

THE EFFECTS OF AMO-1619, B995 AND CCC ON THE GROWTH OF NORMAL
AND DERIVED TISSUES OF NICOTIANA TABACUM L. AND THEIR
INTERACTION WITH AUXIN AND GIBBERELLIN.

by

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A MASTER'S THESIS

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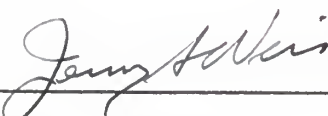
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INTRODUCTION

GROWTH REGULATORS

Hormones are organic substances which are synthesized in minute quantities in one part of an organism and are transported to another site at which they exert their effect. Since plants lack specialized cells for the transmission of stimuli they are almost completely dependent on plant hormones for this purpose. Several growth regulatory substances are produced by plant meristematic tissue, i.e., auxins, cytokinins and gibberellins. All three are necessary for the proper growth and development of the plant.

F. F. Went (1929) devised a method of isolating the growth promoting substance from Avena coleptiles and was able to quantitate the presence of this substance by the Avena coleptile curvature test. This substance was later isolated by Kogl and Haagen-Smit (1934) and identified as indole-3-acetic acid (IAA). While other substances act in a manner comparable to IAA, IAA is the most common naturally occurring auxin.

Auxin is known to bring about an increase in respiration which results in an increase in metabolism and the synthesis of new cell wall material. It is believed that the softening of the cell wall, also accredited to auxin, permits a passive uptake of water which expands the cell, but this has not been proven (Leopold, 1964).

Auxin is influential in promotion of cell division in meristematic tissues, the promotion of root initiation and the inhibition of root growth. Further effects exhibited by auxin are: formation and growth of callus tissues, maintenance of apical dominance, influence on flower formation and leaf, fruit and flower shedding, formation of parthenocarpic fruit and promotion of respiration and protein formation (Linser, 1966).

Kurosawa (1926) observed that sterile filtrates from the "bakanae" fungus exhibited marked growth stimulation in grass including rice. The substance responsible for this marked growth increase was isolated by Yabuta and named gibberellin on the basis of the scientific name of the fungus, Gibberella fujikuroi (Stowe et al., 1961). Today 17 gibberellins have been extracted from fungi and higher plants, the most common of which is gibberellic acid (GA_3) (Brian et al., 1967).

Activities commonly attributed to GA_3 are: increase of internodal length of growing plants, promotion of flower formation, germination, parthenocarpy and hypocotyl growth, dormancy, photoperiodic behavior of flowers and lessening of the activity of IAA oxidase (Linsner, 1966).

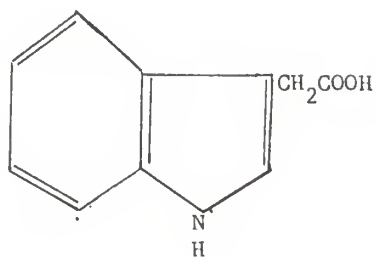
Complementary to the effects of auxins and gibberellins, cytokinins increase mitosis in roots, promote bud growth thus modifying apical dominance, stimulate leaf enlargement and modify polarity, dormancy, stem elongation and bud formation. The chemical structure of kinetin, a cytokinin has been identified as 6-furfuryl adenine (Miller, 1955). A naturally occurring cytolinin, zeatin, has been extracted from corn kernels (Letham, 1963).

TISSUE CULTURE TECHNIQUE

One of the greater tools of the study of phytohormones was the advent of tissue culture. This presented a means of studying chemical reactions in plants on a histological level in a manner conducive to a laboratory situation. Prior to this attempts were made to study plant parts by dissection into smaller and smaller pieces by Vochting in 1878 and through grafting by Carriere in 1875, Vochting in 1892 and Harrison in 1904 (as cited by White, 1963).

The idea of cultivating the vegetative cells of higher plants in

PLANT GROWTH REGULATORS



INDOLE-3-ACETIC ACID (IAA)

FIGURE I

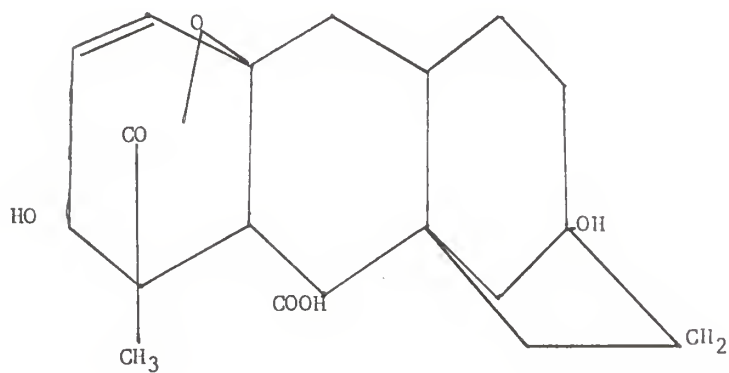
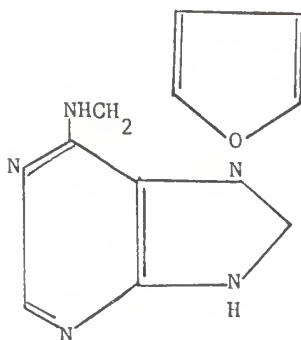
GIBBERELIC ACID (GA₃)

FIGURE II



6-FURFURYL ADENINE (KINETIN)

FIGURE III

suitable nutrients was suggested by G. Haberlandt in 1902. However, the problem of finding a suitable nutrient solution proved difficult and required the study and experimentation of many botanists during the following years. Excised plant roots were grown for several weeks in nutrient solution by Knotte in 1922 and Robbins in 1922 and 1924 (White, 1963), but it was not until 1934 that the field of plant tissue culture was definitely opened. White (1934) demonstrated potentially unlimited growth of excised tomato roots. Also Gautheret succeeded in growing portions of the cambium and phloem of Populus, Acer, Ulmes and Salix on Knop's liquid nutrient medium containing glucose (Gautheret, 1934). Independent tissue growth was reported by White, Gautheret and Nobecourt in 1939 (White, 1939; Gautheret, 1939; Nobecourt, 1939).

Since this time a variety of media have been described for the growth of plant tissue culture (White, 1943; Murashige and Skoog, 1962; Linsmaier and Skoog, 1965). The basic media contain inorganic salts, sucrose, dextrose or levulose as a carbon source and nitrates and urea as a nitrogen source. These have been supplemented with vitamins, growth substances, eg. indole-3-acetic acid, 2-naphthaleneacetic acid, kinetin and 2-4 dichlorophenoxyacetic acid, fruit or other plant extracts, casein hydrolysate, natural endosperms including coconut milk and in some cases have been solidified with agar. Most tissues favor a slightly acidic medium (pH 5.5-6.0).

NATURE OF TISSUE

Tissues of many genera have been used in tissue culture experiments. In cultures of Parthenocissus as well as other systems normal pith cells require for proper growth and development an endogenous source of two growth regulators, namely, auxin and cytokinin (Braun, 1955).

Occasionally, however, autonomous tissues occur spontaneously in

culture from normal tissues as in the case of Nicotiana tabacum L. var. Wisconsin No. 38. These tissues, known as derived tissues, are significant in that they are capable of synthesizing both auxin and cytokinin (Fox, 1962; Weis, 1964). It has been suggested that the normal tissue is inherently autonomous for a cytokinin and an auxin but that inhibitory mechanisms are imposed on systems leading to the synthesis of these growth promoting compounds which, under certain circumstances, can be lost resulting in the production of auxin and cytokinin. This reasoning is made feasible by the fact that a third strain of tissue has been noted in which the mechanisms controlling the production of a cytokinin is independent (Fox, 1963).

Another possibility would be that the IAA oxidase system was destroying IAA in the normal tissue. However, other experimental work has indicated that autonomous O-1 (derived) tissue destroys IAA at a greater rate than that of the auxin-requiring KX-1 tissue (Weis, 1967).

Derived tissues have also been observed to have an increased capacity to take up solutes from dilute solutions. Since a large segment of metabolism is made functional by the presence of certain ions, the derived cell has a selective advantage over the normal cell. One of these functional mechanisms dependent on an ion is the synthesis of meso-inositol which has cytokinin activity (Wood and Braun, 1965).

In the present experimentation two types of cultures derived from the pith of Nicotiana tabacum L. var Wisconsin No. 38 were used. The normal tissue, KX-1, first described by Skoog and Tsui (1948), requires both an auxin and a cytokinin for growth. The derived tissue, O-1, is autonomous for both auxin and cytokinin. The O-1 tissue grows much more rapidly than the KX-1 tissue and forms a friable yellowish callus when grown in the dark. It lack the regular shape of the white circular KX callus tissue.

RETARDANTS

Tissue culture is a convenient means of studying growth regulators in vitro. The purpose of the present experiment is to try to gain insight into the mechanism of action of three growth retardants in relation to their interaction with IAA and GA_3 .

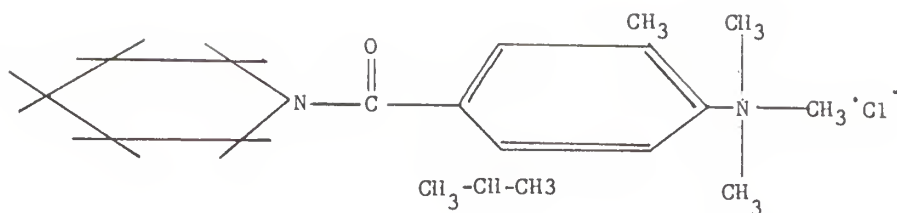
The retardants used in this experimentation were 4-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate (Amo-1618)¹, (2-chloroethyl) trimethylammonium chloride (Cycocel or CCC)² and N-dimethylamino succinamic acid (B995)³.

Amo-1618, (figure 4) a quarternary ammonium carbamate with a molecular weight of 358, was found in screening tests carried out by the National Academy of Sciences, National Research Council, in cooperation with the Growth Regulator and Antibiotic Laboratory of the United States Department of Agriculture and was first cited as an active plant growth retardant by Wirwille and Mitchell in 1950. Each molecule of Amo-1618 consists of four parts; the carbamate nitrogen, the terpene ring, the quarternary nitrogen and halide salt. Each of these four parts is necessary for the proper functioning of the molecule.

CCC, (figure 5) with a molecular weight of 158, is also a quarternary ammonium compound and was first reported by Tolbert (1960). It is an analogue of choline in which the hydroxy group of choline has been replaced by a chlorine atom. The trimethyl ammonium cation is essential for activity. Corresponding tributyl or triethyl derivatives were completely inactive.

¹Chemical obtained from Calbiochem.

^{2,3}Chemical courtesy of U. S. Rubber Co., Naugatuck, Conn.

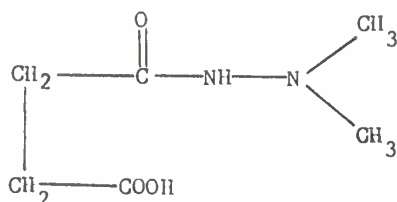


AMO-1618

4-HYDROXYL-5-ISOPROPYL-2-METHYLPHENYL TRIMETHYL AMMONIUM CHLORIDE, 1-

PIPERIDINE CARBOXYLATE

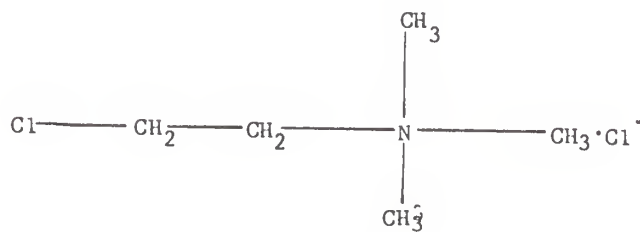
FIGURE IV



B995

N-DIMETHYLAMINO SUCCINAMIC ACID

FIGURE V



CCC

(2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE

FIGURE VI

B995 (figure 6) was first reported by Riddell *et al.* (1962). It is a free ionizable acid with the C-C-N-N system found in beta-hydroxyethyl hydrazine and maleic hydrazine and differs from Amo-1618 and CCC in that it has no benzene ring, quarternary ammonium or phosphonium cation, or substituents that are of small size, nucleophilic and nonionizable. Its molecular weight is 160.

The three retardants differ from one another in stability, plant spectrum and germicidal activity. Cathey states that Amo-1618 persists in soil at least ten years and is relatively stable under steam sterilization whereas B995 and CCC persist only 3-4 weeks in soil and are broken down by steam sterilization. However, experimental results to be presented indicate that Amo-1618 is also broken down by steam sterilization. Cathey did not indicate the pressure used or the length of time during which the retardants were exposed to sterilization. This may be an important factor. B995 and CCC are effective over a wide range of plants whereas Amo-1618 only effects a few plants. B995 is not an effective germicide while the other two are effective (Cathey, 1964).

EXPERIMENTATION

The tissue used for the experiment was obtained from pith cells isolated from the stem of Nicotiana tabacum L. var. Wisconsin No. 38. The origin of the tissue and nature of the growth regulator requirements have been described in the preceeding material.

The constituents of the medium used are listed in Table I. The medium was prepared with glass distilled water, adjusted to a pH of 5.0-5.5 with 0.1M KOH, solidified with 1% Bacto Agar and sterilized 20 minutes at 16 lbs/in² pressure. The medium for the KX-1 tissue was supplemented with 1 mg/l IAA and 0.5 mg/l kinetin. Since the retardants and GA₃ are broken down by autoclaving these were sterilized by passage through a syringe fitted with a Millipore bacteriological filter.

Tissues were grown in 125 ml Erlenmeyer flasks. Three pieces of tissue were aseptically transplanted from stock cultures into each flask. Tissues were grown in the dark or in continuous light (900 ft. candles) in growth chambers at 26°C. After four weeks, tissues were removed from the flasks and fresh and dry weights determined and compared.

TISSUE CULTURE MEDIUM

<u>CONSTITUENT</u>	<u>MG/LITER</u>
NH_4NO_3	1000.0
KNO_3	1000.0
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	500.0
KH_2PO_4	250.0
H_3BO_3	5.0
KI	0.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	300.0
KCl	50.0
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	7.5
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	5.0
THIAMINE HCl	0.1
NICOTINIC ACID	0.5
PYRIDOXINE HCl	0.5
* Na_2 -EDTA	37.0
* $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.0
INOSITOL	100.0
SUCROSE	30000.0

*5 ml/l of a stock solution containing 5.57 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 7.45 g Na_2 -EDTA per liter of water

TABLE I

EXPERIMENTAL RESULTS

Preliminary experiments were conducted to determine the optimal concentration of retardants to be used throughout the experimentation. Concentrations of 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} molar were used. Results of these experimentations for KX-1 tissue in the light and dark are indicated in Figures VII and VIII. A concentration of $10^{-5}M$ proved to cause about 50% inhibition of growth and was used throughout the remainder of the work. As the concentrations decreased so also did the inhibition of growth except a consistent inhibition at $10^{-8}M$ was noted. A feasible explanation for this has not been found. Results of varying concentrations of retardants for O-1 tissue are indicated in Figures IX and X. These results were obscure, probably due to the nature of the tissue which will be discussed later.

Since the effect of autoclaving on the retardants was not known with absolute certitude an experiment was run to determine this. The concentration of retardant used was $10^{-5}M$. Retardants were added to one half of the medium before autoclaving and to the other half of the medium by means of a syringe fitted with a Millipore bacteriological filter after autoclaving. Results are indicated in Table II. A significant decrease in activity of all retardants after autoclaving was noted; thus these additives were filter-sterilized in all future work.

The next and most important phase of the experimentation was to determine whether the inhibitions of the individual retardants were reverted by addition of exogenous GA_3 or IAA separately, whether they were reverted by addition of exogenous IAA and GA_3 together and whether an increased concentration of IAA or GA_3 brought about a proportional change in the reversal of the inhibition. These were studied under both light and dark conditions

with both KX-1 and O-1 tissues. Results which have been averaged from two or more experiments for KX-1 tissue with added Amo-1618, B995 and CCC are indicated in Figures XI, XII and XIII respectively. Statistical levels of significance were determined by means of the t-test and are indicated on the respective figures.

Results of experiments with O-1 tissue were less revealing possibly due to the nature of the tissue. It was impossible to obtain results which were somewhat comparable in running repetitions of the same experiments. Table X shows the average fresh weights for experimental controls in five experiments. It is obvious that the O-1 weight was inconsistent and variation was significantly in excess of variation obtained with KX-1 tissue.

KX TISSUE

DARK

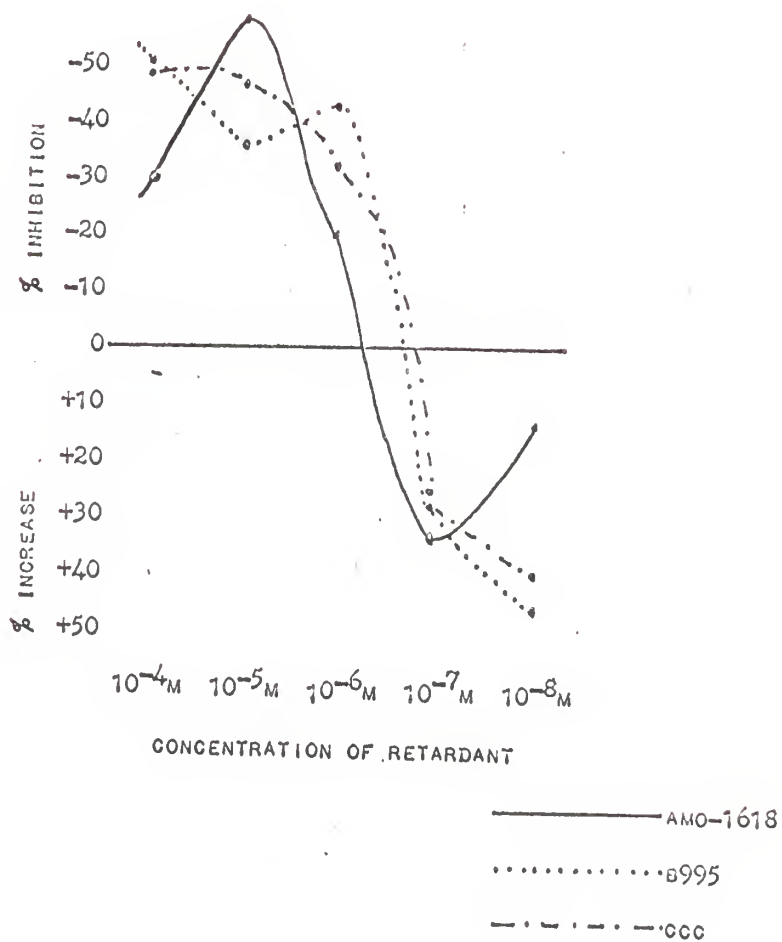
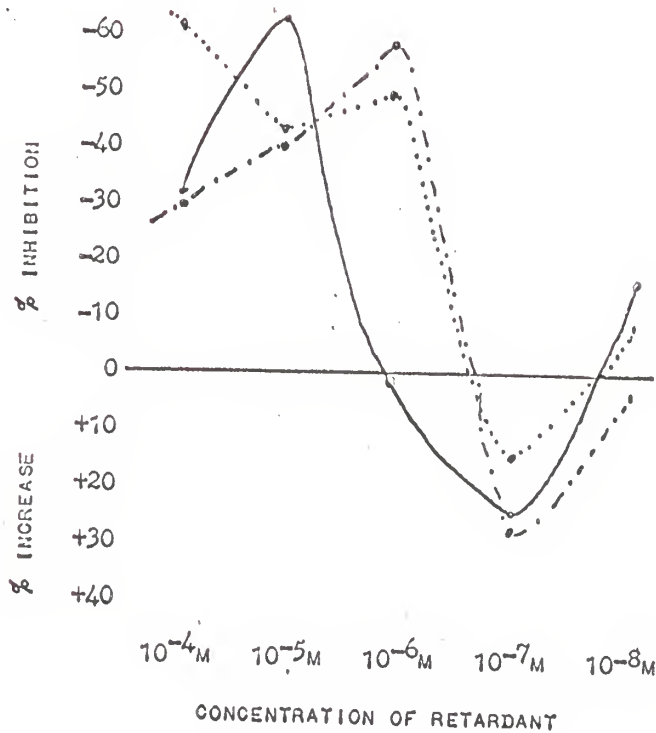


FIGURE VII

KX TISSUE
LIGHT

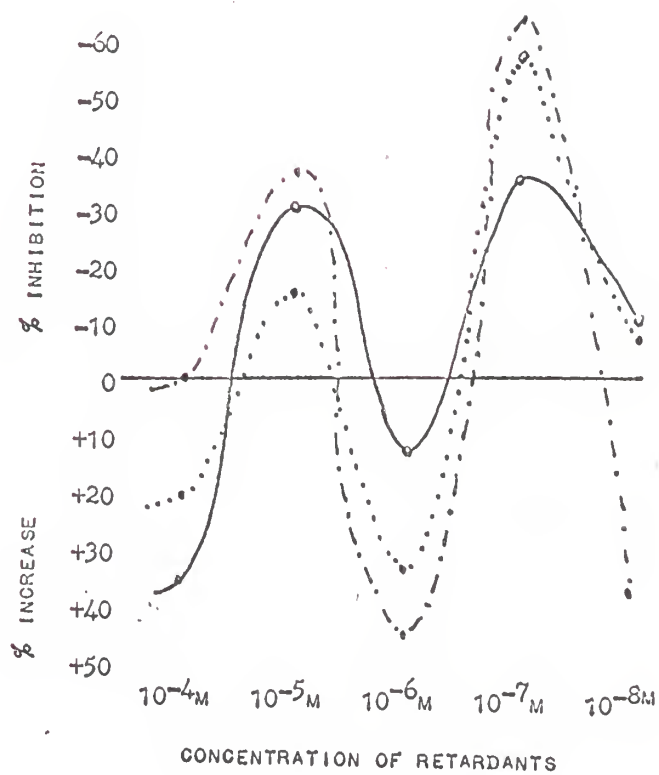


— AMO-1616
..... 8995
- . - . - CCC

FIGURE VIII

O-1 TISSUE

DARK



— AMO-1618
..... B995
- . - . - CCC

FIGURE IX

O-1 TISSUE

LIGHT

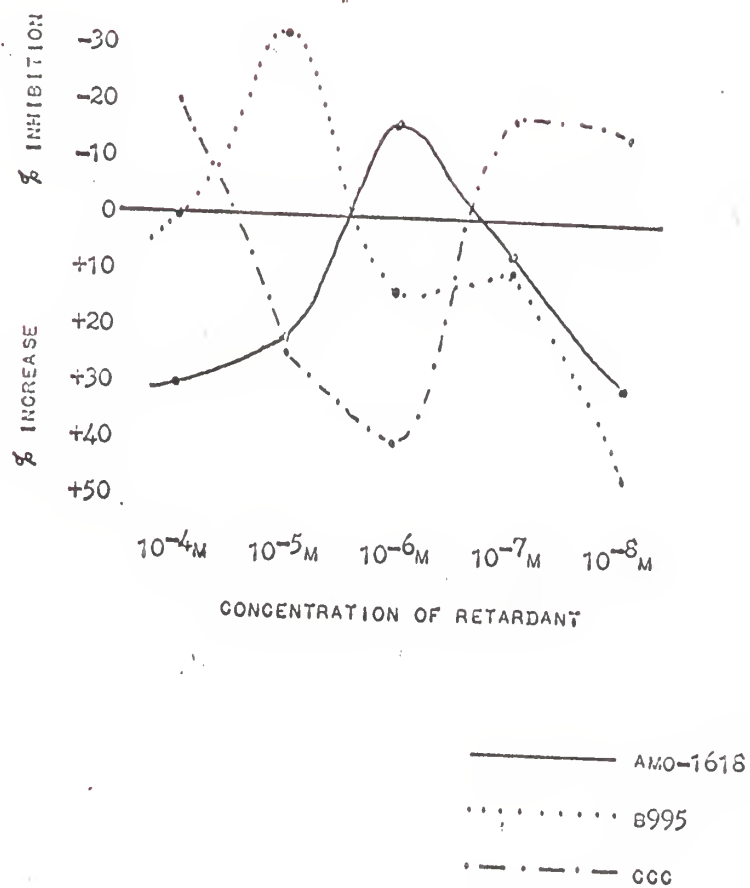
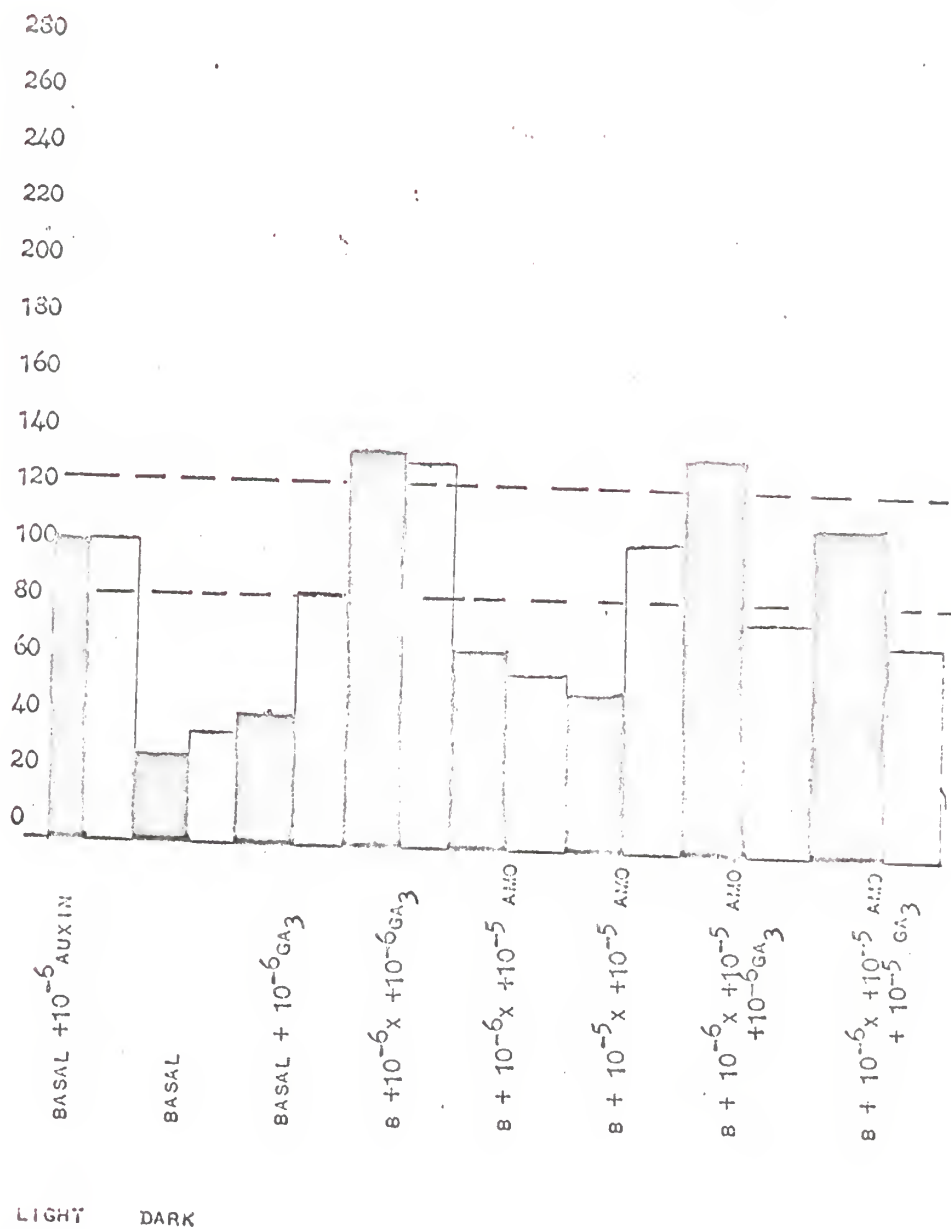


FIGURE X

EFFECT OF AUTOCLAVING ON GROWTH RETARDANTS

<u>RETARDANT</u>	<u>TREATMENT</u>	<u>AVE. WEIGHT</u>	<u>% DIFFERENCE</u>
AMO-1618	AUTOCLAVED	.294 MG.	
AMO-1618	FILTERED	.240 MG.	19%
B995	AUTOCLAVED	.407 MG.	
B995	FILTERED	.268 MG.	35%
CCC	AUTOCLAVED	.305 MG.	
CCC	FILTERED	.271 MG.	12%

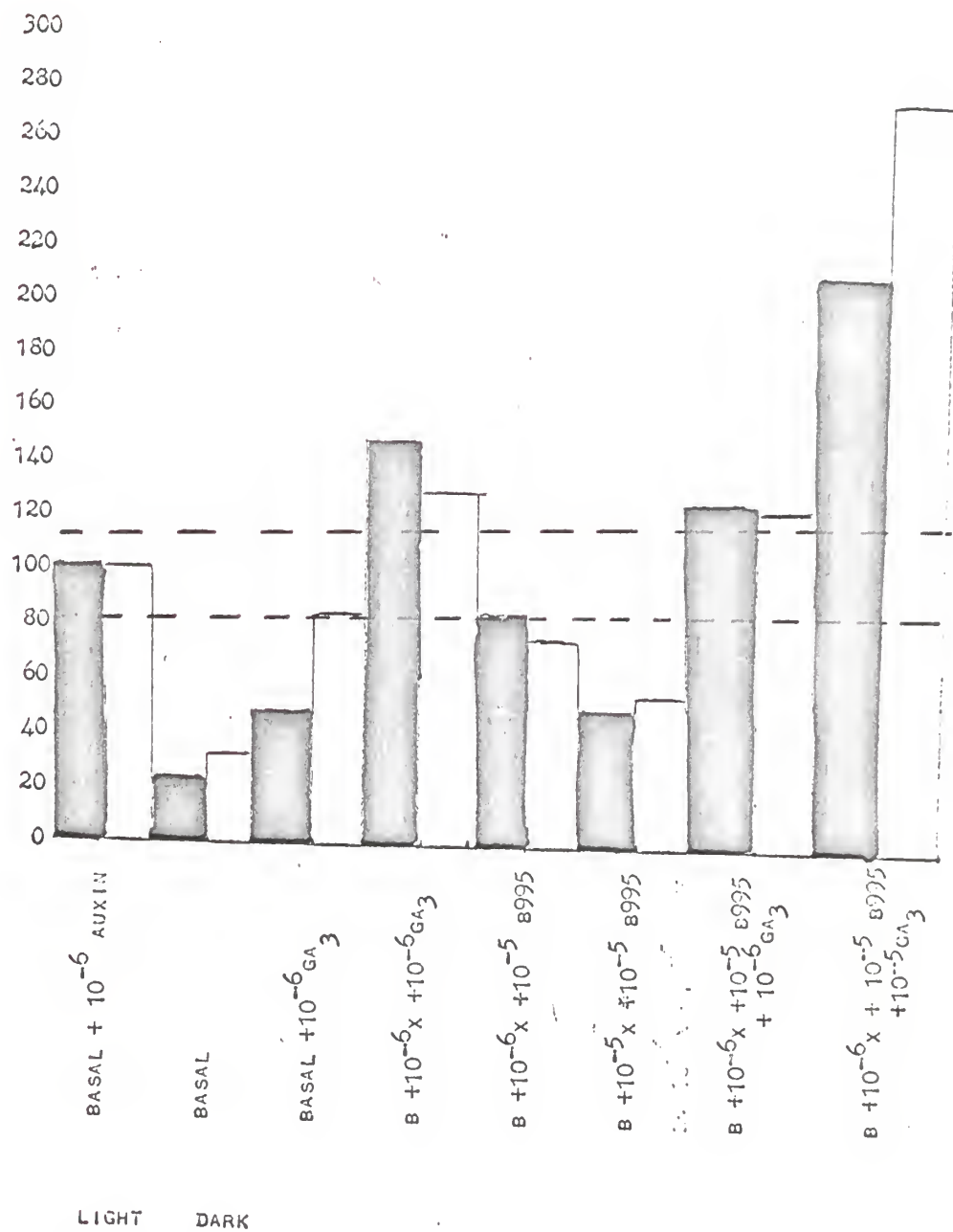
TABLE II



*VARIATIONS OCCURRING BETWEEN THE DASHED LINES ARE NOT STATISTICALLY SIGNIFICANT

CORRELATION DATA FOR AMO-1618

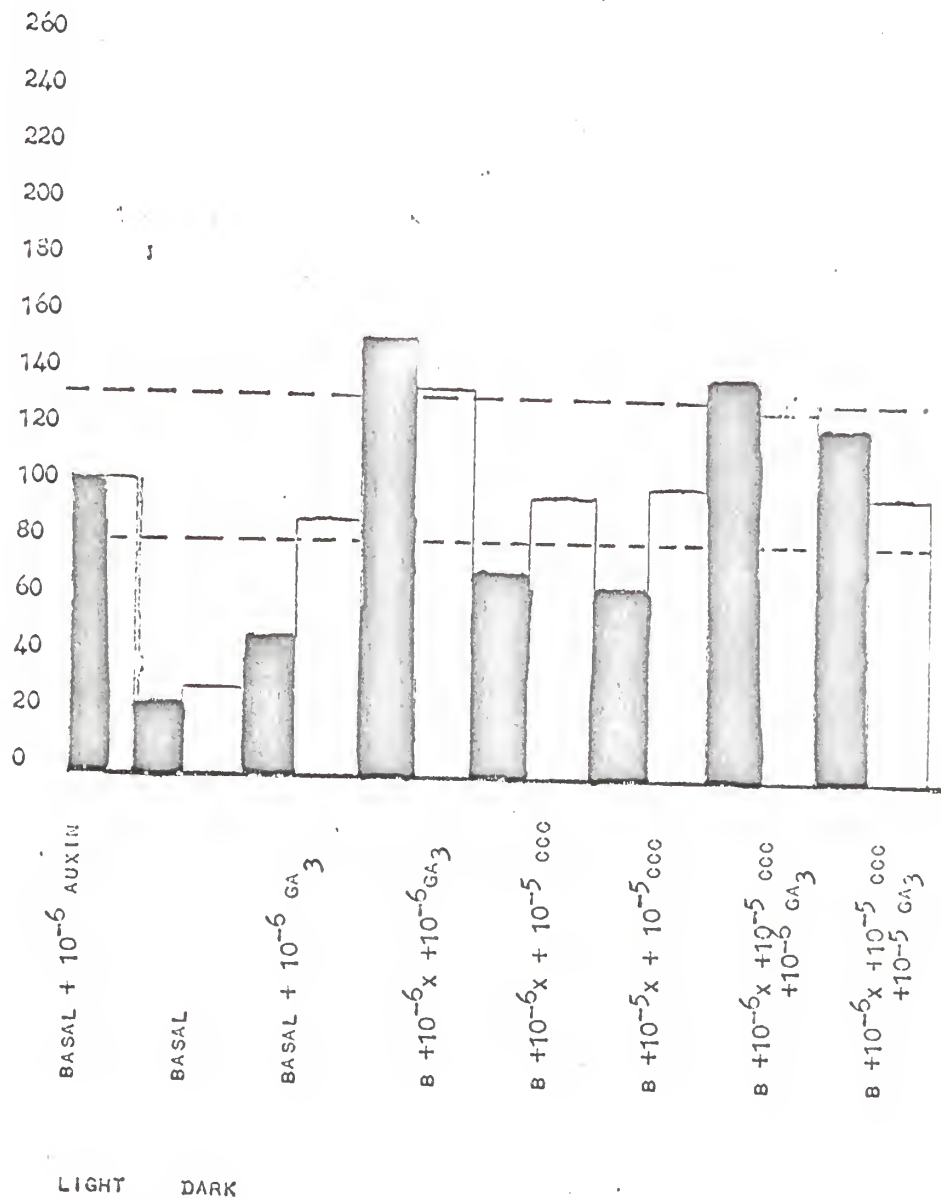
FIGURE XI



* VARIATIONS OCCURRING BETWEEN THE DASHED LINES ARE NOT STATISTICALLY SIGNIFICANT

CORRELATION DATA FOR B995

FIGURE XII



LIGHT DARK

* VARIATIONS OCCURRING BETWEEN THE DASHED LINES ARE NOT STATISTICALLY SIGNIFICANT

CORRELATION DATA FOR CCC

FIGURE XIII

DISCUSSION

Based on broad scale experimentation over a wide range of plants and a variety of plant organ systems there are four possible ways in which the retardants may effect GA_3 -IAA balance and ultimately, plant growth. The first of these is that the retardants may cause inhibitions not directly related to either GA_3 or IAA metabolism. Neither the inhibition by CCC on Raphanus leaf discs nor the inhibitory effects on Avena leaf sections by Amo-1618 were reversed by addition of either GA_3 or IAA (Cleland, 1965). Tissue cultures of Daucus carota, Chrysanthemum, Pelargonium and Nicotiana tabacum which were inhibited with Amo-1618 and CCC separately showed no response to exogenous GA_3 and/or IAA. This indicates that the effects of the retardants are not merely in the prevention of GA_3 or IAA biosynthesis (Sachs and Wohlers, 1964).

A second possibility with regard to the mode of action of the retardants is prevention of GA_3 biosynthesis. If this is the case, the addition of exogenous GA_3 to a retarded system should overcome the inhibition. GA_3 completely reversed flower formation and stem elongation inhibition in Samolus parviflorus brought about by Amo-1618 and CCC indicating these inhibited the synthesis of endogenous GA_3 (Baldev and Lang, 1965). Amo-1618 is observed to reduce stem elongation in caulescent plants without seriously interfering with other growth processes and this effect is counteracted by the addition of GA_3 . Conversely, Hordeum endosperm exhibiting a GA_3 induced growth increase response was not reverted on addition of Amo-1618, B995 or CCC (Paleg et al., 1965).

Further investigations have been conducted on the direct relationship between an exogenous source of a retardant and GA_3 content. The gibberellin content of pea seeds developed in excised pea pods cultured on a medium

containing Amo-1618 was reduced and this reduction increased with increasing concentrations of Amo-1618 (Baldev et al., 1965).

CCC, when repeatedly supplied to the root system of balsam plants, reduces growth in height and the level of gibberellin-like substances in the exudate from the stumps of decapitated plants which seems to indicate a blockage of the normal pattern of GA_3 synthesis (Reid and Garr, 1967). Likewise the GA_3 content of Helianthus apices and root tips treated with CCC was reduced significantly in comparison to controls (Jones and Phillips, 1967).

Kaurene, steviol and kaurenol were not converted to GA_3 in CCC treated cultures of Fusarium moniliforme (Harade et al., 1965) and Amo-1618 was found to inhibit GA_3 biosynthesis in Fusarium (Baldev et al., 1965). Later biochemical studies indicated that kaurene and steviol were also precursors of GA_3 in higher plants (Jones, 1968; Verbiscar et al., 1967) and that the synthesis of GA_3 from kaurene and steviol was inhibited by Amo-1618 (Bennet et al., 1967). CCC, a halogenated analogue of choline, is believed to be involved in lipid metabolism and methylation reactions (Tolbert, 1960; Wittwer and Tolbert, 1960) which are important in the conversion of kaurene to GA_3 .

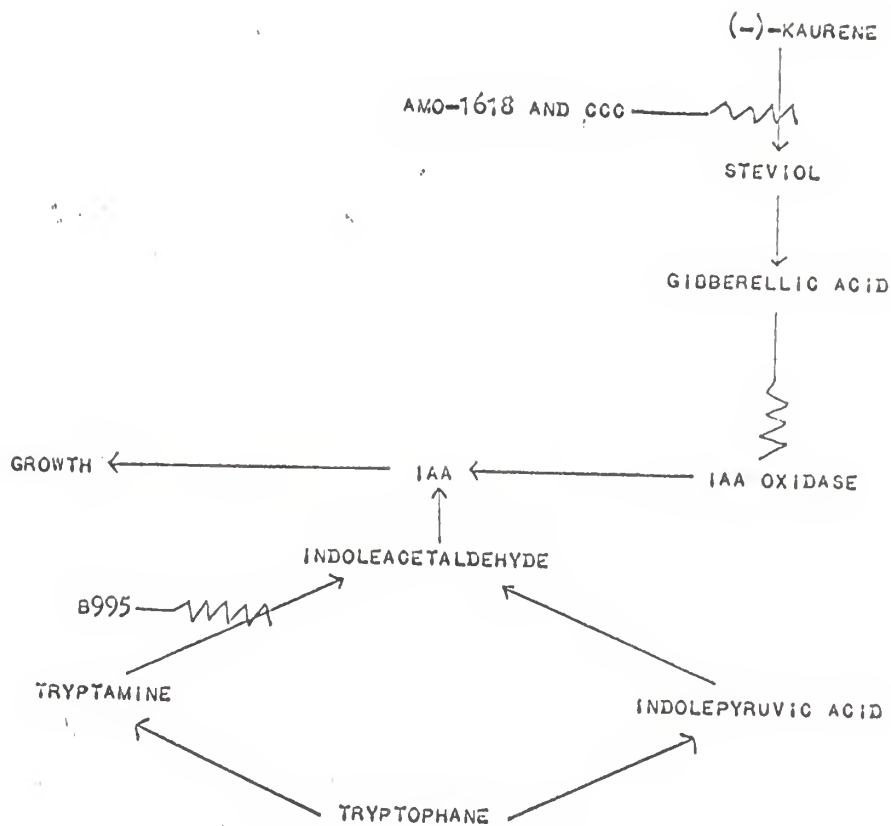
A third mode of action of the retardants is the inactivation of auxin metabolism. CCC inhibited growth of mature Pisum sativum plants. Stem segments were overcome by IAA but not GA_3 . Also the diffusable auxin recovered from pea stem apices was markedly reduced after CCC treatment (Kuraishi and Muir, 1963). B995, a methyl derivative of succinamic acid has given indication, in tests using ^{14}C , of forming 1,1-dimethylhydrazide which inhibits tryptamine oxidation, a necessary step in the formation of IAA from tryptophane (Reed et al., 1965).

A further possibility for the retarding activities is the competition with GA_3 at the site of GA_3 action. This theory, however, was disproven by information presented above (Cleland, 1965; Paleg et al., 1965).

The problem of how the retardants exert their effects is thus obviously complicated by the fact that the same retardants exert varying effects in different plants and tissues in differing concentrations and also by the fact that some retardants manifest no effect at all in certain systems.

Some experimentation has been performed using the same tissue as the experimentation presented here, i.e. tissue of tissue cultures of pith cells of Nicotiana tabacum L., variety Wisconsin No. 38 (Murashige, 1965). Results of this experimentation revealed that retardation induced by Amo-1618, CCC and B995 was not reverted by saturating doses of GA_3 . This is contrary to the results of this presentation. However, the retardants in the work of Murashige were autoclaved and this definitely reduces the inhibitory effects of the chemicals and possibly also destroys them, thus making the results of the previous experimentation questionable.

In the light of the previous experimentation with these retardants plus the present work, two different modes of inhibitory action are apparent; that of B995 and that of CCC and Amo-1618. A theoretical scheme of interactions is proposed in Table III. CCC and Amo-1618 are known to act primarily in the depression of GA_3 biosynthesis (Jones, 1968; Verbiscar et al., 1967; Bennet et al., 1967). Also several workers have propounded the theory that GA_3 suppresses the activity of the IAA oxidase system (Linser, 1966; Leopold, 1964; Havelly, 1963; Das and Shome, 1965). Theoretically, then, a decrease in GA_3 biosynthesis would result in an increase in IAA oxidase activity, a decrease in the amount of functional IAA and a decrease in growth. This proves true experimentally in that an addition of exogenous



BIOCHEMICAL SITES OF ACTION OF GROWTH RETARDANTS

TABLE III

GA_3 overcomes the inhibition in the dark (figures XI and XIII). The lessened response in the light is not clear as GA_3 has been shown to reduce light inhibition (Das and Shome, 1965; Lockhart, 1956).

B995, also included in Table II, is generally cited as suppressing the conversion of tryptamine to indoleacetaldehyde (Reed et al., 1965). In this case the overall effect would be the decrease in the amount of IAA. Therefore, theoretically B995 inhibition should be reverted by GA_3 because of its suppression of IAA oxidase. This was shown true experimentally (figure XII). Light made little significant difference in B995 retardation except in the presence of a high concentration of GA_3 . Others have proposed that GA_3 removes the light inhibition of growth (Leopold, 1964).

The O-1 tissue presents a different picture. While it was impossible to obtain consistent results with this tissue it must be remembered that this tissue is highly sensitive to the presence of exogenous ions which trigger many metabolic processes (Braun and Wood, 1961; Wood and Braun, 1965). The auxins and cytokinins needed for rapid growth of the tissue are produced endogenously by the tissue itself (Schaeffer and Smith, 1963), in amounts varying with the environmental conditions. It is possible that the size of the transplants and the exact age of the tissue being transplanted were important factors and while the size was kept within a definite range the age did vary. Also changes could take place within the tissue itself making it more or less sensitive to the presence of the GA_3 and the retardants.

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LITERATURE CITATIONS

- Baldev, B. and A. Lang. 1965. Control of flower formation by growth retardants and gibberellin in Samolus parviflorus - a long-day plant. Amer. J. Bot. 52(4):408-417.
- Baldev, B., A. Lang and A. O. Agatop. 1965. Gibberellin production in pea seeds developing in excised pods: Effect of growth retardants Amo-1618. Sci. 147:155-157.
- Bennett, R. D., E. P. Lieber and E. Heftmann. 1967. Biosynthesis of steviol from (-)-kaurene. Phytochem. 6(8):1107-1110.
- Braun, A. C. 1955. The activation of two growth-substance systems accompanying the conversion of normal to tumor cells in crown gall. Cancer Res. 16:53-56.
- Braun, A. C. and H. N. Wood. 1961. Studies on the regulation of certain essential biosynthetic systems in normal and crown gall tumor cells. Proc. Nat. Acad. Sci. 47(12):1907-1913.
- Brian, P. W., J. F. Grove and T. P. C. Mulholland. 1967. Relationships between structure and growth promoting activity of the gibberellins in four test systems. Phytochem. 6(11):1475-1699.
- Cathey, H. M. 1964. Physiology of growth retarding chemicals. Ann. Rev. Plant Physiol. 15:271-299.
- Cleland, R. E. 1965. Evidence on the site of action of growth retardants. Plant and Cell Physiol. 6(1):7-15.
- Das, T. M. and A. Shome. 1965. The role of gibberellin and iron in photo-inhibited growth processes in isolated rice tissue. (Printed in White, P. R., Proc. Int. Conf. Plant Tissue Culture. 1965).
- Fox, J. E. 1962. A kinetin-like substance in habituated tobacco tissue. Plant Physiol. 37(Supple.)35.
- Fox, J. E. 1963. Growth factor requirements and chromosome number in tobacco tissue cultures. Physiol. Planta. 16:793-803.
- Gautheret, R. J. 1934. Culture of cambial tissue. Compt. Acad. Sci. 198:2195-2196.
- Gautheret, R. J. 1939. Compt. Acad. Sci. (Paris) 208:118-120.
- Halevy, A. H. 1963. Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. Bull. Res. Council of Israel.

- Harada, Hiroshi and A. Lang. 1965. Effect of some (2-chloroethyl) trimethylammonium chloride analogs and other growth retardants on gibberellin biosynthesis in Fusarium moniliforme. Plant Physiol. 40(1):176-183.
- Jones, K. C. 1968. Time of initiation of the barley endosperm response to GA_3 , GA_4 and Kaurene. Planta. 78(4):366-374.
- Jones, R. L., and I. D. J. Phillips. 1967. Effect of CCC on gibberellin content of excised sunflower organs. Planta. 72:53-59.
- Kuraishi, S. and R. Muir. 1963. Mode of action of growth retarding chemicals. Plant Physiol. 38(1):19-24.
- Kurosawa, E. 1926. Experimental studies on the secretion of Fusarium heterosporum on rice plants. Trans. Natur. Hist. Soc. Formosa. 16:213-227.
- Leopold, A. C. 1964. Plant growth and development. Mc-Graw-Hill Book Company. New York.
- Letham, D. S. 1963. Zeatin, a factor inducing cell division isolated from Zea mays. Life Sci. 8:569-573.
- Linser, H. 1966. The hormonal system of plants. Angew. Chem. 5(9):776-784.
- Linsmaier and Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Planta. 18:100-127.
- Lockhart, J. A. 1956. Reversal of light inhibition of pea stem growth by the gibberellins. Proc. Nat. Acad. Sci. 42:441-448.
- Miller, C. O., F. Skoog, M. Von Salitza, F. Strong. 1955. Kinetin, a division factor from deoxyribonucleic acid. J. Am. Chem. Soc. 77:1392.
- Murashige, T. 1965. Effects of stem elongation retardants and gibberellin on callus growth and organ formation in tobacco tissue culture. Physiol. Planta. 18:665-672.
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Planta. 15:473-497.
- Nobecourt, P. 1939. Compt. Rend. Soc. Biol. (Paris). 130:1270.
- Paleg, L., H. Kende, H. Ninneman and A. Lang. 1965. Physiological effects of gibberellin acid. VIII growth retardants on barley endosperm. Plant Physiol. 40(1):165-169.
- Reed, D. J., Moore and Anderson. 1965. Plant Growth Retardant B995: A possible mode of action. Sci. 148:1469-1471.

- Reid, D. M. and D. J. Carr. 1967. Effects of a dwarfing compound, CCC, on the production of GA like substances by root systems. Planta. 73:1-11.
- Riddell, J. A., H. A. Hageman, C. M. J. Anthony and W. L. Hubbard. 1962. Sci. 136:39.
- Sachs, R. M. and M. A. Wohlers. 1964. Inhibition of cell proliferation and expansion in vitro by three stem growth retardants. Amer. J. Bot. 51(1):44-48.
- Schaeffer, G. W. and Smith, H. H. 1963. Auxin-kinetin interaction in tissue cultures of *Nicotiana* species and tumor-conditioned hybrids. Plant Physiol. 38(3):291-297.
- Skoog, F. C. and C. Tsui. 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultures in vitro. Amer. J. Bot. 35:782-787.
- Stowe, B. B., F. H. Stodola, T. Hayoshi and P. W. Brian. 1961. Fourth Annual Conference of Plant Growth Regulators. Iowa State University Press. Iowa.
- Tolbert, N. E. 1960. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. J. Biol. Chem. 235(2):475-479.
- Veröiscar, A. J., G. Cragg, F. A. Geissman and B. O. Phinney. 1967. Studies on the biosynthesis of gibberellins II. Phytochem. 6(6):807-814.
- Weis, J. S. 1964. A Study of Naturally Occurring Auxins in Autonomous Tobacco Callus Tissue. Doctoral Dissertation. University of Kansas.
- Weis, J. S. 1967. Auxin inactivation by normal and derived tobacco tissue cultures. Advanc. Frontiers Plant Sci. 18:155-162.
- Wirwille, J. W., and J. W. Mitchell. 1950. Bot. Gaz. 111:491-94.
- Wittwer and Tolbert. 1960. 2-chloroethyl trimethylammonium chloride and regulated compounds as plant growth substances. Plant Physiol. 35(1):
- White, P. R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. 9:588-600.
- White, P. R. 1939. Potentially unlimited growth of excised plant callus in an artificial nutrient. Amer. J. Bot. 26:59-64.
- White, P. R. 1963. The cultivation of plant and animal cells. The Ronald Press. New York.
- Wood, H. N. and A. C. Braun. 1965. Studies on the net uptake of solutes by normal and crown gall tissues. Proc. Nat. Acad. Sci. 54(6):1532-1538.

THE EFFECTS OF AMO-1618, B995 AND CCC ON THE GROWTH OF NORMAL
AND DERIVED TISSUES OF NICOTIANA TABACUM L. AND THEIR
INTERACTION WITH AUXIN AND GIBBERELLIN

by

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AN ABSTRACT OF A MASTER'S THESIS

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Tissues which have been isolated from the pith of Nicotiana tabacum L. var. Wisconsin No. 38 may be grown in vitro on a medium containing mineral salts, vitamins, sucrose, indole-3-acetic acid and kinetin. One strain of tissue (O-1) derived from the normal tissue (KX-1) shows complete autonomy for exogenous auxin and kinetin. Both (O-1) and (KX-1) tissues were used in an attempt to determine the biochemical sites of action of three growth retardants, namely, Amo-1618, B995 and CCC.

Experiments were conducted in which tissues were grown on the basal medium containing designated concentrations of indole-3-acetic acid and/or gibberellic acid and $10^{-5}M$ concentrations of the individual retardants for a period of four weeks after which fresh and dry weights were determined and compared.

Amo-1618 and CCC are known to impede the biosynthesis of gibberellic acid from kaurene and experimental results show that an addition of exogenous gibberellic acid overcomes the growth inhibitory response to these respective retardants.

The retarding activity of B995, thought to be a suppression of the conversion of tryptamine to indoleacetaldehyde, a precursor of indole-3-acetic acid, was also overcome by additional gibberellic acid. This verifies the above mentioned theory.