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Effects of Rootone on Shoot and Root Growth of the Soy Bean and the Effects of Nutrient Salts on the Same

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A Thesis submitted in partial fulfillment of requirements for department honors.

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Preface

Plant hormones, as related to commercial crops, is a relatively new field of plant physiology. Recently there has been a trend toward commercialization of plant hormones for agricultural crops, but as yet many farmers are ignorant of the great benefits that are to be derived from plant hormones. Also as in any new field of sceince much tedious experimental work has to be done before such a product for all types of commercial use could possibly be intelligently and efficiently used. With this in mind and with the hope that I could contribute even one small piece to this large puzzle, I choose for my experiment the effects of different concentrations of rootone, a commercially prepared hormone consisting of a-naphthal acetamide, and thiourea in a talc base, on the root and shoot growth of the soy bean. The rootone is produced commercially by the horticultural division of the American Chemical Paint Company in Ambler, Pa.

The following paper is divisible into three parts, the first attempting to give the reader a few general concepts of plant hormones and plant physiology, the second dealing with the actual experiment, and the third, conclustons that are drawn from the actual experiment.

I wish to express my appreciation to Dr. Brownback, head of the Biology Department at Ursinus College, and to Dr. Jones of the American Chemical Paint Company for their helpful criticisms and suggestions.

Introduction

SEEDS

Seeds are among the most variable structures produced by plants. They exhibit a wide range of size, shape, color, and surface anatomy, amount and nature of reserve food, details of the embryo, method of germination, length of life, and so on. In spite of the variety of associated details we may formulate a fairly useful general discussion of seeds. Every normal seed consists of at least two parts, the seed coat and the embryo, and in many seeds a third significant feature, the endosperm, is present.

The seed coat or coats are usually made up of tissues with greatly thickened and hardened cell walls, which are mainly the transformed tissues of the integument or integuments of the old ovule. The principal value of this part of the seed lies in its effective protection. The outer seed coat is often extremely thick and resistant. The inner seed coat, if this coat is present at all, is thin and much less important.

Many seeds show a definite spot or scar on the surface, marking the place at which the seed broke from the funiculus or stalk. This is the hilum.

A copius endosperm is present in many seeds. This tissue is commonly packed with food which may be used by the embryo and seedling during germination.

The rudimentary plant, the embryo of the seed, may be very small and more or less embedded in the endosperm, or in seeds without endosperm the embryo may fill the whole space.

Structually, the embryo consists of a central axis with a rudimentary root, the radicle, and a rudimentary shoot or bud, the plumule, together with an intermediate segment, the hypocotye, and one or two later "seed leaves", the cotyledons. The cotyledons are usually ephemeral (short-lived), and in certain seeds they never escape from the seed coats or reach the light above the ground. The cotyledons of numerous other seeds reach the light thru the elongation of the shoot, and then they usually develope chloroplasts which enable them to photosynthesize sugar as do the us do the usual secondary leaves.

That there are valuable foods in seeds will be recognized when we recall how frequently and in what vast quantities man and other animals utilize seeds as food in daily use. The accumulation of foods in seeds enables plants to live thru periods of inactivity or dormancy and then renew their activities and grow when favorable conditions return.

Among the various carbohydrate foods the starches are the most commonly present in fruits and seeds, and they often occur in large quantity in the cells of either the endosperm or the embryo.

Many plants store sugars in their seeds, but these are not as abundant in seeds in general as are the starches.

Vegetables fats and oils of great variety are abundant in seeds.

These foods are usually most abundant in the embryo, but they also occur in the endosperm.

The proteins are highly complex and are possessed of extremely various chemical and physical properties. They enter into the regular composition of protoplasm, and they are found as stored foods in probably all seeds. The seeds of legumes (soy bean) are often rich in proteins. Stored proteins occur in solution or in the form of grains of crystals. Supplies of stored proteins are of great value to the plant at the time of seed germination and early growth when new protoplasm must be formed in the growing tips of the root and shoot.

GERMINATION

A new plant actually begins with the fertilization of an egg in the ovule and there it continues to grow for a time as the ovule ripens into a seed. Growth of the embryo practically ceases after a certain stage developement, during which period a supply of food accumulates in the seed and the seed coats become thick and resistant. The seed is then mature, and when it finally separates from the parent it is well equipped to endure environmental conditions that may be unfavorable to immediate further growth. When suitable conditions again appear the embryo may resume its growth. Then the seed coats are broken, the radicle and the hypocotyle emerge, and the seed is said to have germinated. Thus we see that seed germination is nothing essentially new in the life of the plant, it is the resumption of activities that had been temporarily reduced or suspended as the seed ripened on the parent plant.

Certain external conditions and materials as well as certain internal changes are necessary to germination. The processes involved occur inside the seed, and have to do with the details of water absorption, digestion by means of enzymes, movement of foods, respiration, and assimilation.

The most important outside conditions which must be met include an adequate supply of oxygen.

The death of all seeds when stored too long, and the fact that some kinds germinate better after moderate storage than when new, show that some processes go on in the seed even while dry. But such processes are slow and inconspicuous. Water is necessary to start the protoplasm and enzymes of the seed into activity, with the consequent taking in of oxygen, and to transport food within the seed to the growing part. Water also softens the seed coats; and as soon as the seed becomes able to hold much more water than it contained during its developement, the bulkiness of the water bursts the coats.

Every vital process of cells and of organisms is closely related to temperature. The temperature may be too low or too high, but there is always a most favorable intermediate, or optimum, for each activity and for growth as a whole. The maximum and the minimum, as well as the favorable temperature for the most active germination of the seeds, vary greatly for different plants.

Respiration is essential to life and activity in every living cell, and a supply of oxygen is absolutely necessary for this vital phenomena. Cellular activities, respiration, assimilation, mitosis, and growth occur vigorously in germinating seeds, and on this account an adequate supply of oxygen is an absolute essential. The proper depth to which seeds should be planted is likely to be closely related to the oxygen supply, other conditions being uniform.

GROWTH OF THE EMBRYO AND SEEDLING

As a result of the above conditions and activities the embryo begins to grow once more. The cells of the embryo are enlarged on account of the absorption of water by the cell walls and vacuoles, and the embryo enlarges rapidly by the formation of new cells by mitosis, particularly in the radicle, hypocotly, and plumule.

The radicle is usually the first structure to emerge from the cleft formed in the seed coats, and it continues at once to develope. The growth of the root is often suprisingly prominent before the cotyledons hypocotyl, and plumule escape from the seed. The elongation of the hypocotyl soon pushes the cotyledons as well as the plumule to the surface where chlorophyll is soon developed in the cotyledons, secondary leaves, and stem of the seedling. The growing plantlet soon becomes independent of the food supply in the old seed and well rooted in the soil, and at that point the seedling stage is ended.

Incidentally, plants must be supplied with the necessary nutrients for metabolic purposes, in my experiment a nutrient solution is added to half the numbers of plants. All the necessary and essential elements for growth of plants are as follows: boron, calcium, iron, magnesium, manganese, phosphorous, potassium, sulfur, carbon, hydrogen, nitrogen, zinc, copper, and oxygen. These elements with some mineral salts fulfill one or more of three functions: first, they are nutritive and used in the chemical composition of the plant body; second, they are catalytic, causing reactions to occur without entering into them, and third, they are balancing, antagonizing harmful effects produced by other salts. Or they may cause a medium of the proper acidity for optimum growth and developement.

HORMONES OR AUXINS

At present, the term "hormone," introduced to science in 1905 by the animal physiologist Starling, designates a fairly large group of substances elaborated by the organism and possessing the capacity of directing and regulaing the course of different vital processes. In common with enzymes, they possess a very high activity at exceedingly low concentrations, but they are distinguished by a greater stability in respect to heat and other destructive agents.

Much experimental work has been done with plant hormones and results indicate the hormones are capable of moving through the plant in only one direction, from the proximal end to the base, and that hormones seem to act best in an acid medium, alkaline soils seem to in activate hormones. At high strengths hormones always stunt growth, while at low strengths they force growth. These hormones are stored in seeds and are of great significance in germination and seedling growth. As growth of the seedling continues the concentration of the store hormone decreases.

DuBuy summarizes most of the work to date in the following statement in a recent magazine article. "The plant 'hormones', also called growth substances, growth regulators or auxins, belong either to the indole-or naphthalene-group such as indole-3-acetic acid, indole-3-propionic acid, etc., and a-naphthyl acetic acid, naphthyl propionic acid and their amides, (such as naphthyl acetamide--Ed.). Or else they belong to the vitamin group, like Vitamin B-1, Vitamin B-6, nicotinic acid and Vitamin C.

Also some other chemicals such as thiourea have similar effects." I

THE EXPERIMENT

The sand culture method of growth was used in this experiment. The sand was thoroughly washed with hot water to remove all ions, and then superheated over a Bunsen flame to make sure that all organism and bacteria present would be destroyed. After the sand cooled it was placed into eight boxes, approximately 4" x 14", and was watered prior to planting.

The seeds used were picked for their uniformity in size and weight. They were then weighted and portions of rootone in proper concentration were prepared. This gave twenty-four treated with 1:960 concentration rootone, twenty-four treated with 1:480 concentration rootone, and twenty-four treated with 1:240 concentration rootone. The remaining twenty-four seeds were used for the control group. In order to treat the seed a standard and accepted method was used-they were placed in a jar with the dry rootone powder and shaken. The powder coated the seed.

Into each sand filled box twelve seeds, two rows of six each were planted 3/4" below the surface. This gave two boxes planted with each type of treated seed mentioned above. In each case one of the boxes was watered with 100 ml. of water, while the second box was watered only on every other day; on the intervening days 100 ml. of an aqueous, nutrient solution was added to these boxes so that the effects of these inorganic ions could be studied.

I. H.G. DuBuy, "Is This New Science Worthwhile?, Southenn Seedsman, 5:2, February, 1942, p. 14.

The nutrient solution used had the following composition:

1 cc. M ammonium acid phosphate ($NH_4H_2PO_4$)
6 cc. M potassium nitrate (KNO_3)
4 cc. M calcium nitrate ($Ca(NO_3)_2$)
2 cc. M magnesium sulfate ($MgSO_4$)
1 cc. H_3BO_3
$MnCl_2$
$ZnSO_4.7H_2O$
$CuSO_4.5H_2O$
986 cc. water

With the addition of this nutrient solution as a factor, the following arrangement existed:

Box 1 - Rootone treated seeds - 1 part:240 parts.
Box 2 - Rootone treated seeds - 1 part:240 parts, plus nutrient.
Box 3 - Rootone treated seeds - 1 part:480 parts.
Box 4 - Rootone treated seeds - 1 part:480 parts, plus nutrient.
Box 5 - Rootone treated seeds - 1 part:960 parts.
Box 6 - Rootone treated seeds - 1 part:960 parts, plus nutrient.
Box 7-- Untreated without Rootone or nutrient.
Box 8-- Untreated without Rootone, but treated with nutrient.

The boxes were placed on a moveable table near a window where plenty of uniform light and heat struck all plants. Each day the table was reversed to neutralize the effect of phototropism on bending of plants. As growth progressed, observations and measurements were made, the measurements being made with a metric rule.

The seeds were planted on February 22 nd and on the fifth day the first sprouts appeared. All seeds seemed to be equal in this their first growth stage. It was observed later that the untreated seeds seemed to be a little better in their top growth; this is due to the fact that all the nourishment of the plant went into top growth and not the root growth. But in the treated seeds and especially the 1:960; the nutrient and nourishment went into root growth and not top growth. Incidentally, the vigor, strength and vitality of the plant depends upon its root system and not upon its top growth.

The charts following this section show the actual measurements on top growth and root growth. A very big diference was also noted between the tootone treated seeds and the untreated ones. The laterals of the treated were longer, thicker, and stronger, had greater number of root hairs, and had greater turgidity as compared with the untreated.

After sixteen days growth observations showed that the rootone treated seeds, 1:960, had by far the heaviest lateral root growth, and most turgid root system. The rootone 1:480, showed the next best developemtnt. Inall observations the nutrient fed plants were about the same as those treated with plain water. This is because the soy bean seed has stored in it enough nutrient for developement and more is not necessary or advantageous as far as growth is concerned in this experiment.

On the whole the rootone treated seeds and especially the 1:960 treated seeds showed the best root growth, this was followed by the 1:480 and in turn was followed in root growth by the 1:240 treated. The untreated showed a definite inferior developement in their root growth, but as concerns their shoot growth they were just about the same as the treated.

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ROOT GROWTH

Days Growth	1:240 Rootone Treated ~ No Nutrient		1:240 Rootone Treated ~ Nutrient		1:480 Rootone Treated ~ No Nutrient		1:480 Rootone Treated ~ Nutrient		1:960 Rootone Treated ~ No Nutrient		1:960 Rootone Treated ~ Nutrient		Untreated ~ No Nutrient		Untreated ~ Nutrient	
	Laterals	Length	Laterals	Length	Laterals	Length	Laterals	Length	Laterals	Length	Laterals	Length	Laterals	Length	Laterals	Length
14.th.	28	7.5	26	4.7	25	4.5	32	5.7	38	7.5	33	6.8	22	6.5	21	6.6
15.th	33	8.0	29	6.5	32	7.0	30	6.3	38	11.5	29	9.6	16	4.1	16	5.2
	30	5.5	26	5.0	31	6.0	27	9.5	16	6.4	27	5.2	18	6.5	16	4.1
16.th.	27	5.5	28	8.9	27	8.2	—	—	19	3.6	28	6.0	19	5.2	12	6.3

TOP GROWTH

Days Growth	1:240 Rootone Treated ~ No Nutrient	1:240 Rootone Treated ~ Nutrient	1:480 Rootone Treated ~ No Nutrient	1:480 Rootone Treated ~ Nutrient	1:960 Rootone Treated ~ No Nutrient	1:960 Rootone Treated ~ Nutrient	Untreated ~ No Nutrient	Untreated ~ Nutrient
5.th	1.4	1.5	3.0	2.9	2.8	3.3	3.0	2.8
6.th	2.8	2.5	3.8	3.3	3.3	4.5	3.4	3.3
7.th	4.2	3.8	4.5	4.0	4.9	4.8	4.7	4.3
8.th	5.9	4.6	5.8	4.5	6.3	6.4	5.5	6.4
9.th	8.3	8.3	8.8	6.8	8.3	8.5	8.8	8.9
10.th	9.1	10.3	11.6	8.3	11.4	10.1	10.5	11.2
11.th	11.1	10.4	11.7	8.7	11.5	10.2	10.7	13.9
12.th	13.3	12.4	13.4	10.0	12.2	12.1	11.1	14.1
13.th	13.9	13.7	14.1	10.4	13.0	12.9	14.3	14.4
14.th	14.6	15.6	14.9	14.0	16.0	13.7	15.4	15.0
15.th	15.2	16.0	15.3	14.4	16.3	14.1	15.9	15.3

* Measurements above are given in centimeters.

GENERAL SUMMARY AND CONCLUSIONS

The advantageous effects of plant hormones are unmistakeably indicated in this experiment. In general hormones usually stimulate shoot and especially root growth. In all cases the rootone treated seeds gave forth plants that had well developed and exceedingly strong root systems. This is an important factor because the plants future developement depends primarily on its root system - especially under adverse conditions or when the growing season is short.

Also in all rootone treated seeds the dicotyledons or embryonic leaves were much stronger, greener, and turgid then the untreated. This means that the rootone seed treated plants have more chlorophyll then those untreated; and as a direct result the rootone treated plants will have extensive shoot growth immediately following its root developement.

This combined growth of first root system and also shoot system will result in a great gain in the amount of plants surviving, in the number of flowers and fruits they bear, and in the general vitality of the complete crop. The benefits of rootone treated (hormones) seeds are clearly indicated in this experiment.

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