SOME INFLUENCES OF ENVIRONMENT AND GROWTH REGULATING SUBSTANCES ON THE STRAWBERRY PLANT

by

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INTRODUCTION

An endogenous growth substance was found by Guttridge (1959) to control photoperiodical induced phenomena, such as flowering and runner formation, in the strawberry plant. This study was conducted in an effort to learn more about the growth substances in the vegetative portions of the strawberry plant. Specifically the identification of naturally occuring growth regulators in the strawberry plant was attempted. Also the relative changes in growth regulator activity during the life cycle of the plant were studied. Further, efforts were made to determine where the growth substances were formed in the plant and to determine, if possible, their role in photoperiodical induced morphological changes.

LITERATURE REVIEW

The life cycle of the strawberry plant was studied by Arney (1956) and Darrow (1929 and 1936). Carlson (1953) determined these 4 chronological stages:

1. Leaf growth, flowering and fruiting
2. Runner initiation and development
3. Crown shoot formation and development
4. Flower initiation and dormant phase.

These phases tend to overlap each other. The various stages are known to be initiated by the length of the day (photoperiodism). Hartman (1947) found that long days inhibit flowering while they promote runner formation. That this phenomenon is triggered by a chemical substance becomes evident from Guttridges (1958) results. He later (1959) demonstrated the transmission of this substance in opposite directions between two plants connected by a stolon (mother and daughter). Guttridge and Thompson (1959a) found a gibberellin like substance present in extracts which had a similar activity to the growth promoting and flower inhibiting hormone, by inducing characteristic responses of long day growth. Prolings and Boynton (1961a) found a gibberellin like substance in parts of the strawberry plant, in particular in the stolon apices, stolon parts, and apices of the main axis; this substance was extracted with methanol and tested in three different bioassays:

1. The dwarf pea
2. The mesocotyl
3. The first leaf.

They collected evidence (1961b) that the strawberry responds to gibberellins in the way other long day plants do, mainly by overcoming unfavorable photoperiodic conditions. In the case of the strawberry plant runner formation was induced.

Kefford and Goldacre (1961) stated that the concept of auxin as a cell enlargement regulator only can no longer account for the variety of growth phenomena controlled by auxin. The interaction with gibberellin in controlling cell enlargement, and auxin-kinin interaction in initiating cell division are the most striking examples of the newer concepts. Van Overbeck (1959) stated that auxins control the growth of lateral buds only in a negative sense (inhibition, as in apical dominance e.g.); on the other hand gibberellins are powerful growth promoters for buds, but their action can be suppressed by auxins.

Brian and Hemming (1958) postulated a three factor system regulating the growth of pea internodes: auxin, an inhibitory system, and a hormone with similar physiologic properties to gibberellic acid. This theory is supported by Galtston and Worborg (1958).

The polar transport of auxin through the tissue as well as the concentration gradient have vital parts in auxin correlative functions.

The materials and methods used in the study of plant growth regulators (auxins) have developed rapidly since 1928, when Went proved the presence of an auxin and developed the Avena curvature test. Nitsch and Nitsch (1955) developed the mesocotyl test which is a sensitive and convenient bioassay and fits the assay requirements of gibberellins and indole compounds. Later in 1959 Harada and Nitsch developed a specific gibberellin test, namely the first leaf test.

Since the nature of the endogenous auxin is not known the methods of extraction are based upon an empiric foundation gained by trial and error rather than sound theoretical knowledge (Bentley, 1958). Many different solvents are used such as water, ether, isopropanol, methanol, ethanol, acetone, and a number of others as reviewed by Fawcett (1961), Kefford (1955), Linser and Kiermayr (1957), Larsen (1955), Sen (1959), and others.

The duration of the extraction period determines the fraction of "free" or "bound" auxin in the extract. This was demonstrated by Skoog and Thimann (1940) who found a gradual liberation of free auxin during a long period of extraction. No specific solvent has yet been found with which only the auxin fraction can be extracted; therefore, the crude extract has to be purified of fats, waxy materials, pigments, and many other substances which might interfere either with chromatography or with the bioassay. Satisfactory methods for purification are described by Fisher (1954), Boysen Jensen (1941), and Nitsch (1956).

The purified extract contains several substances with different growth activities; there are several methods used for separating these substances, one of the most efficient is based upon chromatography and in particular paper chromatography. These methods are reviewed by Sen (1959), Fawcett et al. (1959), Fisher (1956), Linser and Kiermayr (1957), Lederer and Lederer (1957), and others.

The choice of the developing system of the chromatogram is highly important. Although many solvents were tried in different methods, the ideal one has not yet been found for the same reason as mentioned earlier. Sen and Leopold (1954) used isopropyl alcohol, ammonia and water. Nitsch (1956) found that water is essential in any developing mixture and recommended a mixture of isobutanol-methanol-water (in the proportion of 80:5:15) or isopropanol and water (80:20).

No chemical or physical test has been found as sensitive as the bioassays developed by Went (1928), Went and Thimann (1937), Went (1934), Bonner (1933), Thimann and Schneider (1938), Nitsch and Nitsch (1955), and Harada and Nitsch (1959).

These assays utilize parts of plants, mainly coleoptiles, mesocotyls, first leaves of the Avena, or other parts as the ovary of the tomato and the pea stem. Though certain disadvantages of the bioassays are known, they continue to be widely used.

Kefford and Goldacre (1961) listed the factors which they claimed influenced the growth of the Avena coleoptile section in auxin solutions:

1. Influence from seedlings:
   a. size of seed
   b. position on panicle
   c. age of seed
   d. medium of growth
   e. genetics of seed
   f. endogenous system
2. Influence from sections:
   a. physiological age
   b. endogenous auxin
   c. auxin transport

3. <u>Influence from culture medium</u>:

a. sugar content
b. PO_4, Mn, Co, and Ca content
c. chelating agents
d. pH and osmotic reactions
e. amino acids
f. temperature
g. light condition
h. aeration

It can be concluded that the biological systems are highly sensitive to environmental factors as well as to the substances assayed.

MATERIALS AND METHODS

Plant Materials

There were three different phases of the study:

1. Different vegetative parts of five mother and daughter plants connected each with a stolon were assayed for auxins. These plants which were of the Armore variety were grown in 6" pots in the greenhouse. The plants did not get any specific light or temperature treatments and were grown under nearly equal day and night lengths. The plants were sampled in October.

2. The vegetative portions of ten mature plants were assayed for auxin. They were also Armore plants, and were grown at normal temperatures under short day conditions. These plants were harvested in January.

3. In the third study sixty plants of the Surecrop variety were transplanted on the tenth of March from the field where they wintered under mulch, into 8" pots and put into the greenhouse. It was considered that the chilling requirements were adequately satisfied in the field. After three days the plants were divided at random into two groups, one receiving a photoperiod of 10 hours (short) and the other a photoperiod of 16 hours (long). Additional light to lengthen the photoperiod was supplied by four incandescent bulbs, each of 250 watts. The plants receiving the short photoperiod treatments were

put in a dark chamber each afternoon, 10 hours after sunrise. Ten plants in each group were sprayed with 1000 ppm of the K salt of gibberellic acid (GA) three times, at intervals of a week between each spray. Ten additional plants from each group were sprayed with 20 ppm naphthalenascetic acid (NAA), also three times each at weekly intervals. The ten remaining plants in each photoperiod group served as controls.

Sampling

Samples were collected and prepared as follows: In the first experiment the plants were divided into crowns, stolons and leaves. Samples composed of about 20 gr. fresh weight were collected from the composites and were immediately blended with ice cold water and then frozen in the deep freeze (0°F) for a minimum of 24 hours, prior to lyophilization.

Two types of leaves, young light colored ones that had not reached maximum size, and mature dark colored fully developed ones were collected in the second study. Samples were collected from whole leaves as well as selected portions of each young and old leaves. Nearly 3/5 of the outer or marginal portion of each leaflet comprised one sample and the remainder or center portion of the leaflet a second sample. After collection the samples were handled as described for the first experiment. In the third study composite samples of the crowns, apices of young stolons, and mature leaves were collected. These samples were processed the same as for studies one and two.

Lyophilization

After 24 hours the samples were separately dryed by lyophilization, in an Aminco Freeze Dry Apparatus, to prevent oxidation of the auxin. During the lyophilization, which requires from 12-36 hours depending on the samples, the 100 ml flasks were covered with aluminum foil to prevent the destruction of auxin by light. After the samples had dried completely they were kept in a deep freeze at 0^{o} F. until extraction was performed.

Extraction

One-tenth gr. of the dried material of each sample was extracted with 20 cc cold methanol, in darkness at temperatures below the freezing point, for one hour; the solvent was changed three times during this period. The residue was washed with 5 cc of methanol and all of the extract, about 30 cc, was combined and evaporated to dryness in a heating mantle at 58^{o} C.

Purification

A mixture of 1:1 aceto-nitrile and Skellysolve B was used to dissolve the residue. The mixture was then poured into a separatory funnel and shaken. The Skellysolve portion was discarded and the purification repeated with new Skellysolve B. The acetonitrile fraction was then evaporated to dryness in a 50^{o}C. water bath under reduced pressure, by means of a Rinco rotating vacuum type evaporator device.

Paper Chromatography

The dry residue was dissolved in 2 cc of cold methanol; 10 microliters of this solution were applied with a tuberculin syringe as the initial spot on the Whatman No. 1 paper strip. During this operation a steady stream of air was blown on the paper to prevent spreading of the spot and to keep it as small as possible. The paper strip with the initial spot was placed in a glass developing cylinder (40 cm high, 35 cm diameter, and volume graded to 500 ml), one strip in each cylinder. Six chromatograms were prepared from each sample and a control strip as well. Three of the chromatograms were used for the bioassay and the other three in an attempt to identify the growth substances by chemical and physical means. One hundred fifty cc of fresh solvent (isobutanol-methanol and water 80:5:15 or isopropanol 80%) were poured in each cylinder before inserting the strip. The strips were suspended over the solvent in the closed container for 15 hours to equilibrate, then lowered into the solvent for the period of time it took the solvent front to rise 20 cm on the chromatogram (about six hours).

The dried chromatogram was cut into 20 equal strips 1 cm in length.

The Bioassay

The mesocotyl test developed by Nitsch and Nitsch (1956) was adapted for detection of auxins and the gibberellin like substances.

Hulless oat seeds of the James variety were soaked in tap water for two hours and then sowed on vermiculite in trays. The trays were kept in complete darkness for 72 hours at 76-78° F. and a relative humidity of 85%. At the end of this period the seedlings had reached the length of 25 mm and sections of 4 mm in length were cut 2 mm below the coleoptile nodes. These sections were placed in glass distilled water for one hour. Two sections were then placed in test tubes, containing a piece of the chromatogram strip and 1 ml buffer phosphate citrate solution. The test tubes were closed with rubber corks and placed by pairs in 250 ml wide opening Erlenmeyer flasks fastened to the rotation wheel turning on a horizontal axis; the wheel rotated at 1 rpm. After 20 hours of rotation at 76°F. and 85% relative humidity in darkness, section growth was measured with the help of a photomagnifier.

All these operations were conducted under green light furnished by a green 15 watt fluorescent tube (Westinghouse F15T8/G) wrapped with three layers of amber and green acetate window shading.

Since the Avena mesocotyl reacts to both auxin and gibberellins, and Avena first leaf test was also utilized in an effort to distinguish between the two types of substances. This test was developed by Harada and Nitsch (1959) and is a modification of the mesocotyl test.

The oat seeds used for this test were exposed to red light (a fluorescent lamp covered with red cellophane) for a period of 3 hours the first day, and 1 hour the second and third day. The red light prevented the growth of the mesocotyl.

The part of the seedling used for this test was a 4 mm section cut 4 mm above the first node. The section enclosed a segment of the first leaf wrapped in the coleoptile. The measurements of elongation were made after 48 hours. All other procedures and techniques employed were the same as for the mesocotyl test, except that no presoaking of the sections was required and distilled water was used instead of the buffer solution during the incubation period.

RESULTS

First Study

The results of the bioassay of a methanol extract from the daughter-mother plants are concentrated in table 1 and in the histograms following (Fig. 1 and 2).

In table 2 "class" indicates the three parts, stolons, mother plants, and daughter plants, assayed in study one. The term "position" refers to the Rf values of the chromatograms.

In the first study no significant levels of plant growth regulators were found in the whole plant because of the low activity in several parts and the averaging out of inhibitory activity in the crowns and stimulative

Table 1: Total length expressed in 1/100 s of an inch of six mesocotyl sections (initial length 96/100") grown in solutions of chromatographed methanol extracts from mother and daughter plants.

		Mother Plant			Daughter Plant		
Rf	Stolon	Whole Plants	Crown	Leaves	Whole Plants	Crown	Leaves
.05	127	116	133	136	132	114	134
.10	122	145	141	122	125	133	149
.15	119	137	132	123	130	145	123
.20	132	140	121	134	127	139	140
.25	139	146	144	132	136	118	115
.30	126	149	128	134	133	140	139
.35	110	146	124	130	122	131	114
.40	144	144	133	126	134	140	137
.45	121	146	141	134	137	130	135
.50	107	133	134	136	128	119	128
.55	138	110	132	124	122	131	130
.60	127	123	132	145	151	121	127
.65	113	145	134	142	137	127	141
.70	119	135	135	141	132	136	137
.75	119	140	133	123	129	133	130
.80	147	122	142	118	143	118	135
.85	136	129	137	124	124	134	129
.90	126	130	151	124	126	130	133
.95	131	163	131	128	121	141	134
1.00	123	134	134	124	134	147	146
Control	128	119	136	118	125	131	138

Table 2: The analysis of variance of average values of growth increases of mesocotyl sections in methanol extracts of stolons, mother plants and daughter plants.

Source of Variation	D/F	Mean Square
Class	2	94.39**
Position	19	14.22
Class X Position	38	20.15*
Sample Same Class and Position	300	12.90
Total	359	

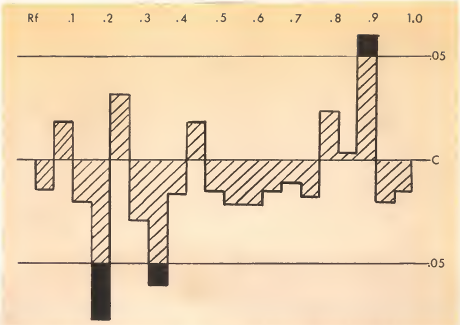


Fig. 1. Histogram showing the growth areas found on paper chromatogram of methanol extracts from the crowns of mother plants assayed with Avena mesocotyls in October.

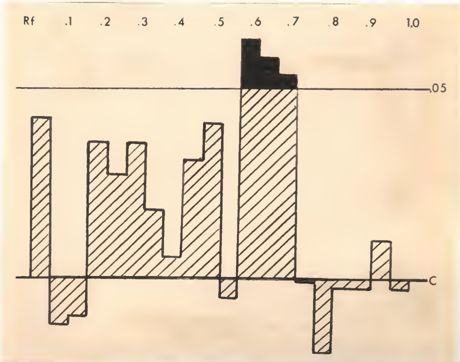


Fig. 2. Histogram showing the growth areas found on paper chromatograms of methanol extracts from the leaves of mother plants assayed with Avena mesocotyls in October.

activity in the leaves. The three main classes, as seen in table 2, however differed significantly from each other.

Second Study

The results of the second study concerning the leaves of mature plants is given in table 3 and 4.

No significant difference (Table 3) was found in plant growth regulator activity among the six classes even though there was an indication of higher activity in the mature leaves and in particular in its center portion. In the younger leaf the margin had higher growth regulating activity. The same substances were active in the extracts of all class and their activity differed significantly from the substances not active.

Significant levels of plant growth regulators within classes were found only in the center portion of mature leaves.

Table 3: The analysis of variance of growth increases of mesocotyl sections in methanol extracts of leaf margins and centers from daughter and mother strawberry plants.

Source of Variation	D/F	Mean Square
Class	5	8.16
Position	19	9.50*
Class X Position	95	6.05
Sample Same Class and Position	600	4.99
Total	719	

In table 3 "class" refers to the different portions of the plant sampled in study two; the whole mother leaves, whole daughter leaves, and margins and center portions of each. "Position" again means the Rf values of the chromatograms.

Table 4: Total length expressed in 1/100 s of an inch of six mesocotyl sections (initial length 96/100") grown in solutions of chromatographed methanol extracts from leaves of strawberry plants.

	Mature Leaves			Young Leaves		
Rf	Whole Leaves	Margin	Center	Whole Leaves	Margin	Center
.05	134	126	128	131	132	130
.10	128	120	134	124	126	122
.15	130	128	133	104	136	124
.20	116	122	126	121	118	124
.25	124	124	129	121	124	114
.30	122	132	124	125	122	124
.35	137	130	130	123	124	124
.40	118	116	133	116	136	138
.45	138	126	139	139	138	128
.50	125	134	124	130	132	118
.55	126	137	128	132	128	130
.60	125	122	130	134	124	120
.65	118	134	124	132	124	124
.70	120	128	130	137	120	122
.75	134	134	123	119	136	118
.80	126	120	122	124	138	134
.85	125	122	134	132	134	126
.90	131	116	126	133	130	132
.95	126	123	126	129	132	112
1.00	140	128	130	130	130	132
Control	118	126	126	114	124	126

Third Study

The results of the third study concerning the changes in endogenous growth regulators induced by photoperiod and by exogenous growth regulators are presented in tables 5, 6, 7, 8, 9, 10 and figures 3, 4, 5, 6, 7, and 8.

Data were collected from plants after the second application of NAA and GA which included the number of inflorescences, the length of the petioles and pedicles, and the number of leaves. These data are presented in table 5, and in a graphic form in fig. 3.

Table 5: Number of leaves, inflorescences and length of petioles and pedicles of 5 strawberry plants grown under short and long day conditions and treated with NAA and GA.

Characteristic	Short Day			Long Day		
	Control	NAA	GA	Control	NAA	GA
Number of Leaves	12	19	28	27	22	27
Number of Inflorescences	0	30	22	33	25	40
Length of Petiole in cm	45	63	80	81	78	84
Length of Pedicle in cm	0	42	94	73	50	124

Table 6: Number of runners initiated by 5 plants each under short and long day conditions with and without treatments of NAA and GA.

Time of Counting	Short Day			Long Day		
	Control	NAA	GA	Control	NAA	GA
3 Weeks after Induction	0	0	0	2	0	3
5 Weeks After Transfer to Natural Day Length	0	4	7	6	4	8

Counting of the runners was done twice, once when the plants were harvested 3 weeks after induction and three weeks after the remaining plants were put under natural day length conditions. These values are given in table 6 and fig. 4.

In table 7 and 8 present the results of the bioassays. Fig. 5, 6, 7 and 8 are histograms derived from these results. The analysis of variance for this study is shown in table 9.

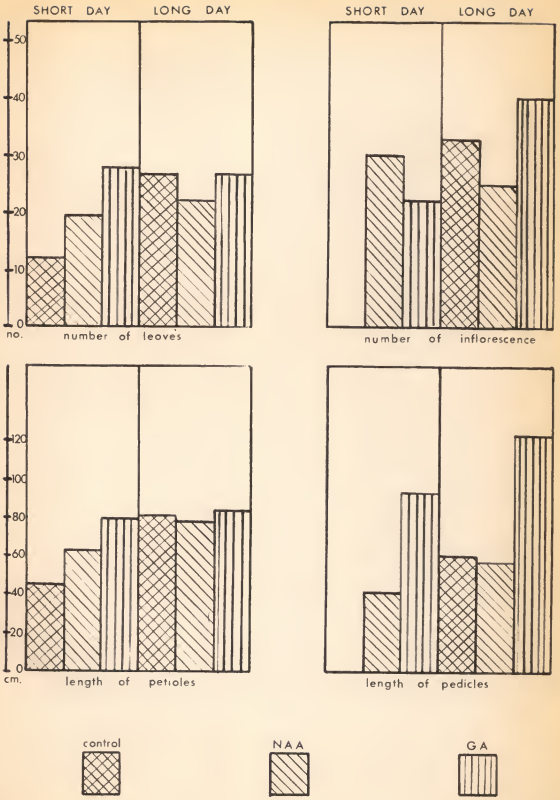


Fig.3. A grophic representation of toble 5.

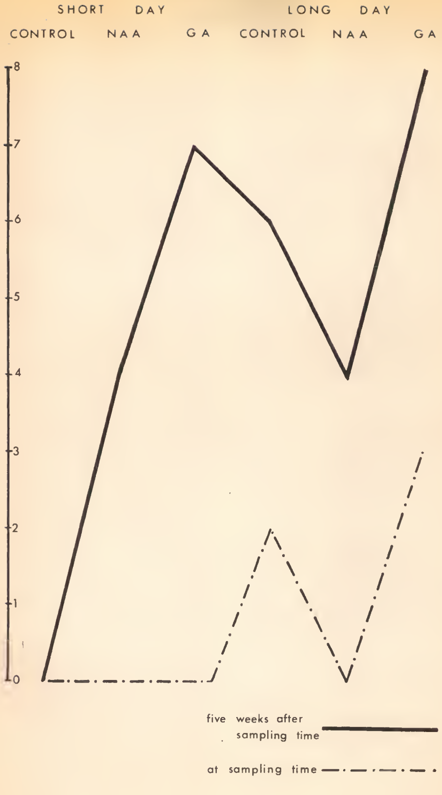


Fig. 4 . A graphic representation of table 6 .

Table 7. Total length expressed in 1/100 s of an inch of six mesocotyl and first leaf sections (initial length 96/100") grown in solutions of chromatographed methanol extracts from strawberry plants grown under long day conditions and treated with GA and NAA.

	Long Day											
	Control				NAA				GA-K			
	Leaves		Crowns		Leaves		Crowns		Leaves		Crowns	
Rf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf
.05	104		118		106		116		114		124	
.10	106		128		108		120		122	154	110	
.15	123		136	160	106	204	112	149	102	156	134	198
.20	116		108		126		122	161	117		110	
.25	106	180	108		112		116		116		104	
.30	100		104		98		134		112		124	
.35	100		110		96		122		108		114	
.40	108		120		120	186	136	162	130	184	110	
.45	120	156	112	186	106		120	182	118	146	124	192
.50	110		112		108		112		110		128	178
.55	120		102		116		130		112		108	
.60	127		104		100		122		98		122	
.65	98		108		134		122		120		114	
.70	127		128		118		136		101		126	
.75	114		118		99		120		108		112	
.80	112		120		122		140		107		122	
.85	116		110		126		114		104		122	
.90	108		128		100		110		104		110	
.95	108		110		118		116		106		126	
1.00	122		128		108		130		118		126	
Cont.	98		103		103		123		100		105	

Table 8: Total length expressed in 1/100 s of an inch of six mesocotyl and first leaf sections (initial length 96/100") grown in solutions of chromatographed methanol extracts from strawberry plants grown under short day conditions and treated with GA and NAA.

	Short Day											
	Control				NAA				GA-K			
	Leaves		Crowns		Leaves		Crowns		Leaves		Crowns	
Rf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf	Mes.	Leaf
.05	106		122		102		124		112		106	
.10	102		110		132		136		127		106	
.15	110		104	142	110	171	126	160	122		128	186
.20	108		116		124	153	116		129		110	178
.25	126	180	121		108		120	162	115		118	175
.30	122		122		105		120	147	122		116	
.35	100		128		116		162		121		110	
.40	120		120		110		110		138	182	116	
.45	128	174	108	158	96		124	176	132	206	114	
.50	98		110		102		146		130		130	
.55	96		104		137		143		130		110	
.60	98		116		103		110		120		128	
.65	120		130		122		112		113		112	
.70	120		104		108		138		110		112	
.75	110		110		105		120		114		128	
.80	114		122		106		144		98		122	
.85	104		136		109		128		118		126	
.90	104		136		102		120		114		128	
.95	126		116		103		166		114		128	
1.00	116		120		125		120		108		123	
Cont.	103		103		111		123		111		105	

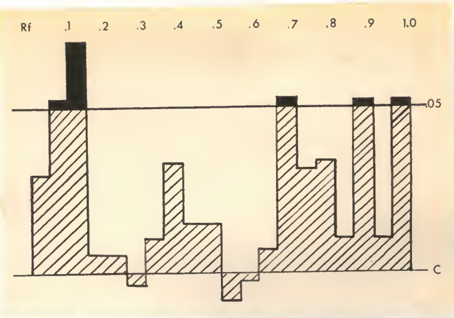


Fig. 5. Histogram showing the growth regulating areas found on paper chromatograms of methanol extracts from crowns of strawberry plants grown under long day conditions.

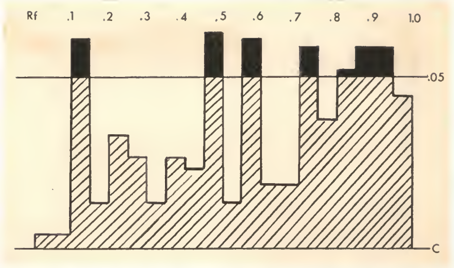


Fig. 6. Histogram showing the growth regulating areas found on paper chromatograms of methanol extracts from crowns of strawberry plants grown under short day conditions and treated with GA.

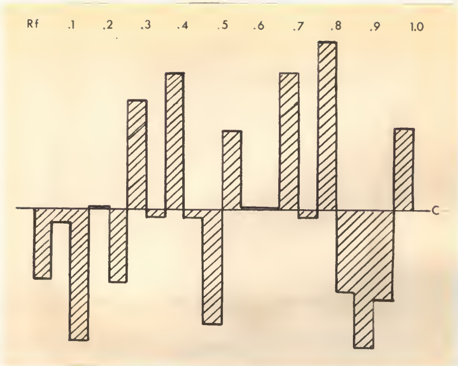


Fig. 7. Histogram showing the growth regulating areas found on paper chromatograms of methanol extracts from crowns of strawberry plants grown under long day conditions and treated with NAA.

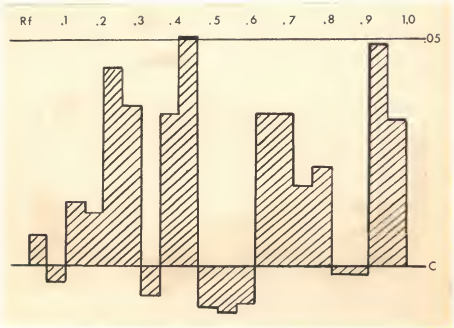


Fig. 8. Histogram showing the growth regulating areas found on paper chromatograms of methanol extracts from leaves of strawberry plants grown under short day conditions.

Table 9: Analysis of variance for the total growth of mesocotyl sections from extracts of leaves and crowns of strawberry plants grown under two different photoperiods and treated with GA and NAA.

Source of Variation	D/F	Mean Square
Treatments	5	44.44**
Positions (Rf)	3	25.00**
Treatments X Position	15	24.76**
Sample	120	2.0
Total	143	

Table 10: Results of the chemical and physical tests of the chromatograms of extracts of methanol from strawberry plants treated with GA and NAA and untreated plants.

	2537 A° Ultra-violet Lamp		
Rf		after spraying w/H_2SO_4 + methanol	dimethylamino-benzaldehyde 1%
.15 - .25	violet-blue greenish-blue	greenish	yellow brown reddish
.45 - .50	faint blue-blue	greenish-blue	violet-dark blue
.65 - .70	light blue	no reaction	blue violet
.85 - .90	orange yellow	no reaction	violet green often no reaction

DISCUSSION

Several substances, separated by paper chromatography from methanol extracts of the strawberry plant, were found to influence growth in sections of the mesocotyl and the first leaf of the oat. Substances appear to be active through parts of the life cycle of the strawberry plant as seen in tables 1, 4, 7, 8, and in figures 1, 2, 5, 6, 7, and 8. The four main activity peaks were observed for the substances with Rf values of .5-.25, .45-.50, .65-.70, and .90-1.0. These substances were active in almost all the extractions from the different samples. No attempt was made for biochemical identification of the substances because of the enormous number of plant growth regulators known today as reported by Leopold and Plummer (1961).

The physiological activity noted in this study was determined by the bioassays, and the substances could be divided into two groups according to their activity:

1. The substances with Rf values of .5-.25 and .45-.50 were active in the mesocotyl bioassay as well as in the first leaf test, therefore believed to be gibberellin like substances according to the definition of Phinney and West (1960).
2. The substances with Rf values of .65-.70 and .90-1.0 were active only in the mesocotyl test, and therefore believed to be auxins according to the identification used by Hillman and Purves (1961).

Similar peaks of activity were obtained by Prolings and Boynton (1961a) with methanol extracts of the strawberry plant and by Harada and Nitsch (1959) with several other plants.

The relative changes of the activity of these substances, during the stages of plant development in a portion of the life cycle, were of interest in this study.

The daughter-mother plant study as represented in table 1 and figure 1 and 2 was conducted in early fall with plants growing in the greenhouse which had not gone into dormancy. High growth inhibitory activity was noted at this time in the crown, which was the greatest amount of inhibition found during all the study. At the same time almost no growth inhibition was found in the solutions from leaves. No inhibitory activity was found in the crowns from plant assayed in the later studies. The relative inhibitory activity in three different parts is illustrated in figure 9. The analysis of variance given in table 2 confirmed the significant difference between the three plant parts.

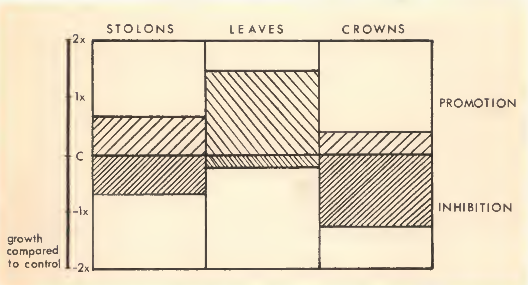


Fig. 9. Illustration of the relative promotive and inhibitory activity found in extraction from leaves, stolons and crowns of a mature strawberry plant in early fall.

No significant difference was found in the consistency of occurence in plant growth regulators between young and mature leaves of plants as shown in table 3; however, the relative activity of the extracts differed. The growth activity extracts of old leaves was higher than that noted for young leaves. A clear gradient in opposite directions was found between the margins and the center portions of the young and old leaves, as illustrated in Fig. 10. This phenomenon is related to the growth habit of the leaves and in particular to the growth zones in the periphery of the leaves (Fitting et al., 1954). These growth zones can be considered to be the site of action for those plant growth regulators while the site of formation, as described by Leopold (1954), is mainly in the mature leaf parts. The latter as well refers to the mature leaf as the main auxin synthesizing organ.

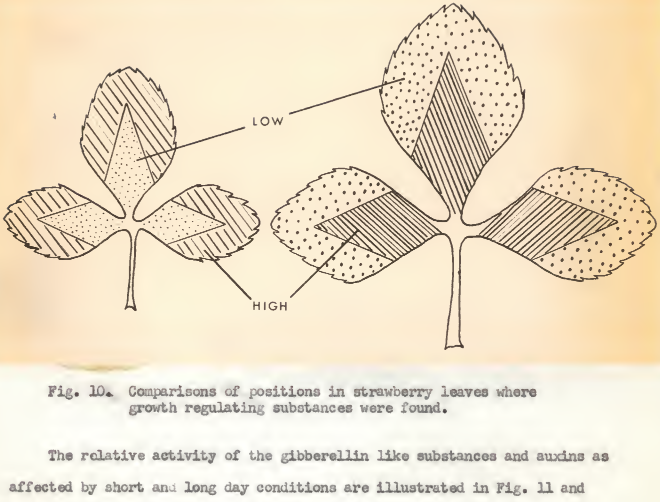
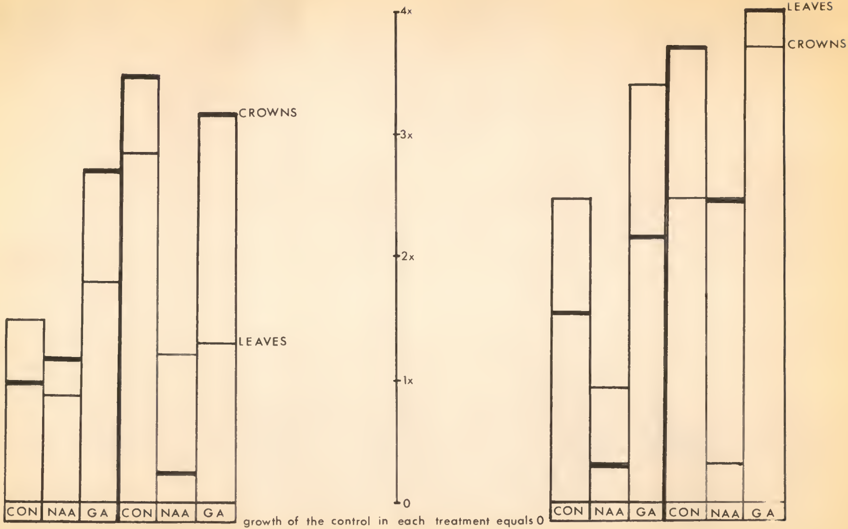


Fig. 10. Comparisons of positions in strawberry leaves where growth regulating substances were found.

The relative activity of the gibberellin like substances and auxins as affected by short and long day conditions are illustrated in Fig. 11 and Fig. 12. In this study it is evident that the gibberellin like activity increased under long day conditions while it was suppressed under short day conditions. The application of sprays GA stimulated the activity of these


CROWNS
LEAVES
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4x
3x
2x
1x
0
LEAVES
CROWNS
CON
NAA
GA
CON
NAA
GA
growth of the control in each treatment equals 0

Fig. 11. Relative activity of the substance with Rf value of .15-.20 found in methanol extracts of crowns and leaves of the strawberry receiving different chemical and photoperiod treatment.

Fig. 12. Relative activity of the substance with Rf value of .65-.70 found in methanol extracts of crowns and leaves of the strawberry receiving different cemical and photoperiod treatment

substances, in particular plants of the short day group. These findings are similar to those found by Harada and Nitsch (1959) in the Rudbeckia speciosa.

Two findings might be related; one in the way the plant responds to GA, and secondly the high activity of gibberellin like substances under long day conditions. If these two factors are related it gives a clue for the possible role of gibberellin like substance such as those with Rf values of .15-.20 and .45-.50. Similar indication for the role of these substances was found by Guttridge (1959) and by Prolings and Boynton (1961 a and b) and is, as well, reviewed by Hillman (1961).

Are these substances essential for the long day response or is their formation induced by such response? This question of "cause or effect" is difficult to answer at this stage and requires further investigations.

The activity of the gibberellin like substances was suppressed in plants treated with NAA (fig. 11). This can serve as additional evidence that the substances found at Rf .15-.20 and .45-.50 are gibberellin like as stated by Van Overbeck and Dowding (1961). It illustrates, as well, the possible interaction between auxin and gibberellin like substances. The mechanism of such an interaction is reviewed by Hillman and Purves (1961); they rejected the theory of gibberellin activity through an auxin mediated mechanism and presented evidence that the interaction is a more complex one. These findings, as shown in Fig. 11 and Fig. 12, indicate that auxin treatments suppressed gibberellin activity, and on the other hand, gibberellin treatments stimulated auxin activity. It is this same line of evidence which caused Kato (1961), and Brain and Hemming (1961) to believe that the interrelation is of a three factor system where auxin and gibberellin like substances are mediated by an inhibitory factor from an unknown nature.

The plant response to NAA treatments, as represented in Fig. 3 and 4 and 11 and 12, shows the inhibitory nature of auxin treatments as related to the inductive photoperiod. This was observed in short day plants as well as in many long day plants as reviewed by Lang (1961) and Hillman (1961).

SUMMARY AND CONCLUSION

Methanol extracts from strawberry plants were separated by paper chromatogram and bioassayed to determine the changes occuring during a portion of the life cycle of the plants as influenced by variable day length and three spray applications each of NAA, and GA.

The findings reported in this study could be summarized as follows:

1. Several growth regulating substances were consistently active in the plant throughout the study. Four main substances were noted, with Rf values of .15-.25, .45-.50, .65-.70, and .90-1.00. The first two were found to posess a physiological activity similar to gibberellin and the two other materials were similar to auxins. Chemical and physical tests confirmed these findings.
2. High inhibitory activity was found in crowns of plants growing in the greenhouse not subjected to low temperatures.
3. In mature leaves higher activity of plant growth substances was noted then in younger leaves.
4. Difference in activity gradients between the marginal portion of the leaves and leaf centers were noted in young and mature leaves. In the first case the gradient progressed from the margin to the center and in the mature leaf from the center to the margin.
5. Long day conditions were found to induce the activity of the gibberellin like substances.

6. GA treatments caused strawberry plants grown under short day conditions to grow similarly to those under long day conditions.
7. GA treatments stimulated auxin activity.
8. NAA treatments suppressed plant growth response to day length.
9. NAA treatments suppressed gibberellin like activity.

These findings support the three factor theory of gibberellin auxin interaction; they are, also, evidences of the possible role of gibberellin in growth promoting and photoperiod induction in the strawberry plant.

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SOME INFLUENCES OF ENVIRONMENT AND GROWTH
REGULATING SUBSTANCES ON THE
STRAWBERRY PLANT

by

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Stages in the life cycle of the strawberry plant were found to be photoperiodically induced and correlated with a growth regulator substance produced in the plant (Guttridge 1960). Prolings and Boynton (1961) reported on the gibberellin like nature of this substance.

The purpose of this study was to identify and determine the role of endogenous plant growth regulators in the strawberry plant. The changes in the activity of these substances as related to the changes in morphological response to photoperiod and endogenous plant growth regulators was also studied.

Three different experiments were conducted. First the relationship of mother and daughter plants, with respect to the production of endogenous plant growth regulators, was studied. For this purpose crowns, stolons, and leaves of these plants were sampled. The growth regulator activity in leaves of two different age groups was studied in the second experiment. In each group samples of the leaf margins and the centers were assayed. In the last study the influence of two photoperiods on the growth and development of strawberry plants was studied. A Short day of 10 hours light and a long day of 16 hours light was compared. In each photoperiod group one third of the plants received 3 sprays of 1000 ppm each of K salt of gibberellic acid, one third was sprayed three times with 20 ppm NAA, and the remainder in each group served as controls. In this study leaves, crowns and stolon apices were sampled.

Each sample was lyophilized and extracted with methanol. The methanol extract was purified prior to paper chromatography. The paper chromatography of the auxin extracts was done by the ascending method in a solvent system of isobutanol-methanol-water (80:5:15) or isopropanol 80%. After developing and drying the chromatograms were cut transversally into twenty strips of 1 cm each and biologically assayed by use of the Avena mesocotyl or Avena first leaf tests.

During the portion of the life cycle studied several endogenous substances showed growth activity. In particular the substances with Rf values of .15-.25, .45-.50, .65-.70 and .90-1.00 were active. The first two substances showed gibberellin-like activity and the other two substances appeared to be auxins. High inhibitory activity was found in October in crowns of plants not exposed to low temperature. The influence of the day length was found to affect the activity of the gibberellin like substances; this activity was higher in plants subjected to long days than in those receiving short day treatments. GA spray treatments caused strawberry plants under short day condition to grow similarly to long day plants. These findings gave additional indication as to the possible role of gibberellins in photoperiodical induction in the strawberry plant. GA application stimulated the auxin activity while NAA treatments suppressed the gibberellin like activity. This suggests that the auxin gibberellin interaction is mediated by a third unknown factor as suggested by Brian and Hemming (1961). NAA treatments were found to suppress the photoperiodical induction under long and short day conditions.