EFFECT OF PHOTOPERIOD AND OTHER FACTORS ON THE DEVELOPMENT OF SOME SHORT-DAY PLANTS

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Signatures have been redacted for privacy

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INTRODUCTION

The development *at* the oonoept of photoperiodism by Garner and Allard $(4, 5, 6)$ reintensified the experimentation on the etfect *ot* external factors on tho development of plants which began with Klebs (11) and was popularized in the United states by Kraus and Kraybl11 (12). Working from the oonoepts of Kraus and Krayb111, Loomis (14) has attempted to 01nsa1fy plant reactions under the two headings of growth, which inoludes cell dlvision and enlargement, and differentIatIon, which covers all other phases of development. Fruiting is considered by Loomis to be an alternation of the differentiation of flower buds, growth of flowers, dlfferentiation of spores, and growth *ot* gametophytes and later fruits.

The concept of photoperiodism does not fit readily into suoh a soheme of plant deVelopment, and this study is intended to explore the interrelatIons of photoperiod, or relative length of day and n1ght, and of other faotors wh10h might be eXpeoted to affeot d1fferentiat1on.

REVIEW OF LITERATURE

Garner and Allard $(4, 5, 6)$ have shown the importance of photoperiodism in relation to the growth responses of plants and performed the basic experiments on the problem. Hamner (7) gives an exoellent review of early work. Since the publishing of his paper, Long (13) haa showed the effeot of variation of light intensity, temperature and hum1d1ty upon the length of the cr1tioal dark period for Biloxi soybeans (Glycine Max). In his experiments Biloxi soybeans flonered only after exposure to conseoutive, long, dark periods, each alternating with a short light period, and plants did not flower regardless of the number of long dark periods they reoeived, unless at least three of the dark periods were in oonsecut1ve order. In order to produoe a seoond group of flower primordia the plants had to be exposed to another induction per10d of three or more long dark periods. Borthwick and Parker (1), illustrating the photoperiodic responses of several soybeans, state that old Biloxi soybean plants initiated flower primordia on light periods longer than 16 hours, but that 12-14 hours of light was the oritical upper limit for normal flowering and fruiting.

The influence of the duration of the daily photoperiod \sim · upon reproductiveness is the outstanding, but by no means the only effect of this climatio faotor on growth. Some of its.

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other more important effects are upon: (1) rejuvenation (Garner and Allard, 5), (2) sexual expression (Schaffner, 17, 18), and (3) production of storage organs (Zimmerman and Hitchcock, 19).

The observations of many investigators indicate that the carbohydrate content of plants, in relation to the amount of nitrogen available, strongly influences the type of growth which is made, particularly since the work of Kraus and Kraybill (12). Working with tomatoes, these investigators found that flowering and fruiting can only take place when the carbohydrate and nitrogen content of the plant lies between certain limits. In their analyses a correlation between the change of the carbohydrate-nitrogen ratio and the sequence of reproduction activities appeared to exist, whether this ratio was the cause or the effect of the reproductive sequence. As a result of their studies, this carbohydrate-nitrogen hypothesis has found wide acceptance. Hightingale (16) and others, however, clearly show that there is no simple or consistent relationship between the ratio of nitrogen to carbohydrate and the growth response of the plant. On the other hand, experience of growers and research workers is adequate evidence that there is a relationship between the commercial fertilization of an apple orchard and its fruiting. Loomis (14) has shown in his work that growth-differentiation balance differs from the carbohydrate-nitrogen balance in (a) assigning an independent

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and major role to moisture, (b) including with nitrogen the other equally essential if not so commonly limiting factors concerned in the synthesis of protoplasm, (c) recognizing the effects of temperature, and (d) emphasizing the importance of active carbohydrates as opposed to storage forms. He states this concept only as a convenient and simplified scheme for predicting or explaining plant behavior.

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MATERIALS AND METHODS

Uater1als

Plant mater1als used 1n these experiments were grown 1n the greenhouse at Ames. e1ther 1n flats. 4 inohes deep or 1n 2&-lnch pots. The Bl10xi soybean seed was furnished by Dr. A. D. Suttle, Professor of Agronomy at Mississippi State College. The Oosmos and Salvia seed were commerolal stooks purohased trom local dealers.

Methods

The purpose *ot* the study was to compare photoperlodism with differentiation induced by stunt1ng. Plants were grown aocordingly in two groups, one wlth fertIle so11, high moisture and a double cheeseoloth shade to reduoe the greenhouse light to 40 per cent, and a second group in poor soil, lower moisture and full greenhouse light. Plants from each group were then given the normal April to August day length of 14 to 16 hours. or were transferred dally to a dark room at 5:00 p.m. and returned to the greenhouse at $$:00$ to $9:00$ a.m. for a short day of g to 9 hours.

The "fertile" soil was composed of $1₂$ bushels of sod and manure compost thoroughly mixed with 2 pounds of superphosphate

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(17 per cent P₂O₅) and one pound of ammonium sulfate. The low fertility soil was prepared by mixing one part of compost and three parts of river sand. High moisture pots were watered daily to optimum moisture. Low moisture pots were watered lightly to prevent wilting.

Observations included:

Height of plant growth Density of plant growth Health Time of flowering Root development Dry weight differences

Quantitative analyses of selected samples

In all there were 5 groups of 8 pots each in the 1935 experiments. 4 groups for the Cosmos and the same number for the soybean. The soybeans were kept on one side of the bench in the greenhouse to themselves while the Cosmos were placed on the opposite side. This arrangement prevented any shading between the different plant groups. However, the plants of all groups were close enough together to have similar exposures of sunlight. The central bench was used for the best light advantage.

Factors Influencing the Growth of Plants

Group I

Low light intensity High soil moisture Fertile soil Long day

Group II

Low light intensity High soil moisture Fertile soil Short day

Group III

High light intensity Low soil moisture Medium fertile soil Long day

Group IV

High light intensity Low soil moisture Medium fertile soil Short day

In addition to the regular groups a second set of experiments was run on the Cosmos and soybean. These experiments used seedlings grown in river bottom sand in flats; three flats of Cosmos and three flats of soybeans. Instead of watering these flats with tap water, a manure compost extract was used. This was prepared by placing a shovel of horse manure in a five gallon crock and covering with tap water. The process was repeated to supply additional water for the flats.

The flats were treated as follows:

Flat I

Optimum amount of water Low light intensity Long day

Flat II

Optimum amount of water High light intensity Short day

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Flat III

Opt1mum amount of water H1gh light intensity Long day

In the early part of this experiment, the seedlings of the Cosmos began to "damp off". This condition was corrected by spraying the soil with a weak solution of organic mercury. The solution was made by dissolving a gram of mercury dust in a gallon of 'water. After an application of this spray to the top soil there was no further evidence of "damping off".

During the spring of 1938 this experiment was repeated except that the growing of plants in flats was omitted. At this ttme Salvia was grown instead of Cosmos.

Sampling and Preserving

The mater1al for chemical analyses was gathered and divided into duplicate samples of 25 to 75 grams. All plant parts, whether leaves, stems, fruit or roots, were handled as rapldly as possible to avoid enzymatic changes. The samples were weighed, out into 2- or 3-mm. sections and dropped into mason jars containing 500 cc. of boiling 95 per cent ethyl aloohol. The jars containing the samples were placed on a boiling water bath and allowed to simmer for 30 minutes. At the termination of this period, the jars were sealed tlghtly and set aside until extractions were made.

The material was extracted 20 times by decentation

with 80 per cent redistilled ethyl alcohol. Further extractions showed the prooess to be complete. The oombined extraots were made to volume (1000 or 2000 cc.) at 20 degrees O. The 1nsoluble residues were dried and welghed, atter whioh they were ground In a Wiley mill and then 1n a ball mIll until the material was in a powdered form. The residues were stored in tIghtly sealed bottles for' polysacoharide and 1nsoluble n1 trogen determinations. The extracts were used for determinations of sugars and soluble or non-ooll01dal n1trogen traotions.

Methods for the Analysis of Plant Material

One-tenth aliquots of the alcoholic extracts were transferred to water, cleared with neutral lead aoetate. deleaded with anhydrous sodium oxalate, and reducIng sugars determIned by a modifioation of the Munson-Walker and Bertrand methods. Suorose was hydrolyzed w1th invertase and determined as Invert sugar (15).

The total nitrogen *ot* the alcoholio extract was determined by the unmodified Kjeldahl method and reported as noncollo1dal or soluble nitrogen. Total nitrogen of the aloohol insoluble residue 1s reported as colloidal n1trogen and oons1dered to be a measure of the protein content of the tissues.

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EXPERIMENTAL RESULTS

Growth and Flowering Responses

Experiments with Cosmos

In a series of experiments with Cosmos plants observations were made on the general growth of the plants in height, branching. first blossom date and color of leaves during a limited growth period.

Plants grown in poor soil watered with liquid manure, and receiving varied treatments of light intensity and photoperiod from June 28 to August 22, 1935, showed differences in their growth responses as shown in table 1. Plants exposed to a 15-16 hour photoperiod at low light intensity elongated most but showed no evidence of flowering. These plants produced the greatest area of leaves. Plants receiving long days but exposed to a greater light intensity did not grow quite as tall, did not produce as much leaf area, nor did they show any indication of flowering during this growth period. Plants given a short photoperiod with high light intensity developed less elongated stems but differed from the above mentioned groups by producing flowers in 46 days.

In another experiment with Cosmos plants given different treatments of light intensity, moisture, fertility and photo-

* Midday with clear sky
t Soil kept moist

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Observations on the Growth of Cosmos. Table 1.

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Midday with olear sky
Soil kept moist
To keep plants from wilting * # }

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period as shown in table 2 , it will be noted that the growth responses of the plants showed more variation. Plants exposed to low light intensity for a long photoperiod were weak and short, while plants given the same low light intensity treatment with a shorter photoperiod grew even less. Plants exposed to high light intensity and subjected to a short photoperiod flowered on August 6, having few leaves and plenty of flowers while plants given the same light intensity but exposed to a long photoperiod flowered 6 days later, having plenty of leaves and the best elongated stems of all groups. The slightly later flowering of this particular group as compared to the earlier flowering of plants subjected to short-day and high light influences may be attributed to the difference in the length of the photoperiod, since both groups grew in poor soil with minimum moisture.

Experiments with Soybeans

A series of similar experiments was conducted with Biloxi soybeans. The treatments given the beans grown in flats and pots were identical to those given the Cosmos plants.

The soybeans grown in flats watered with liquid manure. showed distinct variation in growth and flowering responses as may be seen in table 3. The group of plants given a 15-16 hour photoperiod with low light intensity developed many branches and elongated in height to 45 inches, while the group

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(All plants in flats watered with liquid manure)
(Seed planted June 26; final records Aug. 1935)

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Midday with clear sky
Soil kept moist $\ddot{ }$ ¢

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receiving the same length of photoperiod with high light intensity elongated in height to only 32 inches with no branching. The other group of plants subjected to an&-9 hour photoperiod with high light intensity, grew to be coarse in leaf and stem development, attained a height of 23 inches and flowered after 37 days of growth. The great height attained by the long-day plants is attributed to the influence of the low light intensity while the flowering of the plants subjected to high light intensity might be attributed to the short photoperiod.

Soybeans grown in pots and given different treatments of soil moisture and fertility. light intensity and photoperiod showed additional variation as may be seen in table 4. Those plants given a 15-16 hour photoperiod at low light intensity, growing in rich soil and receiving high moisture, developed to 54 inches in height. This group had leaves much larger and thinner than the leaves of any of the other groups. The low light intensity and higher humidity probably account for this morphological leaf change. The plant internodes were much longer than any of the other groups. Plants receiving a long photoperiod of high light intensity, growing in poor soil and receiving low moisture, developed the second highest growth of 29 inches. The leaves of this group were second largest in size, however, the plant stems of this group were the largest of all groups. Plants given and-9 hour photoperiod of high light intensity, growing in poor soil and receiving

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Midday with clear sky
Soil kept moist
To keep plants from wilting

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 $\hat{\boldsymbol{\theta}}$ \sim low moisture, flowered 1n 31 dayo. These planta were small in leaves and stems. Plants exposed to ang-9 hour photoperiod of low light intensity, growing in rich soil and receiving high moisture, grew to the same height as the shortday, high-light plants but flowered 8 days later. This group of plants had very little lateral growth. Its leaves were the smallest of all the groups. In regard to all the groups it seems that tho large leaf development 1s aauooiatea w1th long photoperlods, however, low light intensity seemed to have augmented the s1ze 01' leaf' evon more. Height *ot* groups may be looked at 1n the same rogard. As to the differenoes in the flowering dates of groups mentioned only plants on short photoperiods flowered, and flowering was retarded 8 days, but not prevented by the low light intensity.

In 1936 the experiment with the Biloxi Soybeans was repeated, using the same treatments and prooedure as in the experIments *ot* 1935. SalvIa was substituted for Cosmos. The flat grown groups were omitted and only pot grown plants were observed. The observations on the bean experiments grown in 1935 were confirmed by the experiments of 1938, as is shown In table 5. Long-day, low-light plants grew vine-like and developed the largest and thinnest leaves of all groups. These plants elongated to a height of 34 lnohea to be the tallest *ot* all the groups. Long-day, high-light plants developed a ooarse type of growth with the lower leaves mostly dying, and those

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To keep plants from wilting

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 $\frac{1}{\sqrt{2}}$

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leaves that matured and remained on the plant were the second largest in size and measured about $3\frac{1}{2}$ x $2\frac{1}{2}$ inches. These plants developed very suall buds during a limited growth period which were not identified as flower buds. This group attained 2g 1nohes 1n height to be the· second tallest. Plants exposed to short days of high light intensity grew 21 inches tall and flowered 32 days after planting of seed. The stens were medium as compared to the other groups. The other group of plants given a short photoper1od of low light 1ntensity devoloped the ana llest and weakest plants in every respeot. At the t1me of harvesting (June 4) the plants of this particular Group bad buds about to open. In regard to these groups, it seems that plants subjected to ahort photoperlods were induoed to flower regardless of the degree of light intensity, amount of moisture or fertility of the soil; however, the influence of high light seems to have forced flowering. Plants subjected to long photoperiods regardless of light 1ntensIty, mo1sture and *ter*tility were muoh taller and larger. Plants of these long photoperiod groups were influenced to grow vine-like by low light intensity, while high light caused a coarse development. Large leaves are also associated with long photoperiods, partioularly when shaded.

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Midday with clear sky
Soil very moist
To keep plants from wilting \bullet \leftrightarrow \rightarrow

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Experiments with Salvia

These experiments were conducted in the same manner as those with Cosmos and Biloxi soybeans grown in pots. Observations were made with the same point of view of noticing the general growth of all the groups as to their height, size of leaf, branching, first blossom date and color of leaves during a limited growth period as shown in table 6. Plants grown in rich soil, receiving plenty of moisture and subjected to long photoperiods with low light intensity, developed stems 10 inches long, being the tallest of all the groups. These plants had the largest leaves measuring $2\frac{1}{2}$ x 3 inches. Other general characteristics observed were that the group had large stems. small leaves in the lower leaf axils and buds in leaf axils higher up on the plant stem. Plants given and-9 hour photoperiod with low light intensity grown in soil with plenty of moisture developed small plants 4 inches tall with leaves $1 x$ $3/4$ inches large. These plants produced buds very slowly. Plants exposed to a 15-16 hour photoperiod with high light intensity grown in poor soil with a minimum moisture, elongated to 5 inches in height. This group had leaves measuring $\mathbf{1}_{2}^{3}$ x 1 inches. There were branches in the lower leaf axils and small vegetative buds in leaf axils higher up the plant stem. Plants subjected to an8-9 hour photoperiod with high light intensity grown in poor soil with a minimum amount of moisture elongated to 3 inches, the least in height of all the Salvia groups.

The leaves measured 1 x $1\frac{1}{4}$ inches. The stems were the smallest. There were buds and small leaflets in the axils of all leaves. This last fact indicates probable early flowering. In regard to all the leaf sizes of the various groups. it seems that the plant subjected to long photoperiods and grown in rich or poor soil developed the largest leaves. The leaves of plants grown in rich soil were considerably larger than those grown in poor soil.

Harvesting Data

In the harvesting of all material the plants grown in one flat were treated as a group. All tops were gathered for the green weight, being handled as rapidly as possible in order to avoid enzymatic changes in the material, and then placed in an oven for 30 minutes at 100 degrees C. This material was then dried at 70 degrees C. for 24 hours to constant weight. All other plant parts were given this same treatment after harvesting. Following this period of dehydration the plant parts were weighed and sealed in separate desicoators for future use. The same treatment was used for obtaining green and dry weights of stems, leaves, roots and nodules of plants grown in pots.

Harvesting data, Cosmos plant material

Harvesting data of Cosmos plants grown in flats and

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac{1}{\sqrt{2}}\sum_{i=1}^n\frac$

Table 7. Harvesting data; Cosmos Grown in Flats - 1935

 $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2\alpha} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{$

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watered with liquid manure revealed some interesting facts in regard to f1nal height at time of harvest, number *ot* buds, flowers, fruit, green weights of tops and roots, average dry weights of tops and roots, average green and dry weights of all plants, and the average individual green and dry weights for plants *at* all groups, as shown in table 7. Those plants subjected to low light intens1ty for a long photoperIod attained the greatest height, produced no buds flowers or fruit, and had an avorage green weight per plant of 0.67 grams which dried out to 0.187 grams. Plants exposed to high light. long day oonditions attained 12 inches in heIght, produced 3 buds, no flowers or fruit, and. had nn average weight per plant of 2.19 grams green and 0.36 grams dry. Plants given a high light and short day treatment remained the shortest of all groups but developed 27 buds, 4 flowers and 15 fruit and had an average green weight *ot* .0.674 grams which dr1ed out to O.118 grams. In regard to the accumulation of dry matter, ; it seems that the plants subjected to long photoperiods regardless of light intensity were most productive; however, the higher light intensity caused an upward trend in dry matter produced.

Oosmos plants grown 1n pots gave the data shown 1n table 8. Plants given long days of high l1ght Intens1ty and grown 1n poor so11 *ot* low moisture grew to be the tallest of all plants. They attained an average height of 17.8 inches.

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 $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\sim 10^{11}$

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Table S. Harvesting Data. Cosmos Grown in Pots - 1935.

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2}d\mu_{\rm{eff}}\,.$

 $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\sim 10^7$

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 $\sim 10^7$

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 $\mathcal{L}^{\text{max}}_{\text{max}}$

These plants developed few buds, flowers and fruit. They had an average green weight of 3.74 grams which dried out to 0.701 grams. Plants given short days w1th high light intensIty and grown In poor so11 with little moisture grew ssoond in height to 16.25 1nohes. These particular plants had an abundance of buds, flowers and fruit as compared to the other groups. Thls group had an 1ndividual green we1ght *ot* 2.45 grams which dried out to 0.337 grams. Plants subJeoted to long days w1th low light Intensity and grown in fertIle soil with high moisture developed to only 8.75 inches in height, Just one 1nch more than plants given the short day, low light intensity treatment. Long-day, low-lIght plants produced no buds, flowers or fruit, as was the oase of the short day, low light IntensIty treated plants; however, long day, low light intensity treated plants had an 1ndiv1dua1 green weight of 1.76 grams wh1ch dehydrated to 0.251 grams while the short-day, low-lIght-IntensIty plants mostly died with only 2 pots remaining wIth one plant each averaging 0.58 grams in green weight and 0.13 grams dry weight. In cons1dering the growth of all these groups, plants of long day, high l1ght 1ntens1ty treatment grown 1n poor 80il with low moisture, accumulated the greatest amount of dry matter, which may be accounted for by the favored position of the group tor maximum photosynthesis. The short-day, high-light-Intensity plants fa1led to produce as much dry matter as the high-l1ght, long-day plants but did produoe more buds,

flowers and fruit which was probably due to the short photoperiod. The other groups as shown in table 8 seemed to be Inh1b1ted 1n dry matter aooumulation beoause *ot* a laok of light intensity, or an increased humidity caused by the cheeseoloth inclosure.

Harvesting data, Biloxi soybean material

B1loxi soybeans grown 1n flats and watered w1th l1quid manure produced nearly the same results as Cosmos but differed somewhat 1n regard to the green we1ght, and dry we1ght, of plant materials as shown in tables 7 and 9 . This difference 1s partioularly noted in the average ind! v1dual plant we1ghts and was due. in part at least, to differenoes 1n stand.

Soybeans grown 1n pots produoed the harvest data shown 1n tables 10, 11, and 12. Plants exposed to long days with low l1ght 1ntens1ty grew to an average heIght of 63.87 1nches at the tIme of harvest as shown 1n table 10. They produoed no buds, flowers or fruIt but had an average green we1ght *ot* 41.85 grams and an average dry weight of 10.62 grams as shown 1n tables 11 and 12. Only one plant in this group had any nodules. Plants g1ven the short day, low light Intensity treatment produoed plenty *ot* buds, flowers and fru1t but were later than plants gIven the short day, hlgh light treatment. ^Aslow rate of photosynthes1s 1n the short-day, low-11ght plants probably aooounts tor th1s delayed bud formatIon,

Table 9. Harvesting Data: Soybeans Grown in Flats-1935
(All plants watered with liquid manure) $\mathcal{L} \rightarrow \mathcal{L} \rightarrow \mathcal{L}$.

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flowering and fruiting. The average weight per plant for ehort-day, low-light plants wes 11.02 grams for green weight and 3.42 grams: for dry weight. This group failed to develop any nodules on its roots. The general failure of nodule formation on the roots of the plants exposed to low light intensities probably may be acoounted tor by the low sugar oontent of the roots of plants grown in a moist, rich soil with a reduced rate of photosynthesis. Fred and his coworkers (2, 3) have emphasized the stimulating effect of carbohydrate synthes1s on nitrogen fixation; and the conolusion maybe reaohed that any factor, e.g., light 1ntensity or day length. that w1ll increase the carbohydrate level in the·plant will tend to inorease. nodule formation and nitrogen fixation. Conversely, any method whioh decreases the carbohydrate concentratIon, suoh as short exposure to light and addit10n of oomblned nitrogen to the substrate, will lower nodule productIon and total n1trogen fixed.

Plants exposed to long day, high light intenslty had elongated stems to a he1ght of 36.75 inohes. There were no buds, flowers or fruit formed; however, with this group, there was the greatest nodule formation as shown in tables 11 and 12 . These plants nveraged 38.69 grams green weight and 12.62 grams dry weight per plant. The increased nodule formation in this group may have been due to higher sugar content of plants grown in poor, low-m01sture so11 (3). Plants given short day, high light intensity treatment had fruit only at the time of

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$

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harvest· whioh showed that the flowering stage had been passed. This group had an abundance of root nodules which at the time of harvest did not waigh quite as muoh as the nodules *ot* the long-day, h1gh-11ght plants. indioating probably that toods . had been translocated to the newly formed fruit. These shortday, high-light plants had a green weight *ot* 21.74 grama and a dry weIght of 6.23 grams per plant.

Soybeans grown in pots 1n 1939 and subJeoted to s1milar growth conditIons as the beans grown in pots 1n 1935 reveal some contradioting results 1n regard to average plant weIghts, but the experiments of different years otherwise agree in harvesting data. Biloxi soybeans grown 1n pots in 1938 were harvested after 43 days of growth. The green we1ght of various plant parts 1e shown In table 13. Plants exposed to long-day, and high light intensity developed the greatest average green weight of 26.180 grams, and this group had more root nodule development than any other group. Long-day, lowlight plants averaged next 1n green weight at 19.43S grams, 1.269 grams more than the average green weight *ot* plants g1ven the short-day, high-light treatment. These short-day, highlight plants were the only other group to have nodules. Plants grown in moist fertile soil with low light and long or short photoperiods produced no nodules. The short-day, low-light plants grown in moist fertile soil developed the least green weight per plant, 8.164 grams.

 $\label{eq:2} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$

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Chemical Analyses

After considering the observable effects caused by the various growth conditions upon the plants, an attempt was made to see what might be revealed biochemically. In the summer of 1935 a study made with plants growing under the various conditions of light intensity, length of photoperiod, soil moisture and fertility, revealed the following biochemical facts:

Experiments with Cosmos

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 $\mathcal{A}=\mathcal{A}^{\mathrm{c}}$, \mathcal{A}^{c} , \mathcal{A}^{c}

Cosmos grown in pots developed slowly, producing only a small amount of plant material for chemical analysis. Only two groups afforded sufficient plant material for the analysis. High-light, short-day plants grown in poor soil low in moisture did not produce the total carbohydrates or total nitrogen that high-light, long-day plants grown under similar conditions produced, as shown in table 14. The high-light, long-day plants contained more carbohydrates and nitrogen generally than the high light, short day plants. This difference in carbon and nitrogen levels of the two groups may be assigned to differences in total photosynthesis.

Experiments with soybeans

Biloxi soybeans grown in flats, watered with liquid manure and given various treatments of light intensity and photoperiods produced the data shown in table 15. Highlight. short-day plants developed the greatest level *ot* total oarbohydrates and nitrogen aooumulation *ot* all groups. Highlight, long-day plante developed a relatively high oarbohydrate aooompanied by a low n1trogen aocumulat1on. Hlgh-light, shortday plants differed cono1derably from the hieh-light, long-day plants 1n having 147 per cent more soluble nitrogon and 26 per cent more residual nitrogen. It is generally assumed that factors that decrease the carbohydrate level 1n plants should inorease the soluble nitrogen level. Low-light, long-day plants, although having the greatest dry weight per single plant tor all the groups grown in tlats, developed the lowest carbohydrate level of all groups, and a relatively high nitrogen level when oompared to the high-light, long-day group. From this oonsideratlon, lt might be assumed that low-light, longday treatment 18 su1table tor slow accumulatlon *ot* oarbohydrates and nitrogen; high-light, short-day treatment is su1table for a taster aooumulation *ot* oarbohydrates and nitrogen and that high-11ght, long-day treatment inoreases the oarbohydrate level while n1trogen aooumulation lags.

A ohemioal analysis was made of Bilox1 soybean stems and leaves comblned. Table 16 reveals the data. The growth *ot* these plants gave 1nteresting results. Plants exposed to high-llgh t, long-day and grown in a poor so11 *ot* low mo1sture developed a very high oarbohydrate level and &lowed

* Mg. of substance per 100 gr. of original green plant material

 $\sigma_{\rm{max}}$

Table 15. Chemical Composition of Soybeans Grown in Flats-1935. (Leaves and stems combined) (All plants in flats watered with liquid manure)

Growth conditions		Carbohydrates		Nitrogen		
	: Reducing: sugars :	Sucrose:	Total :	Soluble	Residual	
High moisture Low light Long day	150.00*	138.00	288.00	85.20	101.31	
High moisture High light Short day	219.00	174.65	393.65	107.71	114.60	
High moisture High light Long day	222.35	160.50	382.85	43.89	90.70	

*Mg. of substance per 100 gr. of original green plant material

 ~ 100

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 $\sim 10^{-10}$

 $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\sim 10^{-11}$

 ~ 100

the highest resldual nItrogen content when compared to the other groups. High-llght, short-day plants revealed a relatively high carbohydrate level but a low nitrogen level when compared to plants grown in fertile soil. Low-light. long-day plants grown in very moist fertile soil developed the lowest carbohydrate level but an Intermediate nitrogen level when considered with all the groups. Low-light, short-day plants grown in a very molst fertile soil developed a higher carbohydrate level than low-light, long-day plants but a lower carbohydrate level than hlgh-light, long-day and highlIght, short-day plants. However, low-lIght, short-day plants showed more soluble nitrogen present than all the other single groups.

Chemical analyses of Biloxi soybeans grown in 1938 with the same treatments as those of the experiments conducted in 1935 are shown in table 17 on the chemical composition of leaves and table 18 on the chemical composition of stems. In regard to the Influenoes of llght 1ntensity and photoperiod on the chemical content of plant material grown in different ferti11ty and mo1sture of so11, the leaves of the Short-day groups had low reduoing sugars, but relatively high levels of total carbohydrates and resldual nltrogen. The long-day groups developed fairly high reducIng sugars and sucrose; however, there was more variation of oontent shown 1n the h1gh-llght, long-day group. In regard to the ni trogen content of all the

- 41 -

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 $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

Table 16. Chemical Composition of Soybeans Grown in Pots-1935. (Stems and leaves combined)

 $\sim 10^{11}$ km $^{-1}$

*Mg. of substance per 100 gr. of original green plant material

 $\sim 10^{-10}$

groups, low light and high soil fertility oausedan aooumulation of soluble as well as residual nitrogen.

The ohemical composition of stems of Biloxi soybeans grown in pots in 1938 is shown in table 18. Stems of longday groups grown in soils of different fertility and moisture indicated a high total carbohydrate content; however, there was a wide d1fference in their reducing sugar percentages. Short-day groups showed very low reducing sugar contents. As to the nitrogen content of all the groupe, they showed a relative even amounts of residual nitrogen, but the low light groups with thelr fertilized soll showed the highest soluble n1trogen oontent.

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	Carbohydrates.			Nitrogen	
Growth conditions	Reducing: sugars:	Sucrose	Total		Soluble : Residual
High moisture Fertile soil Long day Low light	$89.91*$	87.63	177.54	173.32	735.90
High moisture Fertile soil Short day Low light	9.89	188.01	197.90	206.13	545.79
Low moisture Poor soil Long day High light	234.77	162.86	397.63	94.14	621.78
Low moisture Poor soil Short day High light	59.52	172.81	232.33	121.93	560.70

Table 17. Chemioal Composit1on of Leaves of Soybeans Grown in $Pots - 1938.$

-Mg. of substanoe per 100 gr. of or1ginal green plant material

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 $\sim 10^{-1}$

		Carponydrates			Nitrogen	
Growth conditions	Reducing: sugars :	Sucrose	Total	Soluble ÷	Residual	
High moisture Fertile soil Long day Low light	66.60	167.67	234.27	276.05	120.38	
High moisture Fertile soil Short day Low light	24.98	104.40	129.38	315.60	121.85	
Low moisture Poor soil Long day High light	167.75	190.99	358.74	181.47	102.79	
Low moisture Poor soil Short day High light	9.73	185.39	195.12	173.23	120.51	

Table 18. Chemical Composition of Stems of Soybeans Grown in Pots -1938 .

*Mg. of substance per 100 gr. of orig1nal green plant material

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 $\sim 10^7$

DISCUSSION OF' RESULTS

The experiments conducted in the summer of 1935 gave results in agreement with those reported by Garner and Allard (4). Short-day plants were forced into flowering by removing the plants to a ventilated dark chamber in the afternoon and returning them to the greenhouse in the morning for an $8-9$ hour light exposure. The 1938 experiments also were in agreement. Biloxi soybeans were strictly short day in response and were not forced into flower by attempts to increase the oarbohydrate level in the plants by growing them at low water levels on infertile soil. Cosmos, however, were forced into flowering with long days by stunting treatments and prevented from flowering on short days by reducing the light and increasing the soil fertility and moisture (Table 2). Soybeans were not prevented from flowering by shading, and chemical analyses (Table 17) indicated a high sucrose content in the leaves of these plants.

Plant groups subjected to long photoperiods of low light Intensity developed light green colored leaves, while plants subjected to long photoperiods of high light intensity developed yellowish green colored leaves. Plants given short photoperiods with high light intensity developed an ordinary green color of leaves, while plants given a similar' photoperiod with low

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light intensity developed the darkest green leaves of all plant groups. indicating that the deep green of chlorophyl in plant leaves may be a partial compensation for the reduced light intensity. Reducing the light intensity, however. normally brought about a lower dry weight and sugar level. Plants given high light intensity regardless of the photoperiod had higher sugar levels than plants exposed to low light intensities irrespective of the photoperiodic length.

High soluble nitrogen content was generally evident in plants growing under the influence of low light. It will be recalled that these plants also were heavily watered and were fertilized with ammonium sulfate. When a nitrogen salt was added to the substrate of legune plants. there was little evidence of nodule formation. An increased light intensity on plants grown in a soil low in fertility helped nodulation. presumably because of the raised level of carbohydrates. Nodulation was increased still further by a longer photoperiod. A difference in the photoperiod and light intensity caused a variation in the carbon and nitrogen levels in the plant tissues. Klebs (11) arrived at the conclusion that a piling up of carbohydrate food products favored flower production. The work of Kraus and Kraybill (12) indicates that the relationship of carbohydrates to nitrogen is closely associated with the vegetative and reproductive growth of plants. Hen dricks and Harvey (10) found an increased concentration of

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carbohydrates in the leaves of Easter lilles grown under artificial light to be correlated with early flowering. In our experiments we found that plants subjected to an abundance of moisture and mineral nutrients and exposed to long photoperiods at low light intensity failed in the accumulation of the carbohydrate level above the nitrogen level. Plants grown under such conditions were vine-like and non-flowering. Biloxi soybean plants given high moisture and an abundance of available nutrients, but exposed to short photoperiods at low light intensity built up a higher carbohydrate level, and initiated early flowering. It is significant, and characteristic of the photoperiodic response, that plants exposed to light for only half of the normal day should have accumulated higher percentages of carbohydrate than the more rapidly growing plants exposed for the full day. Plants subjected to high light intensity for a long photoperiod and grown in a soil of low fertility developed a still higher carbohydrate level, but failed to flower. Plants exposed to high light intensity for a short photoperiod and grown in a soil low in fertility developed a high level of carbohydrates and flowered earlier than the short-day shaded plants. Thus, in our experiments, it was found that an increased concentration of carbohydrates in plants failed to initiate early flowering in Biloxi soybeans grown with long days.

The results can be explained by assuming that some

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essent1al d1fferentiation produot 1s destroyed by light in ehort-day plants. Hamner (S, 9) has shown, for example, that a short exposure to light during the dark period will prevent flowering of Xanthium. Presumably a lower sugar level than was obtained in our plants would have ohecked flowering on short days because of the lack of this essential material for differentiation (14).

SUMMARY

Three short-day plants, Cosmos (Cosmos bipinnatus), Salvia (Salvia splendens), and Biloxi soybeans (Glycine Max). were grown with $14-16$ hours of daylight (long day) or 8-9 hours (short day) under conditions favorable and unfavorable for differentiation and observed for flowering. Conditions favorable for differentiation were obtained by growing in a sand mixture with full greenhouse light and watering sparingly. Conditions unfavorable for differentiation were obtained by growing in fertilized compost with 40 per cent light and watering liberally.

Flowering of Cosmos and Biloxi soybeans was hastened by shortening a 14-16 hour day to 8-9 hours. Biloxi soybeans did not flower on the longer days. Long-day Cosmos grown under conditions favoring differentiation flowered 6 days after the short-day plants on August 12.

Reducing the light to 40 per cent of normal and simultaneously increasing watering and soil fertility caused a weak. twining growth in long-day soybeans and a short weak growth in short-day beans. Flowering was prevented in Cosmos, but not in short-day soybeans.

Plants grown on long photoperiods were larger, but carbohydrate percentages, particularly of sucrose, were frequently

 $\frac{1}{2} \left(\frac{1}{2} \right)$

higher in short-day plants, even with reduced light and high soil fertility. $\mathcal{O}(\mathcal{O}_\mathcal{O})$, and $\mathcal{O}(\mathcal{O}_\mathcal{O})$, and $\mathcal{O}(\mathcal{O}_\mathcal{O})$ $\sim 10^{-11}$

Soybean plants grown under conditions unfavorable to $\sim 10^{-10}$ differentiation failed to produce nodules while nodulation was heavy under high-differentiation conditions.

 $\label{eq:2.1} \frac{1}{2}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\$

 $\label{eq:2.1} \mathbf{r}^{\text{max}}_{\text{max}} = \mathbf{r}^{\text{max}}_{\text{max}}$

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 \mathcal{L}_{max} , where \mathcal{L}_{max}

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 $\mathcal{A}=\mathcal{A}^{\mathcal{A}}$, where $\mathcal{A}^{\mathcal{A}}$

 $\label{eq:3.1} \mathbf{S}^{\text{in}}(\mathbf{S}) = \mathbf{S}^{\text{in}}(\mathbf{S}) \mathbf{S}^{\text{in}}(\mathbf{S})$

 $\sim 10^{-1}$

 ~ 10

 $\sim 10^7$

 $\label{eq:2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{$

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