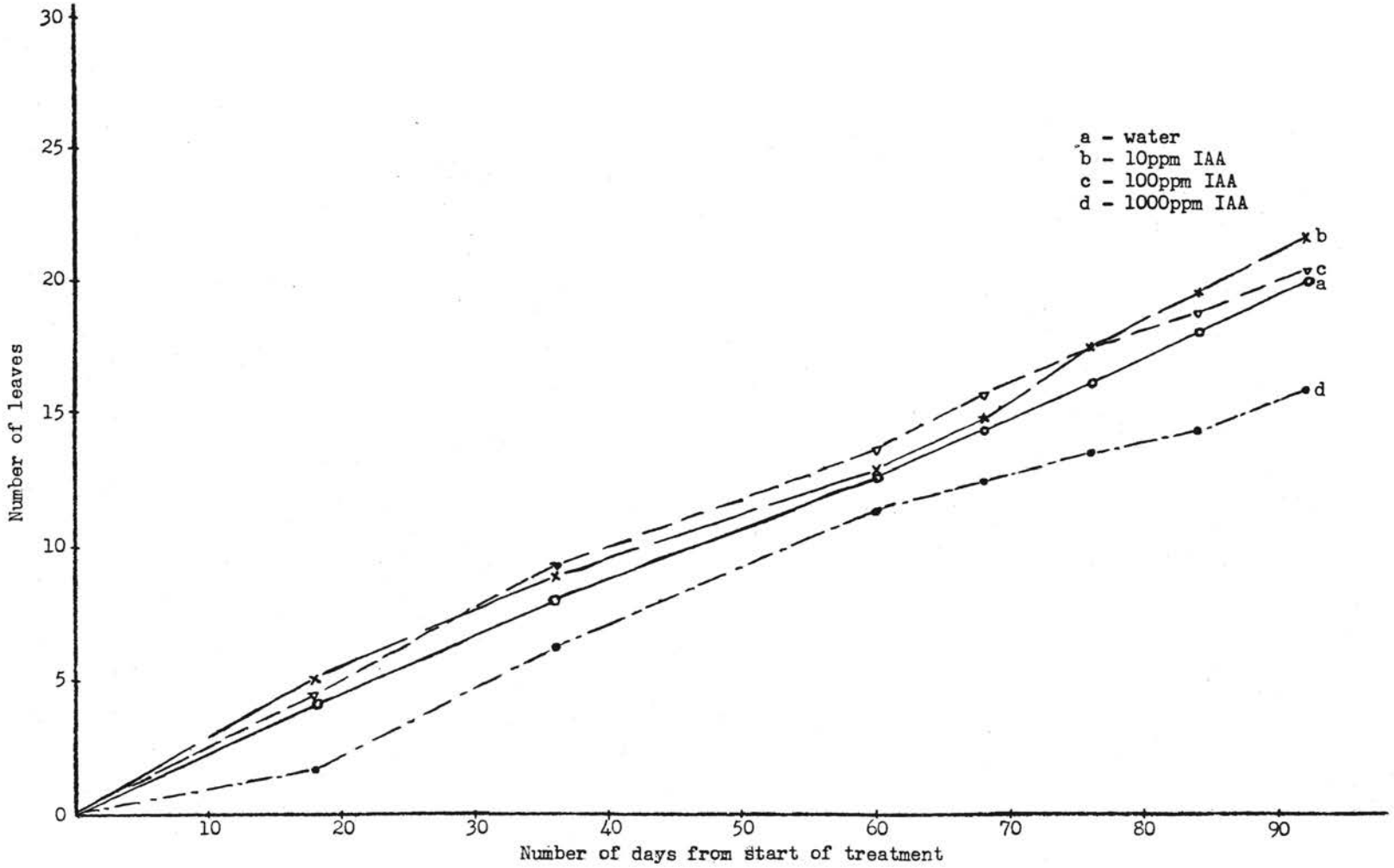

Figure 10. The rate of leaf increase of 7-week old avocado seedlings treated with 3-indoleacetic acid (IAA) at 0 days and 60 days. Each curve represents an average of 8 plants.

increase in leaf number of the 1000 ppm treated plants was markedly reduced from that of the control.

The effect of AS on the number of leaves produced is shown in Figure 11. The number of leaves on both the 10 and 100 ppm AS treated plants increased more rapidly than did the control with the leaf rate increase for the 100 ppm AS treatment being more than 50% greater than with the control plants.

Table I shows the effect of ASCE, IAA, and AS treatments on increase in stem diameter after 92 days. The full strength ASCE treatment markedly increased the stem diameter over the control. ASCE dilutions of 1:10 and 1:100, injected into the plant, had little effect on stem diameter. However, ASCE dilutions of 1:10 and 1:100, applied with a cotton swab, resulted in increases in stem diameter of 0.60 mm and 0.25 mm, respectively, over that of the control. The 10 ppm IAA treatment resulted in a marked stem diameter increase over the control. Treatment with 100 ppm IAA, however, had little effect on stem diameter and the 1000 ppm IAA caused a slight retardation in stem diameter increase when compared to that of the control. Both of the AS treatments, particularly the 10 ppm AS, resulted in marked increases in stem diameter. Over all treatments the cotton swab method of application resulted in a slightly greater stem diameter increase than did the injection method of application.

B. Slit pea bioassay:

Table II shows the average curvature of slit pea sections treated with varying concentrations of ASCE, IAA, and AS. As indicated by an increased curvature both the full strength ASCE and 1:10 dilution of ASCE showed a greater activity than did the water control. Increasing

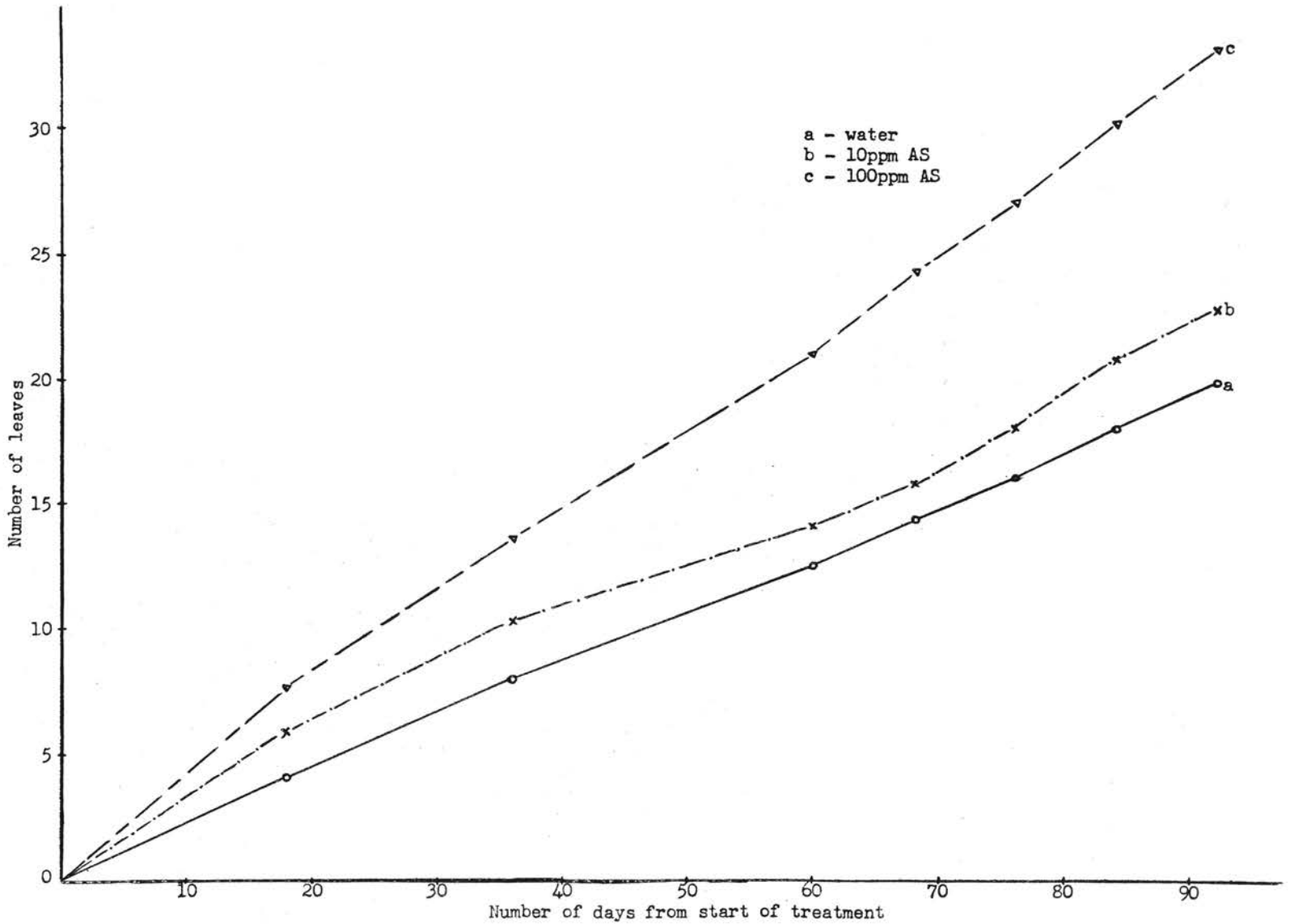

Figure 11. The rate of leaf increase of 7-week old avocado seedlings treated with adenine sulfate (AS) at 0 days and 60 days. Each curve represents an average of 8 plants.

TABLE I

THE INCREASE IN STEM DIAMETER 92 DAYS AFTER TREATMENT OF 7-WEEK OLD AVOCADO SEEDLINGS TREATED WITH AVOCADO SEEDCOAT EXTRACT (ASCE), 3-INDOLEACETIC ACID, AND ADENINE SULFATE (AS) AT 0 AND 60 DAYS. EACH FIGURE REPRESENTS AN AVERAGE OF 4 PLANTS

Treatment	Method of treatment	
	Injection	Cotton swab
	mm.	mm.
Check (water control)	3.85	3.75
ASCE	4.50	4.90
1:10 ASCE	3.83	4.45
1:100 ASCE	3.77	4.00
10 ppm IAA	4.55	4.65
100 ppm IAA	3.85	3.95
1000 ppm IAA	3.72	3.55
10 ppm AS	4.62	5.02
100 ppm AS	3.95	4.37
AVERAGE	4.07	4.29

TABLE II

THE AVERAGE CURVATURE OF ALASKA SLIT PEA STEMS TREATED FOR 24 HOURS WITH AVOCADO SEEDCOAT EXTRACT (ASCE), 3-INDOLEACETIC ACID IAA, AND ADENINE SULFATE (AS). EACH FIGURE REPRESENTS AN AVERAGE OF 10 SECTIONS

Treatment	Degree of curveture
Check (water control)	41.60
ASCE	44.20
1:10 ASCE	45.95
1:100 ASCE	40.10
10 ppm IAA	44.60
100 ppm IAA	46.05
1000 ppm IAA	37.80
10 ppm AS	42.65
100 ppm AS	62.80

concentrations of IAA, and AS, up to 100 ppm, also caused an increased curveture of the slit pea sections, thus indicating considerable biological activity. The 100 ppm AS showed more activity than any other treatment.

C. Germination tests:

The results of the tomato and lettuce seed germination tests are given in Tables III and IV, respectively. All of the ASCE treatments markedly inhibited both tomato and lettuce seed germination. After 10 days neither the tomato or lettuce seeds had started to germinate in the full strength ASCE. A dilution of 1:10 ASCE resulted in 16 percent germination of lettuce seed compared to 62 percent for the control, while a dilution of 1:100 ASCE resulted in 20 percent germination of tomato and 26 percent germination of lettuce seeds. In contrast to the control, which produced vigorous and healthy seedlings, none of the ASCE treatments resulted in satisfactory seedling growth. In most cases the radicle turned brown and died when it came in contact with the moist filter paper. Tomato and lettuce seeds germinated in the 10 ppm IAA treatment resulted in 42 and 50 percent germination respectively. A concentration of 1000 ppm IAA completely inhibited all seed germination. Seeds treated with IAA produced weak spindly seedlings compared to those of the control. The 10 ppm AS treatment caused a slight inhibition in germination of tomato and lettuce seeds. However, increasing the concentration of AS from 10 ppm to 100 ppm had no further effect on seed germination. The seedlings in both AS treatments produced good strong epicotyles although the development of the hypocotyles was rather slow.

TABLE III

PERCENT GERMINATION OF TOMATO SEEDS GERMINATED ON FILTER PAPER MOISTENED WITH AVOCADO SEEDCOAT EXTRACT (ASCE), 3-INDOLEACETIC ACID (IAA), AND ADENINE SULFATE (AS)

Treatment	Total Percent Germination Each Day									
	I	2	3	4	5	6	7	8	9	10
Check (water control)	-	-	-	24	52	64	66	70	72	72
ASCE	-	-	-	-	-	-	-	-	-	-
1:10 ASCE	-	-	-	-	-	-	-	-	-	-
1:100 ASCE	-	-	-	-	-	-	-	12	16	20
10 ppm IAA	-	-	-	18	30	34	36	38	38	42
100 ppm IAA	-	-	-	14	28	46	46	46	46	48
1000 ppm IAA	-	-	-	-	-	-	-	-	-	-
10 ppm AS	-	-	-	24	38	52	52	54	58	58
100 ppm AS	-	-	-	14	32	60	60	62	62	62

TABLE IV

PERCENT GERMINATION OF LETTUCE SEEDS GERMINATED ON FILTER PAPER MOISTENED WITH AVOCADO SEEDCOAT EXTRACT (ASCE), 3-INDOLEACETIC ACID (IAA), AND ADENINE SULFATE (AS)

Treatment	Total Percent Germination Each Day									
	1	2	3	4	5	6	7	8	9	10
Check (water control)	12	32	52	56	60	62	62	62	62	62
ASCE	-	-	-	-	-	-	-	-	-	-
1:10 ASCE	-	-	-	-	-	4	6	6	8	16
1:100 ASCE	-	-	-	-	6	8	18	24	24	26
10 ppm IAA	-	10	32	46	46	48	48	50	50	50
100 ppm IAA	-	4	12	18	24	26	28	30	32	32
1000 ppm IAA	-	-	-	-	-	-	-	-	-	-
10 ppm AS	12	24	42	44	46	46	46	48	48	48
100 ppm AS	-	10	16	40	44	44	48	48	48	48

D. Chromatographic separation:

A substance, or compound, which gives a positive test with 4-dimethylaminocinnamaldehyde was observed when a paper chromatographic separation of the alcohol extract of the avocado seedcoats was made. This compound has an Rf value similar to IAA in the solvent mixture used for this chromatographic separation but the color test shows the substance to be some compound other than IAA. The color with 4-dimethylaminocinnamaldehyde was a pink spot on the paper chromatograph. A ninhydrin spray of the same chromatographed material did not show a positive test for an amino acid.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Although avocado seedcoat extracts (ASCE) had an inhibitory effect on stem elongation this effect was temporary since it was closely followed by a growth rate increase similar to that of non-treated control. There apparently was an inhibitor present in the extract which was either dissipated rapidly in the metabolism of the plant or was overcome by a rapid increase of natural plant growth factors which cause stem elongation to occur. It is presumed that the inhibitor in ASCE probably was composed of an auxin-inhibitor complex. Thus as soon as the effect of the inhibitor ceased the auxin (growth factor responsible for stem growth) resulted in a promotion of stem growth. When the inhibitor was further reduced an accelerated growth occurred and eventually the treated stem attained or surpassed the non-treated control in elongation.

It is interesting to note that the inhibitor in ASCE apparently had an opposite effect on stem enlargement, since the greater the concentration of ASCE the greater the stem diameter in comparison with that of the control. Apparently the auxin-inhibitor complex contained some substance which accelerated the development of xylem and phloem tissue.

This inhibitor in the ASCE was not toxic to leaf development since the number and size of leaves were not markedly different from those of the control. Any inhibiting effect on leaves probably was an indirect effect and thus was expressed only when the stem growth was temporarily retarded. This effect was well demonstrated (Figure 12) since the

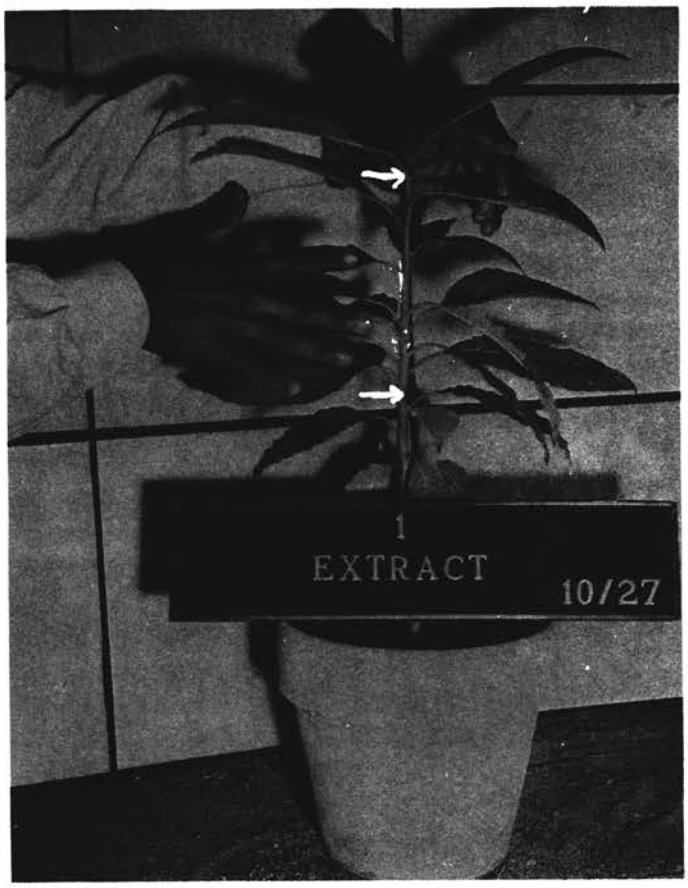

Figure 12. A close-up of the characteristically shortened internodes, on avocado seedling plants, as a result of treatment with avocado seedcoat extract (ASCE). Treatment was given by injecting the ASCE into the stem at 0 days and 60 days, at the point indicated by arrows. Picture taken 92 days after first treatment.

internodes were much closer together immediately above the treated area. The dark green color and the ordinary size of the leaves on the ASCE treated plants also suggests the non-toxic effect on plant growth, other than the retardation of stem elongation.

The higher the concentration of 3-indoleacetic acid (IAA) the greater was the stem retardation, with the 1000 ppm IAA producing a maximum inhibition of stem growth, both in stem elongation and in stem diameter enlargement. It has long been known that IAA generally causes the production of roots and the retardation of stem growth. It also is known that high concentrations of IAA cause the production of formative effects in leaves similar to those exhibited in this experiment. In view of the fact that 10 and 100 ppm IAA had little, if any, retarding effect on stem growth it may be presumed that the concentration of growth factors already in the plant, which promote stem elongation, were sufficiently high in concentration to overcome any retarding effect exhibited by the IAA. It would have been interesting, in the light of these results, to have measured the total amount of root growth in the treated and untreated plants.

In contrast to the ASCE and IAA solutions, increasing the concentration of adenine sulfate (AS) exerted a marked stimulatory effect on stem elongation, stem diameter enlargement, and leaf production. Although AS is not produced naturally in plants its presence enhanced the effects of the growth material(s) responsible for stem elongation, stem diameter enlargement, and leaf production. Although there was a marked increase in stem length over the control in the 100 ppm AS the internode length was not greatly affected due to the production of an increased number of leaves. AS showed the greatest relative activity of any of the growth

materials tested. As expected, increasing the concentrations of growth materials, with the exception of 1000 ppm IAA, resulted in a greater relative activity. Apparently the 1000 ppm IAA was toxic to the slit pea sections since some of them were shrivelled after 24 hours. It is of interest to note that the undiluted ASCE showed approximately as much activity as the 100 ppm AS.

The high concentrations of both ASCE and IAA apparently were toxic to seed germination. As expected, decreasing concentrations of these growth materials reduced their toxic effects and permitted some germination to occur, although there was still enough inhibitory material present in the 1:100 ASCE to cause injury to the radicle. AS slightly delayed the time of germination from that of the control. However, the percent germination of both tomato and lettuce seeds was nearly as good as that of the control after 10 days.

Although the chromatographic separation provided some clues as to the identity of the extracted material the separation was not carried far enough to determine whether it was an inhibitor alone or an auxin-inhibitor complex. Although its identity is not known the material in the extract apparently was related to IAA since it had the same Rf value in the chromatographic separation as did IAA.

In general, it can be concluded that there is present in the avocado seedcoat a growth material(s) which inhibits both seedling growth and seed germination. The inhibitory effect on seedling growth was only temporary and was apparently overcome by the accumulation of naturally occurring growth materials which promote stem growth. The material(s) in the extract were not toxic to leaf development although germinating seedlings were injured when they came in contact with it.

It is presumed that the material(s) exists in the seedcoats as an auxin-inhibitor complex.

CHAPTER VI

SUMMARY

This study was concerned with a comparison of the inhibitory effects of the alcohol soluble avocado seedcoat extract (ASCE), 3-indoleacetic acid (IAA), and adenine sulfate (AS) on plant growth and seed germination.

The ASCE had a temporary inhibiting effect on stem elongation of seven-week old avocado seedlings, which lasted for about three weeks. The ASCE caused an increase in stem diameter over that of the control, but it did not affect the development of leaves. A 1000 ppm concentration IAA produced a temporary inhibiting effect on plant growth and also retarded the increase in stem diameter. The 1000 ppm IAA also caused the productions of leaves with severe formative effects. Increasing the concentration of AS caused a marked increase in seedling growth. It also resulted in the production of larger stems with more leaves than the control.

In the slit pea bioassay all ASCE, IAA, and AS solutions except 1000 ppm IAA showed as much or more activity than the control. The activity of 100 ppm AS treatment was much greater than that of the control.

In germination tests, using lettuce and tomato seeds, the undiluted ASCE and 1000 ppm IAA solutions resulted in the complete inhibition of germination. When the radicles of seedlings which germinated in 1:10 and 1:100 dilutions of ASCE touched the moist filter paper they turned brown

and growth ceased. AS and IAA concentrations of 100 ppm or less had little effect on germination.

A chromatographic separation of the ASCE showed the presence of a substance with a Rf value similar to that of IAA, although the color was different. The presence of IAA, 3-indolepropionic acid, 3-indolebutyric acid, or any of the amino acids was not detected in the ASCE.

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