INTERACTIVE EFFECTS OF COUMARIN, DITHIOOXAMIDE, ASCORBIC ACID AND AUXINS UPON THE EARLY DEVELOPMENT OF ROOTS

By

Jacques Marie Alamercery

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science

in partial fulfillment of the requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

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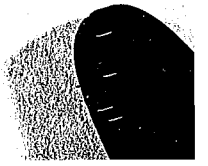

INTERACTIVE EFFECTS OF COUMARIN, DITHIOOXAMIDE, ASCORBIC ACID AND AUXINS UPON THE EARLY DEVELOPMENT OF ROOTS

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AN ABSTRACT

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Approved Charles L. Hamner

Jacques M. Alamercery

The interactive effects of dithiooxamide and of ascorbic acid upon the development of roots and its inhibition by coumarin and by the "auxins" 2,4-D, indole-acetic acid and naphthalene-acetic acid have been investigated in Petri dishes under controlled conditions of light and of temperature, with cucumber for root-elongation and lettuce for germination percentage.

Treatments of dithiooxamide which were stimulative in light were strongly inhibitive in darkness for the elongation of cucumber roots; no stimulation has been observed in darkness; the inhibitions induced by higher concentrations of dithiooxamide, whether in light or in darkness, were not considerable before the third day after that germination had started. Dithiooxamide was able to increase the early low rates of lettuce germination in light at 29°C., or in darkness at 25°C.. Ascorbic acid alone did not produce any significant effects on the development of roots at the concentrations which were used (below 2,000 ppm).

Ascorbic acid was able to reinforce synergistically or to reduce the inhibitions of cucumber root elongation induced by dithiooxamide, depending upon the conditions of illumination, the procedure of testing (simultaneous or successive applications of the chemicals) and the order and the length of the treatments, in the case of successive applications of the chemicals.

Both dithiooxamide and ascorbic acid were able to reduce the inhibition of cucumber root elongation induced by coumarin, in light only in the case of dithiooxamide, in both light and darkness in the case of ascorbic acid. A similar reduction of the inhibition of lettuce germination induced by coumarin was produced by both chemicals, in light and

darkness by dithiooxamide and in light only by ascorbic acid in the case of simultaneous applications with coumarin throughout the experiments; for both chemicals, it took place in darkness only, when they were separately applied after a presoaking of the seeds in a solution of coumarin alone in darkness (case of an artificial dormancy). Ascorbic acid was more potent in the case of cucumber, dithiooxamide was more potent in the case of lettuce.

Both dithiooxamide and ascorbic acid were able to reduce the inhibitions of cucumber root elongation induced by the "auxins" 2,4-D, indole-acetic acid, and naphthalene-acetic acid, both in light and in darkness. In every case, ascorbic acid was more potent than dithiooxamide.

As a tentative interpretation of the interactive effects of dithiooxamide, ascorbic acid, and coumarin, a natural biological system is outlined in which -SH groups and coumarin derivatives may be independently interrelated with the metabolism of Vitamin C in plants, directly or indirectly through the metabolism of -SH enzymes. Two phases of this system appear likely to be interfered with by the "auxins".

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INTRODUCTION

For the past ten years, investigations in the field of plant-growth-regulators have yielded spectacular results, chiefly because of the outstanding properties of a group of synthetic substances, of which 2, 4-dichlorophenoxyacetic acid (2, 4-D) is the best representative. However, up to the present time, little is known concerning the physiological processes which account for this chemical control of growth; also little is known regarding the mechanism by which indole-3-acetic acid (IAA) induces similar but less striking effects.

During the same time, a less spectacular but nevertheless steady advance was achieved in studies dealing with the influence upon plant-growth of the chemicals containing an unsaturated lactone ring; many of those, such as coumarin and some of its derivatives are able (143) both to promote or to inhibit growth at very low concentrations. Meanwhile, it was found that many of these compounds were naturally occurring substances, and some evidences of a relationship between them and compounds containing -SH groups was established.

It is only recently that Van Overbeek (153) put forward the idea that an accumulation of coumarin derivatives in plant tissues might result from the applications of the "auxins", 2, 4-D, naphthalene-acetic acid (NAA), IAA, and account for at least part of their growth-regulating activity.

The initial scope of this work was to investigate the antagonism between some sulfhydryl derivatives and some unsaturated lactones (chiefly coumarin) which induced inhibitions of plant growth, and then to test the possibility that similar antagonisms exist between sulfhydryl derivatives and the "auxins". However, after the discovery of the outstanding properties of dithiooxamide (DTO), theoretical considerations led to think that the effects of DTO might be the result of an interference with the metabolism of ascorbic acid (ASA), (part I). Therefore, the interactions of ASA with coumarin and with DTO were then extensively studied (part II). This provided another tool for testing the analogies between coumarin and auxins by the investigation of the interactions of ASA, as well as of DTO, with 2, 4-D, NAA, and IAA, (part III).

Since the conditions of light were known to be important factors for the growth activity of coumarin, and for the amount and distribution of ASA and of IAA in plant tissues, the great majority of this experimental work has been carried out in varied conditions of illumination.

MATERIALS AND METHODS

A simple and rapid quantitative method was desirable, to determine the effects upon growth of a number of solutions of chemicals. The cucumber test, as used by Ready et al. (125) and germination tests seemed to have both qualities, and were extensively used in this laboratory for screening out a great number of chemicals suspected of having herbicidal properties.

Seeds of cucumber (Cucumis sativus, var. Marketer and Burpee Hybrid) and lettuce (Lactuca sativa, var. Grand Rapids), were used. The seeds were placed in Petri dishes impregnated with a solution of the chemical under investigation. The percentage of germination of lettuce seeds, and the length of the main root of cucumber seeds were found to be good indicators of plant response. A few experiments using the length of the longest root of wheat seeds were also performed.

Throughout the experiments, Petri dishes 100 mm in diameter and, except for the very first experiments, filter paper No 501 from E. H. Sargent and Co. were used.

A. Root Elongation Tests.

The first tests were made with seeds of the Marketer variety of cucumbers, which had been in the laboratory for several years, and were quite satisfactory. However, other samples of the same variety were damaged and unreliable; therefore, the variety Burpee Hybrid was used in later experiments, the seeds of which were

uniform and gave constant results.

For the few tests conducted with wheat seeds (Triticum vulgare) the variety Henry was used. The age of this material is unknown.

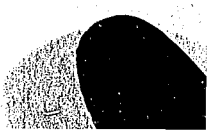
1. Tests using constant solutions.

In these tests, the seeds remained in the original solution until completion of the experiment, that is until the measurements were taken.

The first tests were conducted in tap water, by placing fifteen seeds in a solution of five milliliters of chemical in tap water in a Petri dish with one layer of filter-paper, and allowing them to germinate and grow several days in the laboratory without any special precaution for temperature (fluctuating rather widely around 25° C.) or light.

In these first experiments, it was observed (Appendices 1-A, 1-B, 1-C, and 1-D) that the normal fluctuations of the rate of germination made it difficult to compare quantitatively the effects of the various treatments: the differences had to be large in order to be significant and it also appeared that variations from test to test might be due to variations in the composition of tap water.

Therefore, distilled water was then used for all the solutions and, in an effort to make the test more accurate, the number of seeds for each treatment was increased to 48 (three Petri dishes with sixteen seeds each). Care was taken that the seeds were distributed on the filter-paper uniformly.



All Petri dishes had been previously cleaned together, first by soaking overnight in an activated charcoal suspension, then rinsed and washed with soap, then rinsed again with tap water and then with distilled water. Because of this uniform cleaning, each set of the three Petri dishes was considered as a unit.

After the seeds had been allowed to germinate and grow for a given length of time, the length of each primary root was measured to the closest millimeter with a standard college ruler, and recorded. The measurements of treatments to be compared were always performed in such a way that there was no significant difference in growth during the time of measuring(Usually from 3 to 5 sets of three Petri dishes could be measured in one hour of time).

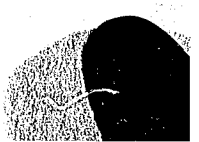
In an attempt to account for an unavoidable proportion of seeds germinating later than the bulk of the seeds, which would induce wide fluctuations in root length, it was decided to consider only the average of the thirty highest figures out of the 48 root lengths recorded for each treatment. It was observed that only small variations from dish to dish occurred within a same treatment in most experiments. If one Petri dish would give an average exceedingly different from the two others in the same set, it was eliminated, and an average based on the 20 highest figures of the two other Petri dishes was used.

The significance of the differences between averages of two treatments was statistically tested by the t test when it was questionable (116). Detailed data and statistical analyses of a few such experiments have been given (Appendices with roman figures) so that the accuracy of the test can be evaluated. Usually, probability higher than 0.99 and 0.95 were obtained for a difference between treatments of 10% and 7% respectively.

The same technique was used in tests with wheat seeds, but experimentation with this material was soon discontinued because of frequent fungus infestation. All experiments reported here are fungus-free.

2. Tests using transfers from a solution to another solution.

Because some of the effects observed in the experiments conducted according to the technique previously decribed might have been caused by a chemical reaction within the solution, making part of one chemical unavailable to the plant, a new technique was designed in which seeds were first germinated in a solution of one of the chemicals under investigation, and then rinsed in distilled water and transferred to a solution of another chemical; in this way, the two chemicals could not react together before getting into the plant.



For each treatment, three Petri dishes containing 20 seeds each received a solution of the first chemical (pretreatment). After a given time, all the Petri dishes receiving a same pretreatment were gathered and 16 of the best germinated seeds from each dish were selected and all put together in the same cheesecloth, rinsed in distilled water according to a standardized procedure, and then transferred to Petri dishes containing the solution of the second chemical (post-treatment), on the basis of three Petri dishes containing 16 seeds each for each post-treatment. Therefore, for all seeds receiving a same pretreatment, the post-treatment was assumed to be the only different factor. A similar procedure was used for all other pretreatments in the same experiment.

All the solutions were made of distilled water, all the operations of rinsing and transferring were done in the controlled room and in light, so that for experiments reported as performed in continuous darkness, and interruption of the dark conditions could not be avoided. This interruption never exceed 20 minutes.

The roots were then measured and averaged in the way previously described.

B. Germination Tests

All the germination tests were done with black lettuce seeds, variety Grand Rapids. Two samples were used; the first one was two years old, whereas the second one was received in June 1951.

In all these experiments, distilled water was used. In order to avoid the floating of the small lettuce seeds over the solution, three layers of filter-paper were used instead of one only. The procedure was first to select a number of lots of 50

or 100 seeds each, then to place the three filter-papers in every dish and to moisten them with five milliliters of the solutions under investigation. The seeds were then rapidly scattered all over in the dish, and immediately put under the environmental conditions to be applied.

In the tests conducted in continuous or alternate illumination the germinated seeds were removed from the dishes every 24 hours, counted and the percentage of germination up to this day was computed. For the tests in darkness, no counting of seeds was done until the end of the first dark period in order to prevent illumination for even a very short period, (because lettuce seeds treated with coumarin appear to be sensitive to a very short period of illumination), after this time, the Petri dishes were moved to an illuminated table and removal and counting of the germinated seeds were then performed in the way previously described.

An experiment was also conducted according to the method of pretreatment used by Nutile (112). A number of seeds were soaked for 24 hours in a coumarin solution (25 ppm) rinsed in distilled water and dried for 4 days in an oven at 43° C., all these operations being done in perfect darkness. After 17 days in a dark drawer in the laboratory, they were put in lots of 50 seeds each and then germinated according to the usual process in various solutions.

C. Environmental Conditions.

Except for those in which Marketer cucumber seeds were used, all the experiments were conducted in a special room where light and temperature conditions were controlled.

Temperature was maintained at 25° C., and there were certainly not more than 1° C. fluctuations. Unless otherwise stated, all the experiments were carried out on a bench receiving from 130 in the center to 85 foot-candles on the edges of a light which was 70% fluorescent and 30% incandescent. Because of this light, the actual temperature on the bench was 26° C.; Petri dishes were so grouped that all treatments received about the same intensity of light.

For experiments in alternate illumination, the Petri dishes were placed on the same illuminated bench, but were covered twelve hours a day with dark paper and cardboard.

For experiments in darkness, the Petri dishes were placed in a drawer and covered with cardboard and dark paper immediately after the solutions were poured into the dishes. For most experiments, treatments were simultaneously applied both in light and darkness; in these cases, we prepared only one solution for each treatment, which was then poured into several sets of three Petri dishes to be placed in the various environmental conditions. The often observed differences of the responses in light and darkness were then a proof that the results were not due to indidental mistakes in the making of the solutions, since the same solution would induce different responses according to the environmental conditions.

The experiments with Marketer cucumber seeds were done in the laboratory without any special attention to light and temperature conditions. For those of these tests which were performed in continuous light, the illumination by night was provided by a few fluorescent tubes giving an intensity of about 300 foot-candles.

There was no control of moisture, but it can be assumed that the atmosphere in the Petri dishes was pretty close to saturation; distilled water was added when necessary in order to keep the filter-papers in a uniformly moist condition.

In the experiments with coumarin and/or DTO, it was observed that the addition of these chemicals had practically no influence on the pH, which would not be changed by more than 0.2 by the highest concentration of either one. For a number of either one. For a number of experiments with ASA, it was necessary to use solutions of low pH in order to have about the same acidity in all the treatments. Accordingly, several Sorensen's buffer solutions were tried, which gave strong inhibitions of growth (see for instance the difference between controls of experiments 24-A and 24-B). The simplest and least harmful way to get the desired pH was to put a drop of dilute sulfuric acid (chemical grade), or 0.05 ml., for each 500 ml. of distilled water. Such a solution was then used for the making of all the chemical solutions of the test and for the controls. (see for instance experiments 24-A and 24-C for comparison of the controls).

The pH of the solutions were thus adjusted as follows:

Distilled water	6.1
Acid water	3.5
ASA 250 ppm	3.5
ASA 500 ppm	3.4
ASA 1000 ppm	3.25
ASA 2000 ppm	3.1

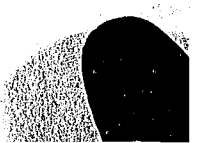
The other chemicals did not induce any significant change in pH at the concentrations which were used.

PART I

DITHIOOXAMIDE

GROWTH-REGULATING ACTIVITY

ANTAGONISM TO COUMARIN



I. INTRODUCTION

In the course of routine experiments carried out in this laboratory, an investigation of the phytocidal activity of coumarin derivatives was started. Because these substances have an unsaturated lactone structure which may react with -SH groups, it was an attractive idea to investigate also some sulfhydryl derivatives; therefore a few of those were selected which, for chemical reasons, appeared most likely to exhibit growth-regulating properties. Out of ten such chemicals, the screening tests showed that only thio-beta-naphthol and, chiefly, dithiooxamide (DTO) were most promising.

Accordingly, the investigation became centered around DTO which was suspected of being a possible antagonist to coumarin.

A. Review of Literature

1. Unsaturated Lactones

Effects on Plant-growth

Klebs (83) seems to have been the first worker to report upon the effect of coumarin application to plants, when he observed its inhibitive activity for the growth of Algae. Even though Cameron (28), in 1910, reported a similar effect of coumarin and of daphnetin for the growth of wheat, it was not until 1943 that the work of Kuhn *et al.* (87) definitely called the attention of plant students

upon unsaturated lactones. These workers demonstrated the inhibitions of germination (cress seeds) and pollen tube development (Antirrhinium) caused by coumarin, and showed that parasorbic acid (synthetic or extracted from malt) had a similar activity. The same year, Veldstra et al. (154) confirmed and extended these results to a few other unsaturated lactones, and some benzo-coumarins.

Since then, compounds of this series have received increasing attention from plant scientists, chiefly as regards their effects on germination and root development. Nutile (112) investigated the artificial dormancy induced by coumarin in lettuce seeds and the influence of light thereupon, pointing to its likeness with natural dormancy. Similar effects were later shown by Weintraub (163) to be also produced by a number of other chemicals, including a few unsaturated lactones, but to a much lesser degree. Cornman (39, 40), treating onions and lily roots with saturated aqueous solutions of coumarin, produced within a few hours a blocking of all mitoses resembling the effect of colchicine, whereas parasorbic acid only slowed down mitosis, causing an accumulation of metaphases in onion roots. Similar activity of protoanemonin has been reported by Erickson et al. (51). Haynes et al. (74) synthesized several alpha-beta-unsaturated lactones which also inhibited the germination of cress, and stressed their outstanding light-absorption capacity. Ciferri et al. (36) indicated that seeds of various species would become photosensitive after treatment with coumarin. Accordingly Lavollay et al. (92), aesculin (6-glucoxy-7-hydroxy-coumarin) presents growth-regulating properties resembling those of IAA in their effects upon germinating peas. Audus et al. (8) observed

a significant herbicidal activity of coumarin, and Audus (6), working with pea and cress, found the first species to be more sensitive to this chemical which, at high concentrations, hastened maturation processes in root meristems.

Dicoumarol, the natural substance responsible for the sweet-clover hemorrhagic disease (29), has been investigated by Marx et al. (101), and found to be a powerful inhibitor of germination, about three times as effective as coumarin itself, but similar investigations of a number of coumarin derivatives failed to show any good correlation betweeen prothrombin inhibition and blastokolin activity.

As for scopoletin, a coumarin derivative widely occurring in plant tissues, which can be determined by fluormetric procedures, Best (15, 16, 17) showed that in tobacco plants its concentration falls off during the rapid growth stages, and reaches its maximum at time of maturity. In plants infested with spotted wilt it occurs at greater concentration than in healthy plants, though with the same relative distribution. Andreae et al. (3, 4) detected similarly the blue fluorescence of scopoletin in tubers and leaves of potatoes infested with leafroll virus, whereas healthy plants did not seem to accumulate any of it. Goodwin et al. (59) observed its fluorescence in five days old Avena roots, and estimated its concentration to be about 10 ppm; this amount varied in different parts of the roots, and since it possesses growth-inhibiting properties, (61), they suggested that its relatively lower concentrations in the most actively growing parts of the roots might be of biological significance.

Scopoletin is not the only coumarin derivative to be present in plants, and lists of naturally occurring coumarins and unsaturated lactones have been given by Goodwin et al. (60) and Geismann (56). Goodwin et al. (61), having further investigated the influence of coumarin derivatives upon root development in Avena, and found only a few of those to be strong inhibitors. The latest known advances in the study of unsaturated lactones have been made in this laboratory, with the discovery of Hamner et al. (67) that beta-methyl-umbelliferone was a selective growth-inhibitor for higher plants and fungi, and with the finding of Alamercery et al. (2) and Hamner et al. (68) of the selective inhibition of chlorophyll by a tetronic acid derivatives.

b. Interactions with sulfhydryl groups.

The first clue regarding the mechanism of action of unsaturated lactones was not to be found in the botanical field. Aside of their effects upon plant growth, it had been known for some time that a number of them possess bacteriostatic properties. In 1944, Cavallito et al. (32) discovered that cystein was able to antagonize the inhibitions of bacterial growth induced by some of them. Similar antagonistic capacity of cysteine was also demonstrated for the growth-regulating activity of hexeno-lactone by Hauschka et al. (71) and the bacteriostatic activity of a number of alpha-beta-unsaturated ketone by Geiger et al. (55). Then, Cavallito et al. (33) gave evidences for the occurrence of a reaction between the -SH groups and the unsaturated lactone ring, probably of the type of the reaction studied by Posner (120) in 1902.

Even before these results were known, Nutile (112) observed that thiourea was able to break the artifical dormancy induced by coumarin in lettuce seeds (as well as the natural dormancy). A few other evidences of interaction of sulfhydryl compounds with unsaturated lactones were then given, Lavollay et al. (93) reported an antagonism of thiourea to the inhibition of root development induced in barley seeds by coumarin. Thimann et al. (143), using Avena coleoptile and pea stem as plant material, found that protoanemonin and coumarin were able to promote or to inhibit growth according to the concentration used, and that some dithiols, and chiefly 2, 3-dimercaptopropanol (BAL), were able to prevent their inhibitive effects. Higher concentrations of BAL were inhibitory. Since they had previously shown (141, 142) that various inhibitors of -SH enzymes (arsenite, indo-acetate, para-chloro-mercury-benzoate) inhibited the growth of the same plant materials, they concluded that the two unsaturated lactones were probably able to attack the essential -SH groups of some enzymes.

2. Sulfhydryl Compounds : Effects on plant-growth.

It is well known that -SH groups are very important for biological processes. Barron (10) has recently given an excellent review of the "Thiol groups of biological importance". They seem to be essential for many enzymes. Hammett (65, 66) claims that the -SH groups of amino- and nucleic-acids accelerate the rate of increase in the number of cells, and that their partial oxidation retards the production of new cells.

It is therefore not surprising that some sulfhydryl derivatives exert some influence upon plant growth. The chemical first and most often tested seems to be thiourea, which has been found in free state in Laburnum (84). Thompson et al. (148, 149) discovered that it was outstanding for breaking the dormancy of lettuce seeds. Allyl-thiourea, thiocyanate, thio-semicarbazide, thioacetamide were less effective; the presence of sulfur was essential for the activity of these compounds. Similarly, thiourea has been reported to hasten and increase the germination and sprouting of Gladiolus (132), but reduces that of several alpine plants (54). Thompson et al. (147) found that it was able to counteract the detrimental effect of too high a temperature for the germination of lettuce. No abnormality resulted of its use. Working with endives seeds in similar conditions, he also demonstrated (146) a similar improvement of germination; but he observed that, in some cases, presoaking with water alone and then drying was almost as beneficial as the same treatment with a solution of thiourea which, however, was much more effective in most cases. Tukey et al. (150) succeeded also in breaking dormancy of peach seeds, with the same substance, but observed some physiological changes: dwarf seedlings, short internodes, abnormal leaves.

Other sulfhydryl compounds have also been tested for their plant-growth activity. Out of a large number of those, Brian et al. (24) found that only thioacetic acid and methyl dithiocarbamate were very toxic to wheat seeds. Dithiobiuret (49) seems to exert various effects upon germination and root growth.

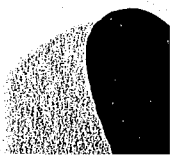
Both gluthathione (42) and cysteine (43) have been reported by Davis to promote healing of wounds, and glutathione (64) has been found to shorten the dormancy of potato tubers; many other chemicals containing -SH groups are quoted by Avery et al. (9) as having the same property.

B. Problem

A common feature of most sulfhydryl compounds exhibiting some growth-regulating activity is the presence of an amino- or an imino-group close to the -SH group, (for instance thiourea, dithiocarbamates, cysteine, dithiobiuret, glutathione...). On the other hand, BAL, of which we have previously mentioned the growth activity and the antagonism to coumarin and protoanemonin (143), is a powerful enzyme inhibitor (162), which seems to be due to the disposition of the two -SH groups on two adjacent carbon atoms. Dithiooxamide combines these two important structural features, two -SH groups on adjacent carbon atoms, each one flanked by an imino-group, as shown by its more reactive tautomeric formula (see appendix, formula)

This chemical has never been extensively tested for biological activity. It came out unnoticed from screening tests for rodenticidal (46) goitrogentic (81), antivesicant (144), bacteriostatic (96, 128) activity; similarly, it was placed in the class of lowest activity after Thompson et al. (145) 's gigantic tests for herbicidal properties.

In spite of these unfortunate precedents, and because its chemical structure allowed to expect that it would exhibit both growth-regulating properties and antagonism to unsaturated lactones, it was decided to study it more thoroughly from these two viewpoints.



II. EXPERIMENTAL RESULTS

A. Preliminary Experiments with old Marketer Cucumber and Wheat seeds.

1. Screening out the most active chemicals.

As previously indicated the first tests with Marketer seeds were performed in tap water and under laboratory conditions. The results are given in tables I and II. Only a few of its derivatives, besides coumarin itself, appeared to be strongly effective in inhibiting root-elongation. Germination apparently takes place, but very soon root-elongation ceases and a bulbous enlargment becomes conspicuous (Fig. 2, 3, 4). At the lower concentrations, after a few days of a retarded elongation, chiefly the main root becomes watery and decayed, sometimes dries out, even though the lateral roots can be unaffected and even sometimes extremely developed.

Two sulfhydryl derivatives, thio-beta-naphthol and chiefly dithiooxamide, were the most effective for inhibition of root elongation. Both compounds were found to produce similar effects on root-development of Alaska peas and Yellow Globe radish, but experiments were continued only with cucumber seeds which are most sensitive. The growth of the seedling was retarded, but no formative effect or toxic symptom other than reduced growth was observed.

TABLE I

PERCENT GERMINATION AND GROWTH RATE OF MARKETER CUCUMBER SEEDS SOAKED IN TAP WATER SOLUTIONS OF VARIOUS COUMARIN COMPOUNDS

Name of Compound	Conc. in ppm	Percent Germination 3 days	Growth Rate	
			Percent of Control 3 days	mm. 3 days
Coumarin	5	97	94	23
	10	90	86	21
	50	70	80	20
	100	35	48	12
	200	30	41	10
	500	28	47	11
	1000	4	33	8
7-n-Propyloxy, coumarin, 3 carboxylic acid	100	90	40	10
7-n-Propyloxy coumarin	100	0	0	0
6,7-Dimethoxy-coumarin, 3-Ethyl-carboxylate	100	42	24	6
6,7-Dimethoxy-coumarin, 3-Carboxylic acid	100	25	8	2
6,7-Dimethoxy-coumarin	100	51	12	3
8-Methoxy-coumarin, 3-Carboxylic acid	100	61	16	4
8-Methoxy-coumarin	100	91	16	4
7-Methoxy-coumarin, 3-Ethylcarboxylate	100	58	20	5
7-Hydroxy-coumarin, 3-Ethyl-carboxylate	100	61	40	10
2,4,5-Trihydroxy Benzaldehyde	100	68	12	3
7-Hydroxy coumarin, 3-Carboxylic acid	100	88	20	5
Benzyl-Beta-Methyl-Umbelliferone	100	100	83	21
5,7-Dihydroxy 4-Methyl coumarin	100	100	41	10
5,7-Dihydroxy-dimethyl-coumarin	100	100	100	25
Control	--	97	100	25

TABLE II

PERCENT GERMINATION AND GROWTH RATE OF MARKETER CUCUMBER SEEDS SOAKED IN TAP WATER SOLUTIONS OF VARIOUS SULFHYDRYL CHEMICALS

Name of Compound	Conc. in ppm	Percent Germination 3 days	Growth rate	
			Percent of Control 3 days	mm. 3 days
2 - Mercaptobenzothiazole	50	98	80	20
	100	45	0	15
B - Dithiodiglycol	50	97	100	25
	100	46	32	8
2 - Thiobarbituric Acid	50	95	80	20
	100	50	32	8
Thioacetamide	50	97	100	25
	100	49	40	10
Thio - β - Naphthol	50	17	8	2
	100	0	0	0
2 - Mercaptoethanol	50	100	140	35
	100	50	80	20
Sodium Thiocyanate	50	97	100	25
	100	45	32	8
Dithiooxamide	50	0	0	0
	100	0	0	0
Acid Thioglycollic (Mercaptoacetic Acid)	50	97	80	20
	100	48	60	15
Thiourea	50	92	40	10
	100	48	32	8
Control --	97	100	25	

2. Effects of DTO on root elongation.

a. Old Marketer cucumber seeds.

with the same sample of old Marketer cucumber seeds which was used in the screening experiments, a study of the effects of various concentrations of DTO on elongation of roots was conducted. Several experiments were run, according to the technique of constant distilled water solutions. The detailed results are given in appendices I, II, 3-A, 3-B, 3-C, and 3-D. Significant promotions of root elongation were observed at low concentrations, whereas inhibition took place above 20 ppm. It was noted, in the case of strong inhibitions, that they could not be detected by eyes before the third day. A curve where elongation (in percent of the controls) is plotted against concentration is given in Fig. 1. Each point represents the average of the responses in the several experiments reported. It should be indicated here that the roots of most seedlings grown in DTO solutions had less root hairs, slightly less lateral roots, and were a little thicker and more ligneous than roots from water controls.

b. Wheat seeds.

A similar investigation was carried out with wheat seeds, the results of which can be found in appendices 2-B, 2-C, 2-D and 2-E. No indication of growth promotion was ever observed; a less abrupt curve of growth inhibition has been deduced from these data and plotted on the same graph than the corresponding curve for cucumber seeds (Fig. 1). The different responses of the two species are evident.

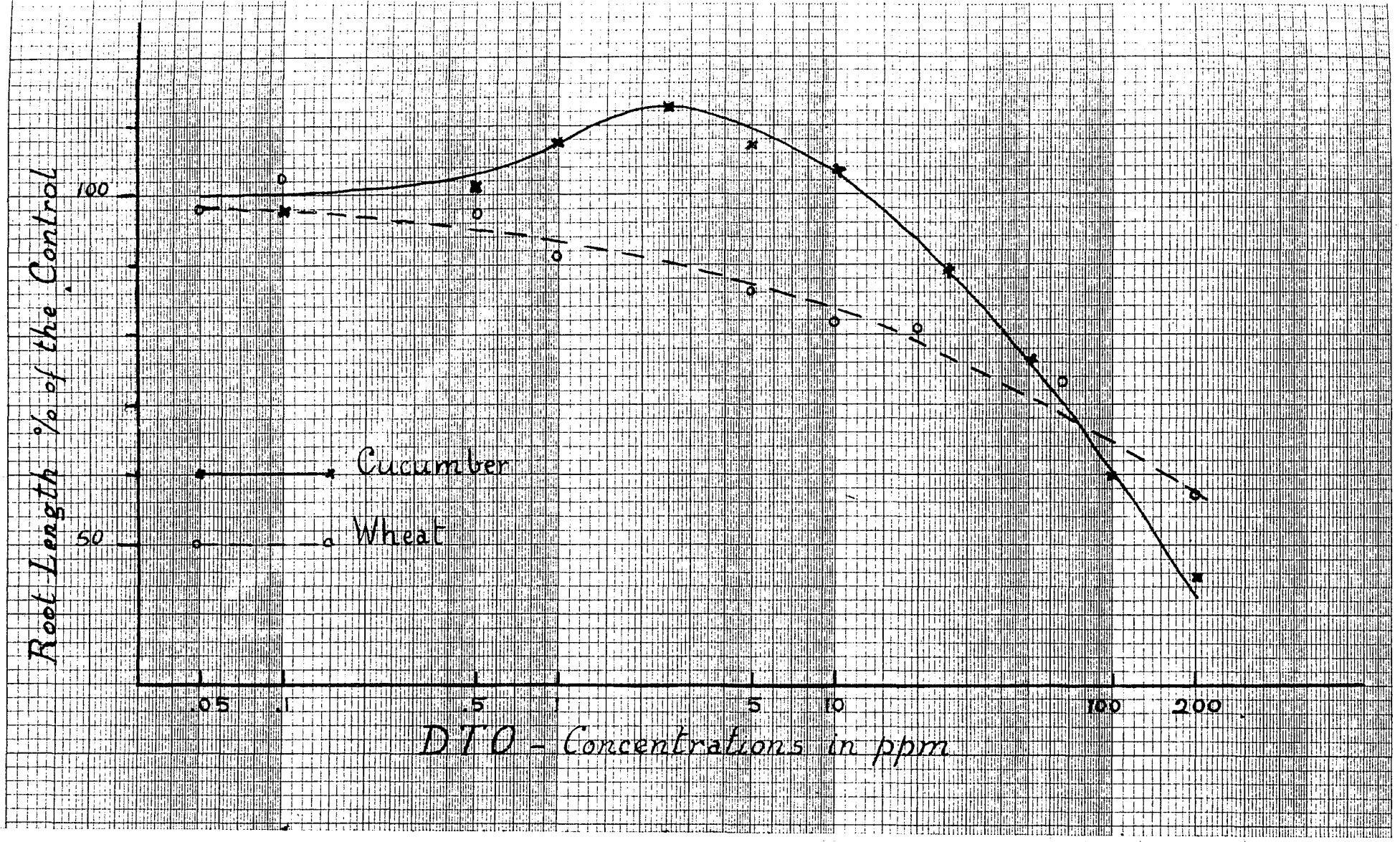


Figure 1. - Effects of distilled water solutions of Dithiooxamide (DTO) on the elongation of the roots of Marketer cucumber and of wheat (var. Henry). Semi-logarithmic scale.

3. Antagonism of DTO to Coumarin.

a. Old Marketer cucumber seeds.

Old seeds from the same sample of Marketer cucumbers, were used under similar conditions (constant solution technique), to investigate the effects of various mixtures of DTO and coumarin.

The first experiment, reported in appendix III was carried out in tap water, and with only one petri dish. After 3 days, the roots of the seeds receiving a mixed solution of coumarin at 150 ppm and DTO at 10 ppm, were clearly longer than those of the seeds receiving only the same concentration of coumarin.

A similar experiment was then run in distilled water, using the technique previously described (constant solutions) with three Petri dishes for each treatment. The roots were measured on the fourth day, and it can be seen in appendix IV and in Fig. 2, 3, 4 how strikingly the inhibitions of root elongation caused by 100 and 150 ppm of coumarin were reduced by low concentrations of DTO. This experiment was repeated with only the concentration of 150 ppm of coumarin and the same concentrations of DTO. Similar results were obtained and were as conspicuous; no picture or measurement was recorded this time. The same experiment was tried in continuous darkness, under similar conditions but no conspicuous difference was observed between seeds receiving coumarin alone and those receiving coumarin and DTO.

Fig. 2. Marketer cucumber seedlings grown in mixtures of Coumarin and Dithiooxamide for five days.

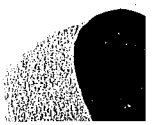
A. Coumarin 150 ppm.
B. Coumarin 150 ppm plus Dithiooxamide 1 ppm.
C. Coumarin 150 ppm plus Dithiooxamide 5 ppm.
D. Coumarin 150 ppm plus Dithiooxamide 7.5 ppm.
E. Coumarin 150 ppm plus Dithiooxamide 10 ppm.

Fig. 3. Marketer cucumber seedlings grown in mixtures of Coumarin and Dithiooxamide for five days.

A. Coumarin 100 ppm.
B. Coumarin 100 ppm plus Dithiooxamide 1 ppm.
C. Coumarin 100 ppm plus Dithiooxamide 5 ppm.
D. Coumarin 100 ppm plus Dithiooxamide 7.5 ppm.
E. Coumarin 100 ppm plus Dithiooxamide 10 ppm.

Fig. 4. Marketer cucumber seedlings grown in mixtures of Coumarin and Dithiooxamide for twelve days.

A. Coumarin 150 ppm.
B. Coumarin 150 ppm plus Dithiooxamide 7.5 ppm.
C. Coumarin 150 ppm plus Dithiooxamide 10 ppm.



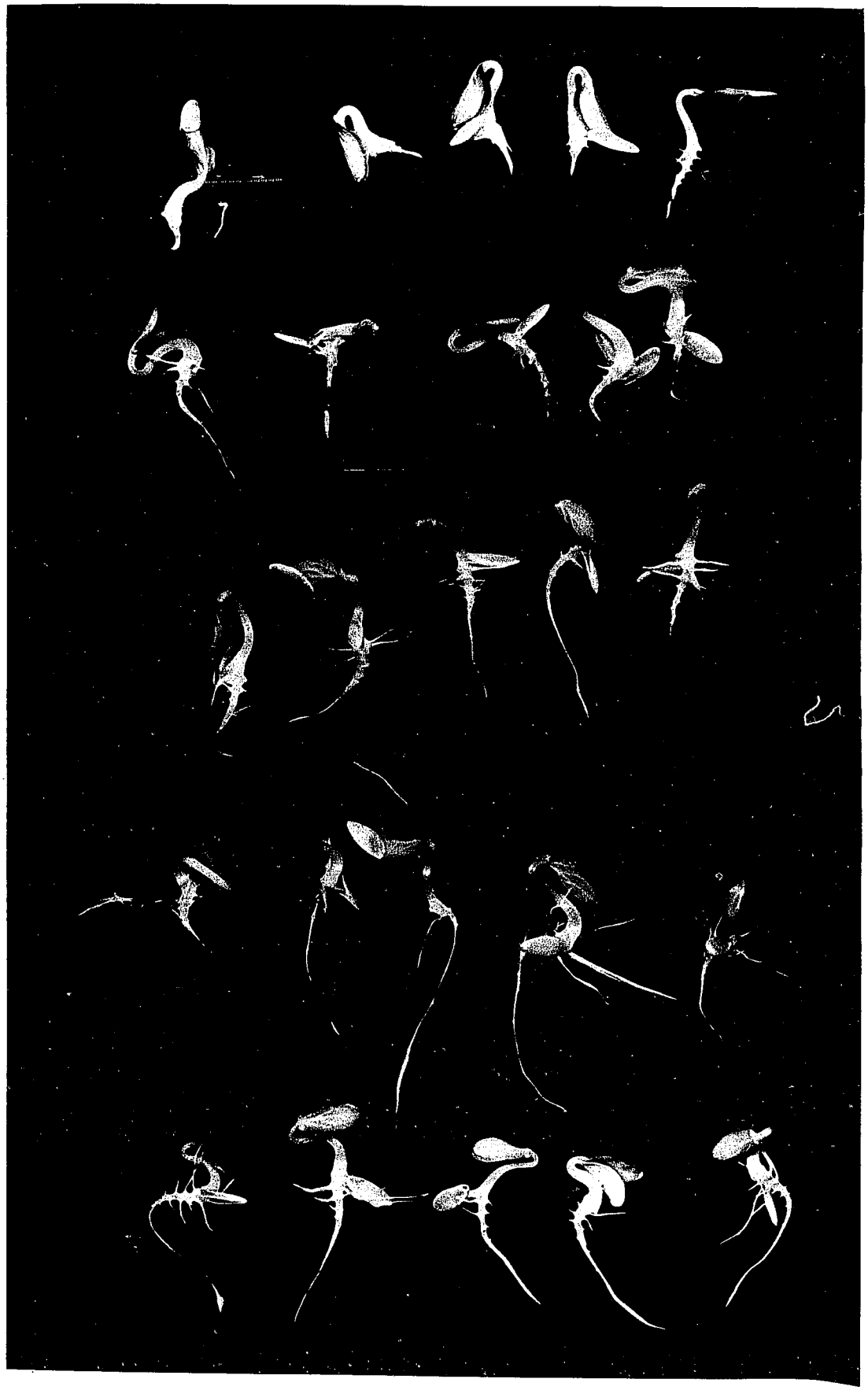


Fig. 2

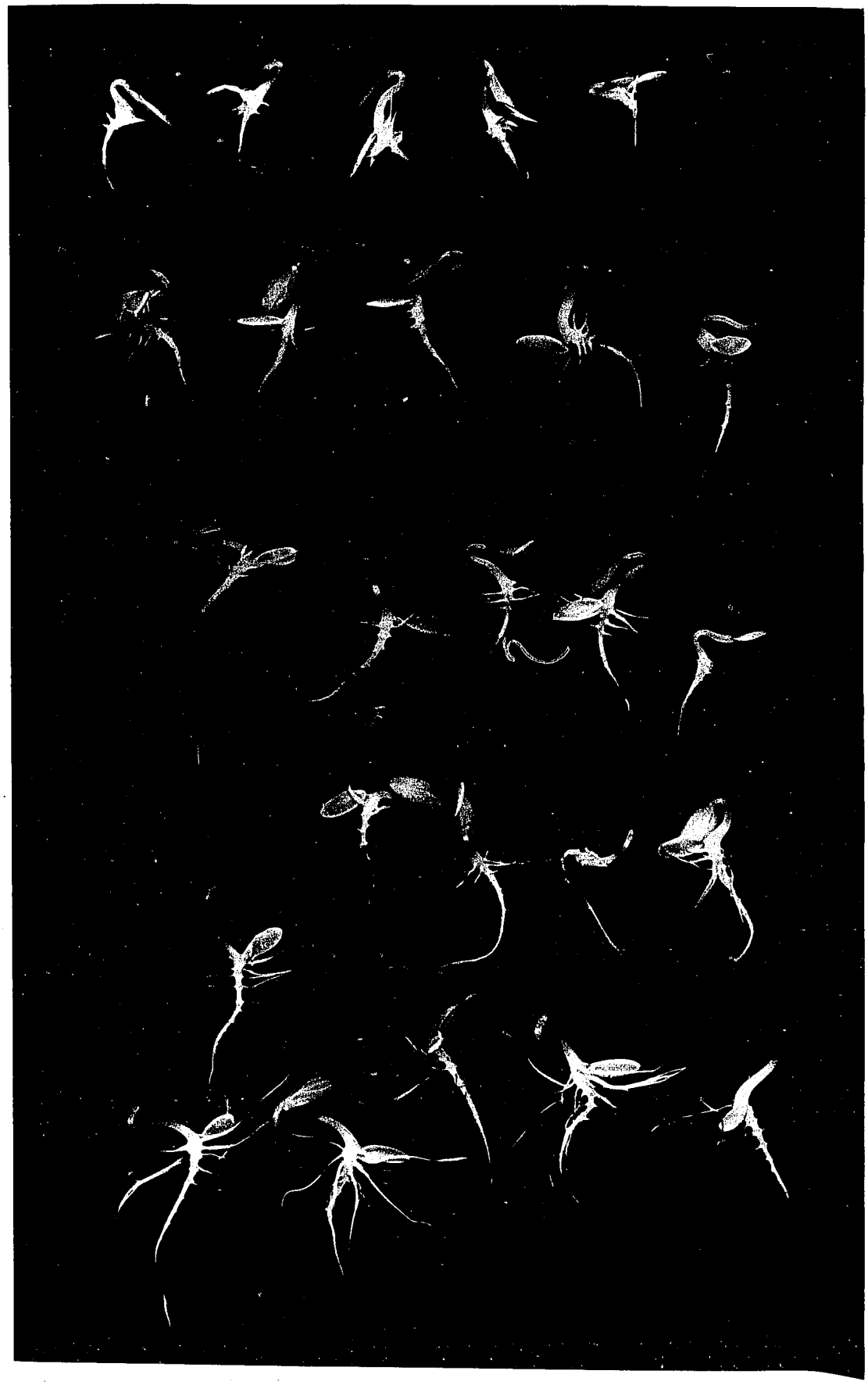


Fig. 3

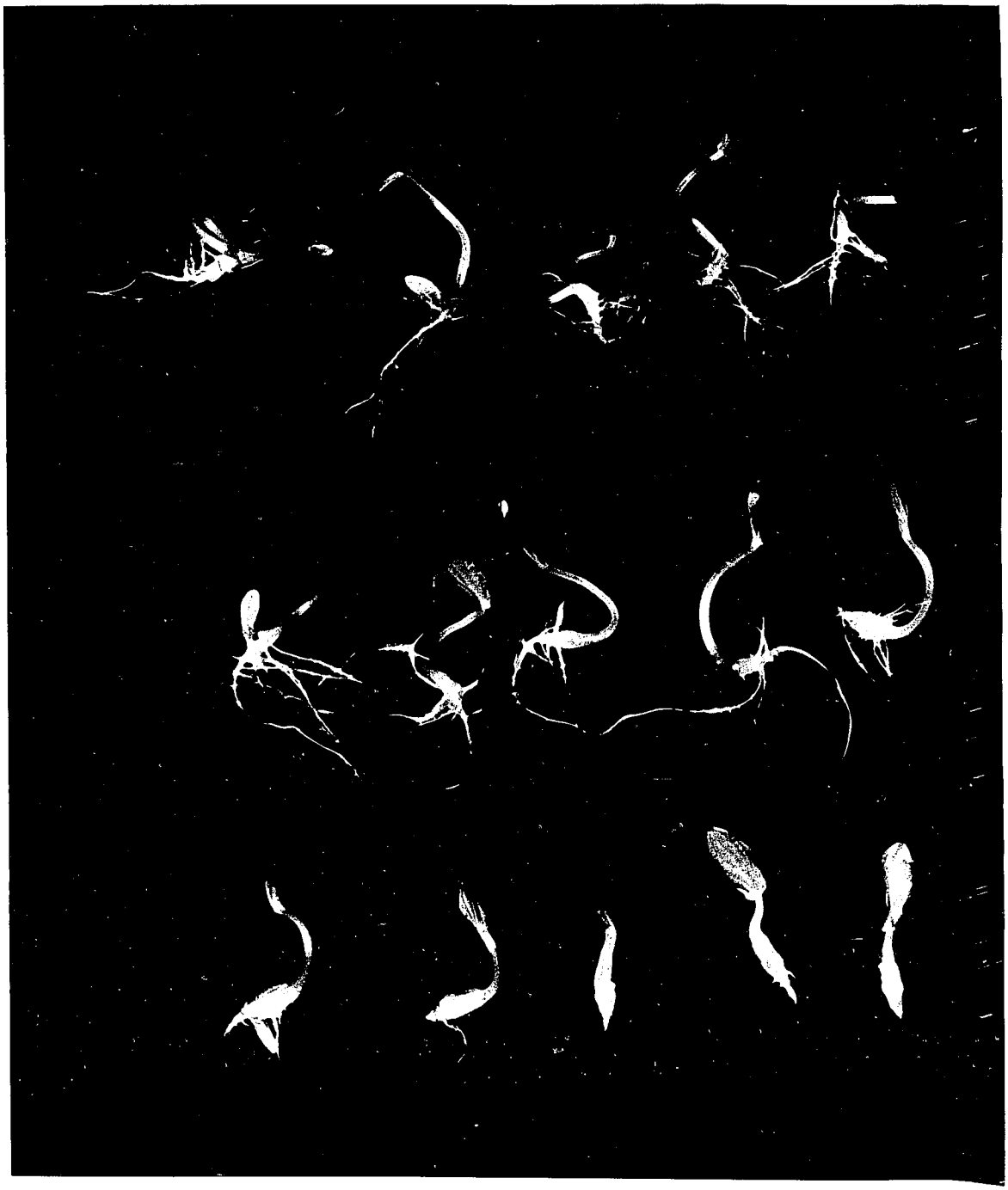


Fig. 4

At the same time, with the same sample of seeds and under the same conditions, an experiment was run to test the possibility of an antagonism of thio-beta-naphthol to the inhibitory activity of coumarin, but no positive result was obtained. It was then decided to investigate more extensively the properties of DTO.

b. Wheat seeds.

Similar experiments were conducted with wheat seeds. However, in the Petri dishes containing a coumarin solution, heavy infestations of fungi occurred. Therefore these experiments were discontinued.

B. Experiments with a new sample of Marketer cucumber seeds.

After our first supply of Marketer cucumber seeds was exhausted a new sample was ordered. The new shipment was less uniform and was different in appearance; many seeds had to be rejected because they were cracked or damaged. A few experiments were carried out in a similar line of investigation, and it soon appeared that the new seeds did not respond in the same way (appendices 4-A and 4-B). Attempts to repeat the results previously obtained with mixture of coumarin and DTO gave erratic and less striking results. Because we had been also using a newer sample of DTO, we thought that a change in the chemical might be responsible for these changes in the results. But a test reported in appendix V showed that both old and new samples of the chemical induced similarly erratic results. Some indications of antagonism by DTO at 3.3 ppm were questionable.

Since winter was over, and the conditions of light and temperature of the laboratory were much changed, it was thought that environmental factors might be involved. Accordingly, it was decided to conduct the next tests in environmental conditions as well controlled as possible. A special room was then used, as indicated on page 10.

However, these new experimental conditions did not change the results appreciably much, as can be seen in appendices 5-A, 5-B, and 5-C. Even though there were some indications of antagonism, in continuous or alternate illumination, this was not consistent and was irregular. From these tests and other tests in which no measurements were taken, it could be concluded that DTO acted more slowly, and was antagonistic only to lower concentrations of coumarin than it was in the tests with the older seeds. After several days, the inhibited main roots of the seeds placed in coumarin alone were not much shorter than in the solutions containing also DTO. Only after about 6 days, when the main roots of the seeds in the coumarin solution ceased growing and started decaying, the main roots of the seeds in the mixed solutions were in better condition. Little or no decay was observed, and growth continued for a few more days, giving then a difference in length.

This was true only of seeds put in continuous or alternate illumination; in darkness, such beneficial effects of DTO were not observed, apparently because the decay of the seeds receiving coumarin alone was not as rapid as in light. The reduction of inhibition of coumarin by DTO at 1 ppm in darkness (new sample), shown in appendix V, seems to have been incidental.

Because of a seed factor obviously involved, as suggested by the changes in the response to DTO and by the difference in age and appearance of the two samples, it was decided to investigate another variety. A Burpee Hybrid variety was chosen which has given satisfaction in every respect.

C. Experiments with Burpee Hybrid cucumber seeds.

1. Antagonism of DTO to Coumarin

a. Tests using constant solutions.

All experiments were conducted in the controlled room. Mixtures of chemicals were tested according to the technique previously described. The aspect of the roots and seedlings grown in solutions of coumarin alone or mixed with DTO was similar to that exhibited by the last sample of Marketer cucumber seeds, except that Burpee seeds in light showed a better response to higher concentrations of DTO, as can be seen in the appendices 6-A and 6-B. There seemed also to be a maximum concentration of coumarin above which the antagonism failed to appear. Experiment 6-B is especially interesting because it demonstrates clearly the importance of the conditions of illumination: DTO did not reduce at all the inhibition of root elongation induced by coumarin in darkness. Since the same solutions were used for each treatment irrespective of the conditions of light or darkness, the same solution induced a positive response to DTO in light, and no response at all in darkness, which rules out the possibility of an error in the making of the solutions.

b. Tests using transfers.

Since Cavallito et al. (33) have shown that unsaturated lactones and sulfhydryl compounds can react together, it was thought that the antagonism between coumarin and DTO might be due to a chemical reaction taking place in the solution and making part of the coumarin unavailable to the seeds receiving mixtures of the two chemicals. We therefore conducted several experiments in which the technique of transfer, previously described, was used, in order to prevent a contact of the chemicals outside of the seeds.

A preliminary experiment (7-A) conducted in both light and dark conditions, indicated that treating first with DTO and then transferring to coumarin was more promising than the other way, from coumarin to DTO. A second one (7-B) showed that concentrations of coumarin higher than 150 ppm seemed to inhibit root elongation irreversibly; in the same experiment, a slight antagonism was observed in darkness. However this can have been incidentally due to a low figure for the control set (transfer from water to coumarin) since this is the only case where some inhibition was observed in darkness, as shown by several tests (7-A, 7-B, 8-A and 8-B) and that it was slight (but significant with a probability of 0.99). Concentrations tested for antagonism in darkness ranged from 5 to 75 ppm for coumarin, and from 25 to 300 ppm for DTO.

Important reductions of inhibition were observed in light during the experiment 8-A, which was confirmed by three other experiments performed in light only (9-A, 9-B and 9-C) with,

each time, similar success demonstrating beyond any doubt the antagonistic action of a pretreatment of 300 ppm of DTO for the inhibitions of root elongation caused by coumarin afterwards.

The data given in 8-A, 9-A, 9-B and 9-C have been averaged and put in table III so that a comparison of the reductions of inhibition at various concentrations of coumarin in light can be made. It is specially interesting to compare them to the promotion of growth (see below) induced by the pretreatment of 300 ppm of DTO over the controls grown in distilled water throughout the experiments; it can be seen that the reduction of the inhibitions caused by coumarin is much larger (both in absolute and in relative values) than the corresponding growth-promotive effect of DTO.

2. Effects of DTO on the elongation of roots.

a. Constant solutions: Inhibition as a function of time.

Because it had been previously observed that the inhibitions of root elongation induced by higher concentrations of DTO were not visible before the third day, an experiment was run in order to determine precisely how soon they become considerable. A concentration of 200 ppm of DTO was used. Burpee cucumber seeds were germinated in the usual way and the root lengths measured after 2, 3, 4, and 5 days(appendices 10-A and VI). No formal measurement was taken after the first day, but a few seeds were measured at the time of several transfers, which fluctuated between 4 and 6 mm in light, and were never longer than 1 mm in darkness (the differance was conspicuous). The results are given in the form

TABLE III

EFFECTS OF SUCCESSIVE APPLICATIONS OF DITHIOOXAMIDE (DTO) AND OF COUMARIN IN DISTILLED WATER SOLUTIONS ON THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS GROWN UNDER CONTROLLED CONDITIONS IN LIGHT FOR FIVE DAYS. TRANSFER AFTER 24 HOURS.

		Treatment averages mm.		General Average mm.		Average Difference in mm.	% of growth (base: water)		
Pretreatment / Post-treatment		Distilled Water	DTO 300 ppm	Distilled Water	DTO 300 ppm		Distilled Water	DTO 300 ppm	% Increase produced by DTO over the check (coumarin alone)
Coumarin 80 ppm	8-A	-	-						
	9-A	41.9	41.6						
	9-B	41.0	52.1	40.1	44.0	3.9	47.6	52.2	109.7
	9-C	37.4	38.2						
Coumarin 40 ppm	8-A	40.5	61.2						
	9-A	43.8	68.1	45.3	64.5	19.2	53.7	76.5	142.4
	9-B	48.9	66.3						
	9-C	48.1	62.3						
Coumarin 20 ppm	8-A	49.4	65.4						
	9-A	47.1	68.1	50.7	72.5	21.8	60.1	86.0	143.0
	9-B	56.7	78.2						
	9-C	49.7	78.3						
Coumarin 10 ppm	8-A	65.4	80.5	59.2	84.7	25.5	70.2	100.5	143.0
	9-A	-	-						
	9-B	-	-						
	9-C	53.1	89.0						
Distilled Water (Controls)	8-A	-	-						
	9-A	80.0	95.7						
	9-B	90.9	100.3	84.3	97.9	13.6	100.0	116.1	116.1
	9-C	81.9	97.7						

of several curves where average root length is plotted against time for DTO and controls in distilled water in both light and darkness (Fig. 5), which shows that the growth inhibitory action of DTO becomes considerable only after the end of the second day. However the differance between controls and seeds treated with DTO are significant at the 0.99 level as soon as the end of the second day, in both cases of light and darkness (appendix VI). Of special interest is the change in the ratio of the length in light to the length in darkness for both controls and treated seeds; it fluctuated between 4 and 6 after the first day, but was already significantly smaller than 1 at the end of the second day (appendix VI).

b. Transfers: Influence of the conditions of illumination.

(1) promotion of growth in light.

In the previously reported experiments 9-A, 9-B and 9-C, conducted in light a promotion of root elongation was observed as a result of the application of a pretreatment of DTO at a concentration of 300 ppm during 24 hours. High statistical significance (probability of 0,99) of the differences between the averages of the treated seeds and of the controls were found in each case (appendix VII). Similar data have been extracted from several experiments which will be reported in part II, and in which the only difference was that the post-treatments were performed in water at pH 3.5 instead of distilled water, as indicated on page 12. Similar statistical comparison with their

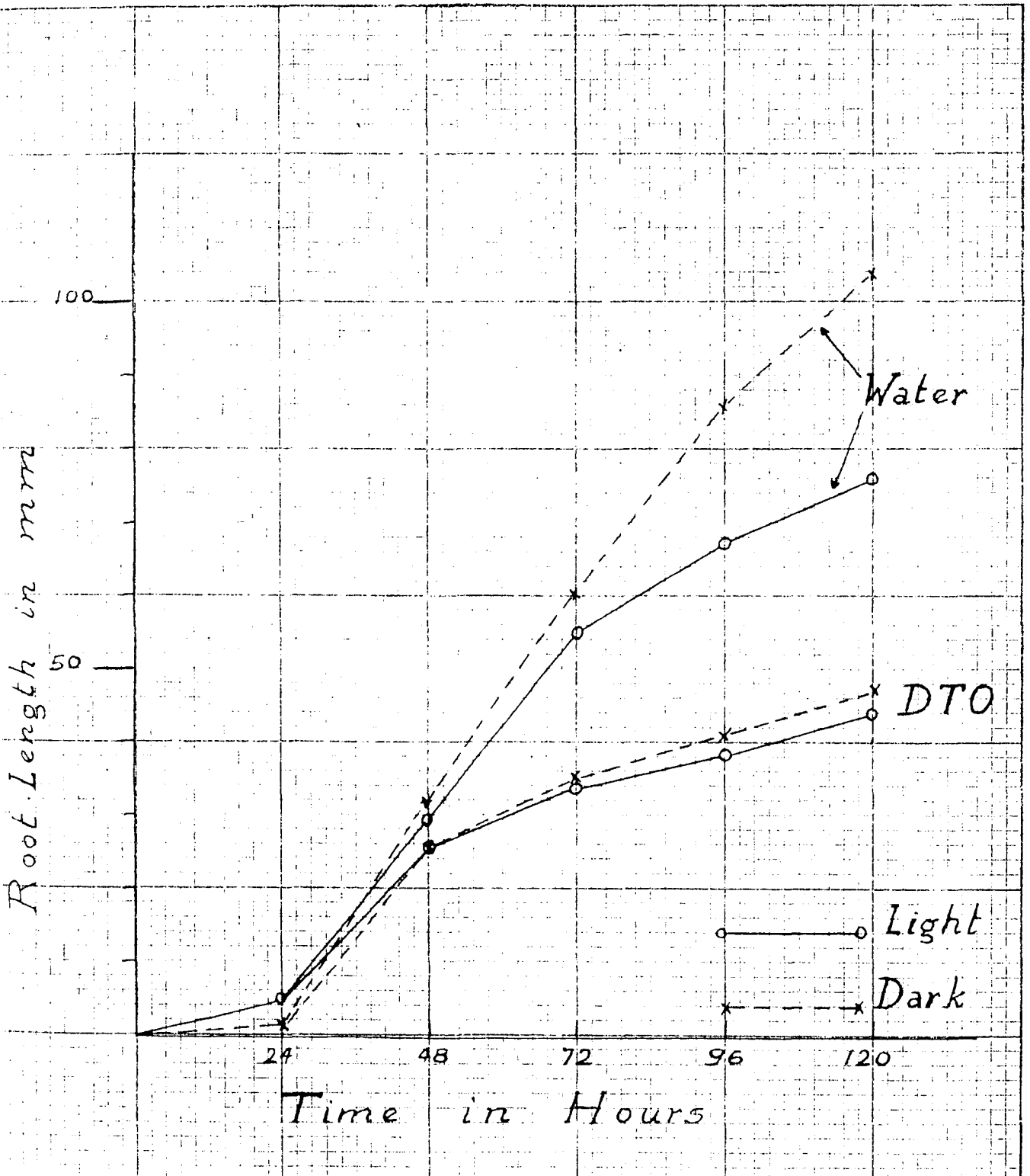


Figure 5.- Elongation of Burpee Hybrid cucumber roots in distilled water and Dithiooxamide (DTO) at 200 ppm, in light and in darkness at various intervals of time.

controls (appendix VIII) yielded the same high significance in each case (0.99). It should be noted that the Burpee Hybrid used in the latter series of experiments were not from the same shipment than those of the first series. The data from the six experiments demonstrating the promotion of root elongation have been gathered in table IV and averaged.

(2) Inhibition of growth in darkness.

No promotion of growth was ever observed in darkness as a result of a pretreatment of DTO. This is demonstrated by experiments 8-B, 25-A, 25-B, 26-A, 26-B, and 27-A, most of them with post-treatments in water at pH 3.5. The concentrations covered a range from 25 to 300 ppm. Comcentrations as low as 25 ppm were tested because the concentration of 300 ppm which promotes root elongation in light gives a strong inhibition in darkness. Since no difference was observed between the controls and the seeds receiving a pretreatment of 25, 37.5, 50, or 75 ppm of DTO in darkness during 24 hours, it seems impossible that any growth stimulation may occur at a lower concentration. From the experiments 26-A, 26-B, and 27-A, a table has been extracted, which shows the difference between the effects of pretreatments with various concentrations of DTO in light or in darkness: concentrations which promote root elongation in light, inhibit it strongly in darkness (table V).

D. Experiments with Lettuce seeds

In order to test the possibility that the antagonistic relationship of DTO and coumarin be exhibited only by cucumber seedlings, it seemed that the investigation of some other plant species

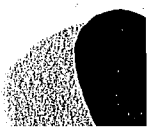


TABLE IV

STIMULATION OF THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS BY A PRETREATMENT (24 HOURS) OF DITHIOOXAMIDE (DTO), UNDER CONTROLLED CONDITIONS IN LIGHT.

Pretreatment / Post - treatment		Root-length averages mm.		General averages mm.		Average % increase in root-length of treated seeds over Water controls.
		Distilled Water	DTO 300 ppm	Distilled Water	DTO 300 ppm	
1st sample Distilled water acidified at pH 3.5	25-B	89.7	100.3			
	26-A	93.0	109.9	89.8	102.8	14.5
	26-B	86.7	98.3			
2nd sample Distilled water	9-A	80.0	95.7			
	9-B	90.9	100.3	84.3	97.9	16.1
	9-C	81.9	97.7			

TABLE V

EFFECTS OF A PRETREATMENT (24 HOURS) OF DITHIOOXAMIDE (DTO) IN DISTILLED WATER SOLUTIONS ON THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS GROWN UNDER CONTROLLED CONDITIONS IN LIGHT OR IN DARKNESS FOR 5 DAYS.
Transfer after 24 hours.

		Treatment averages mm.		General averages mm.	
Post-treatment / Pretreatment		Distilled Water acidified at pH 3.5		Distilled Water acidified at pH 3.5	
		Light	Darkness	Light	Darkness
Distilled water	26-A	93.0	100.1	89.8	101.9
	26-B	86.7	103.3		
	27-A	-	102.4		
DTO 375 ppm	26-A	-	-		
	26-B	-	-	-	103.0
	27-A	-	103.0		
DTO 75 ppm	26-A	-	-		
	26-B	-	104.4	-	99.6
	27-A	-	94.8		
DTO 150 ppm	26-A	97.1	87.6		
	26-B	94.2	81.3	95.6	84.4
	27-A	-	-		
DTO 300 ppm	26-A	109.9	72.8		
	26-B	98.3	67.9	104.1	70.3
	27-A	-	-		

might be of interest. The antagonism between coumarin and thiourea reported by Nutile (112) for germination of lettuce seeds suggested that lettuce might respond to DTO and to coumarin in a way similar to cucumber.

1. Preliminary tests with old seeds.

The first experiments were run with a limited sample of Grand Rapids lettuce seeds which were more than two years old at the time of the tests. Mixed solutions of DTO and coumarin were used, and their effects compared with those given by coumarin alone or distilled water solutions, according to the previously described technique of germination.

It was found in preliminary experiments that as little as 5 or 10 ppm of DTO could produce a considerable reduction of the inhibition of germination induced by 25 ppm of coumarin, and that this antagonism was strongly influenced by the conditions of light (appendix 11-A).

Because of the small number of seeds available, an experiment using only one replicate (100 seeds) for each treatment was conducted to investigate the influence of the duration of an initial dark period upon this antagonism. Each series inclu ded one control in distilled water, one treatment with coumarin alone, and two treatments where coumarin was mixed with 5 and 10 ppm respectively of DTO. All the seven series investigated were run at the same time, in the same environmental conditions of light (70% fluorescent and

30% incandescent) and temperature (26° C. on the illuminated bench, 25° C. in darkness). Fresh solutions were added on the fourth day, then distilled water was used to keep the paper moist. After one series had received a given initial period of darkness, it was moved to the illuminated bench, where it was receiving then an alternate illumination (12 hours of darkness, 12 hours of light). Detailed results are given in appendix 11-B and have been presented in form of graphs where the percent germination is plotted versus time; in these graphs, only one mixture of coumarin (25 ppm) and DTO (10 ppm) has been compared to the corresponding curves for water and for coumarin (25 ppm) alone (Fig. 6).

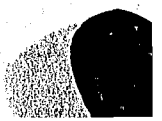
2. Experiments with younger seeds.

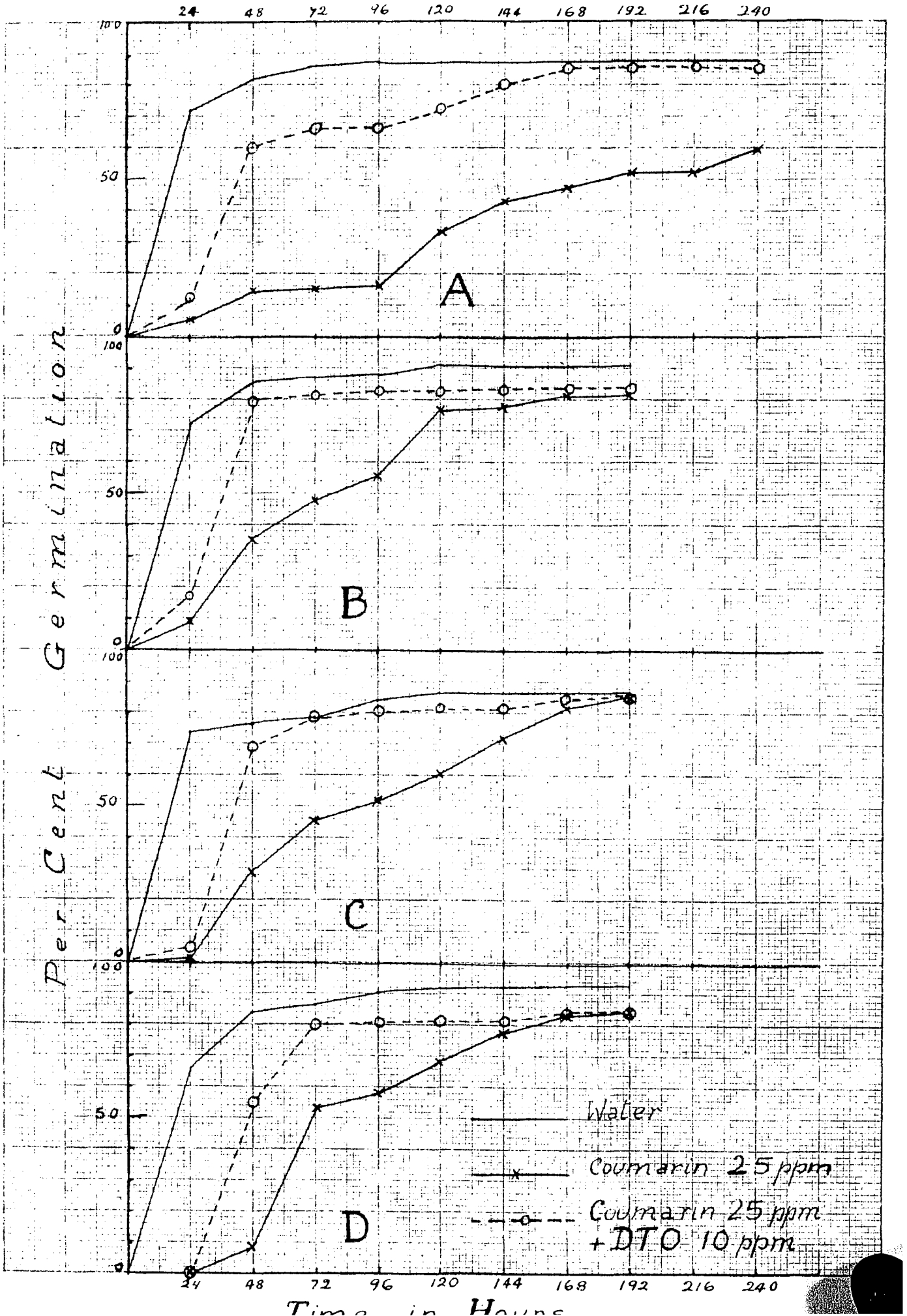
a. Antagonism of DTO to Coumarin.

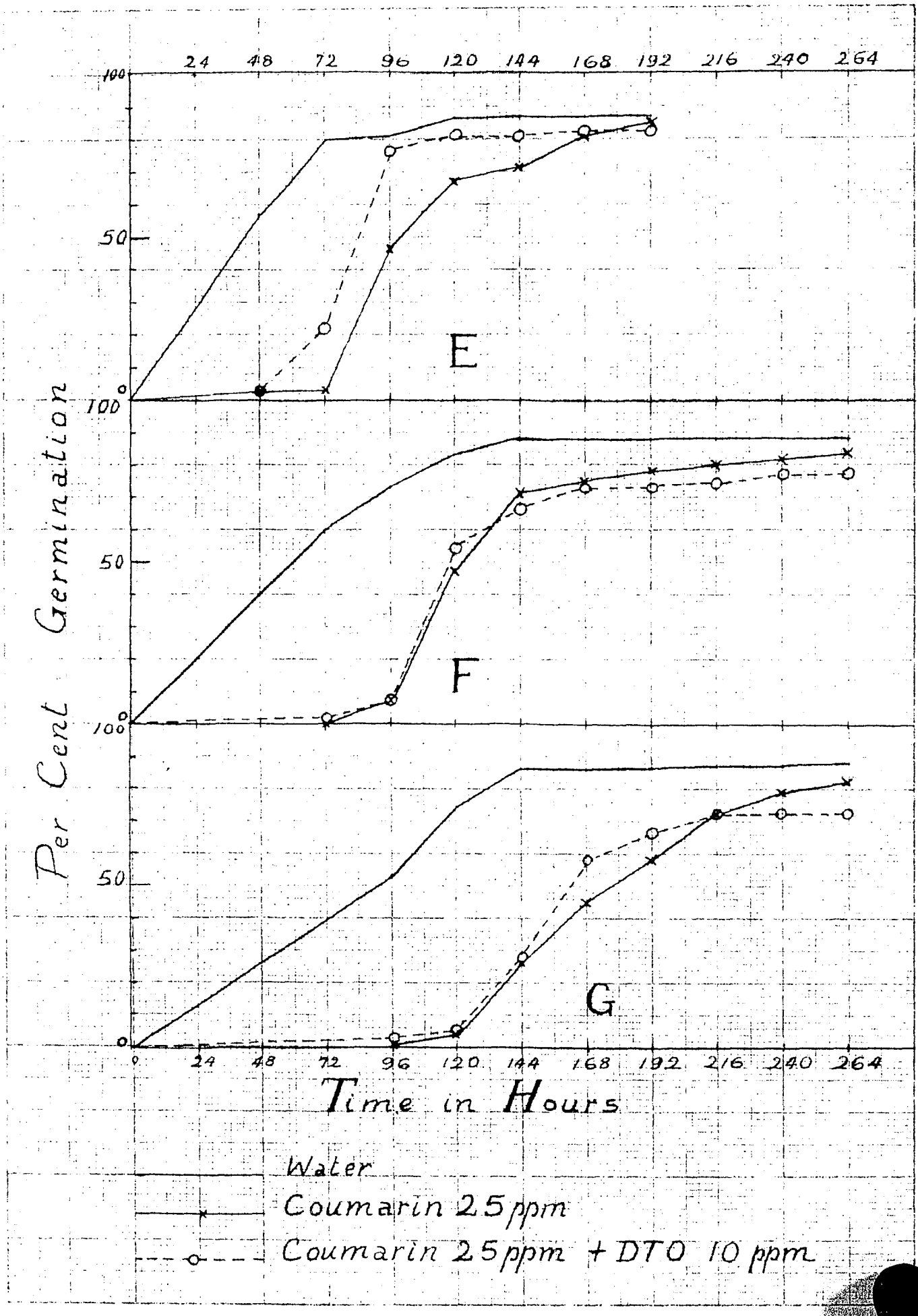
Similar experiments were conducted with a shipment of younger seeds. But, in this case, there was no antagonism to the inhibition caused by coumarin from concentrations of DTO lower than 50 ppm (appendix 12-A). The optimum response took place at 100 ppm, because at higher concentrations, some seeds exhibited an abnormal germination in which cotyledons developed prior to radicle emergence. Some of these experiments were conducted on two benches, one being the bench previously used which received a light of composition: fluorescence 70%, incandescence 30%; the other one received an equal total intensity of light, but was 50% fluorescent, 50% incandescent. On this one a temperature of 29-30° C. was induced by the

Fig. 6. Effects of simultaneous applications of Coumarin and Dithiooxamide (DTO) on the germination of lettuce seeds in distilled water solutions under various conditions of illumination.

A. Continuous illumination.
B, C, D, E, F, G, - Alternate illumination after an increasingly long dark period (B, none; C, 12 hours; D, 24 hours; E, 48 hours; F, 72 hours; G, 96 hours).




24
48
72
96
120
144
168
192
216
240
100
50
0
A
B
C
D
Germination
Per Cent
Time in Hours
Water
Coumarin 25 ppm
Coumarin 25 ppm
+ DTO 10 ppm


24 48 72 96 120 144 168 192 216 240 264
100
50
0
E
F
G
Per Cent Germination
Time in Hours
Water
Coumarin 25 ppm
Coumarin 25 ppm + DTO 10 ppm

increased proportion of incandescent light. It was observed in this case (12-B) that the germination was reduced, as compared with the other bench, except for the seeds receiving high concentrations of DTO (200 ppm). The antagonism between coumarin and DTO was still evident, both the check receiving coumarin only and the treatment with a mixture of coumarin and DTO exhibiting less germination; because the reduction was considerable chiefly for the check, a relatively better antagonism was observed than on the other bench. A much smaller antagonism was exhibited in darkness than in either light in these experiments (12-A and 12-B).

Since it is well known that lettuce germination is reduced by high temperatures, it was thought that the difference of 3-4° C. between the two benches might explain the difference in the results. However, it was also possible that the compostion of light had some influence.

In order to eliminate the factor of temperature, a sheet of plain, ordinary window glass was interposed between the second bench and its lights, just below the incandescent bulbs and the fluorescent tubes. This was enough to cut down the heat radiating from the bulbs, and bring down the temperature to 26-27° C. so that the only difference between the two benches was now only the composition of lights (and a difference of temperature less than 1° C.). Under such conditions, a comparative experiment was run in continuous illumination, with and without a previous dark period of 96 hours. Three replicates were used for each treatment. The seeds were from the same sample which had been used fro the experiments 12-A and 12-B four months before.

The results are given in appendix 13, and have been averaged in table VI for convenience of comparison. Within each series, the antagonism effect of DTO upon the inhibition of germination induced by coumarin was evident. Some influence of light composition was visible only in the treatments receiving coumarin, and only in the case of no former dark period. The influence of temperature can be appreciated by comparing the germination of the controls on the second bench with that of the controls in experiment 14-A. It can be seen also that, in the present experiment, germination after 96 hours was lower in darkness than in continuous illumination for the controls and for the treatments receiving coumarin alone, but was about the same for all the treatments receiving DTO. In this experiment, the antagonism functioned in darkness about as well as in light.

b. Promotion of germination by DTO.

In the preceeding experiments, it was repeatedly observed that, when the germination of the water controls was reduced by either darkness (12-A, 12-B, and 13) or high temperature (12-A, 12-B), a treatment of DTO alone at 100 ppm (13-A) or at 200 ppm (12-A and 12-B) was able to restore the germinating ability completely. Especially interesting was the case of high temperature in light.

In order to ascertain the restoration or germinating ability by DTO in light at high temperature, an experiment was ran on the second bench (without the sheet of glass) in three replicates,

TABLE VI

EFFECTS OF SIMULTANEOUS APPLICATIONS OF COUMARIN AND DITHIOOXAMIDE (DTO) ON THE GERMINATION OF NEW LETTUCE SEEDS UNDER CONTROLLED CONDITIONS. PERCENT GERMINATION AT VARIOUS INTERVALS OF TIME IN HOURS, AVERAGE OF 3 REPLICATES OF 100 SEEDS EACH.

Composition of light	70% Fluorescent + 30% Incandescent									50% Fluorescent + 50% Incandescent								
Illumination	No dark period continuous Illumination					96 hours darkness then continuous Illumination				No dark period continuous Illumination					96 hours darkness then continuous Illumination			
Time (hours)	24	48	72	96	120	96	120	144	168	24	48	72	96	120	96	120	144	168
Distilled water	74	82	87	90	91	50	82	89	89	68	78	83	85	86	58	89	95	95
DTO 100 ppm	77	85	87	89	90	83	91	92	92	74	76	78	82	84	85	91	92	92
Coumarin 25 ppm	10	29	47	63	74	5	5	11	21	0	8	10	18	22	7	12	15	22
Coumarin 25 ppm + DTO 100 ppm	63	78	82	84	85	77	78	82	83	38	63	69	73	76	75	78	79	80

in which germination in distilled water and germination in a solution of 200 ppm of DTO were compared. The results are given in appendix 14-A, showing clearly the improved germination in the solution of DTO. When not in combination with coumarin, 200 ppm of DTO did not seem to induce much abnormal germination of lettuce seeds.

c. Abolition of artificial dormancy by DTO.

Nutile (112) has shown that it is possible to throw lettuce seeds into artificial dormancy by a presoaking of 24 hours in a solution of 25 ppm of coumarin in absolute darkness. Such an experiment was conducted (see technique page 9) and the seeds were then germinated in solutions of DTO at various concentrations in darkness, continuous or alternate illumination, and in water. As shown in 14-B only the seeds germinated in darkness exhibited improvement from the use of DTO, and this improvement vanished after 24 hours in light. This is in agreement with the findings of Nutile relative to the abolition of artificial dormancy by use of thiourea.

III. DISCUSSION

The preceding experiments suggest that DTO has a definite growth-regulating activity, and that this chemical is able to reduce the inhibitions of root development induced by coumarin. As already pointed out, the beneficial effect of DTO in the complex treatments (DTO and coumarin) as compared with the check (coumarin alone), is much greater than the increase in length of the check (DTO alone) over the water controls, as can be seen in table III. Since the reduction of the inhibition due to coumarin is greater than the simple promotion of growth, it can be spoken of a true antagonism of DTO to the effects of coumarin.

Before attempting to interpret the physiological meaning of these phenomena, it seems interesting to emphasize first the strong influence of the factors of both seed and environment.

A. The conditions of the experiments

1. Seed factors

It is remarkable that, for both Marketer cucumber seeds and Grand Rapids lettuce seeds, the older seeds appeared to be the most sensitive to the antagonistic power of DTO against inhibitions induced by coumarin. In the case of Marketer seeds, this antagonism was effective against high concentrations of coumarin able to

suppress completely the elongation of the roots, in sharp contrast with the younger seeds for which DTO was ineffective against such concentrations. In the case of older lettuce seeds, 1/10 as much DTO was required to counteract the effects of the same concentration of coumarin than it was for the younger seeds (5-10 ppm, or 50-100 ppm respectively). However it is possible that the age of the seeds in not the only factor responsible for these differences, and the previous story of the mother plants might also effect the seed properties.

2. Environmental factors.

a. Water.

The differancesin the results of Marketer cucumber seeds germination in solutions of DTO show that purity of the water used for making the solutions is important: the use of tap water in the experiments reported in table II is the only possible explanation for a strong inhibition of germination which was never observed with solutions made of distilled water. The same explanation holds true for the wide variations of the first experiments, as exemplified by the tests 1-A and 1-B.

From table IV, it can be seen easily that the pH of the solutions do not influence much the length of the cucumber roots, at least between 3.5 and 6.1.

b. Illumination

That the conditions of light are of capital importance is visible from experiments with cucumber and lettuce seeds. Whereas

the antagonism between coumarin and DTO functions in light only for cucumber, it works also in darkness for lettuce, although it seems less consistent in the latter case when the chemicals are mixed together. For lettuce, in the experiment by the procedure of pretreatment, the antagonism worked in darkness only, not in light. However, it seems that in the case of lettuce two phenomena are involved. In the case of mixed chemicals in which the seeds are soaked during the full length of the test, it is a matter of toxicity only in light (which breaks dormancy), and of toxicity added to a dormancy in darkness; whereas, in the case of a pretreatment, there is nothing to be antagonized in light since dormancy is destroyed, and in darkness just the dormancy remains, as indicated by Nutile (112).

This matter appears more complex, from the fact that both chemicals individually induce different responses according to the donditions of light. Coumarin is more toxic in darkness than in light for lettuce seeds, which may be due to the existence of an artificial dromancy in darkness (112). However, in all experiments where both alternate and continuous illuminations were tested, coumarin also induced more inhibition in continuous than in alternate illumination!

In the case of DTO, the promotion of elongation of cucumber roots took place in light only; but in darkness, DTO improved the early germination of lettuce seeds, which could not occur in light because the germination of the controls was faster (see for instance appendix 13-A).

c. Time of application of light or chemicals.

The opposite responses of cucumber and lettuce to the variations in the conditions of illumination might be due to, in part, the fact that they differ also according to the stage of development of the seedlings at which the chemicals were applied.

As for cucumber seeds, it is remarkable that a soaking of the seeds in DTO solutions continuously throughout the test induced about the same inhibition of elongation in both cases of light or darkness, whereas a pretreatment of 24 hours with 300 ppm of DTO induced a promotion of elongation in light, and a strong inhibition in darkness. In the continuous treatment, the inhibition does not become considerable before the third day; for both control and treated seeds, root elongation starts faster in light, then becomes slower than in darkness after the first day. This seems to indicate that the conditions of illumination are specially important during the first day of germination. Because cotyledons do not emerge from the seed coat before the middle of the second day, it appears probable that photosynthesis is not concerned with this higher early rate of elongation in light.

As for lettuce seeds, the length of the first dark period appears important for the manifestation of the antagonism between coumarin and DTO. Increasing it depressed the antagonism, as shown in Fig. 6, which is due to the fact that the curve of germination of the seeds in the mixture travels faster towards the right than

does the curve of germination of the seeds in the check solution (coumarin alone). One day added to the length of the first dark period induced a delay of one day and a half in the mixture between D and E, E and F, and F and G, whereas the delay is of one day only for the check (see Fig 6). However the disparition of the antagonism observed after a dark period of 96 hours was never observed with the younger seeds, which may be due to the higher concentrations of DTO used in this case, and to the fact that, they were moved to continuous illumination instead of alternate illumination.

d. Temperature.

Its importance has been investigated with lettuce seeds only. It had been known for a long time that the germination of lettuce drops abruptly above the optimum of 25° C.. Thompson has shown (146, 147) that thiourea was able to counteract this detrimental effect of too high a temperature, DTO has the same ability (14-A); however, the concentration of DTO effective in this test was 200 ppm, which is much lower than the requirement for thiourea (0.5% or 5,000 ppm). Apartial inactivation by heat of an enzyme necessary for germination and its protection by DTO and thiourea might constitute a tentative interpretation of these facts.

B. Interpretation of the Results

Because general effects upon the growth of the whole seedling have been investigated, it is difficult to determine what phsiological processes are more specifically involved in the phenomena previously described.

The exact chemical structure of thioamides such as thiourea and DTO is still disputed by organic chemists. A structure of zwitterion seems to be most probable presently, which is compatible with the great ability of thiourea (34) and thiomides in general (133) to react like thiols possessing the -SH group. As previously indicated, DTO would be in this casepossess two such groups on adjacent carbon atoms. For DuBois et al. (48), studying the inactivations of some enzymes by thioureas, it seemed most likely that the -SH groups of these substances were involved in these biological reactions.

It is therefore reasonable to think that DTO has the properties of a dithiol. The recent investigations performed with BAL have shown that dithiols are outstanding in three respects at least (12):

(a) they are strong, although sluggish oxidation-reduction systems, rapidly oxidized in the presence of a number of catalysts, of which copper and iron-porphyrin are the most powerful;

(b) they combine with a number of heavy metals, forming complex compounds usually insoluble;

(c) in the presence of oxygen, they can destroy iron-porphyrin compounds (haemin, oxy-haemoglobin) by opening the porphyrin ring.

These chemical properties enable the dithiols to inactivate many enzymes; Webb et al. (162) found that, among the most sensitive, are: polyphenol oxidase (a copper enzyme), carbonic anhydrase, catalase and peroxidase(which has an iron-porphyrin prosthetic group), and they concluded that BAL may be considered as a potent inhibitor of metal containing enzymes Barron et al. (13) confirmed and extended these results to other dithiols, adding that the oxidation products of dithiols seem to be able of inhibiting -SH enzymes.

Evidences of the effects of thiols and thioamides upon polyphenol oxidase and peroxidase have been dontributed. DuBois et al. (48) have reported that several thioureas are able to inhibit tyrosinase activity in rat tissues. Jaques (80) observed that several thioureas may inactivate potato phenol oxidase. According to Randall (123), thiourea and other thiols do not inactivate animal peroxidase, but rather serve as a substrate for it, and their ability to reduce hydrogen peroxide is increased by peroxidase.

Among the metal-containing enzymes not tested by the students of BAL, ascorbic acid (ASA) oxidase, a copper enzyme, is prominent in cucumber seedlings. If we recall that both polyphenol oxidase (109) and peroxidase (139)(140) are able to oxidize ASA indirectly, it comes out that dithiols may attack the three most important enzymes which control the metabolism or ASA in plant tissues. ASA possesses an unsaturated lactone structure (see appendix, formula), and is widely occurring in plant tissues, where it may assume a role of redox system. The possibility then is that one

effect of the substances containing -SH groups, and specially of dithiol and thioamides, be an upset of ASA metabolism. Coumarin might interfer with this mechanism by its own unsaturated lactone ring.

However, other biological processes may also be affected by the unsaturated lactones and be sensitive to sulfhydryl compounds at the same time. For instance, since it has been shown (33) that unsaturated lactones can react with natural -SH groups such as those of cystein, the -SH enzymes might be inactivated by a substance like coumarin. Toennies (151) has shown that thioamides also may immobilize the -SH groups of cystein; so the natural -SH groups might be sensitive to both coumarin and DTO. The antagonism of these substances would then be explained by the fact that mercaptide forming substances and reducing agents, such as ASA, may in certain cases reactivate the natural sulfhydryl groups, as indicated by Barron (101).

It is remarkable that in either one of the previously suggested mechanisms ASA seems to be entitled to play a key role on the inhibitory effects of coumarin and of DTO and on their antagonism.

IV. SUMMARY

The effects and interactions of coumarin derivatives and sulfhydryl compounds upon germination of lettuce seeds and elongation of the main root of cucumber seedlings have been investigated in Petri dishes, and under controlled conditions of light and temperature.

(1) Several substituted coumarins, besides coumarin itself, have been found to inhibit root growth of cucumber, with an accompanying bulbous enlargment of the hypocotyl.

(2) Chiefly two sulfhydryl compounds, dithiooxamide and thio-beta-naphthol, were effective in inhibiting root-growth of cucumber.

(3) Dithiooxamide has been found to promote root growth of cucumber in light, but not in darkness. To a promotion of root growth induced by a solution of dithiooxamide (300 ppm) applied as a pretreatment of 24 hours in light, corresponded a strong inhibition of root-growth by the same pretreatment applied in darkness- A treatment of 200 ppm continued for five days produced a strong inhibition of root-growth in both light and darkness, which became apparent only after the third day.

(4) Dithiooxamide was able to reduce considerably the inhibition of root growth of cucumber induced by coumarin in light, but not in darkness.

(5) Dithiooxamide was able to reduce considerably the inhibition of germination of Grand Rapids lettuce seeds induced by coumarin both in light and darkness.

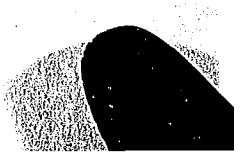
(6) Dithiooxamide was able to increase considerably the germination of lettuce seeds at 29°- 30° C. in light and at 25° C. in darkness.
(7) These results are discussed relatively to the age of the seeds, the conditions of light, and the stage of development at which the chemicals or the conditions of light were applied. A possible biochemical relationship between unsaturated lactone and -SH groups is suggested.

PART II

ASCORBIC ACID

ANTAGONISM TO COUMARIN

INTERACTIONS WITH DITHIOOXAMIDE



I. INTRODUCTION

The experiments reported in part I, which establish the growth regulator properties of DTO and its antagonism to the inhibitions of root development induced by coumarin, thus constitute another evidence for an interaction of unsaturated lactones with dithiols and thioamides.

Looking for the biochemical reasons of this growth-regulating activity and this antagonism, it has been suggested that two mechanisms of physiological relationship chiefly might be involved, in both of which a key role of regulation could be played by ASA.

Because these two hypotheses were both of a highly theoretical character, a thorough search of the literature dealing with ASA, its effects upon plant-growth, and its physiological relationships with the natural -SH groups and with unsaturated lactones was achieved, before going into experimental work for testing the ability of ASA to interact with DTO and coumarin.

A. Review of Literature

1. Ascorbic Acid: Effects on Plant-Growth

a. General growth

In 1935, simultaneously Havas (72) and Hansen (70) reported about the effects of ASA upon plant growth, The first worker, studying the germination and early development of wheat, did not observe

any stimulation of germination by ASA but found an increase and an acceleration of the growth of the seedling, amounting to 25-30% of the shoot weight, and 50% of the root weight after 12-13 days. High concentrations were inhibitory, He observed also that ASA was able to increase the dry weight of tomato plants by 20%, but at the same time decreased the fruiting. Hansen reported similar results with peas. Davies et al. (44), in 1937, made a thorough study of the effects of ASA on various growth phases of several plant species. For generation of buds and roots in willow branches, ASA was beneficial, more than IAA during the first few days, less after the first week. ASA also promoted the germination and the elongation of roots for oats, cress, and mustard seeds in Pfeffer's inorganic nutrient solution. It did not induce bending in the decapitated or the normal oat plant. Applied to tomato petiole, it produced a general growth stimulation with a considerable increase in dry weight, but no local nastic response.

Bonner et al. (21) found that ASA was able to improve the development of pea embryos. This was not true of all pea varieties, which explains the negative results of Kogl et al. (85) working with another variety of the same species. Bonner observed an excellent correlation between the ASA content of different varieties of peas and their response to applications of ASA: the lesser ability to synthesize ASA naturally, the better response to an addition of this vitamin. He also emphasized two of the main difficulties encountered in investigation dealing with ASA:

first, the extreme variability of the responses to ASA applications, due to great variations in ASA content induced in plant tissues by the plant and the environmental (see below) factors, and, second, difficulty in obtaining materials deficient in natural Vitamin C in order to get a response from ASA addition. This probably accounts for many failures to induce plant materials to respond.to ASA applications.

Borgstrom (23) has reported that ASA promotes the growth of Allium Cepa and Allium fistulosum: Onchatschek (113) observed that it increased the growth of eighteen species of mixotrophic algae, chiefly when they were growing in autotrophic conditions. Dennison (45) observed a general increase in growth of tobacco treated with ASA. As for Ulva Lactuca, contradictory results were contributed by Tore Levring (95) and by Harald Kylin (89). Here again, a matter of environmental conditions was involved (namely: the composition of the artificial sea-water used for growing the algae). Raadts et al. (122) found that dehydro-ascorbic acid stimulates the growth of Avena coleoptile. Wetmore et al. (165) observed that the incorporation of ASA in agar blocks receiving natural auxins from foxtail would increase the curvature of Avena coleoptile. Virtannen et al. (155), working with cotyledon-free peas, found that ASA would give a growth comparable to that of normal plants if the nitrogen was supplied in nitrate form. Kunning (88) found ASA to be about as effective as IAA and thiamin in producing cambial activity in decapitated bean plants and sun-flowers.

b. Germination.

A number of workers have investigated the effects of ASA upon germination and root development. After the negative results of Havas (72) with wheat, we have already cited the positive findings of Davies et al. (44). Bonner et al. (22) did not find ASA to be essential for the growth of isolated pea roots in a mineral nutrient solution containing sucrose, vitamin B and Nicotinic Acid. Cooper (38) has reported that ASA is very effective in inducing the germination of pollen grains of Carica papaya. However, Addicott (1) found it to be inactive on the pollen of Tropaeolum and of Milla., and so did Wang (160) for the pollen of Lotus Corniculatus. Germination of rye and barley was accelerated by ASA, according to von Euler et al. (156). Also Rugge (127) found that the germination ability of barley could be increased by addition of ASA, especially for old seeds with a diminished germinative power.

c. Environment and plant factors.

That the age of the seeds influences the response of plants to applications of ASA is only an example of the many factors which interfer with the effects of ASA upon plant growth. Those are probably important because they change the amount of ASA which is normally present in the tissues, as shown by Bonner (21) for several varieties of peas. It is well known that light, temperature, nutrition, trace elements are important in this respect, and the literature dealing with their influence upon ASA content is considerable. An accurate investigation of some of these factors has recently been performed by Somers et al. (135), who have also

listed some of the most important works on this subject (136). It is generally admitted that ASA content is higher in plants grown in light than in plants grwon in shade; Clark (37) has been one of the first workers to remark that gradients of chlorophyll and ASA concentration in plant tissues are similar. However, he recognized also that light was not necessary for ASA synthesis, which has been condirmed (62). The precursor of ASA is still unknown, although there are someindications that hexose sugars might constitute one of the steps of its synthesis (98). Speaking of light, it should be recalled here that ASA solutions deteriorate more rapidly in light (5), chiefly if riboflavin is present (75).

2. Interactions between Ascorbic Acid and Sulfhydryl groups.

The protection of ASA by natural compounds possessing -SH groups (glutathione, cysteine), is among the most conspicuous and, seemingly, the most important processes involved in the metabolism of ASA. It seems that such mechanisms may function directly by chemical reaction with oxidizing agents able to attack ASA, or indirectly, through influence on the enzymes which catalyse the oxidation-reduction reactions of the ASA-dehydroascorbic acid system. Evidences of a direct protection of ASA by synthetic compounds with an -SH group can also be found in the literature, as well as of a working together of natural -SH groups with Vitamin C in some metabolic processes.

a. Physiological protection of ASA by -SH groups.

The first evidence of a connection between the metabolism of ASA and -SH groups appears to have been provided by Pfankuch (118) who reported in 1934 that cysteine was able to reduce dehydroascorbic acid back to ASA in the pressed juice from potato, probably by an enzymatic process. Then Mawson (102) indicated a protection of ASA by glutathione, cysteine, and cystine in animal tissues. Hopkins et al. (76) were apparently the first workers to study in detail the protection of ASA by glutathione in plant tissues. They demonstrated the existence of an enzyme able to oxidize ASA, the oxidation of which was prevented by glutathione. They found that glutathione was able to afford the same protection both in the presence of the enzyme of of copper and also to reduce the oxidized ASA in presence of the enzyme. They furthermore observed that the enzyme would not oxidize glutathione when ASA was not present, but that, in presence of the system ASA, enzyme and glutathione, the last substance was oxidized at the same rate at which ASA would have been oxidized, had glutathione not been present. A protection of ASA by glutathione has also been demonstrated by Barron et al. (11) in animal tissues. Crook et al. (41) discovered that 9 species of plants at least contain an enzyme able to catalyze the reduction of dehydroascorbic acid by reduced glutathione (dehydroascorbic acid reductase) which would thus protect ASA from oxidation by atmospheric oxygen. Synthetic -SH derivatives, such as sodium diethyldithiocarbamate (103, 137) in small concentrations may inhibit the activity of ASA oxidase from cucumbers, therefore they protect ASA from oxidation. The same chemical, thiourea and H_2S markedly

inhibit the activity of soybean ASA oxidase which would induce a reversible oxidation of ASA to the stage dehydroascorbic acid only, according to Rangnekar et al. (124), who also indicated that the enzyme practically developed to a maximum in 48 hours, then remained constant in the cotyledons, but decreased in the sprouts*. Thiourea was also found to cause an extreme reduction in the amount of (reduced) ASA in plasma and tissues of rabbits (82). Thiouracil was less effective. When given high, but not lethal, amounts of thiourea, rabbits could live for a long period of time with an extremely low concentration of plasma-ASA without development of scurvy symptoms.

The previous works show that ASA and sulfhydryl groups are interrelated in plant and animal physiology. However the nature of this relationship is still far from being perfectly clear. It is the opinion of Barron (10) that, most probably, the protection of ASA by glutathione is an indirect one, by combination with copper for instance, although he does not completely rule out the possibility of a direct mechanism. Certainly also, indirect protection by interference with enzyme systems do exist.

* Mapson et al. (99) have recently reported that a complex system involving coenzyme II, glutathione and Ascorbic acid functions in plant tissues for the stranport of hydrogen, in which dehydroascorbic acid is reduced by glutathione independantly of the reductase.

b. Direct protection of ASA by synthetic -SH compounds.

A few examples of direct protection by thiol derivatives exist. Arcus et al. (5), after ascertaining the rapid deterioration of ASA in the presence of ultra-violet light observed that glutathione may protect ASA when the two chemicals were mixed together, or when a glutathione solution was interposed as a screen between thelight and the ASA solution. In 1943, Drake et al. (47) showed that by iodine titration and by change in optical activity of the mixture that ASA may form complex or addition compounds with glutathione, cysteine and thioglycolic acid, on a mole to mole basis. Yusuke Sumiki et al. (166) reported that thiourea, thioglycolic acid and reductic acid were fairly effective agents for the stabilization of Vitamin C in solution. Choten Inagaki (35) found that thiourea was a very good stabilizer of ASA and Campbell et al. (30) reported that it was true also of glutathione and sodium ehtyl dithiocarbamate beside thiourea.

c. Various associations of ASA with -SH groups.

There are a few known facts which strongly suggest that ASA and -SH groups might be associated in various metabolic precesses. As early as 1938, Giri (57) investigated the inhibiton of phosphatase activity (in sprouted soybean) brought about by a complex system ASA-copper. The additon of glutathione,cysteine, cystine, H_2S and some other reducing agents annuled this inhibition. Similar mechanism was then demonstrated by the same author for the animal

phosphatases (58), and he suggested that ASA and glutathione may contribute the two parts (hydrolysis and synthesis) of a system controlling the metabolism of phosphorus.

Pett (117) has shown that, in potatoes, glutathione and ASA contents undergo parallel variations, both rising sharply with sprouting and then declining simultaneously. Kretsovitch et al. (86) demonstrated that similar changes occur in germinating seeds (wheat, rye, and corn). Hopkins et al. (77) for glutathione in a number of seeds, and Cailleau et al. (26)(27) for ASA in wheat and peas confirmed that the amount of these substances, from nil at the start of germination, rises sharply during the earliest stages of the seedling development.

Virtanen et al. (155) found that both ASA and several thiol derivatives (glutathione, cysteine) enabled cotyledon-free peas to use the nitrogen from nitrates and grow almost as well as intact plants; they believed that the -SH compounds reduced dehydroascorbic acid formed and thus allowed the dotyledon-free embryo to use more effectively the small amount of Vitamin C which it contains, and which, they thought, functions as a donor of hydrogen.

It has been recently reported by Carruthers (31) that ASA oxidase, the leading enzyme for the phsiological oxidation of ASA, presents a catalytic wave characteristic of -SH groups in polarographic determinations. It should be recalled here, as stated

by Barron (10) that:

"When the active -SH groups of thiol enzymes are abolished, either by oxidation or by mercaptide formation, they may, under certain conditions, be regenerated with complete restorationof the enzyme activity by the addition of reducing agents (ASA, H_2 S, cyanide) or of mercaptide forming substances".

Thus, the all important -SH enzymes constitute another common ground of action for ASA and -SH compounds, where those have most opportunities to compete and interact with each other.

3. Interactions between ASA and Unsaturated Lactones.

Whereas it was possible to find many cases of interactions of ASA with sulfhydryl compounds, it is more difficult to gather indications of an interrelationship between unsaturated lactones and ASA. Weintraub (163) found that, among many other chemicals, ASA was, like coumarin, able to inhibit the germination of lettuce seeds and this inhibition was also photosensitive. Buston et al. (25), investigating the effects of several unsaturated lactones upon seed germination, did not find any antagonistic action of ASA for the inhibitions induced by hexeno-lactone.

Works in animal phsiology have indicated an antagonism between the effects of dicomarol and Vitamin C. Working with rabbits (115), rats (14) and Guinea pigs (138) Link et al. gave several evidences pointing to the existence of an antagonism between ASA and the sweet-clover hemorrhagic disease induced by dicoumarol; repeated applications of ASA may reduce the symptoms

of the disease; animals suffering with scurvy are more affected by applications of dicoumarol than healthy ones, and in certain cases dicoumarol applications induced a temporary excretion of Vitamin C. Martin et al. (100) confirmed part of these works.

Another indication of a possible relationship, is provided by several works dealing with scopoletin, another coumarin derivative. As previously indicated, scopoletin (4) does not accumulate in healthy potato tubers but is apparently built up in case of leafroll virus infection(3), and is also associated with spotted wilt of tobacco (16). It has been found by Smith et al. (134) that potatoes infested with leafroll virus have also an excessive content of ASA; this has been confirmed by Newton (111) and used as a basis for a method of detection of diseased plants, Further investigation by Andreae et al. (4) led them to discover that potato tubers metabolize scopoletin with production of an unstable blue intermediary product. The reaction, can be accelerated by $H_2 O_2$ and inhibited by a few substances including high concentrations of ASA. There is therefore a possibility that the accumulation of scopoletin in the diseased tissue be due to a blocking of its metabolism caused by the build up of ASA.

B. Problem

The preceding review of literature has procured facts which demonstrate the importance of ASA as a natural growth-regulator for plants; it has revealed substantial evidences indicating that

ASA and natural -SH groups constitute a complex metabolic system widely occurring in plant tissues, and very likely to be sensitive to the applications of synthetic thiol derivatives. Some works also suggest the existence of a connection between the metabolism of coumarin derivatives and ASA.

These evidences are consistent enough to substantiate the hypothesis that ASA might be able to interfer with the growth regulating activity of the chemicals previously studied (coumarin, DTO), such as was suggested in the discussion of the first part of this work.

The purpose of the experiments now reported was to investigate the effects of ASA upon the inhibitions of root elongation and of germination caused by coumarin and by DTO. This investigation has been extended to some chemicals closely related to ASA, and to thiourea which resembles DTO.

II. EXPERIMENTAL RESULTS

A. Effects of Ascorbic Acid on Root Elongation

A few experiments were performed in tap-water with Marketer cucumber and wheat seeds to test the effect of ASA on root elongation. But the results were not consistent and varied widely from test to test and even within a same test (appendices 1-A, 1-B, 1-C, and 1-D). No special experiment has been run in distilled water for the study of the effects of ASA alone on roots, but from the checks used in many experiments for purpose of comparison, some conclusions can be drawn regarding the effects of ASA on root elongation. As a rule, no considerable effect was produced by a concentration of ASA lower than 2,000 ppm. According to the time and the technique of application (constant solution, pretreatment or post-treatment), according to seed factors such as variety and environmental factors, such as air-composition or moisture content which were not controlled, slight variations occurred, seldom higher than 10% of the length of the controls, some times as a promotion, some times as an inhibition.

For instance, in experiment 37-C, ASA concentrations up to 2,000 ppm were not inhibitive and even slightly growth-promotive (500-1,000 ppm), in light; but, in experiment 32-B in light, 250 ppm of ASA were already inhibitive; in experiment 24-D in

light, growth-inhibition was not apparent before a concentration of 1,000 ppm was reached. Slight promotions of growth were observed several times, both in light (experiments 4-B, 26-A, 24-C) and in darkness (24-C). Therefore, in many cases, checks with ASA alone were run, in order to determine accurately what was the contribution of ASA in the results of the complex treatments.

As for the aspects of the roots grown in solutions containing only ASA, they were more twisted, and had generally more root-hairs and more lateral roots than the controls.

B. Antagonism of Ascorbic Acid and Coumarin

1. Preliminary Experiments with Marketer Cucumbers.

The first experiments were donducted with the idea that, if ASA was to exhibit any antagonism to coumarin such as did DTO, the mixture of DTO and ASA might give an antagonism stronger than either chemical alone. Therefore, in experiments 4-A, 4-B, and 5-A, seeds grown in mixtures containing coumarin and DTO or ASA or both chemicals were compared to seeds grown in coumarin alone. No attention was paid to the pH of the solutions at that time, and the Marketer seeds used were from the second sample which did not respond to DTO as well as the first and older sample.

In the tree experiments, clear evidences for an antagonism of ASA to the effects of coumarin were obtained. In spite of some variations from test to test, it was clear also that this antagonism functioned as well in darkness as it did in light. It can be seen in 4-B that the slightly promotive effect of ASA alone was much smaller than the difference between the root length in

the complex treatment (coumarin plus ASA) and the check (coumarin only). The roots from the complex treatment were sometimes twice as long as the roots from the check, and more than half of the inhibition caused by coumarin was suppressed (67% in the case of seeds placed in alternate illumination in experiment 5-A).

As for the addition of the antagonisms of DTO and of ASA to coumarin, the failure of the seeds to exhibit the antagonisms of DTO throws a doubt on the results obtained with mixtures of the three chemicals. Only in the treatments in coutinuous illumination of experiment 4-A did DTO reinforce the effects of ASA. In all the other cases, DTO depressed them, or even suppressed them completely. Special attention will be devoted elsewhere to the interactions of ASA with DTO.

2. Experiments with Burpee Hybrid Cucumbers

a. Constant Solutions

In all the experiments now reported, all the solutions were made using acid water at pH 3.5, so that only the treatments receiving 1,000 ppm or 2,000 ppm of ASA had a slightly different pH (respectively 3.25 and 3.1 as indicated on p.12). The addition of coumarin did not change the pH. Two experiments were conducted, in which the seeds were from different samples of Burpee Hybrid cucumbers. Photographs were taken after six days of germination in experiment 15-B (Fig. 7, 8, 9, 10) and, in each case, measurements were taken which are reported in 15-A and 15-B.

Fig. 7. Burpee Hybrid cucumber seedlings grown in mixtures of Coumarin and Ascorbic Acid for six days in light.

1. Coumarin 150 ppm.
2. Coumarin 150 ppm plus Ascorbic Acid 250 ppm.
3. Coumarin 150 ppm plus Ascorbic Acid 500 ppm.
4. Coumarin 150 ppm plus Ascorbic Acid 1,000 ppm.

Fig. 8. Burpee Hybrid cucumber seedlings grown in mixtures of Coumarin and Ascorbic Acid for six days in light.

1. Coumarin 75 ppm.
2. Coumarin 75 ppm plus Ascorbic Acid 250 ppm.
3. Coumarin 75 ppm plus Ascorbic Acid 500 ppm.
4. Coumarin 75 ppm plus Ascorbic Acid 1,000 ppm.

Fig. 9. Burpee Hybrid cucumber seedlings grown in mixtures of Coumarin and Ascorbic Acid for six days in darkness.

1. Coumarin 150 ppm.
2. Coumarin 150 ppm plus Ascorbic Acid 250 ppm.
3. Coumarin 150 ppm plus Ascorbic Acid 500 ppm.
4. Coumarin 150 ppm plus Ascorbic Acid 1,000 ppm.

Fig. 10. Burpee Hybrid cucumber seedlings grown in mixtures of Coumarin and Ascorbic Acid for six days in darkness.

1. Coumarin 75 ppm.
2. Coumarin 75 ppm plus Ascorbic Acid 250 ppm.
3. Coumarin 75 ppm plus Ascorbic Acid 500 ppm.
4. Coumarin 75 ppm plus Ascorbic Acid 1,000 ppm.

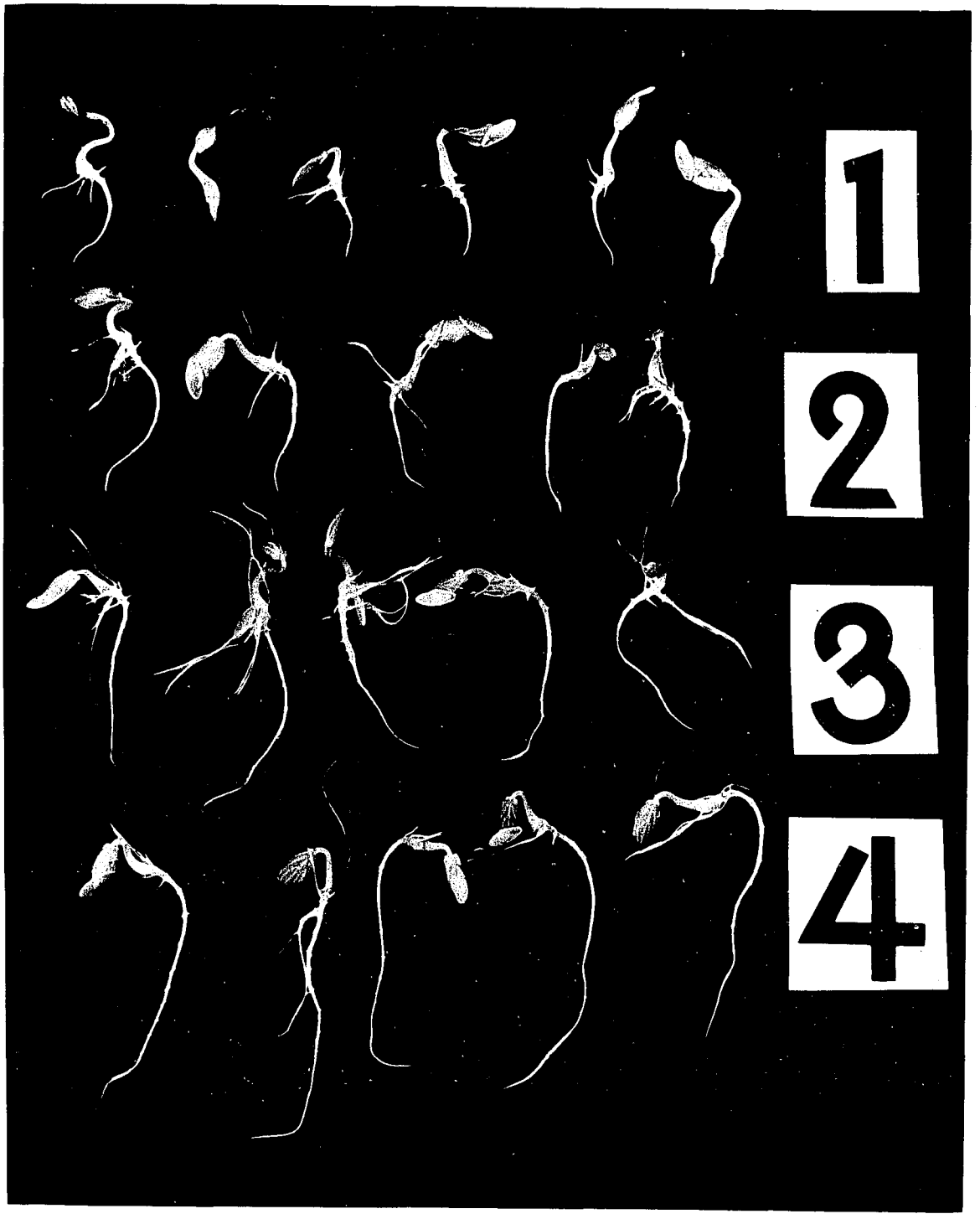


Fig. 7

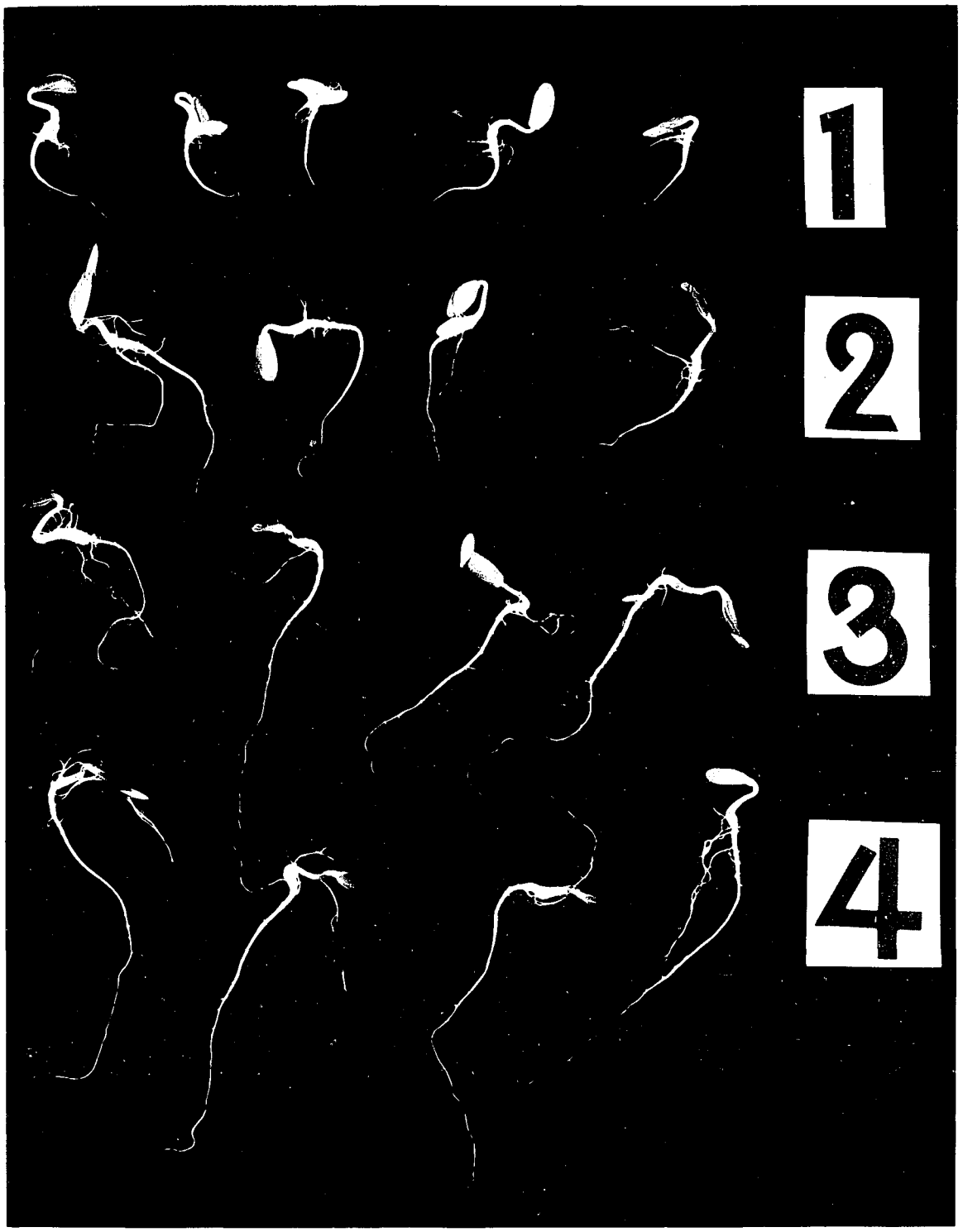


Fig. 8

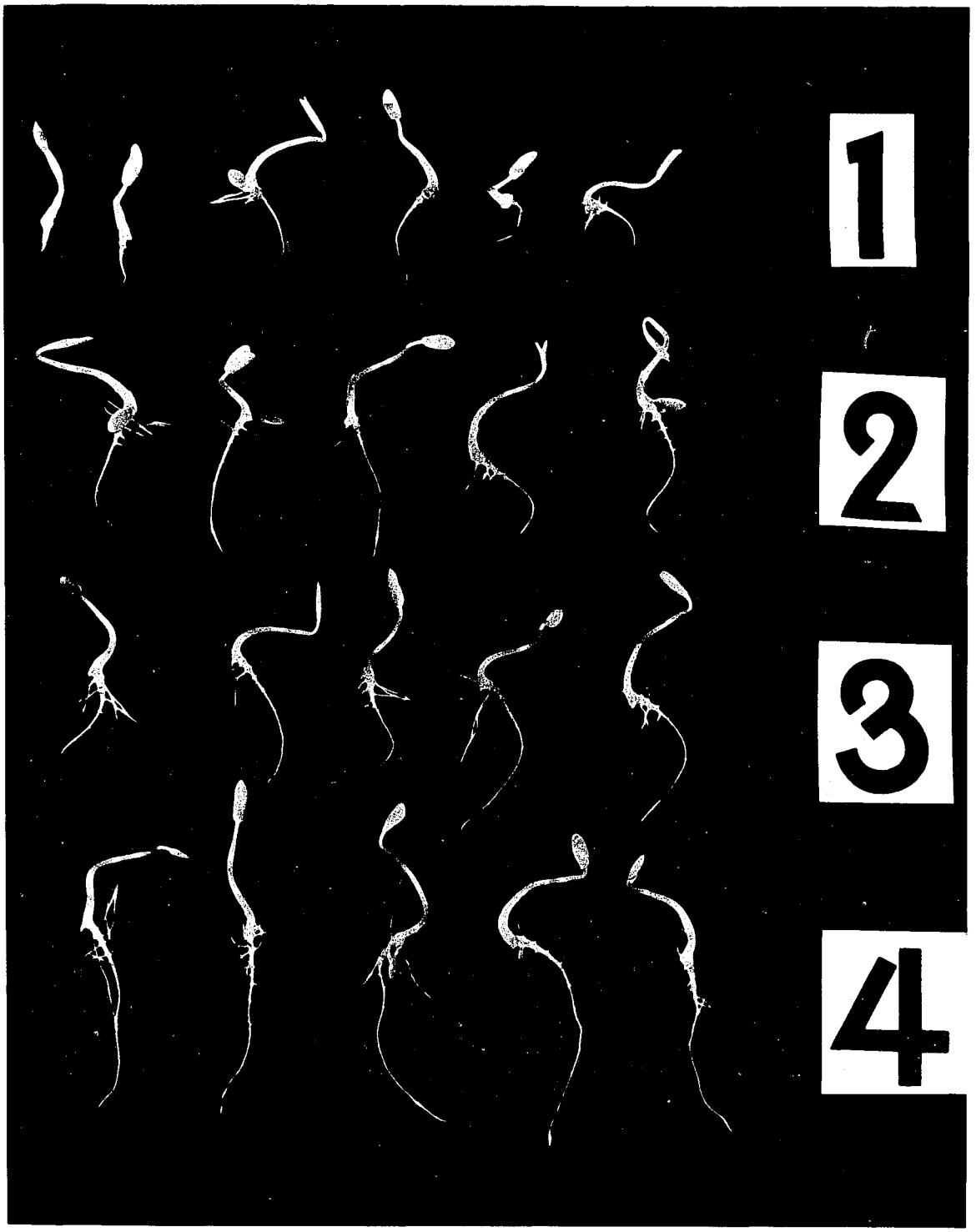


Fig. 9

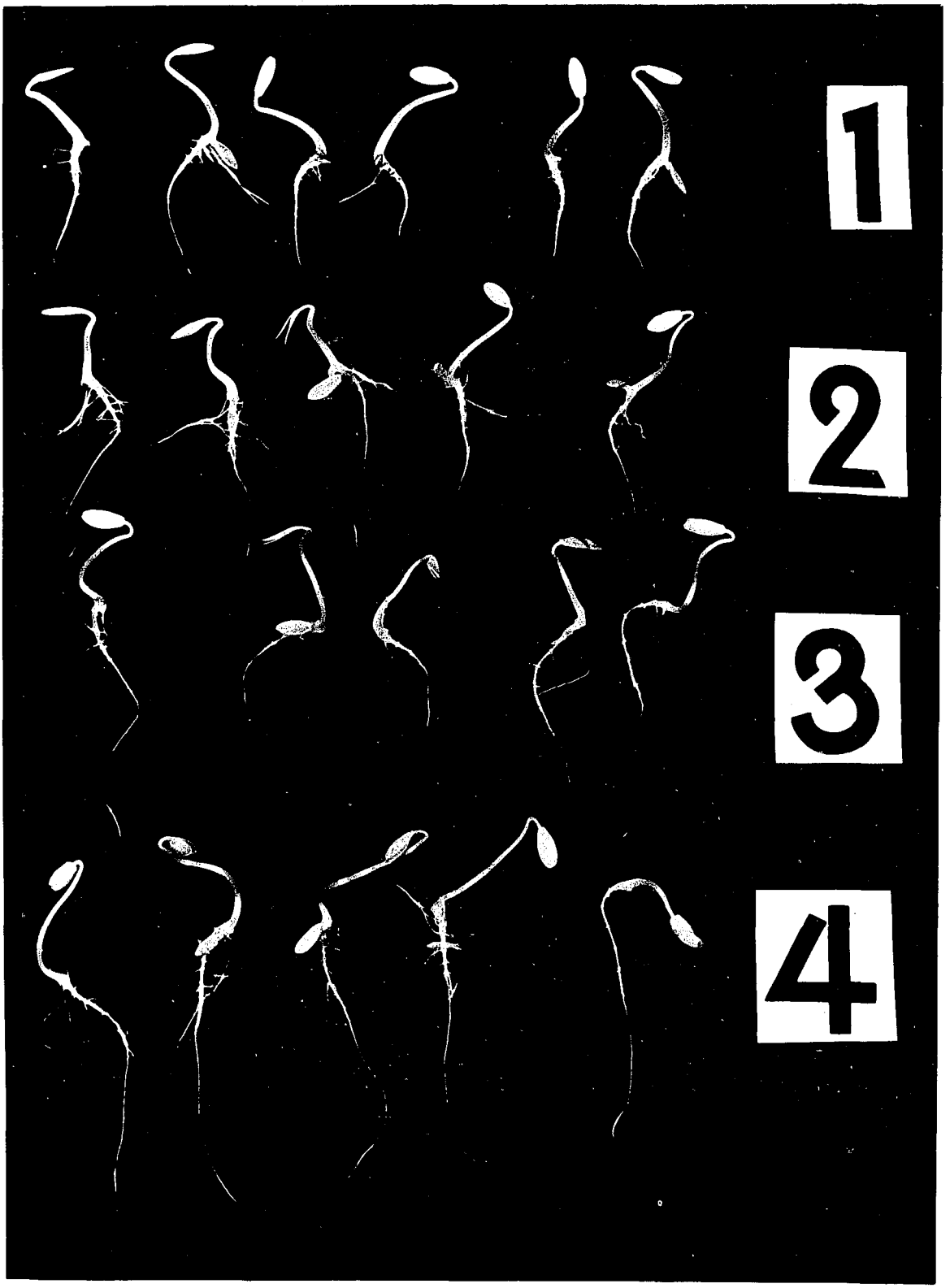


Fig. 10

As can be seen from the pictures and from the data, the antagonism was considerable under all conditions of illumination; no promotion of elongation by ASA alone was observed, and the concentration of 1,000 ppm of ASA, which was slightly inhibitory by itself, gave the better response for the antagonism to coumarin (15-B). Like in the case of Marketer seeds, it was common to observe suppression of more than 60% of the inhibition caused by coumarin (73% in 15-B). Twice the roots of the treated seeds were very close to three times as long as those of the check receiving coumarin only (15-A, in alternate illumination and 15-B in light with 150 ppm of coumarin).

b. Transfers

In order to test the possiblity that the antagonism observed in the experiments conducted in constant solutions be due to a chemical reaction occurring in the solution and making part of the coumarin unavailable to the seeds, the technique of transfers was used in the experiments now reported. The pH was not the same throughout the tests; the solutions of coumarin and their controls were made of distilled water, whereas acid water at pH 3.5 was used for the solutions of ASA and their controls, so, for all the seeds in each test, the pH was the same at a given time, but used to be similarly changed at the time of the transfer from the pretreatments to the post-treatments.

Because it had been found in part I that transfers from DTO to coumarin used to make the antagonism of DTO to coumarin more manifest than transfers from coumarin to DTO, the first experiment

was conducted by applying first ASA for 24 or 48 hours, then transferring to coumarin solutions. This did not give any evidence of antagonism (16-A). Therefore another experiment was run, in which the other way of transferring was used (from coumarin to ASA). Only in the case of a pretreatment for 24 hours in light did we observe a strong reduction of the inhibition caused by coumarin (17-A).

Two other tests were performed in a similar way and with the same results. A new sample of seeds from the same origin was used in experiment 18-A.

(1). Slight antagonism in darkness.

The results of experiments 17-A, 17-B, and 18-A all agreed to indicate that ASA is able to slightly reduce the inhibition caused by coumarin in darkness; on a range of concentrations going from 37.5 ppm to 150 ppm for coumarin, and from 125 ppm to 1,000 ppm for ASA, it was impossible to uncover an antagonism comparable to the antagonism in light. However, many evidences of a slighter antagonism can be found in these tests. The best result was obtained in 17-A with a pretreatment of 48 hours: the root length in the complex treatment was 17% longer than the roots receiving only coumarin.

(2). Strong antagonism in light.

The same experiments showed that, in continuous illumination, ASA gives a considerable reduction of the inhibition of root-elongation caused by a pretreatment of coumarin for 24 hours; the same does not seem to take place if the pretreatment is applied for

48 hours, as shown in 17-A.

In both 17-A and 17-B where a post-treatment of 500 ppm of ASA was slightly but significantly inhibitory, the complex treatment gave a slight promotion of growth over the controls receiving no chemical: The inhibition of elongation induced by the pretreatment of coumarin was not only completely suppressed, it was even reverted into a promotion. This was observed four times (three replicates of 17-B, once in 17-A), but was statistically significant only twice (see appendix IX) at the level of probability 0.95.

In 18-A, the effects of various concentrations of ASA on the inhibitions caused by several concentrations of coumarin are given. Several times, the roots in the dishes receiving the complex treatment, were 30% longer than in the check, and in this experiment also complete suppressions of the root inhibition were observed.

The results of experiments 17-A, 17-B, and 18-A in light have been put together and averaged in table VII.

3. Experiments with Lettuce Seeds

a. Preliminary Experiments with old Lettuce Seeds.

Since DTO was able to reduce the inhibitions of lettuce germination induced by coumarin, it was thought that ASA might be able to do this also.

Accordingly, experiments similar to those which have already been reported in the case of DTO (part I) were run with ASA. Due to the different pH, fungi and molds developed frequently in the solutions, chiefly those of ASA.

TABLE VII

EFFECTS OF SUCCESSIVE APPLICATIONS OF COUMARIN IN DISTILLED WATER SOLUTIONS AND OF ASCORBIC ACID (ASA) IN ACID WATER SOLUTIONS AT pH 3.5 ON THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS GROWN UNDER CONTROLLED CONDITIONS IN LIGHT FOR 5 DAYS

Pretreatments (24 hours)		Average root-length (mm.) Post-treatments: Acid Water	ASA 250 ppm	ASA 500 ppm	Percent average root-length (Water controls: 100) Post-treatments: Acid Water	ASA 250 ppm	ASA 500 ppm	Percent increase produced by ASA over the checks (coumarin alone) Post-treatments: Acid Water	ASA 250 ppm	ASA 500 ppm
Distilled water	17-A	83.6	-	75.5						
	17-B	80.2	78.1	70.9	100	92.0	94.3	100	92.0	94.3
	18-A	90.8	-	93.8						
Average		84.9	78.1	80.1						
Coumarin 75 ppm	17-A	68.7	-	92.3						
	17-B(1)	68.8	79.9	85.2						
	17-B(2)	65.5	77.0	86.3						
	17-B(3)	69.4	73.8	88.3	80.6	90.6	105.3	100	112.4	130.6
	18-A	69.5	-	94.7						
Average		68.4	76.9	89.4						
Coumarin 150 ppm	17-A	58.6	-	81.2						
	17-B	-	-	-						
	18-A	60.2	-	79.5	70.0	-	94.6	100	-	135.1
Average		59.4	-	80.3						
Coumarin 300 ppm	17-A	-	-	-						
	17-B	43.7	53.4	55.1						
	18-A	-	-	-	51.5	62.9	64.9	100	122.1	126.0
Average		43.7	53.4	55.1						

In the preliminary experiments with old Grand Rapids lettuce seeds (19-A and 19-B), no attention was given to pH; molds and fungi occurred only in the solutions receiving ASA. The antagonism was observed in continuous illumination only.

b. Main Experiments with a new Sample of Lettuce Seeds.

Acid water was used in the making of all the solutions, so that molds and fungi were less unevenly distributed, generally, these undesirable organisms did not appear before the third day, so that the results given for the first two or three days may be trusted. In the experiments 20-A and 20-B, some antagonistic effect of ASA (250 ppm and 500 ppm) to the inhibition of germination induced by 25 ppm of coumarin were consistently exhibited in continuous illumination, with two different compositions of light. The antagonism worked in alternate illumination in 20-B only. It was never apparent in darkness.

Looking for a way to make the test last longer, we found that the safest way to get rid of the undesirable organisms was to remove the seeds at the first sign of infestation, and to exert a careful watch for any fungus body that we could detect in the dishes. A little surrounding zone of the filter paper was torn away by means of tweezers each time such seed or fungus body was removed. In this way, it was possible to get clean cultures, chiefly in experiment 22-B where several inspections were made each day. We could not use any other method, such as dipping in solutions of a fungicide, since it has been shown that in some

cases, even a washing in distilled water may affect the germination processes (146). The two experiments conducted in the way previously described (22-A and 22-B) brought the wanted confirmation that in clean cultures also the antagonism works in light, but not in darkness.

c. Experiment using a Pretreatment.

A test similar to test 14-B was also performed, in which seeds were first soaked in coumarin (25 ppm) for 24 hours and then died in perfect darkness before being germinated in ASA solutions. Like in the experiment with DTO, we observed some improvement of germination from the use of ASA in darkness only, and for the highest concentration only and it disappeared after 24 hours in continuous illumination. In light, on the contrary, the highest concentration of ASA was clearly inhibitory, and the lower concentrations did not produce any effect.

C. Interactions of Ascorbic Acid and Dithiooxamide

It has been previously reported (page 61) that DTO usually depressed the antagonistic effects exerted by ASA on the inhibitions induced by coumarin, rather than increased them as was expected (4-A, 4-B, and 5-A). Due to the poor response to DTO of the particular sample of seeds used in these experiments, the conclusion was withheld until better information.

The data presented now deals with the study of the effects directly produced by ASA upon the effects caused by DTO on the

elongation of roots. The fundemental idea was to investigate the possibility that ASA overcame the inhibitions induced by high concentrations of DTO.

1. Constant Solutions: Importance of Illumination

In the first experiments, the seeds used were from the Marketer variety of cucumbers (23-A, 23-B and 23-C); then, seeds from the variety Burpee Hybrid were employed. Mixtures of ASA and of DTO were tested in water solutions and the results were compared with those of checks receiving either chemicals alone.

In most of these experiments, no attention was paid to the pH of the solutions. But, after the first tests, we thought that the differences in acidity might be partially responsible for the effects observed and we began looking for a suitable buffer.

A Sorensen buffer, giving a pH of 4.5 was prepared according to the indications of Gortner (62), but gave deceiving results: a strong inhibition of the controls took place, which caused the disappearence of the inhibition normally produced by DTO (24-B).

Acid water at pH 3.5 was then used for the making of the solutions according to the procedure previously described. One test done at this pH yielded the same general results as the tests performed with solutions made of distilled water (24-C), so that it can be said that the difference observed between the various treatments in those previous tests were not caused by the different pH of the solutions.

In all thses tests, the responses were different according to the illumination given to the growing seedlings.

(1) Antagonism in Light.

All the experiments conducted in continuous or alternate illumination gave the same results, except for 23-B which exhibited, in alternate illumination effects similar to those obtained in continuous darknes. In contrast to this only instance, four experiments (23-A, 23-C, 24-A and 24-C) demonstrated that ASA induces the same reduction of the inhibition caused by DTO, as well in alternate illumination as in continuous illumination.

As shown by experiment 24-C, where all treatments were at the same pH, a check receiving 250 ppm of ASA alone gave a slight promotion of root elongation over the controls, which was definitely smaller than the reduction of inhibition obtained with the mixture of DTO and ASA over the check receiving DTO only.

In this experiment, in alternate illumination, 250 ppm of ASA alone gave a 9% promotion of root growth over the controls (total lengths 98.1 and 89.9 mm respectively), 200 ppm of DTO alone gave 45% inhibition whereas the mixture gave only 23% inhibition (respectively 49.7 and 69.4 mm) which represents a suppression of half of the inhibition caused by DTO. In continuous illumination, the antagonism was still stronger: DTO alone gave 43% inhibition, which was reduced to less than 15% when 500 ppm of ASA were added, which means that more than 60% of the inhibition was suppressed.

Usually, 250 ppm of ASA was the most effective concentration for suppressing the inhibitions of DTO.

It was commonly observed that a slight predipitate would appear in the Petri dishes receiving the mixture of ASA and DTO after a few hours in light, usually not more than 12 hours. In the liquid left over in the flasks where the solutions had been prepared, when these flasks were left in a darker spot of the laboratory, a similar precipitate used to appear more slowly, usually not before a day or so. The same precipitate was practically never observed in the dishes receiving the dark treatments.

(2) Synergism in Darkness.

The concentrations of ASA which were able to overcome the effects of DTO in light did not have the same effect in darkness, but on the contrary they augmented the inhibitory effects of DTO. Increasing concentrations of ASA gave the increased aggravations of the inhibitions, as can be seen specially clearly in 23-C, 24-A and 24-C.

Taking the same experiment as before, 24-C, it was found that 250 ppm of ASA alone, in darkness, induced 9% promotion of root growth over the controls, 200 ppm of DTO gave almost 46% inhibition, and the mixture gave about 60% inhibition, or an increase of $\frac{60-46}{46}$ = 30% in the inhibition.

No indication of antagonism was observed in any of the six experiments 23-A, 23-B, 23-C, 24-A, 24-B, and 24-C in darkness.

However, after the transfer experiments had shown that antagonism may also take place in darkness (see below), another test was ran, where a higher concentration of DTO (300 ppm) and a wider range of concentrations of ASA were used. The seeds tested were from a newer sample of Burpee Hybrid cucumbers. The results, given in 24-D, showed that the antagonism of ASA to DTO may be exhibited in darkness also at lower concentrations of ASA. The synergism, in this experiment, was less evident, and exhibited at the highest concentrations of ASA only.

2. Transfers: Importance of the Sequence of the Chemicals.

The different responses exhibited in light and in darkness to mixtures of ASA and DTO, and the observation that the antagonism seemed to be accompanied by a precipitation in light, suggested that a photo-chemical reaction in the illuminated complex solutions might be responsible for this antagonism, by making part of the DTO unavailable to the seeds. Such reaction between an unsaturated lactone ring and sulfhydryl groups is possible on the ground of the recent findings of Cavallito et al. (33).

Therefore several experiments were performed in which Burpee Hybrid cucumber seeds were transferred from a solution of one chemical, to a solution of the other chemical, As previously indicated (page 62), the pH of the pretreatments was not the same as that of the post-treatments, the solutions of DTO and the corresponding controls being made of plain distilled water, whereas

ASA solutions and their controls were made of acid water at pH 3.5.

a. Preliminary Experiments.

Those were accomplished simultaneously in continuous illumination and darkness; the transfers were made after 8, 24, 48, or 72 hours; both ways, from ASA solution to DTO solutions (group I) and from DTO solutions to ASA solutions (group II) were investigated. In these first tests, reported in 25-A and 25-B, important differences in the responses were already apparent, according to the sequence of the solutions, the time of the transfer, and the conditions of light or darkness.

(1). The sequence of the solutions.

When the order of the treatments was ASA first then DTO (group I of 25-A and 25-B), ASA did not induce much change over the check receiving a post-treatment of DTO only, but there were indications that the inhibitions induced by DTO would be reduced.

When DTO was first applied (group II, 25-A and 25-B), the inhibition was stronger for the roots receiving the complex treatment than it was for the check (DTO alone).

(2) The time of the transfer.

In group I (ASA first), no antagonism was apparent if the transfer was made after 24 hours only; some was visible if it was done after 48 hours or 72 hours.

For the synergism obtained in treatments of group II (DTO first), it was not visible in light if the transfer was done after

8 hours only, but was visible after 24 hours (group II, 25-B).

(3). The conditions of illumination.

It can be seen, from the group II of 25-B, that the checks receiving only DTO as a pretreatment promoted growth in light, whereas they inhibited it is darkness. At the same time, the reinforcement of inhibition was more apparent in darkness than in light for the complex treatments.

From these first experiments, it was demonstrated that only the order in which the chemicals were applied seemed to influence the type of the interaction, and to determine whether it would be an antagonism, or an increased toxicity. The other factors appeared to affect only the intensity of the response in a somewhat secondary way.

Therefore, in the next experiments, both sequences of the transfer were successively investigated; because a new sample of ASA and seeds from a new shipment were used, some slight differnece in the results were observed, as compared with the preliminary tests, although the general trend of the results remained unchanged.

b. Transfers from Dithiooxamide to Ascorbic Acid: Synergism.

Two main experiments were conducted in which DTO was first applied for 8 or 24 hours, and then the seeds were transferred to ASA solutions until the end of the test. Both conditions of continuous illumination and continuous darkness were investigated. The results are given in appendices 26-A, and 26-B.

In addition, three smaller experiments were performed, one in darkness only, with transfer after 24 hours (27-A); the second was carried out in light, with transfer after 48 hours (27-B); in the last one (27-D) the interactions at lower concentrations of ASA were investigated.

(1). The synergism in darkness.

The increased inhibition resulting from the complex treatment as compared with the checks receiving only one chemical has been found to be specially considerable in darkness, and was repeatedly observed on a wide range of concentrations of DTO and of ASA.

In the experiment 26-A, some inhibition was visible in the check ASA (400 ppm) alone, but it can be seen that the inhibition caused by the complex treatment is much higher than the sum of the inhibitions cause d by either chemical independantly, as follows:

Inhibition caused by ASA (400 ppm) alone.....13.3
Inhibition caused by DTO (150 ppm) alone.....12.5
Sum of these inhibitions.....25.8
Inhibition caused by DTO (150) plus ASA (400 ppm).....47.3

Inhibition caused by ASA (400 ppm) alone.....13.3
Inhibition caused by DTO (300 ppm) alone.....27.3
Sum of these inhibitions.....40.6
Inhibition caused by DTO (300 ppm) plus ASA (400 ppm).....59.0

In the case of a pretreatment length of 8 hours, less reinforcement of the inhibition was obtained.

To get a still better evidence of the increased toxicity, the next experiment was conducted with a lower concentration of ASA (250 ppm) which did not induce any significant inhibition alone. Also, a wider range of concentrations of DTO was tested (from 37.5 to 300 ppm), so that the lowest concentrations were even not toxic in darkness.

In this way, it was possible to demonstrate that a non toxic concentration of ASA was able to reinforce the inhibition caused by a toxic pretreatment of DTO; but it was also several times observed that the successive application of DTO at a nontoxic concentration and then ASA also at a nontoxic concentration resulted in a significant inhibition, amounting once to 38% of the total length of the controls (26-B).

These results are summarized in table VIII where data from 26-B and 27-A have been gathered and averaged in order to give the scale of the effects of a post-treatment of 250 ppm of ASA on the inhibitions induced by a wide range of concentrations of DTO. The data from the other similar experiments have not been entered in this table, because of differences in the concentration of ASA and in the origin of the chemical and of the seeds. It can easily be seen however that the results of these experiments are in excellent agreement with those of table VIII

From experiements 27-D, it can be seen that no interaction between ASA and DTO took place at lower concentrations of ASA,

TABLE VIII

EFFECTS OF SUCCESSIVE APPLICATIONS OF DITHIOOXAMIDE (DTO) IN DISTILLED WATER SOLUTIONS AND OF ASCORBIC ACID (ASA) IN ACID WATER SOLUTIONS (pH 3.5) ON THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS GROWN UNDER CONTROLLED CONDITIONS IN DARKNESS FOR 5 DAYS.

Pretreatment		Post-treatments: Detailed Average Root-length (mm) Acid Water	Detailed Average Root-length (mm) ASA 250 ppm	General Average (mm) Acid Water	General Average (mm) ASA 250 ppm
Distilled Water	26-B	103.3	98.4		
	27-A	102.4	100.7	102.8	99.6
DTO 37.5 ppm	26-B	-	-		
	27-A	103.0	83.4	103.0	83.4
DTO 75 ppm	26-B	104.4	65.6		
	27-A	94.8	72.0	99.6	68.8
DTO 150 ppm	26-B	81.3	58.3	81.3	58.3
	27-A	-	-		
DTO 300 ppm	26-B	67.9	48.3	67.9	48.3
	27-A	-	-		

so that the concentration of ASA at which the synergism disappears is somewhere between 100 ppm and 250 ppm.

(2). The synergism in light.

The evidences pointing to the existence of a similar reinforcement of DTO toxicity by post-treatments of ASA are less consistent in light than in darkness. Some can be found in 25-B, group II, and in 26-A, but no positive result was observed in 26-B with three concentrations of ASA and two of DTO.

However, it was thought that a longer pretreatment might be more successful, as was suggested by the results given in 25-A, group II where the pretreatment was applied for 48 hours. Therefore, experiment 27-B was conducted in light only, and the pretreatments of DTO were applied for 48 hours. It was observed that, in this case, the successive application of solutions of DTO and then of ASA which are not toxic by themselves, would result in a significant inhibition of root elongation. Moreover, examples can be found of tests where a nontoxic concentration of ASA, applied after a promotive concentration of DTO, was able to change this promotion effect into an inhibition (25-B, groupII, and 27-B).

(c). Transfers from Ascorbic Acid to Dithiooxamide; Antagonism.

From the preliminary experiments (25-A, group I and 25-B, group I) some evidences for an antagonism between ASA and DTO have already been reported, both in light and in darkness.

The longer the pretreatment, the stronger the antagonism observed.

In experiment 28-A, ASA solutions were first applied for 72 hours and the seeds were then transferred to solutions of DTO. The best antagonism was observed in darkness, for a concentration of 250 ppm of ASA. This experiment was repeated with two replicates, in darkness only, and with only a concentration of 250 ppm of ASA. Comparable results were obtained (28-B). Statistical analyses (appendix X) showed that the three values obtained for the complex treatment were significantly different (probability 0.99) from those given by the checks receiving only a post-treatment of DTO. These results have been averaged in table IX.

As for the antagonism in light, which appears to be less considerable than in darkness, it was possible to extract from experiments 25-B, group I, and 28-A at least three cases where the complex treatment gave a highly significant reduction of the inhibition induced by DTO (appendix XI, probability 0.99).

In all the cases (both light and darkness) but one (25-B, in light, group I, 48 hours: no check), the corresponding checks receiving ASA alone were not significantly longer than the controls; this rules out the possibility that the antagonism be only the result of a growth-promoting activity of ASA alone.

As for experiment 28-C, where a high concentration (2,000 ppm) of ASA was used, it shows that in darkness a complex treatment involving toxic concentrations of both ASA and DTO induces an inhibition which is almost the mathematical addition of the two inhibitions caused by the chemicals alone. For instance:

TABLE IX

EFFECTS OF SUCCESSIVE APPLICATIONS OF ASCORBIC ACID (ASA) IN ACID WATER SOLUTIONS (pH 3.5) AND OF DITHIOOXAMIDE (DTO) IN DISTILLED WATER SOLUTIONS ON THE ELONGATION OF BURPEE HYBRID CUCUMBER ROOTS GROWN UNDER CONTROLLED CONDITIONS IN DARKNESS FOR 5 DAYS

Pretreatment (72 hours)		Post-treatments (48 hours)			
		Detailed Average Root-length (mm)		General Average (mm)	
		Distilled Water	DTO 300 ppm	Distilled Water	DTO 300 ppm
Water pH 3.5	28-A	83.4	58.6	85.0	59.4
	28-B (1)	86.4	62.8		
	28-B (2)	84.7	56.7		
ASA 250 ppm	28-A	84.8	70.2	84.5	71.6
	28-B (1)	84.7*	73.4		
	28-B (2)	83.9	71.2		

* Average of 20 seeds.

Inhibition caused by DTO (100 ppm) alone.....19.0 mm
Inhibition caused by ASA (2,000 ppm) alone.....17.1 mm
Sum of these inhibitions.....36.1 mm

Inhibtion caused by ASA (2,000 ppm) plus DTO (100 ppm).....34.1 mm

Similar calculations can be made for the treatments in which a concentration of 200 ppm of DTO was used. Neither synergism, nor antagonism were observed in this experiment.

d. Effects of changing Light Conditions at Time of Transfers.

It has been previously seen that the conditions of illumination in which the seeds were grown had a great influence upon their response to the chemical treatments; the best example is the difference of root length of the checks receiving only a pre-treatment of DTO for 24 hours (300 ppm): a strong inhibtion was observed in darkness, whereas a promotion was observed in continuous illumination (table V).

The importance of the time and of the sequence of the transfers in determining the type and the intensity of the plant responses suggested the existence of several differnet phases in the early physiological processes of growth. Therefore, there was a possibility that these phases also be differentially sensitive to the light conditions; more specifically, it was thought that the conditions of light during the pretreatment might be of a major importance for the type of response to be obtained.

Accordingly, an experiment by transfer was run, in which the pretreatment of DTO (300 ppm), and the post-treatment of ASA (250 ppm); the dishes in light during the pretreatment were

moved to darkness at the time of the transfer, and vice-versa. The results are given in 27-C. Both checks receiving either chemical alone gave some inhibition in this case, and the complex treatments gave inhibitions which were about the addition of these individual inhibitions.

For instance, in the case of transfer from light to darkness:

Inhibition caused by DTO (300 ppm) alone.....20.3 mm
Inhibition caused by ASA (250 ppm) alone.....22.4 mm
Sum of these inhibitions.....42.7 mm

Inhibition caused by DTO (300) plus ASA (250)....46.8 mm

In the case of transfer from darkness to light:

Inhibition caused by DTO (300 ppm) alone.....9.4 mm
Inhibition caused by ASA (250 ppm) alone.....7.2 mm
Sum of these inhibitions.....16.6 mm

Inhibition caused by DTO (300) plus ASA (250)....22.3 mm

Neither antagonism nor synergism seems to take place in this case.

Specially interesting is the inhibition caused by ASA (250 ppm) in the first case, contrasting with its neutrality in both light and darkness (26-B, 27-A).

D. Interactions of some Chemicals related to Dithiooxamide or to Ascorbic Acid.

A few tests were performed with some chemicals whose structures are close to that of ASA or of DTO to investigate the generality of the results previously reported. In all these tests, Burpee Hybrid cucumber seeds were the material tested, and the

experiments were conducted according to the technique of constant solutions, previously described and extensively used.

1. Interactions of d-Iso-Ascorbic Acid with Dithiooxamide.

The first chemical investigated was the d-isomer of ASA, which has no Vitamin C activity and is not present in plant tissues, but is known to exert upon plants the same effects as does Vitamin C.

As it was expected, mixtures of d-iso-ascorbic acid and of DTO produced the same effects asdid mixtures of ASA and DTO: antagonism was observed in light, and synergism in darkness. Experiments 29-A and 29-B gave the same general results.

2. Interactions of Gluco-Ascorbic Acid with Dithiooxamide.

During the past few years, gluco-ascorbic acid has been sometimes considered as a metabolic antagonist of ASA in animal physiology, although recent works do not support this idea (63).

Gluco-ascorbic acid and DTO were observed to interact together in a way similar to the interactions of DTO and ASA in the same conditions (30-A and 30-B).

In light, an overcoming of the inhibition caused by DTO was observed as a result of mixing with gluco-ascorbic acid. Some growth promotive effects were produced by gluco-ascorbic acid alone in experiment 30-B, but not in 30-A, which were however much less considerable than the difference between the length in the mixture and the length in the check (DTO alone).

In darkness, a synergism similar to that of ASA with DTO takes place even though gluco-ascorbic acid alone was sometimes slightly growth-promotive.

3. Interactions of a Tetronic Derivative with Dithiooxamide.

The compound 3-(alpha-imino-ethyl)-5-methyl tetronic acid has been recently found to induce a strong inhibition of chlorophyll in various plants (2)(68). It can also inhibit root-elongation of cucumber seedlings (31-A and 31-B). Its structural formula contained an unsaturated lactone ring which closely resembles that of ASA, as seen in appendix-formulas.

In similar experiments with mixtures of this substance with DTO, no overcoming of the inhibition caused by DTO was ever observed. Only reinforcements of this inhibition seem to occur, as well in light as in darkness. On the range of concentations tested, these reinforcements of inhibition were roughly proportional to the inhibitions caused by the tetronic derivative alone.

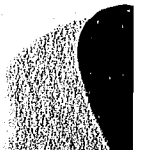
Some promotion of root elongation by this substance was observed in darkness in the first test (31-A) but was not duplicated in 31-B.

4. Interactions of Thiourea with Ascorbic Acid.

Thiourea is a thioamide which resembles DTO, and has been shown to exert some antagonistic effects upon the inhibitions of root development induced by coumarin. It was therefore interesting

to investigate how ASA would effect its influence on the elongation of cucumber roots.

Two tests, in which mixtures of ASA and t hiourea were compared to checks receiving either chemical alone, did not reveal any antagonism of the two chemicals. On the contrary, both in light and darkness, synergistic effects were observed (32-A and 32-B), which were specially evident in 32-B in darkness and 32-A in light, becuase ASA alone was not inhibitory in these cases. In the other cases, it is easy to see that the inhibition caused by a mixture of ASA and thiourea was greater than the sum of the inhibitions induced by either chemical alone (synergism).



III. DISCUSSION

One of the most conspicuous features of the results reported in this work is the deep influence of the environmental factors on the results.

As for the fluctuations of the effects of ASA alone on root elongation, only little comment can be added to the data. The previously reported (21, 37, 135, 136) variations of ASA content in plant tissues may help to understand them, but do not throw much light, as such, upon the fundamental mechanisms involved.

Before attempting any interpretation of the physiological meaning of the interactive effects of ASA with coumarin, DTO, and other substances, it seems of interest first to examine shortly the influence of the circumstances and environmental factors of the experiments upon these phenomena.

A. The Conditions of the Experiments

1. Antagonism of Ascorbic Acid and Coumarin

The spectacular results obtained with mixtures of the chemicals applied in constant solutions need hardly any comment (Fig. 7, 8, 9, 10), as far as cucumber seedlings are concerned; the antagonism occurred as well in darkness as it did in light.

In the case of lettuce seeds, good evidences for an antagonism of ASA to the inhibitions induced by coumarin were observed in light but not in darkness for treatments with mixed constant solutions of these chemicals.

It is only normal that the experiments in which a technique of transfer was used gave less striking results: both chemicals had less time to act upon the seeds, and consequently, their effects were less intense.

In transfer experiments with cucumber seeds, it was surprising that the antagonism was much weaker in darkness than in light, since it was of about the same strength in both cases in the experiments with constant solutions. However, this resembles the antagonism of DTO to coumarin, and may suggest that both antagonisms are related; but this does not explain the differnece between the results obtained with the two techniques in the present case. It is possible that other conditions of application, such as a longer pretreatment, or different concentrations of the chemicals, would have revealed a stronger antagonism in darkness. However, the purpose of showing that the antagonism was not the mere result of a chemical reaction taking place in the solution had been attained beyond question with the results obtained in light.

For lettuce seeds, on the contrary, the pretreatment revealed an antagonism in darkness only, whereas the constant solution method gave one in light only. This may probably be explained by the existence of a dormancy induced by coumarin as follows: since light breaks dormancy (112), there is nothing for ASA to antagonize, in the case of a pretreatment in light, but in darkness ASA antagonizes the dormancy; in the case of constant solutions, in darkness ASA has no power against the addition of the dormancy and of the toxicity both caused by coumarin, whereas in light it can partially

overcome the toxic effects of coumarin, the dormancy being destroyed by light. It is interesting to recall here the results obtained with DTO in part I: ASA appears to be generally less potent than DTO to overcome the inhibition of lettuce germination caused by coumarin.

2. Interactions of Ascorbic Acid and Dithiooxamide and some Related Chemicals.

As for the interactions of ASA and DTO which can work as well as an antagonism or as a synergism, other factors than the conditions ofillumination are also extremely important: the type of experiments, the time when the chemicals are applied, and their concentration.

In the experiments where mixtures of chemicals were applied for the full length of the test, the important factor was illumination, which determined what type of interaction would take place at given concentrations of the chemicals. The antagonism of ASA to the effects of DTO was evident in light only, which resembled the antagonism of DTO to the inhibitions caused bycoumarin. ASA concentrations which were antagonistic in light, became synergistic in darkness, although some antagonism was detected at lower concentrations in darkness.

In the experiments where transfers of the seeds were effected from one chemical solution to another, the important factor for deciding what the interaction would be, was the sequence of the chemicals. Pretreatment of DTO induced synerigsm, pretreatment of ASA yielded antagonism in either light or darkness, provided some delays of

transfer were observed, in the second case chiefly. In both cases, the interactions were stronger in darkness than in light. It can be recalled at this point that stimulation of root elongation and antagonism to coumarin were produced by DTO, only it was applied as a pretreatment (part I); it has been previously indicated also that DTO is effective in breaking chemical and thermal dormancies; in the transfers, ASA overcomes the inhibition induced by DTO only if DTO is applied as a post-treatment after at least 48 hours. All these facts indicate that DTO is specially effective upon the very earliest stages of root development.

To this remark, it should be added that the experiment where light conditions were changed at the time of transfer (27-C) indicated clearly that the later stages of cucumber seedling development were much more fragile in darkness than in light (see page 77) and that light throughout the test was essential for the promotive activity of DTO to take place.

Bringing together these remarks and recalling that the very earliest developmental stages are much more rapid in light than in darkness (10-A), one may get a hint to what takes place in the constant solutions receiving mixtures of ASA and DTO.

In light, DTO is less injurious to the earliest stages of growth because they are rapid, in fact it is even beneficial to them, provided the seedling remains in light; a secondary inhibition takes place then which is overcome by ASA, similarly to what happens when a pretreatment of ASA for 48 or 72 hours protects the plant against a

post-treatment of DTO, both in light and in darkness.

In darkness, more damage is caused by DTO to the slower earliest stages of growth, and the plant so weakened becomes sensitive to ASA besides being poisoned by the lengthy presence of DTO in the solution. In case of a transfer from DTO to ASA, the processes are involved, except for the inhibition due to the lengthy presence of DTO. As seen by experiment 27-D where low concentrations of ASA were used for the post-treatment, the inhibition of the early growth by DTO is not reversible, whereas that of the later processes is (28-B).

It is not clearly understood why a stimulating pretreatment of DTO becomes inhibitory when it is followed by a post-treatment of ASA. The explanation might be that ASA, being antagonistic to the inhibition caused by DTO in light (constant solutions), may also be antagonistic to the stimulation produced by DTO in light (transfers).

In constant solutions in darkness, the reversion of the synergism to the antagonism by low concentrations of ASA indicates that, at higher concentrations, the antagonistic part of the system was also functioning, but was masked by the synergistic part (earlier processes of germination). This suggests that the concentrations of both chemicals need to be carefully adjusted if one wants to obtain either effect.

Before leaving the study of these complex interactive effects of two chemicals which can be synergistic or antagonistic, the strangest part of these phenomena should be emphasized: in constant solutions ASA alone is practically ineffective on the growth processes below a concentration of at least 1,000 ppm, therefore high above those where

the interactive effects take place (250 ppm). These figures make possible to estimate what a considerable upset of ASA metabolism may be caused by DTO.

As for the other chemicals tested, no extensive conclusion can be drawn from the few experiments performed. It can be said however, that d-iso-ascorbic acid and gluco-ascorbic acid appear to interact with DTO just as ASA did.

The tetronic acid derivative tested and thiourea were much less sensitive to changes in illumination conditions, at least as far as were concerned their interactions with DTO and ASA respectively.

B. Interpretation of the Results

The present experiments demonstrate that ASA is able to reduce the inhibitions of root elongation induced by coumarin, and to interact in a complex way with the effects of DTO. Experiments involving transfers of the seeds have shown that these interactions actually take place inside the plant tissues. This suggests that the metabolism of ASA is involved in the phytocidal effects of coumarin, and can be strongly effected by DTO, as was suggested in Part I of this work.

However, whole seedlings are complex organisms; that their general growth responses to ASA, DTO and coumarin are connected does not bring much information as regards the physiological mechanisms involved in these interactions. Since sulfhydryl groups, unsaturated lactones and ASA are natural substances, all of wide occurrence in plant tissues, it is logical, after the results of this work, to suspect that all of them are constituents of one natural complex system, which might be of general importance for plant growth processes.

For instance, it has been wondered about the lower ASA content of the plants grown in shade or darkness. The physiological importance of this can be understood if it is assumed that natural -SH groups interact with ASA in the same way as does DTO, and with the same sensitiveness to light conditions: if ASA content was not decreased in darkness, the combination of it with the natural -SH groups would be harmful to the plant. Of course, this foes not explain the immediate mechanism involved, although some works with dehydro-ascorbic acid reductase (41) and glutathione (99) indicate that a mechanism of this kind is actually at work in plants, which could be sensitive to light conditions (5).

A coordination of the results obtained in various conditions and by differne t techniques made it possible previously to outline an hypothetical complex mechanism of interaction between ASA and DTO, the physiological nature of which will now be discussed.

Two phases were discerned in this mechanism; the first one, which takes place in the early stages of growth, is a sensitization of the plant to ASA, it is therefore an influence of DTO upon the metabolism of ASA, whereas the second one, occurring during later stages of growth, is an antagonism of ASA to the effects of DTO. It is remarkable that, in part I, two kinds of physiological relationships between ASA and -SHgroups were suggested, which exactly fit this pattern of a reversible connection. The influence of DTO, and other thiols, upon ASA metabolism may have various causes; it may be for instance an inactivation of some enzymes controlling the oxidation of ASA, such as

oxidase or polyphenol oxidase (139), or it may be a direct stabilization (30) or immobilization (47) of ASA by sulfhydryl compounds. The other phase, (influence of ASA upon the effects of DTO and upon the -SH groups), would be caused by the reducing properties of ASA which enable it to reactivate the sulfhydryl enzymes in certain conditions (10).

As regards the chemical requirements of these processes, the failure of thiourea and of the tetronic derivative to exhibit the antagonism with ASA or DTO respectively, whereas gluco-ascorbic acid reacted with DTO as did ASA, suggests that the complete mechanism cannot work if there are not two -SH groups (like DTO) and two -OH groups (like in the ascorbic acids) present in the solutions (See formulas).

As for the antagonism of ASA to the effects of coumarin, the finding of Andreae et al. (4) should be recalled before any attempt of interpretation can be done. These workers have observed that high concentrations of ASA inhibit the metabolism of scopoletin in healthy potato tissues; scopoletin is a natural, toxic coumarin derivative. If it was assumed that ASA may also inhibit the metabolism of coumarin, it might then be possible that ASA antagonizes the effects of coumarin by arresting the mechanism by which coumarin is introduced into the processes of growth. Being left unused, coumarin probably should not be poisonous.

Another possibility is that coumarin be inhibitive by inactivation of some -SH enzymes (oxidation) as suggested by the finding of Calallito et al. (33) that unsaturated lactone can react with sulf-

hydryl groups. ASA, being a reducing agent, would then be able to restore the activity of these enzymes (10).

An indirect relationship between ASA and coumarin might involve tyrosine and the enzyme polyphenol oxidase, of which tyrosine and ASA are substrates, among many others. Best (17) has suggested that tyrosine may be the presursor of coumarin derivatives in plants, which, on chemical and biochemical grounds, is possible as indicated by Haworth (73). Even (SEE appendix, formulas) if there is no kinship between them, it is possible that coumarin, and especially its hydroxy-derivatives such as scopoletin and umbelliferone act as substrates for polyphenol oxidase, since this enzyme is known to catalyze the oxidation of various phenols and polyphenols: coumarin is the lactone of o-hydroxy-cinnamic acid, which possesses a phenolic hydroxyl group. However there is no experimental work to support or disprove this hypothetical suggestion, except that it is known from the work of Onslow (114) that caffeic acid (3-4-dihydroxy-cinnamic acid) acts as a substrate for tyrosinase.

From the data presented in this work and the interpretation which was then suggested, it seems therefore possible to conceive that a complex natural system involving -SH groups, ASA, and coumarin derivatives may exist in plant tissues, which can be represented as follows, in Fig 12. Arrows should be read "affects"; the dotted line indicates that the process suggested is merely hypothetical without any experimental referance.

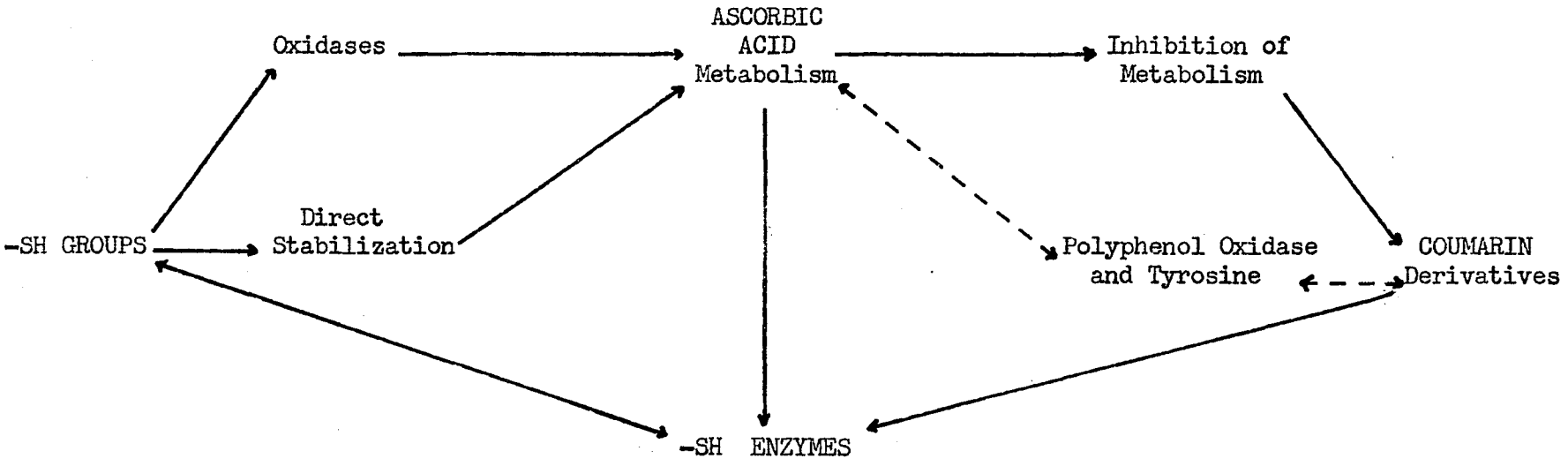


Figure 11

Suggested interrelationships between coumarin, -SH groups and ascorbic acid.

IV. SUMMARY

The effects of ascorbic acid on the inhibitions of root development induced by solutions of coumarin and of dithiooxamide have been investigated under controlled conditions of light and temperature in Petri dishes.

(1) Ascorbic Acid has been found to exert no significant effect upon the elongation of cucumber roots at the concentrations used in this work (below 2,000 ppm).

(2) Ascorbic Acid was able to overcome the inhibitions of cucumber root development induced by solutions of coumarin both in light or in darkness; it was equally potent in either case when the chemicals were simultaneously applied throughout the tests; it was more potent in light than in darkness when the seeds were successively treated with a solution of coumarin first, and then with a solution of ascorbic acid.

(3) Ascorbic acid was able to overcome the inhibitions of lettuce germination induced by coumarin; this took place in light only and not in darkness when the chemicals were simultaneously applied throughout the test; it occurred only in darkness when the seeds were first soaked in a coumarin solution for 24 hours in darkness, and then germinated in ascorbic acid solutions (breaking of artificial dormancy).

(4) Ascorbic Acid, simultaneously applied with dithiooxamide throughout the tests, reduced the inhibition of cucumber root devel-

opment induced by dithiooxamide in light, but increased it in darkness.

(5) Ascorbic acid, applied as a post-treatment after a pretreatment of dithiooxamide, increased the inhibition of cucumber root elongation induced by dithiooxamide in darkness, and changed into inhibition the stimulative effects of dithiooxamide in light.

(6) Ascorbic acid, applied as a pretreatment for at least 48 hours before a post-treatment of dithiooxamide was able to reduce significantly the inhibition of cucumber root elongation induced by dithiooxamide both in light and in darkness.

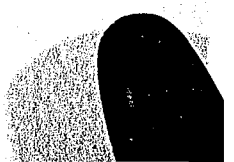
(7) These interactive effects of ascorbic acid were always much more considerable than the slight stimulations or inhibitions of cucumber root elongation which were sometimes induced by ascorbic acid alone in solution, so that it can be truly spoken of antagonisms or synergisms of ascorbic acid with coumarin or with dithiooxamide.

(8) Gluco-ascorbic acid and d-iso-ascorbic acid exhibited interactive effects similar to those of ascorbic acid upon the inhibition of cucumber root elongation induced by dithiooxamide. 3-(alpha-iminoethyl)-5-methyl tetronic acid increased this the inhibition approximately in proportion of its own inhibitory effects. Addition of ascorbic acid to solutions of thiourea produced a synergistically increased inhibition of cucumber root elongation in either light or or darkness. No antagonism was observed.

(9) These results are discussed relatively to the conditions of illumination and to the time and the order of application of the chemicals. A complex biochemical system of interrelationships between the metabolism of Vitamin C in plants and -SH groups on the first hand and coumarin derivatives on the other hand is suggested.

PART III

THE ANTAGONISMS OF DITHIOOXAMIDE AND ASCORBIC ACID TO THE AUXINS



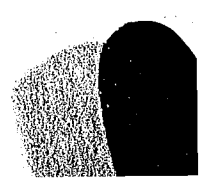
I. INTRODUCTION

For several years, plant physiologists have wondered about the physiological changes responsible for the spectacular effects caused in plants by the natural hormone IAA and by some synthetic growth-regulators which are now extensively used in agriculture and the objects of an important business.

It was a logical idea to try to tie them up with some naturally occurring plant growth substances. Coumarin derivatives and other unsaturated lactones have outstanding growth properties, and are of wide occurrence in plant tissues. Recent discoveries may allow to think that auxins induce deep changes in the natural distribution of these substances, which may then account for their ability to control plant growth mechanisms, as has been actually suggested by Van Overbeek in a recent paper (153).

We should indicate at this point that it is commonly considered, (but not proven) that all auxins act upon plants in some fundamentally similar way. In the further progress of this work, it will be spoken of the auxins as of a class of substances presenting the same general physiological activity.

If Van Overbeek's idea is right, and since the data of the two first parts of this work indicate that ASA and DTO are interferring with the metabolism of coumarin, it seems that these two substances might also be able to influence the still mysterious fate of the so-called "auxins" inside of the plant tissues.



Therefore, after showing how this concept of interrelationship between natural unsaturated lactones and auxins became progressively elaborated, and what few experimental works support Van Overbeek's attractive hypothesis, the following review of literature has been extended to the facts suggesting a similar connection between auxins on the first hand, and thiol groups and ASA on the other hand.

A. Review of Literature

1. The Relationships between Unsaturated Lactones and Auxins

The possibility of an interrelationship between unsaturated lactones has apparently not been seriously considered before 1943. This year, Veldstra et al. (154), after reporting an antagonism of coumarin to NAA in the pea test, advanced the idea of a connection between unsaturated lactones and the natural auxins a and b which can readily assume a saturated lactone structure. This might in turn give an unsaturated lactone ring which, in the case of auxin a would be the same as that of hexenolactone (parascorbic acid) and coumarin, both of which are physiologically active.

Following his own observations that the phytostatic action of coumarin closely resembles the effects produced by 2,4-D, (6, 8), Audus, in an extensive review of the field of plant growth-regulators activity, (7) suggested that unsaturated lactones and auxins may act on a same metabolic process, namely the dehydrogenases (-SH enzymes). Larsen (90), in the course of an investigation of IAA precursors in plants, observed that mixtures of unsaturated lactones and synthetic

indole-3-acetaldehyde give the same curvatures in the Avena test than the neutral growth-substance present in certain plant extracts. Out of these lactones only parasorbic acid give a slight response in the Avena test. From these data, Larsen suggested that a double system of growth controlling substances may exist in plants, of which the auxins would constitute the growth-promoting complex, and the unsaturated lactones, the inhibiting part. Moewus (107) in 1949, reported an antagonism of coumarin and heterauxin. In the inhibitory zone of IAA, the effects of the two inhibitors were additive. Buston et al. (25) also reported an antagonism of dl-hexenolactone to IAA in the Avena test, and to natural auxins contained in plant extracts, although it did not exhibit any auxin activity (the d-idomer, parasorbic acid has some according to Larsen). The antagonism to auxins was overcome by beta-alanine.

Van Overbeek et al. (152) observed an antagonism of trans-cinnamic acid to auxin in the pea stem test; cis-cinnamic acid exhibited auxin acitivity. These properties of the cinnamic acids are of interest because they are structurally related to coumarin (see appendix, formulas).

The works previously cited afford only indications of a rel-ationship which is deduced from theoretical considerations, from the observations of similar effects upon plants, or, more convincingly, of direct antagonisms or interactions.

Fortunately, a direct evidence of a connection between a synthetic auxin, 2,4-D, and a natural coumarin derivative, scopoletin, has been recently contributed by Fults et al. ( ) who discovered

that 2,4-D induced accumulation of scopoletin in several plants, and suggested that an excess of this toxic substance might be the cause of the phytotoxic effects of 2,4-D. Van Overbeek then gave its present form to this hypothesis. Bringing together several facts, such as the natural occurrence of small amounts of scopoletin in many plant tissues, and its toxicity, the resemblance of the phytocidal symptoms of coumarin and of these of 2,4-D, the similar herbicidal selectivity of the later and of beta-methyl-unbelliferone (another coumarin derivative) he suggested that 2,4-D induced a deep change in the metabolism of plants, as indicated by the works of Hamner, Sell and other workers of Michigan State College (67, 97, 108, 130), resulting in the accumulation of toxic coumarin derivatives.

2. The Relationships between Thiols, ASA and the Auxins.

Most of the works dealing with ASA have been devoted to its influence on physiological processes rather than to the external effects produced by its application to plants, which is quite logical since, due to its presence in all of th em, plant tissues do not respond very clearly to external treatments with ASA in every case. Besides, its determination in vivo being relatively easy provides a useful tool for detecting metabolic changes.

a. Distribution of Auxins, Vitamin C and -SH groups in Plants.

Soon after the discovery of its growth-regulating properties, Clark (37) investigated ASA distribution in Avena Coleoptile and compared it with those of auxin and chlorophyll. He found that the

tip was richer than the base in ASA (reduced form), and the opposite for dehydroascorbic acid (oxidized form of ASA); there seemed to be a correlation between chlorophyll and ASA content in Avena tissues from seedlings grown in light. He observed also that extracts from basal sections of coleoptile would oxidize ASA mor than extracts from apical sections, the same occurs for the destruction gradient of auxin the coleoptile.

These observations have been confirmed by several workers. Reid (126) found a correlation between dry weight, ASA content and activity of the cells in cowpea seedlings. Shaw et al. (131) found the highest concentrations of ASA in the most actively growing parts of broad bean seedlings. As precedently indicated, it is well known that the amount of ASA, usually nil at the start of germination, rises sharply during the early most active stages of development of the seedling, then drops off rapidly. The occurrence of the highest concentrations of ASA in the most actively growing parts of plants has been also observed by Lebrec (94), who furthermore showed that it is not due to a greater stability of ASA in these tissues, but to a greater capacity of younger tissues for synthesizing ASA. As pointed out by Clark, the gradient of ASA concentration in plant tissues suggestively resembles the corresponding gradient of auxin. It is interesting to recall here the already cited works of Pett (7) and Kretsovitch et al. (86), and others who have shown that the amount of natural -SH groups in young seedlings undergoes variations quite similar to those of ASA. Thus, ASA and -SH groups are concentrated in acitvely growing tissues, the same as for the natural auxins.

However, it should be pointed out that the similarities of amount and distribution of ASA, IAA and -SH groups in plant tissues do not allow any positive conclusion regarding an interaction between them. It would probably be wiser to consider a complex of growth substances, working together, as suggested by Kunning (88) after his study of the similar stimulation of cambial activity induced by IAA, ASA and other growth-factors in decapitated bean and sunflower.

b. Interactions of ASA and thiols with auxins.

Immediate evidences of interactions of ASA and of sulfhydryl derivatives with auxins have been contributed by two kinds of works: observation of antagonism of these chemicals to the effects of auxins, and study of the inactivations by one group of chemicals of the enzymes controlling the metaboism of the other group of substances.

Zopf (167) observed that the toxic effects of NAA were antagonized by a mixture of Vitamin B_1 and C; however he did not determine exactly what part Vitamin C played in this antagonism. Podesva (119) reported that the toxic effects of naphtyl-octanoic acid upon radish were decreased by addition of thiourea, and completely suppressed by a mixture of thiourea and vitamin C. In contrast, Dykyj et al. (50) did not find any decrease in NAA acitvity from the presence of either thiourea or ASA in "extensive vegetation experiments". Bonner (19) investigating the effects of amino-acids upon the growth of excised sections of Avena Coleoptile in sucrose medium found that cysteine was outstanding for the inhibition of the growth due to IAA.

Speaking of cysteine, we may mention here that Hansch et al. (69), in a recent paper, advanced the hypothesis that auxins may attack the cysteil group of proteins by an "ortho-reaction", which seems highly theoretical.

Recent work by Mitchell et al. (106) indicates that application of para-chlorophenoxyacetic acid just before harvest preserves a relatively high content of Vitamin C in bean pods; however, since this is accompanied by a better storage quality, the higher ASA content may just be the result of this improved keeping quality. Similar effect of 2,4-D for Red McLure potatoes was observed by Fults et al. (53). West et al. (164) observed that the inhibitions of oxygen uptake induced by 2,4-D in the roots of lupine was significantly reduced only by ASA out of a number of chemicals, from what they deduced that ASA oxidase is attacked by 2,4-D.

The effects of auxins upon ASA oxidase have been the object of several recent investigations, together with other enzymes systems likely to be attacked, or to be interfered with by auxins. Wagenknecht (157) in 1947 did not find any interaction between ASA oxidase and auxins. Negative results were also obtained by Mitchell et al. (105), but these workers observed that IAA did not induce the same effects as other auxins on the respiration of bean roots. Respiration was stimulated by IAA, but depressed by NAA and by 2,4-D at high concentrations; since Wagenknecht (158) found that bean roots contained an enzyme capable of oxidizing IAA which was inactivated by diethyl-dithiocarbamate, they tested

a mixture of diethyl-dithiocarbamate and IAA at high concentration and found it to behave similarly to the other auxins. Then Wagenknecht et al. (159) demonstrated that in crude bean-leaf and bean-root juices, the oxygen uptake was stimulated by the presence of ASA, and that the stimulation was significantly reduced by addition of auxins, although they had no inhibitive effect on oxygen uptake in the absence of ASA (except for NAA and 2,4-D, in the only case of juice from roots). Similar observations were made by Miller et al. (104) with crude juice expressed from barley seedlings. Finally Newcomb (110) demonstrated recently that IAA at low concentrations stimulates the ASA oxidase activity of tobacco pith cells (up to 1900 %) and inhibits it at higher concentrations. These effects had some bearing upon cell growth and respiration.

There are also indications that ASA and -SH compounds may interfer with auxin metabolism through a mechanism involving polyphenol-oxidases. Wetmore et al. (165) found that NAA checked the growth of shoot apices of foxtails, on agar blocks. This inhibition was somewhat reduced by phenylthiourea, diethyl-dithiocarbamate and, more effectively, by ASA. A polyphenol oxidase present at the cut surface seems to oxidize the auxin in the medium as well as that which diffuese from the apex. Similar results were obtained with fern apices; curvatures of Avena coleptile could be obtained by auxin diffusion technique from foxtails-shoots only when ASA was incorporated in the agar block. For Larsen (91), these results may indicate that ASA interfers with an oxidative step in the conversion of the precursor tryptophane to IAA.

The enzyme concerned in this process would be a polyphenol oxidase, of which we already know that it can indirectly oxidize ASA (139), which has even been suggested to be part of its coenzyme fraction (18, 129).

B. Problem.

A few evidences supporting Van Overbeek's attractive hypothesis can be found in the literature pertaining to plant-growth-substances. Since this hypothesis assumes that auxin phytotoxicity is the result of a disturbance of metabolism which produces an accumulation of toxic coumarin derivatives, it was reasoned that chemicals able to reduce the inhibitory effects of coumarin should also overcome those of the auxins, if van Overbeek's idea was right.

It has been demonstrated in the two first parts of this work that DTO and ASA were able to overcome the inhibitions produced by coumarin. A few examples of antagonistic interactions of thiols and of ASA with auxins have also been found in the literature, as well as some indications that auxins may be able to affect ASA oxidase; it seems also that thiols and ASA may interfer with the normal metabolism of IAA, and the transformation of neutral to active natural auxin in plant tissues.

It was therefore an attractive idea to investigate the effects of DTO and of ASA, both antagonistists of coumarin, upon the inhibitions of root-elongation induced by the auxins in cucumber seedlings.

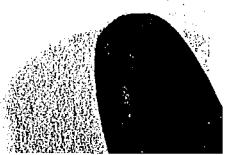
II. EXPERIMENTAL RESULTS

Only the three most important so-called "auxins" presently known have been investigated, namely: 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), and alpha-naphthalene-acetic acid (NAA).

Because 2,4-D produces the most spectacular effects on plants, is the most important commercially, and has actually been shown to induce accumulation of ASA and of coumarin derivatives (scopoletin) in plant tissues, this work was first and most extensively devoted to its interactions with DTO and with ASA. The natural growth-regulator, IAA, was then investigated; the last tests were devoted to a rapid study of NAA to show that the same results could be extended from 2,4-D and IAA to this chemical.

Only concentrations of the auxins giving moderate inhibitions were tested, so that the physiological processes of growth were not irreversibly suppressed, and could therefore be restored by the antagonist substances under investigation, if they were to be effective.

In all the experiments now reported, a technique of mixed constant solutions has been used as it has been previously described.



A. Reduction of the Inhibitory Effects of 2,4-D

1. The Effects of Dithiooxamide

a. Experiments with Marketer Cucumber Seeds.

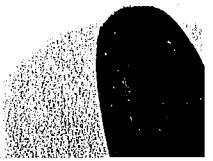
The first experiments were conducted with Marketer cucumber seeds and the same troubles were encountered as in the case of the antagonism of DTO to coumarin in Part I of this work.

Only low concentrations of DTO were used in this experiment, because they were believed to be specially effective at that time. The experiments were carried out in the laboratory, without any attention to the conditions of light or temperature.

In a preliminary test (33-A), only two Petri dishes with ten seeds from the old sample each were used. Slight indications of an antagonism were observed at the concentration of 0.1 ppm of 2,4-D.

Experiment 33-B, performed in the usual way with measurement of the seeds after 4 and 7 days, confirmed these results.

However, in experiment 34-A designed for a preliminary investigation of the interaction of ASA with 2,4-D and of its possible synergism with the antagonism of DTO to 2,4-D, the later antagonism was questionable; two sets of checks receiving 2,4-D alone had been used in this test, and they gave different results, so that, according to one or the other, this antagonism did or did not function. Because one of the checks had been readied at the beginning of the setting up of the experiment, whereas the other one had been prepared at the end, just before the lights were turned off, a difference in



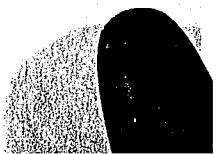
the illumination during the very earliest stages of germination was tentatively made responsible of this discrepancy.

Therefore in the next tests run in alternate illumination, two replicates were started: one in the morning, the other just before turning the lights off. In 35-A, performed with seeds from the old sample, both replicates gave evidences of antagonism of DTO to the inhibition induced by 2,4-D. But in the experiments 35-B, 35-C, 36-A and 36-B, where new seeds were used, erratic results were obtained. The antagonism was sometimes visible in alternate illumination, slightly in darkness, never in continuous light.

b. Experiments with Burpee Hybrid Cucumber Seeds.

The failure of the precedent tests to give consistent results was blamed upon the poor quality of the new sample of seeds. Therefore, the investigation was carried over with seeds from the variety Burpee Hybrids, in the controlled conditions of the special room previously described. All the solutions were made of acid water at pH 3.5.

A first experiment (37-A) with this new material gave good evidences that the antagonism functions as well in continuous illumination, in alternate illumination or in continuous darkness. The higher the concentration of DTO, the better the antagonism, which suggested that part of the failure of the previous experiments with Marketer seeds may have been due to the use of too low concentrations of DTO.



Two other experiments were run later with an other sample of seeds from the same origin, and higher concentrations of DTO were tested, both in continuous light and in continuous darkness, (37-B and 37-C), and with the same success. A concentration of 100 ppm of DTO was found to be most effective to antagonize the effects of 2,4-D, in all cases. As can be seen in 37-B, the better antagonistic effects were obtained at concentrations of DTO which were slightly toxic by themselves; the concentrations which promoted the root elongation were less effective for the antagonism.

The best reduction of inhibition was given by 100 ppm of DTO in experiment 37-C in light, exactly 50% of the inhibition was suppressed.

Inhibition caused by 2,4-D (o.1 ppm).....32.3 mm
Inhibition caused by 2,4-D (0.1 ppm) plus DTO (100 ppm).16.1 mm

2. The Effects of Ascorbic Acid.

a. Experiments with Marketer Cucumber Seeds.

The preliminary experiment (34-A) has already been mentioned and the discrepancy between the two sets of checks has been reported (page104). However, clear indications of an antagonism of ASA to the inhibition induced by 2,4-D can be found within each series receiving mixtures of DTO, and 2,4-D with (treatments) or without (checks) ASA.

In another experiments (35-A), the seeds of the older sample gave further evidences for the existence of this antagonism.

Erratic results were obtained in experiments 35-B, 35-C, 36-A and 36-B where new Marketer seeds were used. Once only (35-B) the antagonism was apparent as well in darkness as in light, but three times (35-C, 36-A and 36-B) it did not function in darkness. Opposite trends were manifest in continuous or alternate illumination for experiments 36-A and 36-B.

b. Experiments with Burpee Hybrid Cucumber Seeds.

In the controlled conditions of the special rood previously described, the investigation of the antagonism of ASA and 2,4-D was carried over with Burpee seeds. All the following experiments were done at pH 3.5.

From the three experiments 37-A, 37-B, and 37-C, it can be seen that ASA reduced the inhibition induced by 2,4-D as well in darkness as in continuous or alternate illumination. From 37-B and 37-C, in which a check was run for each concentration of ASA tested, it is evident that the difference in root length is much larger between check (2,4-D alone) and complex treatment than between check (ASA alone) and controls.

The best results were obtained for a concentration of 1,000 ppm of ASA in light (37-C), as follows:

Effect of 2,4-D (0.1 ppm).....-32.3 mm
Effect of ASA (1,000 ppm).....+6.9 mm
Sum of these effects.....-25.4 mm
(-Inhibition) (+Promotion)

Effect of 2,4-D (0.1 ppm) plus ASA (1,000 ppm).....-11.3 mm

so that the addition of ASA (1,ooo ppm) to the solution of 2,4-D resulted in the loss of almost two thirds of its inhibitory power.

B. Reduction of the Inhibitory Effects of Indole-Acetic Acid.

1. The Effects of Dithiooxamide

The interactions of DTO and IAA have been investigated along two lines of research: the effects of low concentrations of IAA upon the inhibitions caused by high concentrations of DTO, and the effects of DTO upon the inhibitions caused by higher concentrations of IAA.

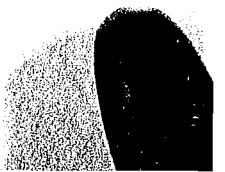
In all the following experiments, seedsof the variety Burpee Hybrid were grown in mixed constatn solutions of the chemicals in the special room where light and temperature were automatically controlled.

a. Indole Acetic Acid against the Inhibitions caused by Dithiooxamide.

Two experiments were conduceted where it was tried to overcome with IAA the inhibition induced by a high concentration of DTO.

In the first one, 38-B, concentrations of IAA ranging from 0.1 ppm to 2 ppm were used; they exhibited no antagonistic effect to the inhibition or root elongation caused by 200 ppm of DTO. On the contrary, the trend was rather of an increased toxicity of the mixture over the check receiving DTO only.

Another test was run to ascertain that a higher concentration of IAA would not produce the desired antagonism, but here also (38-D), it was found that the inhibitions induced by IAA (5 ppm) alone and DTO (150 ppm) alone were additive rather than antagonistic, in light and there was no interaction in darkness.



b. Dithiooxamide against the Inhibitions caused by Indole Acetic Acid

An antagonism of DTO to the inhibitions induced by IAA was repeatedly observed, which is apparently quite similar to the antagonism of DTO against 2,4-D.

In the case of IAA, the most effective concentrations of DTO for antagonism were low. In experiments, 38-A and 38-C, 20 ppm of DTO gave the best reductions of inhibition, in all cases of continuous or alternate illumination and of darkness. Only one concentration of IAA was used, 5 ppm, which gave moderate inhibitions. The best results were obtained in light in 38-C, where the roots grown in the mixture were about twice as long as the roots receiving IAA alone, which corresponded also to a suppression of half of the inhibition induced by IAA, as indicated below:

Inhibition caused by IAA (5 ppm) alone.....48.5 mm
Inhibition caused by IAA (5 ppm) plus DTO (20 ppm).....23.0 mm

In two other experiments, in which another sample of Burpee seeds was used as material, the best results were obtained with about the same concentration of DTO (10-20 ppm). In 39-A, all the solutions were made of acid water at pH 3.5, two concentrations of IAA (5-10 ppm) were used; in 39-B, where the solutions were made of distilled water like in the previous experiments, two concentrations of IAA were also used (2, 5 ppm); in both cases, the best results were obtained with 5 ppm of IAA, and 10 or 20 ppm of DTO. Here agin, the antagonism worked about as well in darkness as in light.

2. The Effects of Ascorbic Acid

Two experiments (40-A and 40-B) were performed with mixtures of IAA and ASA in light and in darkness. In both cases, clear reductions of the inhibitions induced by IAA alone were observed, specially in light.

In experiment 40-A for instance, in light 2 ppm of IAA induced 45% of the root length to be inhibited (42.8 mm), which were reduced to 14% (12.8 mm) by addition of 750 ppm of ASA, that is: 70% of the inhibition was overcome by ASA.

In darkness, less striking results were obtained. However, in experiment 40-B, the roots from seeds grown in mixtures of ASA (750 ppm) and IAA (10 ppm) were more than one and a half times as long as those from seeds grown in IAA alone (44.6 and 29.3 mm respectively). The results were less coherent than in light, and no sharp optimum of ASA concentrations was visible, whereas the most consistently effective concentration in light was 750 ppm of ASA.

B. Reduction of the Inhibitory Effects of Naphthalene-Acetic Acid

1. The Effects of Dithiooxamide

From the experiments 41-A, 41-B, and 42-A, evidences were obtained that DTO may reduce the inhibitions of root-elongation induced by NAA, provided these inhibitions are moderate.

This was especially visible in experiment 41-A, in light in which distilled water solutions were used. The reduced root growth obtained from seeds treated with NAA was more improved

by addition of ASA when the inhibitory treatment was of 0.1 ppm of NAA than when it was of 0.3 ppm or 1.0 ppm of NAA. In the same experiment in darkness, DTO did not produce any beneficial effect.

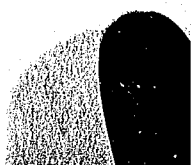
However in experiments 41-B and 42-A performed with acid water solutions, good evidences were obtained that in darkness also DTO may reduce the inhibition of cucumber root elongation induced by NAA at lower concentrations (0.1 and 0.05 ppm).

In experiment 42-A in light it was also clear that DTO could not overcome significantly the inhibition caused by 0.25 ppm of NAA, but was effective in the case of a treatment of 0.1 ppm of NAA. In all cases the optimum concentration of DTO appeared to be around 10-20 ppm.

2. Effects of Ascorbic Acid.

ASA was observed to be more potent than DTO to overcome the inhibition of NAA, which is similar to the results obtained in the cases of coumarin and of the other auxins.

This was observed as well in darkness as in light (42-A and 42-B). In experiment 42-A, 0.1 ppm of NAA alone induced an inhibition of cucumber root elongation which was 36.3% of the total root-length (30.8 mm out of 84.9 mm) in light; this was reduced to 6.5% (5.5 mm) by the addition of 1,000 ppm of ASA, which was a suppression of more than 80% of the total inhibition. In darkness in the same experiment, 0.05 ppm of NAA gave an inhibition of growth which was 43.8% of the total root length (42.2 mm out of 96.4 mm); this was reduced to 9.6% (9.3 mm) by addition of 1,000 ppm of ASA, so that more than 75% of the inhibitions used by the auxin was suppressed.



III. DISCUSSION

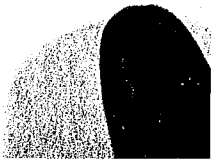
The objective of the experiments reported in the precedent pages was to investigate the effects of DTO and of ASA on the inhibitions of root elongation induced by the auxins.

The results show that these inhibitions were significantly reduced by the addition of both substances to the solutions of the auxins, in a way which was similar to the antagonisms that they opposed also to the effects of coumarin. Such similarity was expected on the grounds of Van Overbeek's hypothesis that at least part of the toxic effects of the auxims are caused by metabolic changes which result in the accumulation of toxic coumarin derivatives: coumarin antagonists were therefore expected to be also antagonistic to the auxins.

The only important difference between the two kinds of antagonisms is that DTO seems to reduce the inhibition induced by coumarin only in light whereas it antagonizes the auxins as well in darkness as in light.

In all cases, for the auxins as for coumarin, it was observed that ASA was a more potent antagonist than DTO, as shown by the technique of constant solutions of mixtures of chemicals, the only one used in part III.

Although the general results of these experiments bring forth good evidences in favor of Van Overbeek's theory, it does not seem

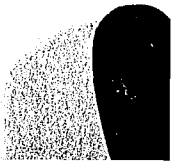


essential to consider an intermediary poisoning by coumarin in order to understand how the auxins can arrest growth and be antagonized by DTO and ASA.

There are evidences that the auxins may directly affect the enzymes controlling ASA metabolism, such as ASA oxidase (110, 159) chiefly and also polyphenol oxidase (165). Quite a few evidences point to the importance of polyphenol oxidase for ASA metabolism (121) and for respiration (20); ASA oxidase also seems to be important for respiration (161, 110); ASA oxidation-reduction system, besides a certain importance in respiratory processes (78), may play a key role as a bridge between oxidative and glycolitic processes through its influence on phosphatase activity (57, 79).

That these effects of auxin or others may induce an accumulation of coumarin derivatives is possible, although only few evidences support it; by themselves, however, they afford an explanation quite sufficient for the growth inhibitory effects of auxins and their antagonism by DTO and by ASA. An addition of ASA would for instance supplement the natural Vitamin C immobilized by oxidase inactivation, and thus allow the terminal oxidation to take place, whereas the dithiols might interfer directly with the inactivation of the enzymes by the auxins and thus slow down their effects. Such a mechanism is most likely to function in cucumber seedlings which are known to be especially rich in ASA oxidase.

It may be temporarily concluded that probably both indirect and direct mechanisms may account for the inhibitions of root elongation caused by the auxins, and their overcoming by DTO and ASA.



Because coumarin is involved in this matter and therefore it is impossible not to refer to the data disclosed in the first parts of this work, a general discussion will now be presented, in an attempt to tie together all the interactions investigated in this work.

IV. SUMMARY

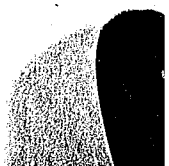
The effects of dithiooxamide and of ascorbic acid upon the inhibitions of cucumber root-elongation induced by the "auxins" 2,4-D, indole-acetic acid and naphthalene-acetic acid have been investiagetd in Petri dishes under controlled conditions of light and temperature.

In every case, both in light and in darkness, dithiooxamide and ascorbic acid were able to reduce the inhibitions induced by the auxins, and sometimes to suppress them almost completely.

In every case it was observed that ascorbic acid was more effective than dithiooxamide in overcoming these inhibitions.

A possible mechanism for an action of the "auxins" upon the metabolism of Vitamin C in plants is suggested.

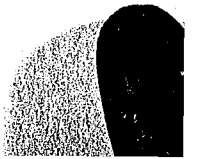
GENERAL DISCUSSION



The various interactions occurring between several natural or synthetic substances which possess outstanding plant-growth-regulating properties have been experimentally studied, and their meaning separately interpreted in the three parts of this work. At the end of this investigation, it seems worthy to bring together all these data, and to coordinate them in a synthetic discussion, where the results obtained in the first and the third parts should become integrated in the scheme elaborated at the end of part II, which appears to be the very knot at which the physiological effects of the substances tested are entagled.

Experimentally, ASA is the heart of the interactions observed since it antagonizes the effects of coumarin, those of the auxins, and antagonizes or reinforces those of DTO according to the circumstances.

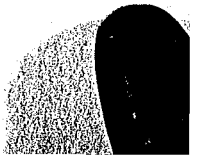
Therefore the complex system is centered around the metabolism of ASA, which, applied alone, does not affect much root development. Thiols compounds and sulfhydryl groups may int erfer with its metabolism in two ways: by attacking the oxidases enzymes which control its oxidation-reduction-activity, or by stabilizing it directly; ASA, in return, may reactivate the -SH enzymes which may be inactivated by the thiols (formation of dissulfide) or the unsaturated lactones, and so constitute another pole of the system directly connected with the three other constituents of the system: -SH groups, ASA and coumarin. A last process connects ASA to the



coumarin derivatives, the normal metabolism of which can be blocked by high concentrations of ASA. (Fig. 11)

The antagonism of DTO to the inhibition induced by coumarin may enter into the scheme in two ways. One would be a direct protection of the -SH enzymes from the inactivation by coumarin; the second one, would indirectly function through an intermediate upset of the metabolism of ASA which would for instance accumulate and arrest the poisonous metabolism of coumarin, or protect the -SH enzymes.

In the present status of our knowledge, both mechanisms should be held for equally probable. Analogies between the antagonisms of DTO and of ASA to coumarin are arguments for the indirect mechanism (such as the general similarity of the effects of DTO and of ASA upon the lettuce germination inhibited by coumarin, the lesser antagonism of ASA to coumarin in the transfer experiments in darkness resembling the failure of DTO to antagonize coumarin in darkness in case of cucumbers); the fact also that light conditions affect the interaction of DTO with ASA would make possible to understand why the antagonism of DTO to coumarin does not function in darkness, because the intermediate step involving ASA would thus be changed; the greater potency of ASA for all the antagonisms would also suggest that ASA acts more directly than does DTO. However, the direct mechanism has the advantage of explaining why coumarin is toxic, besides its simplicity and its great likelihood of actual existence.



As for the intergration of the results obtained with the auxins into the scheme of interactions previously described, some difficulties arise, cheifly as regards the accumulation of coumarin derivatives.

The direct mechanism suggested in part III and by which the auxin would affect the oxidases controlling ASA metaboilsm fits well into the system. By blocking these oxidases, the auxins may be able to affect the plant respiration, and the metabolism of ASA, which are important for the growth precesses; addition of ASA to the solution would then supplement the ASA previously immobilized and addition of DTO would directly interfer with the attack of the oxidases by the auxins.

At first, the accumulation of coumarin deriva t ives induced by the auxins seems easy to understand. One can imagine that the auxins, by blocking the oxidases, may induce an i mmobilization and an accumulation of ASA (which been actually observed twice, 53, 106), which in turn would inhibit coumarin derivatives normal metabolism (4) and therefore cause them to accumulate. According to Van Overbeek, those would then be toxic to plants. Auxin toxicity would thus be the indirect result of ASA accumulat ion, which is difficult to be conciliated with the antagonisms between auxins and ASA which have been observed in the present investigations, when more ASA was supplied to the plants.

It would then become necessary to suppose that ASA is able to induce two opposite effects at the same tim e (accumulation of coumarin derivatives, and counteraction of t h eir toxic effects,

such as protection of -SH enzymes), and to assume that, according to the level of its concentration (increased indirectly by addition of DTO, directly by addition of ASA) one or the other becomes proeminent. This seems to be quite improbable.

However, it may be questionable that the accumulated coumarin derivative be toxic, if their accumulation is due to the mechanism previously imagined, because this mechanism implies a suppression of their natural metabolism and therefore of their utilization, rather than an increased manufacture: it is doubtful that they may be harmful if they remain unused. The antagonistic effects of ASA and of DTO in this case might be explained by the direct mechanism suggested in Part III.

It is also possible that the accumulation of ASA induced by the auxins is not large enough to inhibit the metabolism of coumarin derivatives and cause their accumulation. In this case, the accumulations of coumarin derivatives and of ASA produced by the auxins would be independant; the accumulated coumarin derivatives would be toxic until an increased amount of ASA (directly supplied by addition of ASA or indirectly by addition of DTO) suppresses their metabolism or counteracts their effects by one of the mechanisms previously included in the general scheme of interactions.

The auxins appear therefore to interact with the substances previously coordinated in the complex natural system by two main processes. One involves an attack of the enzymes (oxidases) controlling ASA metabolism; the second one, which is an accumulation of coumarin derivatives, is largely unknown, but there is a possibility that it be a consequence of of the metabolic upset caused by the first one.

These two lines of auxin activity are included in the definitive scheme presented in Fig. 12. It should be emphasized at this point that this scheme does not pretend to account for all the growth regulating properties of the substances investigated, but is only a coordination of those processes which are most likely responsible for their multiple interactions reported in the present work.

Beyond the hypothesis and the suggestions which are the only tools of the human mind for a better understanding of complex natural processes, some facts should remain from this work: ascorbid acid and dithiooxamide exert upon root development very complex interactive effects (synergistic or antagonistic according to the circumstances of the experiment) and both substances antagonize the similar inhibitions of root growth induced either by coumarin or by the so-called "auxins" 2,4-D, indole-acetic acid and naphthalene-acetic acid.

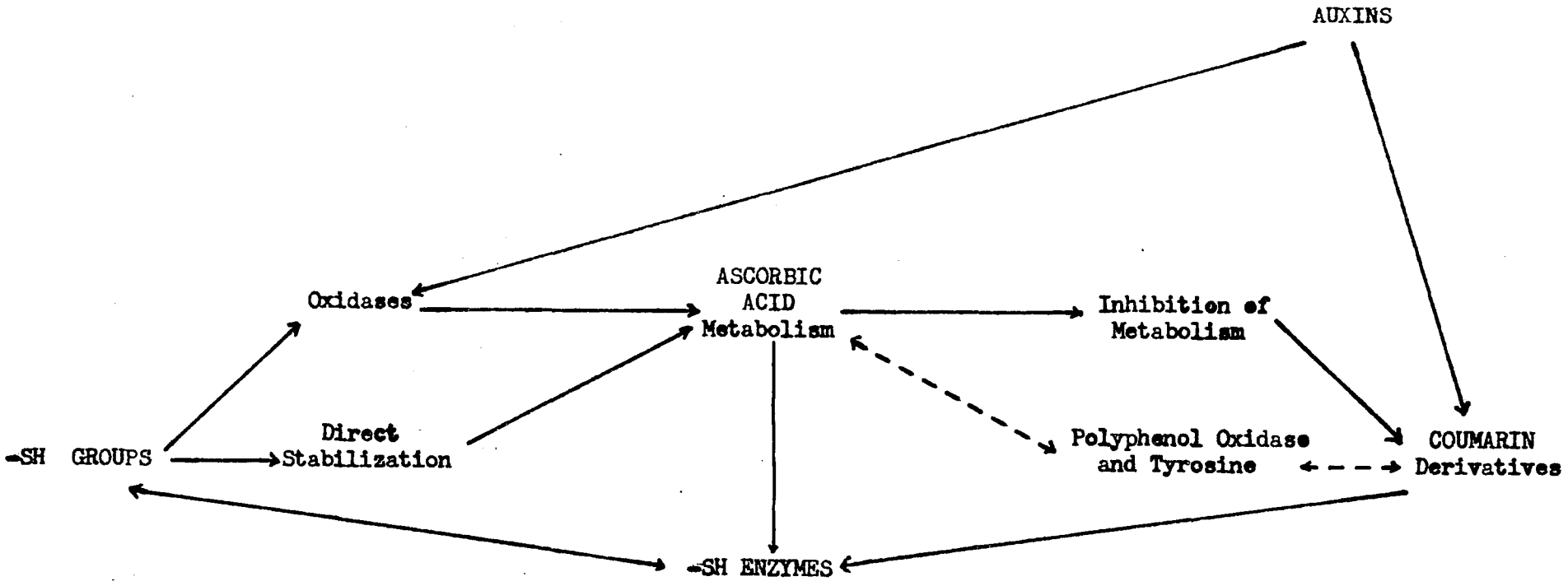


Figure 12

Suggested interrelationships between coumarin, -SH groups ascorbic acid and the auxins.

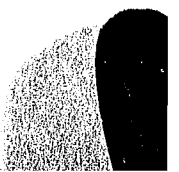
GENERAL SUMMARY

The interactive effects of dithiooxamide and of ascorbic acid upon the development of roots and its inhibition by coumarin and by the "auxins" 2,4-D, indole-acetic acid and naphthalene-acetic acid have been investigated in Petri dishes under controlled conditions of light and of temperature, with cucumber for root-elongation and lettuce for germination percentage.

(1) Treatments of dithiooxamide which were stimulative in light were strongly inhibitive in darkness for the elongation of cucumber roots; no stimulation was observed in darkness; the inhibitions induced by dithiooxamide, whether in light or in darkness, were not considerable before the third day after germination was started. Dithiooxamide was able to increase the early low rates of lettuce germination in light at 29°C. or in darkness at 25° C.. Ascorbic Acid alone did not produce any significant effects on the development of roots at the concentration which were used (below 2,000 ppm).

(2) Ascorbic acid was able to reinforce synergistically or to reduce the inhibitions of cucumber root elongation induced by dithiooxamide depending upon the conditions of illumination, the procedure of testing (simultaneous or successive applications of the chemicals) and the order and the length of the treatments, in the case of successive applications.

(3) Both dithiooxamide and ascorbic acid were able to reduce the inhibitions of cucumber root elongation induced by coumarin, in light only in the case of dithiooxamide, in both light and darkness



in the case of ascorbic acid. A similar reduction of the inhibition of lettuce germination induced by coumarin was produced by these chemicals, in light and darkness with dithiooxamide, in light only with ascorbic acid when they were simultaneously applied with coumarin throughout the experiment; with both chemicals, it took place only in darkness when they were separately applied after a presoaking in a solution of coumarin alone in darkness (case of an artificial dormancy). Ascorbic acid was more potent in the case of cucumber, dithiooxamide was more potent in the case of lettuce.

(4) Both dithiooxamide and ascorbic acid were able to reduce the inhibitions of cucumber root-elongation induced by the "auxins" 2,4-D, indole-acetic acid and naphthalene-acetic acid, both in light and in darkness. In every case, ascorbic acid was more potent than dithiooxamide.

(5) As a tentative interpretation of the interactive effects of dithiooxamide, ascorbic acid and coumarin, a natural biological system is outlined, in which -SH groups and coumarin derivatives may be independently interrelated with the metabolism of Vitamin C in plants, directly or indirectly through the metabolism of -SH enzymes. Two phases of this system appear likely to be interferred with by the "auxins".

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Appendix I. Effects of distilled water solutions of Dithiooxamide (DTO) on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days.

2/14/1951

Root-length expressed in mm.			
Water (control)	DTO 1 ppm	DTO 5 ppm	DTO 10 ppm
60	66	70	71
78	79	77	56
51	87	87	71
66	75	90	62
70	65	73	67
64	56	77	53
63	73	74	56
65	68	79	75
54	68	69	53
71	63	66	62
69	59	72	66
56	79	58	60
72	68	63	85
70	66	63	71
73	66	73	61
68	67	77	67
58	59	81	78
84	74	76	70
62	79	77	76
73	70	72	58
74	83	87	60
73	66	56	57
57	78	55	79
67	66	74	61
60	76	58	53
60	65	74	54
62	58	56	65
67	76	68	74
55	62	74	74
57	55	72	70
Total (30) 1959	2072	2148	1965
Average 65.3	69.1	71.6	65.5
Sy^2 129,665	145,038	156,250	
S.S. 1742	1932	2453	
t (treatment-water control)	1.85 Not Significant	2.87 Highly Significant	

Appendix II. Effects of distilled water solutions of Dithioxamide (DTO) on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days.

3/27/1951

Root-length expressed im mm.					
Water (control)	DTO 1 ppm	DTO 2.5 ppm	DTO 5 ppm	DTO 10 ppm	DTO 20 ppm
77	85	79	92	85	74
78	72	90	75	76	66
74	84	80	82	92	71
55	80	93	73	78	73
63	96	80	95	79	77
60	75	86	63	64	63
59	76	84	65	64	85
93	82	72	64	72	77
50	73	78	58	62	62
76	76	73	92	90	60
75	88	84	81	76	74
82	74	77	77	77	65
63	73	92	70	79	81
68	70	76	83	83	69
65	92	77	77	66	73
65	71	87	91	67	70
62	72	84	66	59	69
55	70	70	61	57	70
58	69	73	59	87	74
81	86	88	83	77	69
71	76	82	83	78	66
71	90	76	75	71	92
66	88	78	70	71	73
90	80	107	96	82	64
75	75	86	94	82	72
78	90	72	87	68	83
71	72	102	87	64	67
70	81	71	79	89	60
70	84	91	73	65	73
62	90	72	64	55	76
Total (30) 2083	2390	2460	2315	2215	2148
Average 69.4	79.7	82.0	77.2	73.8	71.6
Sy^2 147,607	192,152	204,114	182,491	166,523	
S.S. 2977	1719	2394	3850	2982	
t (water control-treatment)	4.4 Highly Significant	5.06 Highly Significant	2.785 Highly Significant	1.68 Not Significant	

Appendix III. Effects of simultaneous applications of Coumarin (COU) and Dithioxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days, concentrations expressed in ppm.

1/10/1951

	Root-length expressed in mm.				
	Water	COU 150	COU 150 & DTO 0.1	COU 150 & DTO 1	COU 150 & DTO 10
	38	8	6.5	7	8.5
	31	6	7	8.5	10
	15	8	10	7	13.5
	32	6	8	10.5	8
	24	8.5	8.5	5	8
	24	7.5	8.5	8	5.5
	35	7.5	8	7.5	15
	33	8	6	9	12.5
	39	-	8	7	19
	35	-	8	-	14
	45	-	7	-	-
	37	-	11	-	-
Total	388	59.5	96.5	69.5	114.0
No. of seeds	12	8	12	9	10
Average	32.3	7.4	8.0	7.7	11.4

Appendix IV. Effects of simultaneous applications of Coumarin (COU) and Dithioxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days, concentrations expressed in ppm.

1/28/1951

Root-length expressed in mm.					
Water	COU 100	COU 100 & DTO 1	COU 100 & DTO 5	COU 100 & DTO 7.5	COU 100 & DTO 10
71	14	39	30	42	42
78	11	26	29	35	31
71	15	14	27	27	30
76	9	10	31	24	30
70	11	21	37	21	33
64	10	16	29	19	38
78	14	11	29	30	34
75	15	14	36	23	32
80	11	11	29	29	36
72	14	21	28	25	30
75	8	18	26	28	32
65	14	18	27	38	32
88	8	27	31	24	31
73	13	31	23	23	27
78	12	17	25	21	34
75	14	17	26	29	31
70	11	13	30	20	36
63	13	9	28	25	36
82	9	10	32	28	34
74	12	14	29	35	30
59	8	19	36	27	42
66	14	22	31	27	43
68	22	25	34	25	33
59	17	23	35	27	28
55	19	21	39	24	27
56	21	20	32	21	29
66	12	20	35	24	32
73	12	14	24	18	34
55	14	14	24	17	33
55	13	11	25	17	30
Total (30) 2090	406	546	907	779	990
Average 69.7	13.5	18.2	30.2	26.0	33.0

Appendix IV. (Continued)

Root-length expressed in mm.				
COU 150	COU 150 & DTO 1	COU 150 & DTO 5	COU 150 & DTO 7.5	COU 150 & DTO 10
6	13	27	47	23
6	11	29	36	31
6	20	19	27	17
7	13	21	34	19
9	7	13	25	24
13	7	13	21	23
9	8	15	20	9
8	13	10	18	19
6	10	8	14	15
7	17	7	15	8
7	11	8	20	9
10	8	19	35	13
5	10	19	24	27
6	13	23	26	20
6	14	18	27	25
5	12	24	19	23
7	9	25	22	14
10	8	10	14	14
7	8	17	12	13
5	9	7	17	15
10	16	39	11	18
8	22	21	19	14
8	7	17	12	30
6	10	17	23	30
6	12	19	21	18
8	7	9	19	22
6	12	11	16	17
7	13	8	16	23
7	7	6	11	16
6	7	7	10	8
Total (30) 216	334	486	631	557
Average 7.2	11.1	16.2	21.0	18.6

Appendix V. Effects of simultaneous applications of Coumarin (COU) and old or new Dithiooxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 5 days, concentrations expressed in ppm.

5/26/1951

	Root-length expressed in mm. Alternate Illumination						Root-length expressed in mm. Darkness					
	COU 100		COU 100 & old DTO 3.75		COU 100 & new DTO 3.75		COU 100		COU 100 & old DTO 1		COU 100 & new DTO 1	
	29	40	40	88	68	69	22	17	21	20	57	99
	27	39	39	94	41	92	24	16	19	17	25	69
	27	33	32	55	32	91	24	25	23	22	38	68
	17	31	26	42	28	89	20	16	31	11	31	35
	32	40	22	52	21	76	32	28	21	25	20	48
	39	22	25	27	24	66	31	18	21	20	29	45
	23	30	23	62	21	77	30	30	20	17	26	58
	23	20	24	64	24	72	25	23	14	15	34	34
	17	32	24	47	21	74	17	26	17	20	20	27
		28	26	39	20	39		19	12	14		21
		32						21				19
Total	234	347	281	640	300	745	225	239	199	181	280	521
No. of seeds	9	11	10	10	10	10	9	11	10	10	9	11
Average	26.0	31.5	28.1	64.0	30.0	74.5	25.0	21.7	19.9	18.1	31.1	47.4

General Results:

Illumination	Average of root-length of 20 seeds expressed in mm. Control	Old Sample of DTO				New sample of DTO			
	COU 100	COU 100 & DTO 1	COU 100 & DTO 3.7	COU 100 & DTO 7.5	COU 100 & DTO 15	COU 100 & DTO 1	COU 100 & DTO 3.7	COU 100 & DTO 7.5	COU 100 & DTO 15
Alternate	29.0	27.6	46.0	26.0	21.7	29.9	52.2	23.6	22.5
Darkness	23.2	19.0	20.0	20.7	19.0	40.0	24.4	19.2	18.9

Appendix VI. Effects of distilled water solutions of Dithiooxamide (DTO) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 48 hours.

8/4/1951

	Root-length expressed in mm.			
	Light		Darkness	
	Water	DTO 200 ppm	Water	DTO 200 ppm
	32	26	29	29
	33	28	38	28
	27	27	33	27
	29	30	33	24
	32	26	37	24
	27	23	33	28
	29	25	28	27
	32	23	32	25
	33	32	30	26
	31	29	28	24
	30	26	36	28
	25	27	32	26
	31	25	27	25
	31	26	33	27
	28	25	29	23
	36	25	32	24
	34	25	31	25
	28	23	27	24
	29	23	29	22
	27	31	38	23
	28	30	34	29
	27	26	35	27
	29	29	33	27
	30	27	31	27
	26	27	29	24
	34	29	33	23
	31	24	32	25
	28	23	30	25
	29	23	33	24
	26	23	28	23
Total (30)	892	786	953	763
Average	29.7	26.2	31.8	25.4
				-
Sy^2	26.736	20.792	30.549	-
S.S.	214	199	276	-
t (comparison with water in light)		5.07 Highly Significant	2.8 Highly Significant	

Appendix VII. Stimulation of the elongation of Burpee Hybrid cucumber roots induced by an application of Dithiooxamide (DTO) in distilled water solution for 24 hours followed by a transfer to distilled water for 4 days in continuous illumination, concentrations expressed in ppm.

Root-length expressed in mm.					
Test 9-A		Test 9-B		Test 9-C	
Water then water	DTO 300 then water	Water then water	DTO 300 then water	Water then water	DTO 300 then water
82	97	80	96	91	104
74	98	79	93	88	84
62	97	77	92	83	104
102	79	96	116	92	102
62	88	88	98	106	99
97	77	82	95	92	103
85	99	117	102	79	93
82	88	109	97	64	103
56	77	105	113	90	100
96	108	88	97	66	98
99	96	79	90	76	109
78	99	123	117	74	90
87	100	107	105	96	83
66	87	90	90	91	110
103	78	92	101	94	107
64	108	85	124	88	94
92	96	75	109	84	103
65	82	111	110	62	101
59	117	91	102	91	94
64	116	82	88	78	107
66	108	84	125	80	103
79	114	79	93	68	96
119	99	99	93	78	110
69	97	85	98	88	92
103	122	85	96	91	81
82	112	118	105	87	98
108	84	91	93	73	97
72	80	86	91	65	88
52	82	73	88	72	96
65	86	71	96	71	81
Total (30) 2390	2871	2727	3013	2458	2930
Average 79.7	95.7	90.9	100.4	81.9	97.7
Sy^2 199,412	279,767	253,671	305,683	204,946	288,198
S.S. 14,021		8,865		5,589	
t 3.98		2.97		6.23	
Highly Significant		Highly Significant		Highly Significant	

Appendix VIII. Stimulation of the elongation of Burpee Hybrid cucumber roots induced by an application of Dithiooxamide (DTO) in distilled water (DW) solutions for 24 hours followed by a transfer to acid water at pH 3.5 (P) for 4 days in continuous illumination, concentrations expressed in ppm.

Root-length expressed in mm.						
Test 25-B		Test 26-A		Test 26-B	
DW then P	DTO 300 then P	DW then P	DTO 300 then P	DW then P	DTO 300 then P
80	115	66	115	75	103
88	113	101	91	92	99
73	92	80	105	80	88
130	115	91	121	127	91
99	96	77	134	107	115
87	95	82	91	103	106
72	103	71	96	99	105
63	111	91	106	66	103
89	96	95	100	90	92
90	111	90	96	94	88
93	88	114	120	79	88
106	95	107	114	76	86
80	87	126	106	92	98
104	107	125	111	79	89
107	106	97	96	93	88
73	98	82	113	87	100
89	95	97	130	87	93
79	94	111	107	98	104
80	94	107	121	75	102
93	102	73	106	71	95
103	99	104	99	89	102
85	114	104	124	93	103
111	94	92	127	89	86
77	94	96	120	77	101
92	103	77	114	97	104
93	110	126	101	77	108
67	109	86	96	99	106
85	95	92	126	72	106
115	81	66	104	73	106
89	87	65	103	66	93
Total (30) 2692	3009	2791	3293	2602	2948
Average 89.7	100.3	93.0	109.8	86.7	98.3
Sy^2 248,064	304,053	268,483	365,661	230,856	291,488
S.S. 8,752		13,026		6,974	
t 3.31		4.36		4.09	
Highly Significant		Highly Significant		Highly Significant	

Appendix IX. Effects of successive applications of Coumarin (COU) in distilled water (DW) solutions and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days in continuous illumination, concentrations expressed in ppm.

	Root-length expressed in mm.				
	Test 17-A		Test 17-B		
	DW then P	COU 75 then ASA 500	DW then P	COU 75 then ASA 500	COU 75 then ASA 500
	93	106	69	93	89
	92	86	106	91	106
	91	81	93	100	103
	90	80	66	91	106
	85	75	71	108	75
	70	100	80	96	88
	67	72	66	89	90
	55	125	77	100	84
	66	124	65	101	96
	62	106	87	76	96
	62	100	75	84	79
	112	96	97	78	80
	108	92	101	78	101
	98	101	81	85	100
	95	98	70	78	86
	80	92	80	91	79
	86	95	94	80	80
	81	73	78	85	75
	74	89	64	96	70
	73	110	80	73	85
	62	101	70	94	72
	117	98	124	92	76
	95	91	70	80	78
	96	95	68	84	86
	91	85	79	98	90
	89	80	86	78	70
	86	95	62	91	73
	95	76	72	79	98
	75	75	91	100	103
	61	72	85	80	70
Total (30)	2509	2769	2407	2648	2584
Average	83.6	92.3	80.2	88.3	86.3
Sy^2	217,295	261,309	199,005	236,339	226,470
S.S.	13,189		5,883	2,609	3,902
t (treatment-water control)	2.24		2.59		1.82
	Significant		Significant		Not Significant

Appendix X. Effects of successive applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) and Dithiooxamide (DTO) in distilled water solutions (DW) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days in darkness, concentrations expressed in ppm.

Root-length expressed in mm.						
Test 28-A		Test 28-B		Test 28-B		
P then DTO 300	ASA 250 then DTO 300	P then DTO 300 (1)	ASA 250 then DTO 300	P then DTO 300 (2)	ASA 250 then DTO 300	
68	75	91	79	60	75	
63	66	70	74	57	67	
61	62	61	69	54	66	
64	84	76	75	53	81	
56	62	70	72	50	72	
51	68	70	67	50	72	
51	64	66	79	63	76	
47	72	60	72	50	70	
81	65	56	70	47	66	
67	74	54	75	69	68	
58	72	52	60	68	67	
72	63	67	68	62	72	
58	72	56	73	62	65	
55	70	55	96	47	65	
62	82	66	75	60	80	
53	68	63	77	46	66	
55	69	58	74	49	74	
52	60	63	74	69	71	
62	66	58	84	63	68	
74	81	65	82	67	66	
56	67	59	69	61	65	
51	73	59	68	62	81	
57	62	77	68	57	79	
50	79	66	67	54	73	
50	83	59	64	49	84	
59	79	66	75	56	76	
57	81	63	75	73	73	
59	68	55	64	50	70	
55	62	50	79	48	64	
54	63	52	69	46	65	
Total (30) 1758	2112	1883	2193	1702	2137	
Average 58.6	70.4	62.8	73.1	56.7	71.2	
Sy^2 104,784	150,224	120,369	161,723	98,386	153,169	
S.S. 3,304		3,594		2,769		
t 6.05		5.05		8.13		
Highly Significant		Highly Significant		Highly Significant		

Appendix XI. Effects of successive applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) and Dithiooxamide (DTO) in distilled water solutions (DW) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days in continuous illumination, concentrations expressed in ppm.

	Root-length expressed in mm.					
	Transfer after 48 hours Test 25-B		Transfer after 72 hours Test 25-B		Transfer after 72 hours Test 28-A	
	P then DTO 300	ASA 500 then DTO 300	P then DTO 300	ASA 500 then DTO 300	P then DTO 300	ASA 500 then DTO 300
	53	68	87	76	71	72
	65	68	69	69	63	75
	60	73	71	73	61	87
	51	59	68	71	71	83
	67	62	77	81	68	92
	61	72	67	72	67	75
	57	60	65	72	78	77
	50	64	78	70	77	70
	54	57	68	76	75	68
	55	59	69	74	79	71
	52	58	74	83	65	89
	53	57	70	80	69	78
	57	63	64	85	66	73
	60	76	68	73	63	85
	61	67	75	81	73	76
	52	64	64	71	64	72
	55	61	76	74	74	81
	67	58	65	70	64	68
	54	64	65	73	62	65
	54	76	65	71	61	76
	61	73	74	75	79	75
	59	65	74	78	68	70
	53	78	83	80	98	87
	63	88	80	80	74	81
	60	69	65	73	66	69
	60	59	71	77	65	68
	52	67	73	75	66	78
	69	70	65	82	73	74
	65	82	63	85	64	69
	56	64	64	82	61	77
Total (30)	1736	2001	2117	2282	2085	2281
Average	57.9	66.7	70.6	76.1	69.5	76.0
Sy^2	101,274	135,245	150,501	174,244	146,665	174,845
S.S.	2,596		1,771		3,171	
t	5.09		3.85		3.40	
	Highly Significant		Highly Significant		Highly Significant	

Appendix 1. Effects of tap water solutions of Coumarin (COU) and Ascorbic Acid (ASA) on the elongation of Marketer cucumber and wheat roots (var. Henry) grown under laboratory conditions. Concentrations expressed in ppm.

A. 1/14/1951 Marketer cucumbers - Length of the test: 3days

Treatments	Water	ASA 10	ASA 100	ASA 1000
Number of seeds	10	10	8	13
Average root length (mm.)	23.3	25.8	23.9	34.0

Treatments	COU .03	COU .1	COU .3	COU 1	COU 3
Number of seeds	10	12	12	10	14
Average root length (mm.)	26.2	24.1	25.8	25.9	24.7

B. 1/18/1951 Wheat - Length of the test: 3 days

Treatments	Water	ASA 10	ASA 100	ASA 1000
Number of seeds	13	12	12	11
Average root length (mm.)	32.3	35.2	30.2	23.0

C. 2/3/1951 Wheat - Length of the test: 3 days

Treatments	Water	ASA 10	ASA 100	ASA 500	ASA 1000
Number of seeds	30	30	30	30	30
Average root length (mm.)	23.2	21.8	31.8	36.1	36.5

D. 2/3/1951 Marketer cucumbers - Length of the test: 4 days

Treatments	Water	ASA 10	ASA 100	ASA 500	ASA 1000
Number of seeds	30	30	30	30	30
Average root length (mm.)	40.7	49.0	33.2	41.6	45.2

Appendix 2. Effects of distilled water solutions of Ascorbic Acid (ASA) and Dithiooxamide (DTO) on the elongation of wheat roots (var. Henry) grown under laboratory conditions for 3 days. Concentrations expressed in ppm. Average of root length of 30 seeds expressed in mm.

A. 2/7/1951

Treatments	Water	ASA 10	ASA 100	ASA 500	ASA 1000
Average length (mm.)	48.3	53.8	53.8	41.0	35.9
Percent of controls	100.0	111.4	111.4	84.9	74.3

B. 2/15/1951

Treatments	Water	DTO 20	DTO 66	DTO 200
Average length (mm.)	57.6	46.8	42.4	32.7
Percent of controls	100.0	81.2	73.6	56.8

C. 3/3/1951

Treatments	Water	DTO 1	DTO 5	DTO 10
Average length (mm.)	53.3	48.7	46.1	43.6
Percent of controls	100.0	91.4	86.5	81.8

D. 3/7/1951

Treatments	Water	DTO .1	DTO .5
Average length (mm.)	49.6	51.0	48.3
Percent of controls	100.0	102.8	97.4

E. 3/12/1951

Treatments	Water	DTO .01	DTO .05
Average length (mm.)	54.0	50.0	52.8
Percent of controls	100.0	92.6	97.8

Appendix 3. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days. Concentrations expressed in ppm. Average of 30 root lengths expressed in mm.

A. 2/24/1951

Treatments	Water	DTO 25	DTO 50
Average length	83.3	73.8	60.1
Percent of control	100.0	88.6	72.1

B. 3/3/1951

Treatments	Water	DTO 50	DTO 100	DTO 200
Average length	73.1	59.0	43.6	32.9
Percent of control	100.0	80.7	59.6	45.0

C. 3/7/1951

Treatments	Water	DTO .1	DTO .5
Average length	65.9	64.3	66.2
Percent of control	100.0	97.6	100.5

D. 4/2/1951

Treatments	Water	DTO 1	DTO 2.5	DTO 5	COU 2	COU 5	COU 10
Average length	75.5	76.7	79.0	75.7	71.7	70.4	58.7
Percent of control	100.0	101.6	104.6	100.3	95.0	93.2	77.7

Treatments	COU 10 & DTO 5	COU 5 & DTO 1	COU 5 & DTO 2.5	COU 5 & DTO 5	COU 2 & DTO 1	COU 2 & DTO 2.5	COU 2 & DTO 5
Average length	56.8	70.2	63.8	66.7	68.2	67.6	78.3
Percent of control	75.2	93.0	84.5	88.3	90.3	89.5	103.7

Appendix 4. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) and/or Ascorbic acid (ASA) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 7 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 4/23/1951

Conditions of Illumination	COU 100	COU 100 & DTO 7.5	COU 100 & ASA 500	COU 100 & DTO 7.5 & ASA 500
Continuous	33.6	31.9	79.9	93.0
Alternate	41.3	41.3	92.6	54.1
Darkness	36.8	32.9	42.2	38.6

B. 5/16/1951

Conditions of Illumination	COU 100	COU 100 & DTO 7.5	COU 100 & ASA 500	COU 100 & DTO 7.5 & ASA 500	Water	ASA 500
Continuous	27.4	23.4	54.6	43.7	80.4	85.3
Alternate (starting with light)	26.3	25.9	45.9	25.6	114.1	130.1
Alternate (starting with darkness)	30.6	25.7	50.7	22.2	105.9	Decay
Darkness	20.8	19.3	34.4	21.9	Decay	Decay

Appendix 5. Effects of simultaneous applications of Coumarin (COU and Ascorbic Acid (ASA) and/or Dithiooxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 6/1/1951 — Length of the test: 6 days.

Conditions of Illumination	Water	COU 100	COU 100 & DTO 3.3	COU 100 & ASA 500	COU 100 & DTO 3.3 & ASA 500
Continuous	103.8	42.9	41.4	72.3	53.5
Alternate*	108.1	33.6	49.7	84.4	47.8
Darkness	109.1	33.9	27.8	62.7	37.2

B. 6/16/1951 — Length of the test: 5 days.

Conditions of Illumination	Water	COU 100	COU 100 & DTO 7.5
Continuous	80.1	41.9	40.6
Alternate	102.5	47.2	40.6
Darkness	110.4	35.7	32.5

C. 12/26/1951 — Length of the test: 5 days.

Conditions of Illumination	COU 37.5	COU 37.5 & DTO 15	COU 37.5 & DTO 50	COU 37.5 & DTO 150	COU 75	COU 75 & DTO 15	COU 75 & DTO 50	COU 75 & DTO 150
Continuous	41.9	49.6	40.1	24.8	32.5	31.0	27.6	19.5
Darkness	31.1	25.9	24.1	14.5	19.1	11.9	12.0	-

*Composition of light: 50% Fluorescent
50% Incandescent

Appendix 6. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) in distilled water solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 7/13/1951 — Length of the test: 6 days.

Conditions of Illumination	COU 75	COU 75 & DTO .8	COU 75 & DTO 2.5	COU 75 & DTO 7.5	COU 75 & DTO 22.5
Continuous	31.6	37.7	36.0	36.5	36.0
Alternate	35.0	35.0	33.7	39.4	37.1
Darkness	28.1*	29.6	31.6	30.7	29.1

	COU 150	COU 150 & DTO .8	COU 150 & DTO 2.5	COU 150 & DTO 7.5	COU 150 & DTO 22.5
Continuous	15.8	18.0	14.6	16.9	13.6
Alternate	13.4	17.4	17.1	11.6	-
Darkness	19.3	16.5	15.4	15.1	-

B. 9/28/1951 — Length of the test: 7 days.

Conditions of Illumination	COU 75	COU 75 & DTO 1	COU 75 & DTO 3.3	COU 75 & DTO 10	COU 75 & DTO 25	COU 75 & DTO 75	COU 75 & DTO 150
Continuous**	35.2	31.8	39.9	36.0	36.4	39.2	54.9
Alternate**	39.2	36.2	35.2	-	42.8	51.2	50.1
Darkness	38.7	33.0	38.3	39.6	35.6	35.1	32.5

*Average of 20 root lengths
**Composition of light: 50% Fluorescent
50% Incandescent

Appendix 7. Effects of successive applications of Dithiooxamide (DTO) and Coumarin (COU) in distilled water solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 10/14/1951 — Transfer after 24 hours.

Pre-treatments	Water	DTO 300	COU 75	
Post-treatments	COU 75	COU 75	Water	DTO 150
In light	42.0	45.1	64.1	52.5
In darkness	25.0	24.1	56.6	45.2

B. 10/30/1951 — Transfer after 24 hours.

	In light				In darkness			
Pre-treatments:	Water		DTO 300		Water	DTO 75	DTO 150	DTO 300
Post-treatments:	COU 150	COU 37.5	COU 150	COU 37.5	COU 37.5	COU 37.5	COU 37.5	COU 37.5
	17.2	41.1	18.2	53.4	36.7	38.4	40.5	40.2

Transfer after 48 hours.

Pre-treatments	Water		DTO 150	
Post-treatments	COU 150	COU 75	COU 150	COU 75
In light	32.7	50.8	33.5	54.8

Appendix 8. Effects of successive applications of Dithiooxamide (DTO) and Coumarin (COU) in distilled water solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 11/17/1951

	Post-treatments	Pre-treatments (24 hours)			
		Water	DTO 300	DTO 150	DTO 75
In light	COU 10	65.4	80.5		
	COU 20	49.4	65.4		
	COU 40	40.5	61.2		
In darkness	COU 5	84.1		70.4	77.0
	COU 10	71.4		67.5	69.0
	COU 20	54.5		49.7	51.4

B. 1/29/1952

	Post-treatments	Pre-treatments (24 hours)		
		Water	DTO 25	DTO 50
In darkness	Water	87.8	88.2	81.8
	COU 15	55.1	55.8	55.1

Appendix 9. Effects of successive applications of Dithiooxamide (DTO) and Coumarin (COU) in distilled water solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions in continuous illumination for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 12/6/1951

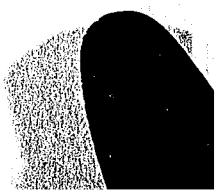
Pre-treatments (24 hours)	Post-treatments			
	Water	COU 20	COU 40	COU 80
Water	79.7	47.1	43.8	41.9
DTO 300	95.7	68.1	64.0	41.6

B. 12/27/1951

Pre-treatments (24 hours)	Post-treatments			
	Water	COU 20	COU 40	COU 80
Water	90.9	56.7	48.9	41.0
DTO 300	100.4	78.2	66.3	52.1

C. 1/14/1952

Pre-treatments (24 hours)	Post-treatments				
	Water	COU 10	COU 20	COU 40	COU 80
Water	81.9	53.1	49.7	48.1	37.4
DTO 300	97.7	89.0	78.3	62.3	38.2



Appendix 10. Effects of distilled water solutions of Dithiooxamide (DTO) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions at various intervals of time. Average of 30 root-lengths expressed in mm.

A. 8/4/1951

Illumination	Treatments	After 48 Hours	After 72 Hours	After 96 Hours	After 120 Hours
Continuous	Water	29.7	54.7	67.0	77.6
	DTO 200 ppm.	26.2	34.0	38.3	44.2
Darkness	Water	31.8	59.6	85.8	102.8
	DTO 200 ppm.	25.4	34.9	40.7	47.0

Appendix 11. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) in distilled water solutions on the germination of old lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 6/2/1951

	Percent germination			
	Continuous illumination 4 days	Alternate illumination 4 days	Darkness 4 days	Darkness 4 days then alternate illumination 7 days
COU 25	20	66	1	43
COU 25 & DTO 5	64	74	2	77
COU 25 & DTO 10	65	74	6	83

B. 6/20/1951

		Percent germination at various intervals of time in hours.									
Illumination	Treatments	24	48	72	96	120	144	168	192	216	240
	Water	72	82	86	87	87	87	87	87	87	87
Continuous	COU 25	5	14	15	16	33	43	47	53	53	59
	COU 25 & DTO 5	1	45	52	52	66	72	79	82	82	84
	COU 25 & DTO 10	12	60	66	66	72	79	85	85	85	85
Alternate	Water	72	85	87	88	91	91	91	91		
starting with	COU 25	9	35	47	55	76	77	81	81		
light	COU 25 & DTO 5	11	77	81	81	82	83	85	87		
	COU 25 & DTO 10	17	79	81	82	82	83	83	84		
Alternate	Water	73	76	78	84	86	86	86	87		
starting with	COU 25	0	29	48	52	60	71	81	85		
12 hours	COU 25 & DTO 5	1	73	83	85	87	88	88	91		
darkness	COU 25 & DTO 10	4	68	78	80	81	81	84	85		

Appendix 11. B. (Continued)

Illumination	Treatments	Percent germination at various intervals of time in hours									
		24	48	72	96	120	144	168	192	216	240
Alternate	Water		57	79	81	86	87	87	87		
starting with	COU 25		2	3	46	67	71	81	86		
48 hours	COU 25 & DTO 5		0	12	64	74	75	78	79		
darkness	COU 25 & DTO 10		3	22	76	81	81	82	82		
Alternate	Water			59	73	83	88	88	88	88	88
starting with	COU 25			0	7	47	71	75	78	80	82
72 hours	COU 25 & DTO 5			1	6	46	64	68	70	71	74
darkness	COU 25 & DTO 10			1	7	54	66	73	73	74	77
Alternate	Water				53	73	86	86	87	87	88
starting with	COU 25				1	4	26	45	58	72	79
96 hours	COU 25 & DTO 5				3	6	27	35	36	36	36
darkness	COU 25 & DTO 10				3	5	28	58	67	72	73

Appendix 12. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) in distilled water solutions on the elongation of new lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 7/20/1951

		Percent germination at various intervals of time in hours											
		Continuous light 50% Fluorescent - 50% Incandescent					Alternate light and dark 50% Fluorescent - 50% Incandescent					96 hours dark then alternate light	
Treatments		24	48	72	96	120	24	48	72	96	120	96	120
Water		46	62	65	65	65	74	81	82	-	-	30	31
DTO 200		74	84	84	-	-	89	94	94	-	-	78	83
COU 25	(1)	0	0	0	0	0	8	15	18	18	18	2	2
	(2)	0	0	0	1	1	18	23	25	25	25	2	2
COU 25 & DTO 25	(1)	1	2	2	2	2	37	51	52	53	54	3	3
	(2)	0	1	1	1	1	27	36	39	39	39	5	5
COU 25 & DTO 50		0	7	12	17	17	62	74	75	-	-	5	6
COU 25 & DTO 100		11	44	48	48	48	58	78	87	-	-	12	13

B. 7/28/1952

			Percent germination at various intervals of time in hours									
Illumination	Treatments		24	48	72	96	120	144	168	192	216	240
Continuous light:	Water		86	91	91	91						
	DTO 200		82	85	91	91						
50% Fluorescent	COU 25	(1)	4	16	43	66	75	87				
50% Incandescent		(2)	2	10	16	30	55	77				
	COU 25 & DTO 100	(1)	72	84	84	84						
		(2)	70	83	84	85						

Appendix 12. B. (Continued)

Illumination	Treatments		Percent germination at various intervals of time in hours									
			24	48	72	96	120	144	168	192	216	240
	Water		56	63	73	86						
Continuous	DTO 200		80	88	88	88						
light:	COU 25	(1)	0	1	2	2	5	13	18	36	53	85
50% Fluorescent		(2)	0	0	2	4	14	27	32	50	52	64
50% Incandescent	COU 25 &											
	DTO 100	(1)	17	51	54	59	61	67	70	72	72	72
		(2)	37	58	67	69	72	79	82			
Dark (96 hours)	Water					31	62	79				
then continuous	DTO 200					76	95	95				
light:	COU 25	(1)				3	3	7	18	41	59	72
50% Fluorescent		(2)				4	5	7	13	17	27	63
50% Incandescent	COU 25 &											
	DTO 100	(1)				6	9	20	35	58	69	79
		(2)				6	6	14	24	36	59	84

Appendix 13. Effects of simultaneous applications of Coumarin (COU) and Dithiooxamide (DTO) in distilled water solutions on the elongation of new lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 12/16/1951

Treatments		Percent germination at various intervals of time in hours																	
		Continuous light										96 hours dark, then continuous light							
		70% Fluorescent 30% Incandescent					50% Fluorescent 50% Incandescent					70% Fluorescent 30% Incandescent				50% Fluorescent 50% Incandescent			
		24	48	72	96	120	24	48	72	96	120	96	120	144	168	96	120	144	168
Water	1	75	81	90	92	94	72	83	87	88	90	50	84	91	91	58	87	91	91
	2	77	85	86	90	92	65	72	81	84	85	48	75	85	85	63	86	94	94
	3	73	81	86	87	88	67	79	82	84	84	52	86	92	92	54	93	99	99
Average		74	82	87	90	91	68	78	83	85	86	50	82	89	89	58	89	95	95
DTO 100	1	79	85	86	88	89	70	75	77	78	79	84	89	90	90	85	87	88	88
	2	67	78	83	86	88	84	86	87	90	92	80	91	92	93	87	94	94	94
	3	86	93	93	94	94	67	67	71	79	82	84	92	93	93	83	92	93	93
Average		77	85	87	89	90	74	76	78	82	84	83	91	92	92	85	91	92	92
COU 25	1	9	23	39	56	66	1	14	18	31	36	2	3	8	18	5	7	8	10
	2	9	23	42	59	72	0	5	6	9	11	4	4	8	13	8	15	18	21
	3	12	42	59	74	85	0	5	7	15	18	8	9	17	31	7	15	19	35
Average		10	29	47	63	74	0	8	10	18	22	5	5	11	21	7	12	15	22
COU 25 & DTO 100	1	65	82	86	87	89	24	48	51	57	62	68	70	75	76	70	73	73	74
	2	51	70	75	78	78	44	69	75	79	81	81	81	85	85	78	80	81	81
	3	72	83	86	88	89	45	71	80	82	86	83	83	85	87	78	81	83	85
Average		63	78	82	84	85	38	63	69	73	76	77	78	82	83	75	78	79	80

Appendix 14-A. Effects of applications of Dithiooxamide (DTO) on the germination of new lettuce seeds under controlled conditions at 29°C. in distilled water solutions.

A. 8/4/1951

	Percent germination at various intervals of time in hours					
	Distilled water			DTO 200 ppm.		
Replicates	24	48	72	24	48	72
(1)	31	54	61	69	77	86
(2)	24	60	71	71	81	86
(3)	43	68	76	75	84	88
Average	33	61	69	72	81	87

Appendix 14-B. Effects of applications of Dithiooxamide (DTO) after a presoaking in Coumarin (COU 25 ppm) for 24 hours in darkness, on the germination of new lettuce seeds, under controlled conditions in distilled water solutions. Concentrations expressed in ppm.

B. 8/21/1951

	Percent germination at various intervals of time in hours										
	In continuous illumination					In alternate illumination				96 hours darkness then continuous illumination	
Treatments	24	48	72	96	120	24	48	72	96	96	120
Water (1)	0	52	80			0	78	86		48	76
(2)	2	42	88			4	82	92		38	80
DTO 50 (1)	0	28	76			0	78	86		56	88
(2)	0	34	76			0	76	88		72	86
DTO 100 (1)	4	28	72			0	72	84		82	82
(2)	0	42	76			0	76	88		82	88
DTO 200 (1)	0	30	66	74	80	0	46	72	82	76	80
(2)	0	40	64	72	82	0	50	72	80	66	74

Appendix 15. Effects of simultaneous applications of Ascorbic Acid (ASA) and Coumarin (COU) in acid water solutions at pH 3.5 on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 7/17/1951 — Length of the test: 4 days.

Illumination	Water	COU 75	COU 75 & ASA 500	COU 150	COU 150 & ASA 500
Continuous*	68.8	32.8	54.2	15.9	35.5
Alternate*	78.1	34.0	58.7	13.8	37.7
Darkness	86.6	29.6	47.9	13.1	28.3

B. 3/8/1952 — Length of the test: 5 days.

Illumination	Water	ASA 250	ASA 500	ASA 1000
Continuous	81.5	78.8	78.3	65.4
Darkness	92.2	93.7	89.3	81.7

Illumination	COU 75	COU 75 & ASA 250	COU 75 & ASA 500	COU 75 & ASA 1000
Continuous	32.4	54.5	65.9	68.3
Darkness	28.5	50.2	48.5	55.5

Illumination	COU 150	COU 150 & ASA 250	COU 150 & ASA 500	COU 150 & ASA 1000
Continuous	13.5	24.9	38.0	43.2
Darkness	13.0	23.3	28.3	33.9

*Light compositions: 50% Fluorescent, 50% Incandescent

Appendix 16. Effects of successive applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) and Coumarin (COU) in distilled water (DW) solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 2/20/1952

	Post-treatments	Pre - treatments 24 hours		Pre - treatments 48 hours	
		P	ASA 500	P	ASA 500
In light	DW	83.6	78.7	97.6	74.3
	COU 40	48.9	40.7	56.3	51.8
	COU 150	19.7	21.4	29.9	33.1
In darkness	DW	104.7	100.3	101.1	87.9
	COU 40	35.1	38.1	40.4	44.0
	COU 150	13.4	14.0	25.9	28.6

Appendix 17. Effects of successive applications of Coumarin (COU) in distilled water solutions (DW) and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 2/16/1952

	Post-treatments	Pre-treatments 24 hours			Pre-treatments 48 hours		
		DW	COU 75	COU 150	DW	COU 75	COU 150
In light	P	83.6	68.7	58.6	99.7	60.0	35.6
	ASA 500	75.5	92.3	81.2	85.1	58.8	37.8
In darkness	P	107.3	67.8	54.7	98.8	79.7	48.9
	ASA 500	92.4	71.8	58.6	84.1	73.5	57.4

B. 2/27/1952

	Post-treatments	Pre-treatments 24 hours						
		DW	COU 37.5	COU 75 (1)	COU 75 (2)	COU 75 (3)	COU 150	COU 300
In light	P	80.2	-	68.8	65.5	69.4	-	43.7
	ASA 250	78.1	-	79.9	77.0	73.8	-	53.4
	ASA 500	70.9	-	85.2	86.3	88.3	-	55.1
In darkness	P		79.4		67.5		48.2	
	ASA 250		82.6		71.9		54.1	

Appendix 18. Effects of successive applications of Coumarin (COU) in distilled water solutions (DW) and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average root-length of 30 seeds expressed in mm.

A. 4/4/1952

	Post-treatments	Pre-treatments (24 hours)				
		DW	COU 37.5	COU 75	COU 150	COU 300
In light	P	90.8	85.3	69.5	60.2	47.1
	ASA 500	93.8	97.7	94.7	79.5	
	ASA 1000	95.6	92.9	93.5	78.6	
	ASA 2000	81.1	81.1	80.7	65.8	49.4
In darkness	P	96.0	84.3			
	ASA 500	95.7	83.4			
	ASA 1000	91.6	87.4			

Appendix 19. Effects of simultaneous applications of Coumarin (COU) and Ascorbic Acid (ASA) in distilled water solutions on the germination of old lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 5/28/1951

	Percent germination			
	Continuous illumination 4 days	Alternate illumination 4 days	Darkness 4 days	Darkness 4 days then alternate illumination 5 days
Water	88	85	64	81
COU 25	23	40	5	9
COU 25 & ASA 250	21	32	8	51
COU 25 & ASA 500	53	50	6	72

B. 6/13/1951

		Percent germination at various intervals of time in hours								
Illumination	Treatments	36	60	84	108	132	156	180	204	228
Continuous	Water	82	84	86	86	87	88	88	88	
	COU 25	2	5	7	7	38	53	63	64	
	COU 25 & ASA 250	7	11	22	40	59	76	87	88	
	COU 25 & ASA 500	4	16	33	49	75	77	81	84	
Alternate starting with light	Water	84	90	93	93	93	93	93	93	
	COU 25	8	25	51	54	68	74	78	87	
	COU 25 & ASA 250	15	27	38	60	74	74	80	81	
	COU 25 & ASA 500	13	34	56	71	80	80	84	85	

Appendix 19-B. (Continued)

Illumination	Treatments	Percent germination at various intervals of time in hours								
		36	60	84	108	132	156	180	204	228
	Water	82	88	90	90	90	91	91	91	
Alternate	COU 25	17	45	67	70	74	77	83	83	
starting with	COU 25 &									
12 hours	ASA 250	27	36	38	52	69	83	86	88	
darkness	COU 25 &									
	ASA 500	21	41	59	74	80	80	82	82	
	Water	80	87	90	90	90	92	92	92	
Alternate	COU 25	0	33	63	74	78	81	85	88	
starting with	COU 25 &									
24 hours	ASA 250	2	29	42	46	63	72	77	80	
darkness	COU 25 &									
	ASA 500	0	30	59	70	73	73	73	73	
	Water		77	88	88	89	90	90	90	
Alternate	COU 25		1	43	65	76	79	82	88	
starting with	COU 25 &									
48 hours	ASA 250		1	29	62	75	82	85	88	
darkness	COU 25 &									
	ASA 500		1	31	62	71	73	77	82	
Alternate	Water			51	85	85	85	85	85	85
starting with	COU 25			3	11	35	44	49	61	65
72 hours	COU 25 & ASA 250			0	18	45	79	86	87	88
darkness	COU 25 & ASA 500			1	27	64	77	84	87	87
Alternate	Water				69	91	93	93	93	93
starting with	COU 25				2	5	22	52	66	67
96 hours	COU 25 & ASA 250				1	7	27	45	59	71
darkness	COU 25 & ASA 500				2	11	42	69	81	84

Appendix 20. Effects of simultaneous applications of Coumarin (COU) and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 on the germination of new lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 7/17/1951

	Percent germination at various intervals of time in hours											
	Continuous illumination					Alternate illumination				96 hours dark, then continuous illumination		
	24	48	72	96	120	24	48	72	96	96	120	144
COU 25	0	5	6	46	53	11	22	60	86	2	2	6
COU 25 & ASA 250	4	17	23	42	49	10	21	69	90	0	29	51
COU 25 & ASA 500	11	29	34	61	70	19	27	53	88	4	26	57

B. 8/18/1951

			Percent germination at various intervals of time in hours											
			Light: 70% Fluorescent and 30% Incandescent						Light: 50% Fluorescent and 50% Incandescent					
Illumination	Treatments		24	48	72	96	120	144	24	48	72	96	120	144
Continuous	COU 25	(1)	0	10	34	38	46	48	2	6	8	8	14	24
		(2)	2	10	30	44	52	54	0	0	2	4	14	40
	COU 25 & ASA 500	(1)	2	36	76	-			0	8	32	-	-	-
		(2)	6	28	72	88			0	6	24	-	-	-
	COU 25 & ASA 2000	(1)	0	26	32				0	0				
		(2)	0	32	62				0	0				

Appendix 20-B. (Continued)

Illumination	Treatments		Percent germination at various intervals of time in hours: Light: 70% Fluorescent and 30% Incandescent 24	48	72	96	120	144	Light: 50% Fluorescent and 50% Incandescent 24	48	72	96	120	144
Alternate	COU 25	(1)	0	10	36	54	56		0	8	22	26	30	46
		(2)	0	18	34	38	40		0	0	22	44	44	64
	COU 25 & ASA 500	(1)	4	44	80	84			0	30	70	80		
		(2)	0	44	86				0	42	58	68		
	COU 25 & ASA 2000	(1)	0	30	42				0	12	16			
		(2)	0	28	42				0	8	18			
96 hours darkness then alternate	COU 25	(1)				0	4	14						
		(2)				6	6	6						
	COU 25 & ASA 500	(1)				2	4	6						
		(2)				6	6	10						
	COU 25 & ASA 2000	(1)				4	4	8						
		(2)				0	0	14						

Appendix 21. Effects of simultaneous applications of Coumarin (COU) and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 on the germination of new lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 2/2/1952

		Percent germination at various intervals of time in hours													
		Continuous light								96 hours dark, then continuous light					
		70% Fluorescent 30% Incandescent				50% Fluorescent 50% Incandescent				70% Fluorescent 30% Incandescent			50% Fluorescent 50% Incandescent		
		24	48	72	96	24	48	72	96	96	120	144	96	120	144
Treatments															
Water	(1)	51	71	79	80	21	45	66	69	37	88		42	78	
	(2)	49	71	83	88	29	66	77	78	53	79		33	79	
	(3)	55	69	84	86	30	52	69	69	38	92		57	84	
Average		52	70	82	85	27	54	71	72	43	86		34	80	
ASA 500	(1)	74	82	92	92	59	72	77	80	30	88		47	80	
	(2)	60	73	78	78	23	55	71	72	42	92		42	88	
	(3)	71	83	91	92	48	56	70	72	63	87		58	80	
Average		68	79	87	87	43	61	73	75	45	89		49	73	
COU 25	(1)	0	0	25	37	0	0	5	11	4	4	9	2	2	2
	(2)	0	2	19	33	0	0	0	2	0	0	4	3	3	3
	(3)	0	0	14	22	0	1	4	10	1	1	2	6	6	6
Average		0	1	19	31	0	0	3	8	2	2	5	4	4	4
COU 25	(1)	1	6	65	71	1	1	11	28	2	2	33	3	3	8
&	(2)	0	16	57	74	0	0	4	13	1	1	21	3	3	16
ASA 500	(3)	1	16	56	76	0	1	11	31	5	5	17	0	0	8
Average		1	13	59	74	0	1	9	24	3	3	24	2	2	11

Appendix 21. (Continued)

B. 3/11/1952

Treatments		Percent germination at various intervals of time in hours Continuous illumination (70% Fluorescent, 30% Incandescent) 24	48	72	96	120	144	168
Water	(1)	56	75	81				
	(2)	52	75	85				
	(3)	53	76	86				
Average		54	75	84				
ASA 500	(1)	34	71	80	81			
	(2)	14	57	74	79			
	(3)	50	84	91	91			
Average		33	71	82	84			
COU 25	(1)	0	0	3	7	16	27	51
	(2)	0	2	6	10	17	24	34
	(3)	0	0	2	11	19	30	49
Average		0	1	4	9	17	27	45
COU 25 & ASA 500	(1)	0	5	33	53	68	98	81
	(2)	0	3	9	35	58	68	77
	(3)	0	3	28	46	58	68	73
Average		0	4	23	45	61	71	77

Appendix 22. Effects of applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5, after a presoaking in Coumarin (COU) (25 ppm) in distilled water solutions for 24 hours in darkness, on the germination of new lettuce seeds under controlled conditions. Concentrations expressed in ppm.

A. 8/21/1951

Treatments		Percent germination at various intervals of time in hours											
		Continuous illumination					Alternate illumination					96 hours darkness, then alternate illumination	
		24	48	72	96	120	24	48	72	96	120	96	120
Water	(1)	0	26	76			0	82	90			62	84
	(2)	2	50	88			0	64	70			44	82
ASA 200	(1)	2	52	86			0	76	86			40	68
	(2)	0	12	74			0	64	78			70	82
ASA 650	(1)	6	34	72	78		0	74	76			64	86
	(2)	0	24	72	90		0	80	88			52	74
ASA 2000	(1)	0	6	12	28	44	0	40	48	64	64	80	82
	(2)	0	0	2	12	20	0	40	54	60	62	76	80

Appendix 23. Effects of simultaneous applications of Dithiooxamide (DTO) and Ascorbic Acid (ASA) in distilled water solutions on the elongation of Marketer cucumber roots grown under controlled contitions. Concentrations expressed in ppm.

A. 5/31/1951

Length of the test: 4 days.
Average of 20 root-lengths expressed in mm.

Illumination	Water	DTO 200	DTO 200 & ASA 250	DTO 200 & ASA 500
Continuous	67.0	34.9	54.8	-
Alternate	87.3	37.7	40.2	46.4
Darkness	66.4	34.5	26.8	29.5

B. 6/5/1951

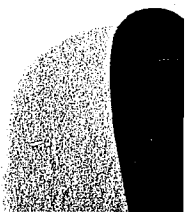
Length of the test: 4 days.
Average of 30 root-lengths expressed in mm.

Illumination	Water	DTO 200	DTO 200 & ASA 250	DTO 200 & ASA 500
Continuous	67.9	21.0	28.1	27.3
Alternate	64.7	26.3	22.8	22.6
Darkness	66.3	23.7	22.4	19.7

C. 6/16/1951

Length of the test: 5 days.
Average of 30 root-lengths expressed in mm.

Illumination	Water	DTO 100	DTO 100 & ASA 250	DTO 100 & ASA 500
Continuous	75.4	54.1	70.9	68.7
Alternate	95.3	58.6	74.4	72.4
Darkness	102.7	48.7	38.5	31.2



Appendix 24. Effects of simultaneous applications of Dithiooxamide (DTO) and Ascorbic Acid (ASA) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 6/26/1951

	Distilled water solutions				
Illumination	Water	DTO 100	DTO 100 & ASA 100	DTO 100 & ASA 250	DTO 100 & ASA 500
Continuous	87.7	69.1	88.2	98.4	85.0
Alternate	93.3	70.5	79.9	94.9	84.9
Darkness	114.8	60.8	57.4	49.1	38.1

B. 7/2/1951

	Buffered solutions, pH 4.5 (Sorensen)-				
Illumination	Water	DTO 100	DTO 100 & ASA 100	DTO 100 & ASA 250	DTO 100 & ASA 500
Continuous	43.8	42.2	53.9	56.3	51.9
Alternate	50.3	51.3	64.8	66.1	66.6
Darkness	51.3	50.8	48.2	52.0	43.9

Appendix 24 (Continued)

C. 7/14/1951

Acid water solutions, pH 3.5

Illumination	Water	ASA 250	DTO 200	DTO 200 & ASA 100	DTO 200 & ASA 250	DTO 200 & ASA 500
Continuous	77.4	85.3	44.5	62.6	61.3	65.9
Alternate	89.9	98.1	49.7	57.8	69.4	62.8
Darkness	98.1	107.2	52.5	47.0	38.5	35.1

D. 4/5/1952

Acid water solutions, pH 3.5

		Water	ASA 125	ASA 250	ASA 500	ASA 1000	ASA 2000
In Continuous Illumination	Controls	93.0	92.8	90.8	93.3	80.1	65.5
	+ DTO 200	32.7	33.8	39.5	49.2	41.5	31.1
In Darkness	Controls	98.8	-	96.6	93.5	84.5	47.5
	+ DTO 200	33.4	40.2	33.6	33.5	24.7	19.5

Appendix 25. Effects of successive applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) and Dithiooxamide (DTO) in distilled water (DW) solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 9/18/1951

		Group I			Group II		
	Pretreatments	P		ASA 500	DW	DTO 300	
	Post-treatments	DW	DTO 300	DTO 300	P	P	ASA 500
In light	Transfer after 24 hours	81.5	46.7	45.8	-	-	-
	Transfer after 48 hours	93.0	60.5	65.1	100.2	78.9	47.7
In darkness	Transfer after 24 hours	113.9	53.7	50.8	121.2	91.4	46.9
	Transfer after 48 hours	123.3	67.4	72.9	126.0	77.3	46.2

B. 10/20/1951

		Group I			
	Pretreatments	P		ASA 500	
	Post-treatments	DW	DTO 300	DW	DTO 300
In light	Transfer after 48 hours	-	57.9	-	66.7
	Transfer after 72 hours	106.1	70.6	102.1	76.1
In darkness	Transfer after 48 hours	-	59.0	-	67.5
	Transfer after 72 hours	110.3	68.3	105.9	83.0

		Group II			
	Pretreatments	DW		DTO 300	
	Post-treatments	P	ASA 500	P	ASA 500
In light	Transfer after 8 hours	-	78.2	89.6	89.4
	Transfer after 24 hours	89.7	91.8	100.3	81.1
In darkness	Transfer after 8 hours	-	93.8	73.8	54.9
	Transfer after 24 hours	102.2	95.0	67.5	48.6

Appendix 26. Effects of successive applications of Dithiooxamide (DTO) in distilled water (DW) solutions and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 10/11/1951

	Pretreatments	DW		DTO 150		DTO 300	
	Post-treatments	P	ASA 400	P	ASA 400	P	ASA 400
In light	Transfer after 8 hours	90.2	91.2	-	-	93.4	79.9
	Transfer after 24 hours	93.0	69.1	97.1	57.9	109.8	62.4
In darkness	Transfer after 8 hours	96.9	85.2	-	-	69.6	45.9
	Transfer after 24 hours	100.1	86.8	87.6	52.8	72.8	41.0

B. 10/20/1951

Illumination	Post-treatments	Pretreatments 24 hours				Pretreatments 8 hours	
		DW	DTO 75	DTO 150	DTO 300	DW	DTO 300
In light	P	86.7	-	94.2	98.3		
	ASA 100	85.1	-	-	94.3		
	ASA 250	82.2	-	78.7	93.6		
	ASA 400	75.9	-	-	83.1		
In darkness	P	103.3	104.4	81.3	67.9	96.6	81.7
	ASA 250	98.4	65.6	58.3	48.3	98.3	72.9

Appendix 27. Effects of successive applications of Dithiooxamide (DTO) in distilled water (DW) solutions and Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 11/20/1951

Pretreatments (24 hours)	DW		DTO 37.5		DTO 75	
Post-treatments	P	ASA 250	P	ASA 250	P	ASA 250
In darkness	102.4	100.7	103.0	83.4	94.8	72.0

B. 2/9/1952

Pretreatments (48 hours)	DW		DTO 100		DTO 200	
Post-treatments	P	ASA 250	P	ASA 250	P	ASA 250
In light	93.9	96.3	104.1	87.8	96.8	73.6

C. 11/1/1951

Pretreatments (24 hours)	DW		DTO 300	
Post-treatments	P	ASA 250	P	ASA 250
Light then darkness	99.0	76.6	78.7	52.2
Darkness then light	87.8	80.5	78.4	65.5

D. 5/12/1952

Pretreatments (24 hours)	DW	DTO 300			
Post-treatments	P	P	ASA 25	ASA 50	ASA 100
In light	80.6	91.0	86.3	88.0	91.8
In darkness	-	57.8	59.4	59.7	57.3

Appendix 28. Effects of successive applications of Ascorbic Acid (ASA) in acid water solutions at pH 3.5 (P) and Dithiooxamide (DTO) in distilled water (DW) wolutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 12/10/1951

Illumination	Post-treatments	Pretreatments (72 hours)			
		P	ASA 250	ASA 500	ASA 6000
In light	DW	90.9	85.1*	91.1	88.6
	DTO 300	69.5	67.5	76.0	71.9
In darkness	DW	83.4	87.4	90.4	73.7
	DTO 300	58.6	70.4	66.9	64.7

B. 1/16/1952

Illumination	Post-treatments	Pretreatments (72 hours)			
		P (1)	ASA 250 (1)	P (2)	ASA 250 (2)
In darkness	DW	86.9	84.7*	84.7	83.9
	DTO 300	62.8	73.4	56.7	71.2

C. 2/6/1952

Illumination	Post-treatments	Pretreatments (72 hours)	
		P	ASA 2000
In darkness	DW	94.1	77.0
	DTO 100	75.1	60.0
	DTO 200	70.9	52.2

*Average of 20 root-lengths.

Appendix 29. Effects of simultaneous applications of Dithiooxamide (DTO) and d-Iso-Ascorbic Acid (DIA) in acid water solutions at pH 3.5 on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

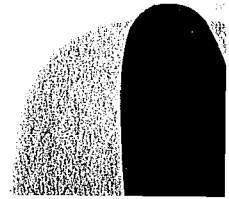
A. 12/13/1951

	Water	DIA 500	DTO 200	DTO 200 DIA 125	DTO 200 DIA 250	DTO 200 DIA 500
In light	75.5*	78.6	47.1	48.8	56.6	56.8
In Darkness	67.8	70.3	44.6	41.6	36.1	34.5

B. 12/21/1951

		Water	DIA 20	DIA 50	DIA 250	DIA 1000
In light	Control	82.3			81.8	88.0
	+ DTO 200	46.3			55.8	34.1
In darkness	Control	82.5	85.3	85.1	78.2	77.0
	+ DTO 200	46.4	46.9	45.2	35.9	30.9

*Average of 20 root-lengths



Appendix 30. Effects of simultaneous applications of Dithiooxamide (DTO) and Gluco-Ascorbic Acid (GAS) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 12/18/1951

	Water	GAS 500	DTO 200	DTO 200 GAS 125	DTO 200 GAS 250	DTO 200 GAS 500
In light	91.5	78.5	48.6	53.4	53.9	56.8
In Darkness	81.0	72.6	47.5*	45.1	39.2	35.3

B. 2/6/1952

		Water	GAS 125	GAS 250	GAS 500
In light	Control	84.7	86.9*	91.2	97.2
	+ DTO 200	43.4	53.5	66.8	72.2
In darkness	Control	89.1	83.4	94.7	93.1
	+ DTO 200	50.3	44.4	40.2	32.6

*Average of 20 root-lengths

Appendix 31. Effects of simultaneous applications of Dithiooxamide (DTO) and 3-(Alpha-Imino-Ethyl) -5-Methyl Tetronic Acid (T) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled contions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 12/19/1951

		Water	T 25	T 100	T 500
In light	Controls	82.1	76.9	60.6*	40.8
	+ DTO 200	43.5	39.6	34.5	24.5
In darkness	Controls	80.9	99.6	90.6	58.4
	+ DTO 200	48.0	46.5	39.5	25.0

B. 12/28/1951

		Water	T 5	T 12.5	T 25	T 50	T 100	T 250	T 500
In light	Controls	85.4	82.0	75.9	69.7	59.7	46.6	36.6	31.4
	+ DTO 200	44.3	31.9	27.1	32.5	25.0	27.0	22.3	20.5
In darkness	Controls	90.7	90.3	94.3	90.5	96.1	85.2	75.8	59.3
	+ DTO 200	46.1	51.3	47.4	48.8	44.4	39.9	36.5	24.7

*Average of 20 root-lengths.

Appendix 32. Effects of simultaneous applications of Thiourea (TU) and Ascorbic Acid (ASA) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 12/15/1951

	Water	ASA 500	TU 600	TU 600 & ASA 125	TU 600 & ASA 250	TU 600 & ASA 500
In light	74.2	83.8	50.4	35.4	40.0	33.6
In darkness	79.1	76.9	52.3	26.3	29.7	26.1

B. 2/9/1952

		Water	ASA 25	ASA 50	ASA 125	ASA 250	ASA 500
In light	Control	80.1	79.0	77.1	77.5	72.2	69.6
	+ TU 600	46.1	47.3	45.5	41.4	38.2	26.6
In darkness	Control	102.6*	105.0	100.8	96.5	94.8	99.0
	+ TU 600	51.8	49.2	49.3	46.1	38.1	33.2

*Average of 20 root-lengths

Appendix 33. Effects of simultaneous applications of 2,4-D and Dithiooxamide (DTO) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions. Concentrations expressed in ppm.

A. 4/2/1951

Length of the test: 5 days.

	2,4-D 0.1	2,4-D 0.1 + DTO 2.5	2,4-D 0.1 + DTO 5	2,4-D 0.5	2,4D 0.5 + DTO 2.5	2,4-D 0.5 + DTO 5
Number of seeds	17	17	17	18	18	18
Average length (mm)	29.0	34.1	36.8	11.3	13.4	13.2

B. 4/9/1951

	Average of 30 root-lengths expressed in mm.			
	2,4-D 0.1	2,4-D 0.1 + DTO 5	2,4-D 0.1 + DTO 7.5	2,4-D 0.1 + DTO 10
After 4 days	23.8	36.7	39.4	37.7
After 7 days	24.4	48.5	44.0	45.7

Appendix 34. Effects of simultaneous applications of 2,4-D, Dithiooxamide (DTO) and Ascorbic Acid (ASA) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days. Concentrations expressed in ppm. Average of 20 root-lengths expressed in mm.

A. 4/15/1951

2,4-D 0.1	2,4-D 0.1 + ASA 25	2,4-D 0.1 + ASA 100	2,4-D 0.1 + ASA 250	2,4-D 0.1
31.4	37.5	36.3	42.8	37.2

2,4-D 0.1 + DTO 2.5	2,4-D 0.1 + DTO 2.5 + ASA 25	2,4-D 0.1 + DTO 2.5 + ASA 100	2,4-D 0.1 + DTO 2.5 + ASA 250	
36.7	39.0	35.6	40.0	

2,4-D 0.1 + DTO 5	2,4-D 0.1 + DTO 5 + ASA 25	2,4-D 0.1 + DTO 5 + ASA 100	2,4-D 0.1 + DTO 5 + ASA 250	2,4-D 0.1 + DTO 5 + ASA 500
39.1	41.0	39.3	49.0	47.2

2,4-D 0.1 + DTO 7.5	2,4-D 0.1 + DTO 7.5 + ASA 25	2,4-D 0.1 + DTO 7.5 + ASA 100	2,4-D 0.1 + DTO 7.5 + ASA 250	2,4-D 0.1 + DTO 7.5 + ASA 500
38.0	41.0	42.6	50.1	46.8

2,4-D 0.1 + DTO 10	2,4-D 0.1 + DTO 10 + ASA 25	2,4-D 0.1 + DTO 10 + ASA 100	2,4-D 0.1 + DTO 10 + ASA 250	2,4-D 0.1 + DTO 10 + ASA 500
38.4	44.6	40.8	47.8	46.7

Appendix 35. Effects of simultaneous applications of 2,4-D, Dithiooxamide (DTO) and Ascorbic Acid (ASA) in distilled water solutions on the elongation of Marketer cucumber roots grown under laboratory conditions for 4 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 4/21/1951

Illumination	2,4-D 0.1	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 250	2,4-D 0.1 ASA 250 DTO 7.5
Alternate (started with light)	33.7	41.0	51.7	52.3
Alternate (started with darkness)	31.9	38.2	39.2	48.4

B. 4/23/1951

Illumination	2,4-D 0.1	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 250	2,4-D 0.1 DTO 7.5 ASA 250	Water
Continuous	44.6	42.0	54.5	56.7	62.6
Alternate	42.4	41.9	51.0	44.6	72.9
Darkness	31.8	33.6	44.0	34.3	66.7

C. 5/7/1951

Illumination	2,4-D 0.1	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 250	2,4-D 0.1 ASA 250 DTO 7.5	Water	ASA 250
Continuous	55.2	53.4	60.3	67.0	67.7	78.5
Alternate (started with light)	38.8	49.4	46.2	47.1	72.7	78.1
Alternate (started with darkness)	37.8	45.5	43.1	49.0	74.3	73.9
Darkness	38.5	37.1	38.9	39.6	82.5	82.3

Appendix 36. Effects of simultaneous applications of 2,4-D, Dithiooxamide (DTO) and Ascorbic Acid (ASA) in distilled water solutions on the elongation of Marketer cucumber roots grown for 4 days under laboratory conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 5/21/1951

Illumination	Water	ASA 500	2,4-D 0.1	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 500	2,4-D 0.1 ASA 500 DTO 7.5
Continuous	71.0	79.3	52.8	55.5	67.1	63.0
Alternate (started with light)	83.3	82.5	53.8	51.9	45.1	48.9
Alternate (started with darkness)	78.9	89.0	45.5	52.5	54.8	57.6
Darkness	90.1	87.2	42.3	32.9	42.3	34.5

B. 6/8/1951

Illumination	Water	2,4-D 0.1	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 250	2,4-D 0.1 ASA 250 DTO 7.5
Continuous	53.9	27.7	25.4	30.1	30.6
Alternate	66.2	21.1	28.7	35.1	30.2
Darkness	65.8	27.5	31.9	26.5	25.7

Appendix 37. Effects of simultaneous applications of 2,4-D, Dithiooxamide (DTO) and Ascorbic Acid (ASA) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 8/9/1951

Length of the test: 4 days.

Illumination	Water	2,4-D 0.1	2,4-D 0.1 DTO 3.75	2,4-D 0.1 DTO 7.5	2,4-D 0.1 ASA 500
Continuous	63.6	35.6	44.3	46.7	54.4
Alternate	82.6	34.2	38.3	47.2	54.6
Darkness	84.2	34.5	38.7	45.5	44.0

B. 2/23/1952

Length of the test: 5 days.

		Water	DTO 10	DTO 30	DTO 100	ASA 250	ASA 500
In light	Controls	75.8	85.8	80.1	66.2	77.4	78.5
	+ 2,4-D 0.1	49.6	50.2	56.0	61.7	58.1	64.0
In Darkness	Controls	92.9	81.9	68.1	60.5	85.5	77.8
	+ 2,4-D 0.1	37.8	46.3	46.9	55.1	47.4	61.2

Appendix 37. (Continued)

C. 3/1/1952

Length of the test: 5 days.

		Water	DTO 100	DTO 300	ASA 500	ASA 1000	ASA 2000
In light	Controls	82.7	78.6	28.9	89.9	89.6	85.3
	+ 2,4-D 0.1	50.4	66.6	34.8	68.1	71.4	68.7
In darkness	Controls	88.9	60.0	33.5	85.5	80.0	41.8
	+ 2,4-D 0.1	35.4	52.8	31.0	50.7	61.9	40.8

Appendix 38. Effects of simultaneous applications of Indole Acetic Acid (IAA) and Dithiooxamide (DTO) in distilled water solutions on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions. Concentration expressed in ppm. Average of 30 root-lengths.

A. 7/20/1951

Length of the test: 4 days.

	IAA 5	IAA 5 DTO 2	IAA 5 DTO 8	IAA 5 DTO 20	IAA 5 DTO 50
In light	35.7	38.9	41.4	46.6	42.9
In darkness	27.7	33.5	27.8	41.6	40.2

B. 7/27/1951

Length of the test: 4 days.

	DTO 200	DTO 200 IAA .1	DTO 200 IAA .3	DTO 200 IAA 1	DTO 200 IAA 2
In light	49.0	43.7	48.6	50.9	42.6
In darkness	49.6	50.3	52.4	49.7	44.4

C. 8/17/1951

Length of the test: 4 days.

Illumination	Water	IAA 5	IAA 5 DTO 3.3	IAA 5 DTO 20	IAA 5 DTO 100
Continuous	74.9	26.4*	34.7*	51.9	33.2*
Alternate	89.9	25.3	37.6	40.2	36.2
Darkness	88.4	20.0	24.5	36.4	34.1

D. 9/13/1951

Length of the test: 4 days.

	Water	DTO 150	IAA 5	DTO 150 IAA 5
In light	73.1	49.2	52.7	40.3
In darkness	88.0*	48.4	33.8	34.4

*Average of 20 root-lengths.

Appendix 39. Effects of simultaneous applications of Indole Acetic Acid (IAA) and Dithiooxamide (DTO) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root lenths expressed in mm.

A. 4/9/1952*

Acid water solutions at pH 3.5

	IAA 5	IAA 5 DTO 1	IAA 5 DTO 5	IAA 5 DTO 20	IAA 5 DTO 75	IAA 5 DTO 150
In light	37.3	39.8	41,7	62.7	41.6	38.3
In darkness	27.8	34.6	34.6	38.5	29.8	29.5

	IAA 10	IAA 10 DTO 1	IAA 10 DTO 5	IAA 10 DTO 20	IAA 10 DTO 75	IAA 10 DTO 150
In light	30.5	33.2	39.7	37.4	36.1	36.7
In Darkness	24.9	21.6	-	32.8	27.8	24.5

*Temperature 21°C.

B. 4/17/1952

Distilled water solutions

	Water	IAA 2	IAA 2 DTO 10	IAA 2 DTO 20	IAA 2 DTO 40
In light	81.9	51.7	60.5	55.6	48.9
In darkness	100.5	38.9	55.0	52.1	42.2

	IAA 5	IAA 5 DTO 10	IAA 5 DTO 20	IAA 5 DTO 40
In light	33.1	42.9	48.9	42.3
In darkness	25.1	42.1	37.6	35.4

Appendix 40. Effects of simultaneous applications of Indole Acetic Acid (IAA) and Ascorbic Acid (ASA) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths.

A. 4/24/1952

	Water	IAA 2	IAA 2 ASA 100	IAA 2 ASA 250	IAA 2 ASA 750	IAA 2 ASA 1500
In light	94.2	51.4	54.1	73.6	81.4	69.8
In darkness	86.1	45.3	55.4	55.3	52.4	52.1

	IAA 5	IAA 5 ASA 100	IAA 5 ASA 250	IAA 5 ASA 750	IAA 5 ASA 1500
In light	37.7	71.3	67.8	68.7	62.8
In darkness	37.0	44.0	39.7	43.6	38.1

B. 5/14/1952

	Water	IAA 10	IAA 10 ASA 100	IAA 10 ASA 250	IAA 10 ASA 750	IAA 10 ASA 1500
In light	77.7	-	58.3	65.9	70.2	58.4
In darkness	92.7	29.3	40.5	37.0*	44.6	39.8

*Average of 20 root-lengths.

Appendix 41. Effects of simultaneous applications of Naphthalene Acetic Acid (NAA) and Dithiooxamide (DTO) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root lengths expressed in mm.

A. 4/25/1952

Distilled water solutions

In light

	Water	DTO 5	DTO 20	DTO 100
+ NAA .1	46.2	53.1	61.0	43.6*
+ NAA .3	37.2	48.0	46.6	41.6
+ NAA 1.0	30.2	35.6	34.1	30.5

In darkness

	Water	DTO 5	DTO 20	DTO 100
+ NAA .1	41.0	42.5	41.8	41.4
+ NAA .3	29.5	29.0	31.4	32.3
+ NAA 1.0	23.8	22.6	25.8	26.0

B. 5/15/1952

Acid water solutions (pH 3.5)

Illumination	Water	NAA .1	NAA .1 DTO 1	NAA .1 DTO 2.5	NAA .1 DTO 7.5	NAA .1 DTO 20
Darkness	90.6	43.0	48.1	47.2	48.1	52.6

*Average of 20 root-lengths.

Appendix 42. Effects of simultaneous applications of Naphthalene Acetic Acid (NAA) and Dithiooxamide (DTO) or Ascorbic Acid (ASA) in acid water solutions (pH 3.5) on the elongation of Burpee Hybrid cucumber roots grown under controlled conditions for 5 days. Concentrations expressed in ppm. Average of 30 root-lengths expressed in mm.

A. 5/5/1952

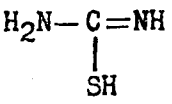
		Water	DTO 5	DTO 10	DTO 20	DTO 40
In light	+ NAA .1	54.1	53.7	58.1	64.4	59.5
	+ NAA .25	45.9	42.8	51.6	50.8	48.3
In darkness	+ NAA .1	48.8	51.1	48.6	49.8	47.6
	+ NAA .05	54.2	64.2	62.7	60.8	55.2

		ASA 250	ASA 500	ASA 1000	Water Controls
In light	+ NAA .1	65.8	68.5	79.4	84.9
	+ NAA .25	62.1	66.4	71.0	
In darkness	+ NAA .1	55.1	72.5	69.2	96.4
	+ NAA .05	76.8	83.3	87.1	

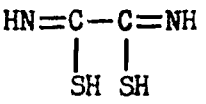
B. 5/14/1952

Illumination	Water	NAA 2	NAA 2 ASA 100	NAA 2 ASA 250	NAA 2 ASA 750	NAA 2 ASA 1500
In darkness	92.7	25.0	26.5	34.2	41.0	40.8

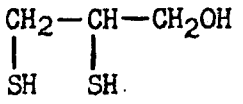
FORMULAS



Thiourea



Dithiooxamide



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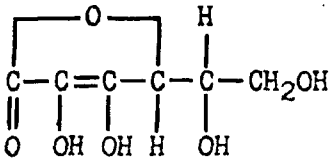

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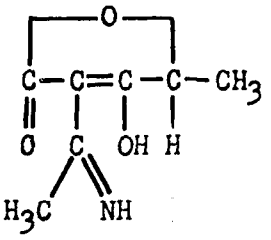
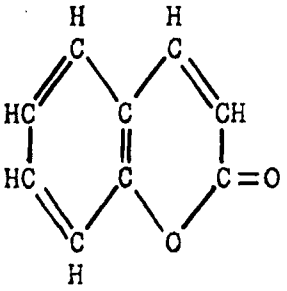
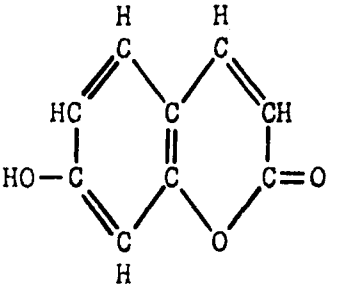


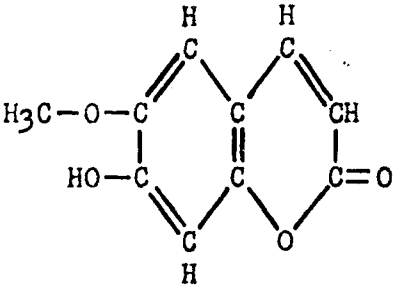
3-(alpha-Iminoethyl)-5-Methyl Tetronic Acid



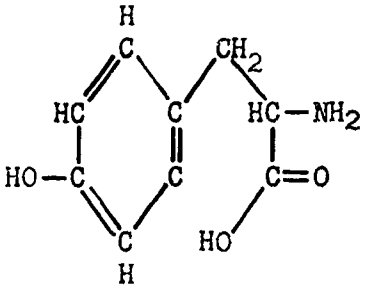
Coumarin



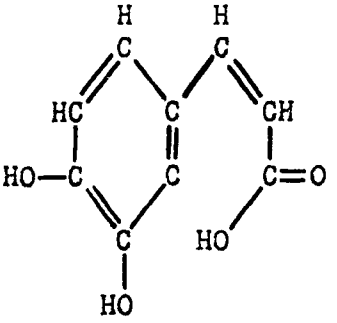
Umbelliferone



Scopoletin



Tyrosine



Caffeic Acid
(3,4-Dihydroxycinnamic Acid)

ERRATA

Page 11. Line 14 from the top : omit " For a number of either one ".

Page 42. Line 11 from the top : omit " be ".

Page 56. Line 5 from the bottom : read " dicoumarol " instead of " dicomarol ".

Page 60. Line 6 from the bottom : read " three " instead of " tree ".

Page 66. Second paragraph, line 3 : read " dried " instead of " died ".

Page 85. Line 4 from the top : read " only when it was applied " instead of " only it was applied ".

Page 86. Line 5 from the top : read " the same processes " instead of " the processes ".

Page 95. Line 9 from the bottom : read " parasorbic acid " instead of " parascorbic acid ".

Page 98. Line 7 from the top : read " of auxin in the coleoptile " instead of " of auxin the coleoptile ".