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Biochemical Studies of Photoperiodism in Plants

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ABSTRACT

When soybean plants, var. Biloxi, are exposed to a photoperiod conducive to sexual reproduction (7-hour day) two significant effects on development were recognized: (1) *Photoperiodic induction* of reproduction, which occurs during the first 4-14 days and (2) *photoperiodic inhibition* or reduction of growth in height, most conspicuous soon after induction is completed. The two phenomena appear to be brought about independent of each other. Their causal mechanisms have not been discovered.

During the period of induction a relatively higher nitrogen metabolism and lower carbohydrate concentration is maintained by the reproductive or short-day plants than the vegetative plants exposed to a long (14-hour day). Stems of reproductive plants were higher in all determined forms of nitrogen (total, coaguable, soluble, proteose, basic, ammonia, amid, humin and amino), excepting nitrates. In the light of our present knowledge of nitrogen and carbohydrate metabolism in sexual reproduction, no specific dynamic function can be ascribed to any particular form or group of substances, either directly or in their relationship, in the initiation of floral organs. Present evidence points to the action of a specific flower producing "hormone," whose function appears to be independent of the general nutritional state of the plant.

A higher respiration rate (CO_2 output) is maintained at the time of photoperiod induction by the short-day (subsequently reproductive) than the long-day (vegetative) plants. A similar increase in respiration occurs when plants are moved from a long to a short photoperiod. Growth promoting substances—indole acetic, indole propionic and phenyl acetic acids—do not have an effect on initiation of sexual reproduction.

Due to photoperiod inhibition, growth in height of short-day soybean plants is retarded after 10-day exposure and ceases almost completely by the 20th day. As a result of curtailed growth, nitrogen and especially carbohydrates accumulate in the stems and other structural parts. The relatively higher concentration of carbohydrates than of nitrogen, at the time of full bloom and thereafter, may be expressed by a higher C-h/N ratio. These groups of organic substances are utilized by the long-day plants for vegetative growth and by the short-day plants (whose growth is inhibited) for the formation of flowers and the development of fruits and seeds.

The carotene and xanthophyll concentration of the leaves of soybean plants exposed to a 7-hour day reaches a maximum at the approximate time of flowering and then decreases. Buds, flowers and young fruit tissues have a comparatively high carotene content.

Biochemical Studies of Photoperiodism in Plants

A. E. Murneek

Although the effect of photoperiodism in plants has been known for almost twenty years and its importance is now widely appreciated, the mechanism through which this phenomenon expresses itself is still unknown. Several suggestions have been made as to the possible causal metabolic changes in plants when they are exposed to and respond in a characteristic way to a definite length of day (photoperiod). None of these proposed ideas, however, can be considered at present as basic and none seems to have gained general recognition.

Photoperiodism offers a very desirable approach to the study of "physiology of reproduction of higher plants." Quite independent of the extent of their growth, many plants respond strikingly in the rapidity with which sexual reproduction is initiated and maintained under the proper length of day. Of the many other environmental factors influencing plant development, the photoperiod has frequently the greatest effect. This appears to be a very desirable situation in all such instances where, for various reasons, it is not possible to have a more or less complete control of the environment under which the plants are grown. Moreover, few experimental treatments can be given to large numbers of plants as conveniently and uniformly as exposure to a definite length of day.

The object of the present investigation was to conduct a detailed study of the metabolic changes in certain selected types of plants coincident with their photoperiodic response. While several species have been taken into consideration, by far the largest amount of work was done with the soybean (*Soja max* (L.) Piper). Though not ideal in every respect, this species has been found to be a desirable material for the requisite culture and treatment under a greenhouse environment in this locality.

The method of approach in this investigation has been almost entirely physiological, with particular emphasis on biochemical analysis of the experimental material. It is our belief, that by detailed studies of the chemical changes in various parts of the treated plants, closer approach may be made to the ultimate mechanism of photoperiodism.

GENERAL REVIEW OF LITERATURE

The rather extensive literature on photoperiodism has been reviewed periodically by Maximov—1925 (122),* Kellerman—1926 (90), Reddington—1929 (187), Schick—1932 (201), Garner—1936 (58), and Burkholder—1936 (28). No attempt, therefore, will be made to give here a detailed account of all the experimental work in this field of investigation. Specific publications will be referred to, however, in reviewing certain physiological studies and in connection with the introduction and discussion of the present experimental work.

While the formative effects of diurnal length of exposure to daylight and artificial illumination have been noted by several students of plant life (Schübeler—1880, Kjellmann—1885, Bonnier —1895, Tournois—1912), all credit is due to Garner and Allard (59) for the discovery and demonstration of the phenomenon of *photoperiodism.* They were also the first investigators to study extensively the response of various plants to relative lengths of day and night (photoperiods), especially in relation to sexual reproduction (59, 60). Since then a large number of tests and observations have been made with the object of determining the extent of this reaction in various species, varieties and strains of higher plants (1-9, 16, 23, 40, 45-46, 52, 56, 57, 71, 72, 77, 100, 108, 124, 127-128, 103, 133, 152, 179, 183, 203, 215, 217, 227). Concomitantly investigations have been conducted on the practical application of photoperiodism, with particular reference to greenhouse crops (69, 106-108, 164-166, 169-172, 223, 225), to breeding, selection and inheritance (6, 27, 39, 53, 57, 73, 74, 120, 121, 191) and to plant adaptation, distribution, origin and related problems (5, 42, 44-46, 55, 59, 105, 111, 133, 219).

Some of the above and several other contributions (20, 38, 41, 48, 63, 85-87, 89, 111, 113, 123, 147, 157, 159, 161, 188, 192-194, 200, 216, 221, 228-229) deal not only with the effects of length of day on the gross development of certain parts of the experimental plants but to some extent also with the detailed morphological and anatomical structure. This phase of the subject matter has been summarized recently by Garner (58).

The numerous species and types of plants studied so far seem to differ greatly in their development under various photoperiods. In respect to reproduction, higher plants may be roughly grouped into "long-day" and "short-day" types and those not susceptible

*Numerals refer to "Literature Cited," page 76.

to differences in length of day. It must be remembered, however, that in addition to reproduction, most other functions and practically all structural parts may be strikingly affected by the photoperiod.

Much research work has been done on the internal conditions of plants as influenced by the duration of exposure to light. By studying the assimilation-respiration balance, Lubimenko and Sceglova (111) came to the conclusion that a fundamental difference exists between "long" and "short" day plants in respect to the magnitude of oxidation (respiration) and reduction (synthetic) processes, the ratio being higher in the long-day plants. It would appear, therefore, that the relative amount of dry matter produced per hour of light exposure is greatest under a short day. But by determining the rate of CO_2 assimilation, as regards intensity and daily course, Tageeva (207-208) was unable to find any conspicuous differences between the two groups of plants. According to this author, photosynthetic processes evidently do not play a determinable rôle in the differences induced by various photoperiods, though plants passing over to the stage of reproduction show a somewhat increased photosynthesis. Apparently several other environmental factors, especially temperature (66-67, 87-88, 47, 222), light intensity (88, 203), humidity (67), soil nutrition (115, 123, 222) and carbohydrate concentration (104) affect markedly the assimilation-respiration balance and the resultant accumulation of dry matter in plants exposed to various photoperiods.

The concentration and distribution of carbohydrates in relation to definite light regime have been determined by several workers (15, 41, 64, 88, 14, 47, 77, 218, 221-222). The accumulation of various forms of carbohydrates is in a large measure a function of the amount of light received by the plant. Their accretion in certain parts of organs is determined not only by the rate of photosynthesis but also by respiration, and is affected by growth and removal to storage organs. Under extremely short daily exposure to light, and with relatively high temperature, very little storage of carbohydrates can take place, while with a long day and low temperature, they will be stored in greater abundance. But short-day plants often accumulate more of these organic substances in a short than a long photoperiod. This appears to be frequently due to the direct inhibiting effects (named by the writer *photoperiodic inhibition*, 148) by short days on vegetative extension with consequent reduction in utilization and an increase

in accumulation of carbohydrates. Of significance in this connection is the fact that in their chemical studies Arthur, *et al* (14) obtained various values for the carbohydrate fractions in plants under changed light exposures, depending on the way the plants were sampled for analysis.

Much of the chemical work dealing with photoperiodism has been influenced by or interpreted by means of the carbohydrate-nitrogen relationship concept, which was promulgated by Klebs (92-93) and emphasized by Kraus and Kraybill (102-103) about the time of discovery of photoperiodism. The results of several of the earlier investigations, notably those by Auchter and Harley (15), Nightingale (149-150), Gilbert (66), Maximov (123), Tincker (216) and Roberts (190), and some comparatively recent ones by Haut (77), Werner (222), and Hurd-Karrer and Dickson (88), have been analyzed and explained on the basis of a relative concentration of carbohydrates and nitrogen. In most instances this interpretation of the possible correlation between chemical composition and photoperiodic response has been rather general, although in certain instances the chemical data have been expressed in the form of quantitative ratios between total (analyzed) carbohydrates and total nitrogen (C/N), or as $\frac{C}{\text{soluble N}}$, $\frac{C}{\text{insoluble N}}$, $\frac{\text{starch}}{N}$, $\frac{\text{sugar}}{N}$, etc. Frequently this has left in the reader's mind a desire for greater certainty in such an explanation.

The extensive studies by Arthur, Guthrie and Newell (14), Eaton (48), Borodina (24), and Purvis (177) tend to show that photoperiodism cannot be placed in causal relationship with the carbohydrate-nitrogen concentration in the plants. The chemical composition of the material would seem to vary tremendously, subject to other environmental factors than the photoperiod and depending upon the age of the plants and the tissues included in the samples.

Because of the variability in results of the chemical work and lack of definiteness in their interpretation, it would appear very desirable to analyze in greater detail the relation of organic food reserves to vegetative and reproductive development of plants as influenced by definite lengths of day. Such an investigation may be profitable even if one should agree with Arthur, *et al*, Borodina and others, that the C/N ratio is not the immediate cause of a change from the vegetative to the reproductive state but only a possible attendant phenomenon or perchance even a result.

In studies of the mechanism of photoperiodism the biochemical approach has been influenced to a large extent by the fact that the response can be definitely localized. This was beautifully demonstrated by Garner and Allard with *Cosmos* (61). Knott (94) and others (229) have supplied additional evidence that photoperiodic reception may be restricted to the apex of the stem, but concluded from further studies (97) that the leaves appear to function in some way to hasten the effect. Rasumov (184, 186), however, is of the belief that, though the growing point is very sensitive to light, there is no special plant organ that receives the stimulation induced by length of day. By his work with tuber bearing plants he seems to have demonstrated that the effect travels more readily down than up the stem. But the recent investigations of Moshkov (135) and Cajlachjan (33) emphasize again leaves as the receptors, whence the effect is transmitted to the stem. When plants are exposed to the proper length of day, with increasing leaf area, there was a proportional acceleration in time of flowering (33).

The demonstration of localization of the response to relative length of day has led to chemical studies of a more restricted nature, especially to testing for enzyme activity in certain organs.

By transferring a short-day type of plant from a long to a short exposure Garner, *et al* (64) have shown that after 3-5 days there is a sharp temporary rise in P_H value. This may indicate the time of physiological transition from the vegetative to the reproductive state, although this change in acidity may not stand in any causal relationship to initiation of sexual reproduction. Lubimenko and Sceglova (112) have expressed the idea that differences in reduction-oxidation enzymes may determine the reaction of plants to length of day. One of the enzymes related to oxidative processes, catalase, has been studied intensively by Knott (95-96), who has shown that its activity in the apex of stems of spinach plants decreases during a change from vegetative to reproductive type of growth. In apical bud tissue differences were noted as early as 16-40 hours after the change. This indicates that the developing meristems of spinach respond rapidly to alterations in day length. But Cajlachjan and Alexandrova (32) could not observe any difference in catalase activity of leaves of millet and barley when exposed to various lengths of day, though peroxidase was highest in the long-day (vegetative) millet and short-day (vegetative) barley, but also highest in the short-day (reproductive) soybean plants. From their recent studies with beans and

chrysanthemums, Krassinsky, *et al* (101) conclude that the shortening of the day (leading to reproduction) resulted in a "very considerable" and "regular" increase in activity of both catalase and peroxidase. Eckerson (49) found in the tops of Biloxi soybeans grown in an 8-hour day only 1-5 per cent as much reducase as in tops of long-day plants. It is difficult to say whether the reduced reducase activity, or changes in oxidation enzymes, stand in any direct or causal relationship with the formative effect of a shortened photoperiod.

When plants are exposed to various lengths of day, changes in chloroplast pigments are often quite apparent. By studying certain species Lubimenko and Sceglova (110-111) came to the conclusion that, excepting under a very short daily exposure (4-6 hours), there does not seem to be any marked difference in chlorophyll content of leaves. Murneek (145) has called attention to the conspicuous changes in greenness of soybean plants during the course of their development when subjected to a short-day and to an increase in carotine and xanthophyll in leaves about the time of development of the reproductive organs. The chlorophyll concentration of 15-day old plants of 4 species has been determined by Cajlachjan (31). With increasing age there was a marked increase in chlorophyll under a short day (10 hour) exposure.

In studies of the mechanism of photoperiodism two major features seem to have gained importance at present: (a) The influence of temperatures on initiation and development of sexual reproduction and (b) the induction of reproduction (or vegetation) by exposure to an appropriate light period, which is retained as an after-effect when the plants are subsequently moved to an opposite photoperiod. Both of these factors have recently been united into a treatment known as *vernalization.* And the extensive investigations of vernalization (*yarovization*), and speculation as to the essential nature of the response of plants to this treatment, has given rise to the conception known as *"phasic development."*

During the past few years much activity has been displayed, especially by agronomists and horticulturists, in research on the relation of temperature to photoperiodism, and more lately on the direct effect of temperature on sexual reproduction. It would seem that of the greatest interest at present is not the fact that temperature may influence and modify the formative effect of the photoperiod (12, 40, 47, 54, 62, 66-67, 87-88, 131, 163, 177, 212, 220, 222) but that this environmental factor (temperature) itself may be as potent in induction of reproduction as the daylight period. The

extensive investigations by Thompson (210-211), Boswell (26), Miller (132), Platenius (162), Peto (158), Chroboczek (37), and Post (173-175), primarily on vegetables and to some extent on ornamental plants, show that as small differences as 6-10°F. will either suppress or stimulate the formation and development of floral organs. Evidently the length of day is not the only potent external factor influencing reproduction. Plants seem to be more sensitive to environmental conditions than we have suspected. Thus it is probable that there may be other external factors, yet to be discovered, which, at least under certain circumstances, and with particular groups of plants, may be as effective as the photoperiod and the temperature in their influence on growth and development.

While studying the effects of various photoperiods on reproduction of soybeans, Garner and Allard (60) noted (page 884) that the initial 10 short days may be sufficient to bring about flower formation, which was maintained when the plants were thereafter exposed to a long day. But when given an increasing number of short days, more fruits were successfully developed. Since then numerous workers have observed that when certain plants are grown for a definite time under one photoperiod and then transferred to another, the effect of the first exposure will become manifest in later development. This phenomenon has been named *"photoperiodic after-effect"* or *"photoperiodic induction."*

A preliminary exposure of short-day plants to a short diurnal period of light hastens markedly the time of appearance of flowers (29-30, 32, 51, 60, 98, 110, 112-113, 123-124, 177, 181-183, 191). Sometimes as few as 4-6 days are sufficient for the induction of reproduction (112, 182). Such a preliminary exposure is frequently as effective as the growing of plants continuously under a short photoperiod. It is probable, however, that a certain length of day is more conducive to the initiation of floral primordia, while a different length of day is best for further development and function of the reproductive organs (177). A similar pretreatment of long-day plants is also effective, though possibly to a smaller degree (112, 177, 182-183). And it has been suggested that short-day photoperiodic after-effects are possible only for short-day plants and long-day after-effects for long-day plants (32). The causal mechanism of photoperiodic induction, as that of photoperiodism itself, is unknown. Irreversible changes evidently are brought about by induction in the protoplasm of the growing point which speed up the ontogenic development of the plant.

By exposing typical short-day species to a lengthened daylight period early in their growth, a more extensive development of tops may be obtained, which, when the plants are thereafter given a short photoperiod, will lead to the production of larger yields of seeds, fruits or tubers. Thus in their seasonal growth and development plants with a relatively long life cycle will attain maximal size and greatest production only under a varying photoperiod. This fact should be always borne in mind in studies of the adaptation of species and varieties to certain latitudes. It seems to be of considerable importance in greenhouse culture of certain vegetables and ornamentals.

A rather unexpected approach to physiology of reproduction of plants, with a special bearing on photoperiodism, has been made through studies of vernalization—a treatment given to seeds previous to sowing to hasten the time of flowering.

As early as 1918 Gassner (65) demonstrated that when winter wheat is exposed during germination to a temperature little above freezing, it will flower and fruit the same year though sown in the spring. Hence an early exposure of the seed to low temperature *predetermines* reproductive development. Gassner's results seem to have remained of theoretical interest only till its practical importance and general relation to physiology of reproduction was emphasized by Lysenko and his coworkers (114-119). Since then this field of investigation has developed rapidly (22, 109, 123-124, 129, 131, 167, 177, 206, 214, etc.) and a new general idea of plant development has been proposed (11, 119, 126).

In coordinating the results of his own and those of other recent investigators on vernalization and photoperiodism, Lysenko has postulated the following theoretical conceptions of growth and development of plants (11, 119, 126, 209).*

> 1. *Growth and development are not identical phenomena and they are independent of each other.* Growth is an increase in size of a plant, without any profound qualitative changes in the growing parts. By development is understood the transition of a plant into a new stage, qualitatively differing from the preceding one.
>
> 2. *The entire process of development of an annual seed plant consists of individual stages or phases.* Actual "stages of development" may or may not be associated with visible morphological changes, though there may have occurred profound internal alterations.
>
> 3. *The phases always proceed in a strict sequence and a subsequent phase canot set in until a preceding one has been completed.* So far only two, the thermo- and the photo-phase have been intensively studied. There may be others.

*For reasons of accuracy, some of the statements hereunder (indented part) are produced *verbatim*.

4. *Different stages of development of the same plant require for their completion different external conditions.* A plant cannot proceed to reproduction unless the environmental factors permit it to pass through the various phases of development. Lacking this, it will remain in a vegetative state.

Stress is placed on the fact that growth and development are distinct phenomena. By growth is understood the multiplication of cells and increase in size of a plant. Development, on the other hand, produces qualitative changes in the growing parts. It is possible to have growth without development and development without growth. In vernalization, growth is minimized or kept at a standstill while development is made to proceed rapidly.

The general subject of vernalization and most of the important experimental work in this field have been reviewed and brought up-to-date by several investigators (Maximov—1933 (125), Whyte and Hudson—1933 (224), Maximov—1934 (126), Lysenko—1935 (119), Thomson—1936 (209) and Purvis—1936 (178). Hence no detailed consideration will be attempted of the numerous papers dealing with this problem. The present discussion is confined to the more or less direct bearing of vernalization on photoperiodism.

By exposure of the partly swollen seeds to definite temperature (vernalization), depending on the species, an induction of reproduction is obtained, which is very similar to photoperiodic induction or after-effect. Seeds of long-day temperate zone plants are usually subjected to a comparatively low temperature and those of more southern habitat, or short-day plants, to relatively high temperature. This treatment itself, however, will not make plants reproductive unless during their subsequent development they are exposed to a required length of day. Many plants, therefore, have to pass through two, possibly more, stages (phases) of internal readjustment—the thermo- and the photo-phase at least—before they can become sexually reproductive. Lacking the requisite external environment for the completion of these phases, vegetative growth will be more or less continuous. In soybeans, for instance, a comparatively high temperature (20-25°C.) will complete the thermo-phase in moistened seeds and a short day (long night or even continuous darkness, according to some)—the photo-phase. Though Lysenko postulates a succession in the phasic development of plants, it would seem that in the soybean at least vernalization implies the completion of both phases (thermo and photo) more or less concurrently. Of probable significance in this connection is the suggestion that it may be possible to "vernalize" by light only (29) and that some seeds may become vernalized while still connected with the mother sporophyte (98).

From all these studies the striking fact emerges that plants may be induced to become reproductive in a very early stage of their development—while still in the seed. Hence some phases of the physiological studies of photoperiodism and sexual reproduction, perforce, will have to be moved back to the seed stage of the plant.

Since no flower primordia are present in this extremely early period of development, the after-effect from vernalization, and most probably from photoperiod also, must express itself through alteration in the physiological state of the whole or some part or parts of the embryo plant. This would suggest a chemical substance, possibly of catalytic nature, as the active agent bringing about these striking results.

Changes brought about in cells of plants in the embryonic stage as a result of vernalization seem to be transferred to other cells produced from them, but not translocated to other parts of the same plant. This appears to be true for both the thermo- and the photo-phase. Plants developing from vernalized seeds will become independent of temperature or photoperiod or both, depending upon whether vernalization has been for one or both environmental factors.

Vernalization during the thermo-stage may be given in installments as it were: half of the total exposure now, then seeds dried, and the other half later. The results from the treatment are additive. Consequently the influence exerted by temperature appears to be of the nature of gradual quantitative effects and not trigger-like in its action. Recent evidence (20) would suggest that the effects from a photoperiod are also of a "quantitative" nature and that a time factor exists for the accentuation of the induced changes. By a careful regulation of the period of exposure to certain lengths of day, it is possible to obtain interphasic development between vegetative shoots and normal flowers. This, too, seems to point to a chemical substance as the causal agent.

While Lysenko has postulated two important phases (thermo and photo) in the development of higher plants, a suggestion has been made of the probable existence of a third phase associated with gametogenesis (99). In its development the gametophyte will not pass through the necessary qualitative changes (will not produce normally functioning eggs and fertile pollen) unless, after the completion of the first two phases, the third is fostered by a proper photoperiod. In the case of wheat, for example, the photo-phase requires a long day, while the gametophyte phase may be

completed under a relatively short photoperiod. Before it is accepted, further proof is necessary for the existence of the "third phase."

In studies of the influence of length of day on plant development, very limited work so far has been done on the effects of intensity and quality of light. Observations, in connection with artificial illumination to lengthen the day, seem to point to the fact that, within reasonable limits, it makes very little and possibly no difference as regards the amount of light given the plants (59, 226). Intensities even as low as 5-10 foot candles may produce in some plants typical results under definite photoperiods. However, some investigators have noted that the intensity of light is of some importance in obtaining the desired results from exposure to definite light periods (14, 195, 204). The longer wave lengths, i. e. red and orange, may be most effective in inducing photoperiodic response in respect to earliness of blooming and the production of the largest number of flowers (153, 185, 195, 226). This is in agreement with the discovery by Klebs (93) that red light is most conducive to flowering of plants. In order to understand more thoroughly the mechanism of photoperiodism, more intensive studies would seem to be desirable on light as the causal agency.

To avoid possible confusion and misunderstanding in the use of certain technical words and phrases in connection with the discussion of photoperiodism and related phenomena, a list of the more popular terms with their definitions is presented herewith.

TERMINOLOGY

1. Photoperiod.—Length of daily exposure to light. (Garner and Allard).

2. Photoperiodism.—Response of plants to photoperiod. (Garner and Allard).

3. Long-day plants.—Species, varieties and strains in which the flowering period is accelerated by a *relatively* long daily exposure to light, usually more than 12 or 14 hours. (Garner and Allard).

4. Short-day plants.—Species, varieties and strains in which the flowering period is accelerated by a *relatively* short daily exposure to light, usually less than 12 or 14 hours. (Garner and Allard).

5. Photoperiodic induction.—The carry-over effect of a photoperiod conducive to sexual reproduction to one opposite to it and *vice versa*. Also the transfer of photoperiodic stimulation to a non-treated part of the same plant. (Lubimenko and Sceglova).
6. Photoperiodic after-effect.—The same as photoperiodic induction (Maximov). Plants may exhibit also "temperature" and possibly other "after-effects."
7. Photoperiodic adaptation.—The adaptation of plants, in their native or artificial habitat, to a definite length of day or to latitude (Lubimenko). Sometimes confused with photoperiodic "induction" or "after-effect."
8. Thermoperiodic adaptation.—The adaptation of plants, in their native or artificial habitat, to periodic changes in temperature. (Lubimenko).
9. Photoperiodic inhibition.—Inhibition or retardation of growth, primarily of the main axis, by certain photoperiods. (Murneek).
10. Yarovization.—A preliminary treatment of seeds (with cold, heat, darkness, light, etc.) to induce early reproduction in crop plants. (Lysenko).
11. Vernalization.—English equivalent of the word yarovization. (Whyte and Hudson).
12. Physiological predetermination.—Effect from treatment or condition of seed which influences the future development of the plant. (Kidd and West).
13. Phasic development.—A theory that in their development plants pass through definite successive stages or phases. (Lysenko).
14. Carbohydrate-nitrogen ratio (relationship).—A concept that vegetative development and sexual reproduction are controlled by the relative concentration of carbohydrates and nitrogen in the plant. (Kraus and Kraybill).

CULTURAL AND EXPERIMENTAL METHODS

This investigation was conducted during the years 1930-1936, inclusive, in greenhouses of the Missouri Agricultural Experiment Station, Columbia, Missouri.

In the first or preliminary year, several species of plants were observed and studied as to their general adaptability and response to certain photoperiods. Cosmos (*Cosmos bipinnatus*), Salvia (*Salvia splendens*) and Soja (*Soja max*) were found to be most desirable

for the contemplated studies. Of the soybeans the following varieties were compared: Peking, Tokyo, Minsoy, and Biloxi. As the work progressed, during the succeeding years the material was limited almost entirely to the Biloxi variety.

Beginning in 1931 all of the plants were raised in a greenhouse specially constructed for this purpose and containing an ample darkroom built within it. This room is forcibly ventilated by means of a powerful fan and, when used, its temperature does not differ from the greenhouse proper by more than ± 1°C.

Long and deep wooden boxes of the type used by Garner and Allard (59-60) were the equipment for growing all plants. These boxes were kept either on benches or steel trucks, which were moved into the darkroom as needed. Good potting soil of light texture was found the best cultural medium. Soil used for soybeans was inoculated with a suitable strain of *Rh. japonicum*. All plants were grown from seed in their permanent places and maintained under uniform nutrition and exposure and, of course, a definite photoperiod. Those to receive a short day were given 7 hours of daylight, between 8:00 a. m. and 3:00 p. m. The rest of the time they were kept in the ventilated darkroom. They will be referred to hereafter as "short-day" plants. The "long-day" plants were oxposed to the full length of day and, if necessary, additional electric illumination in the evening to bring the light period to 14 hours. Mazda bulbs in reflectors supplying about 250-foot candles near the tops of plants were used for the artificial lighting (147).

With this treatment groups of plants could be raised repeatedly that were quite alike in size, appearance and general chemical composition. Of the over 80 series of soybeans grown for various studies, only 6 or 7 plantings were not strictly to type because of extreme weather conditions. Naturally such cultures were discarded promptly to give room for new ones.

A few groups of soybeans were grown in sand for the purpose of testing, in connection with photoperiodism, the possible effects on reproduction of some of the synthetic "growth substances" or "plant hormones" (82). The seeds for these cultures were germinated in sphagnum moss and planted in new 5-inch pots containing moistened quartz sand. Other groups of plants were grown similarly in soil of the same volume.

These sand cultures received the following nutrient solution (213):

Partial volume molecular concentration and grams per liter of salts in nutrient solution.

KH_2PO_4		$CaCl_2$		$Ca(NO_3)_2$		$MgSO_4.7H_2O$	
P. V. M.	Gms	P. V. M.	Gms	P. V. M.	Gms	P. V. M.	Gms
.0063	.862	.0015	.162	.0044	1.038	.0024	.584

This solution was adjusted to approximately P_H 6.0 and used liberally. Periodically 5 cc of each of weak solutions of iron, manganese and boron were also supplied. Plants thus treated grew quite normally—as well as those in soil.

A few series of soybean plants, var. Biloxi were raised in sand cultures with $Ca(NO_3)_2$, $(NH_4)_2SO_4$ or NH_4OH as source of nitrogen. Large glazed containers were used for this purpose. The quartz sand was thoroughly washed. Composition of the nutrient solutions, adjustment of P_H and other procedure was that used by Tiedjens and Robbins (213). The sand was flushed thoroughly at intervals of a few days.

A few words should be said about the Biloxi variety of soybeans, which served as material for practically all of the detailed chemical studies. It is strictly a short-day plant. With a 7-hour exposure to light, the plants become sexually reproductive after a comparatively brief period of vegetative growth. Normally the first initiation of flower buds begins about 14 days after the seedlings emerge, which is usually 4-5 days after seeding. Flowering begins in 30-45 days, depending to a considerable extent upon the prevailing weather and, primarily, the temperature of the greenhouse (Murneek and Gomez, 147). The long-day plants, receiving a diurnal exposure to light of 14 hours, remained strictly vegetative, with a more or less indefinite delay in flowering. For a detailed description of the soybean material the reader is referred to Missouri Agricultural Experiment Station Research Bulletin 242.

CHEMICAL METHODS

Sampling and Preservation

Plant material used for chemical analysis or for determination of enzyme activity was sampled as a rule either at sunrise or at 8:00 a. m., when the short-day plants were taken out of the darkroom. It was thought that these precautions helped to obtain more uniform material than when the sampling was done at other times of the day.

It was noted that in collecting whole plants or parts thereof for chemical analyses, the greatest care must be exercised that

the material is representative and consists of physiologically related more or less uniform tissues. Differences in results of biochemical work of this type seem to be due more to variations in age of plants, time of sampling, and kind and amount of tissues analyzed than to differences in precision of the chemical methods used.

Plants selected for analysis were cut near the ground and taken to the laboratory at once. When roots were also included in the sample, the soil was run through a coarse sieve and all roots collected, washed carefully and dried between layers of absorbent paper. The number of plants per sample varied greatly, from a few dozen to several hundred, depending upon the use to which the material was put. In most instances several men asssisted in the laboratory in dissecting, cutting and weighing the material preparatory to its preservation. This work was executed with dispatch and usually completed in one-half to two hours. When for some reason there was a delay, as in cases where the fresh tissue had to be ground and thoroughly extracted with water, the plant material was frozen promptly at -14°C. and then used at one's convenience.

For most of the chemical analyses the cut up parts of the plants were dried at 75°C. in a ventilated oven. The desiccated material was ground to a fine powder, first in a Wiley and then a Merker mill and preserved in stoppered bottles.

Carbohydrate Analyses

The various carbohydrates were determined on 2-gram samples by the method and procedure described in detail by the writer elsewhere (137). Included were the reducing and total sugars, starch, and the acid hydrolyzable residue known as "hemicellulose" (143).

Nitrogen Analyses

Total N.—It was determined on one gram samples of the air dry powder by the official Kjeldahl-Gunning-Arnold method to include nitrates, as modified by Ranker (180).

Nitrogen Fraction. Extraction.—In order to study nitrogen metabolism more closely in the experimental plants, fresh and frozen (151) plant material was used for the determination of the various nitrogen fractions.

Two hundred grams of the fresh tissue was cut into very fine pieces by means of a sharp knife and then ground with 25-50

grams of washed quartz sand in a large mortar. After 20 minutes of thorough maceration, distilled water was added to the pulp-sand mixture, allowed to soak, and the whole washed upon lawn cloth and extracted with water. This operation, grinding for 20 minutes and washing, was repeated 3 times, resulting in extraction of practically all of the soluble fraction of the cells. Kjeldahl determinations of the residue showed that in the case of soft tissue, such as leaves and the more succulent parts of the stem, all but about 10 per cent of the nitrogen was removed. In more lignified tissue there remained approximately 10-30 per cent of the nitrogen unextracted (34-35, 150, 154). Almost all of this, undoubtedly, was in the insoluble or protein form. The total water extract amounted to 2 liters.

Coaguable N.—Four 500 cc aliquots of the water extract were heated to boiling in 8 minutes, 5 cc of 10 per cent acetic acid added and boiled for 1 minute longer. The coagulum was filtered immediately with suction over nitrogen-free paper pulp through large Büchner funnels and the precipitate washed with water. After thorough drying at 80°C., the nitrogen content of the coagulum was determined by the usual Kjeldahl method.

Soluble N.—The protein or coagulum-free filtrate was made up again to 2 liters. Duplicate aliquots, slightly acidified, were evaporated with aspiration to dryness in Kjeldahl flasks. Great care must be exercised in this procedure, else the nitrogen content of the residue may vary.

Protein N.—The unextracted plus coaguable nitrogen may be designated by this name (36, 150).

Proteose N.—Aliquots of soluble nitrogen were made slightly acid with H_2SO_4 and then saturated with $ZnSO_4$ at room temperature. After standing for 12 hours, the solution was passed through quantitative filter paper, which was washed with saturated solution of $ZnSO_4$. Nitrogen was determined on the air dry precipitate.

Proteose nitrogen is supposed to consist of partial hydrolytic decomposition products of proteins, soluble in H_2O, non-coaguable by heat and precipitated by certain salts (156).

Basic N.—This fraction was determined by Osborn's method. Aliquots of 100 cc of the soluble nitrogen solution were made acid with 5 cc of concentrated H_2SO_4. When cooled to room temperature, 30 cc of phosphotungstic acid solution (20 grams phosphotungstic acid and 5 grams of H_2SO_4 in 100 cc H_2O) was added,

1-2 cc at a time. After standing for 24 hours, the solution was filtered and nitrogen determined on the precipitate.

Basic nitrogen is made up to a large extent of diamino acids—arginine, histidine, lysine, cystine, etc. (155).

Since phosphotungstic acid precipitates also proteose, the latter, if considered separately, must be deducted from the basic nitrogen figures.

Ammonia N.—Aliquots of 250 cc of the soluble nitrogen solution were made alkaline with magnesium oxide cream and ammonia distilled into normal .02 acid. To remove carbonates, which sometimes interfere with the titration, the magnesium oxide was burned for a prolonged time in a muffle furnace.

Amid N.—The resultant solution from ammonia distillation was made acid to litmus and enough H_2SO_4 added to make a 5 per cent concentration. After refluxing for 2 hours on a water bath, it was made neutral to methyl red and alkaline with magnesium oxide and the ammonia was distilled off.

Humin N.—After amid nitrogen was removed the resultant solution was filtered while hot and nitrogen determined on the dry residue.

Humin nitrogen is considered as being made up largely of derivatives of tryptophane and other soluble nitrogenous substances difficult to hydrolyze (68, 156). On acid hydrolysis most proteins will give rise to humin through condensation of certain amino acids with glucose and other aldahydic compounds.

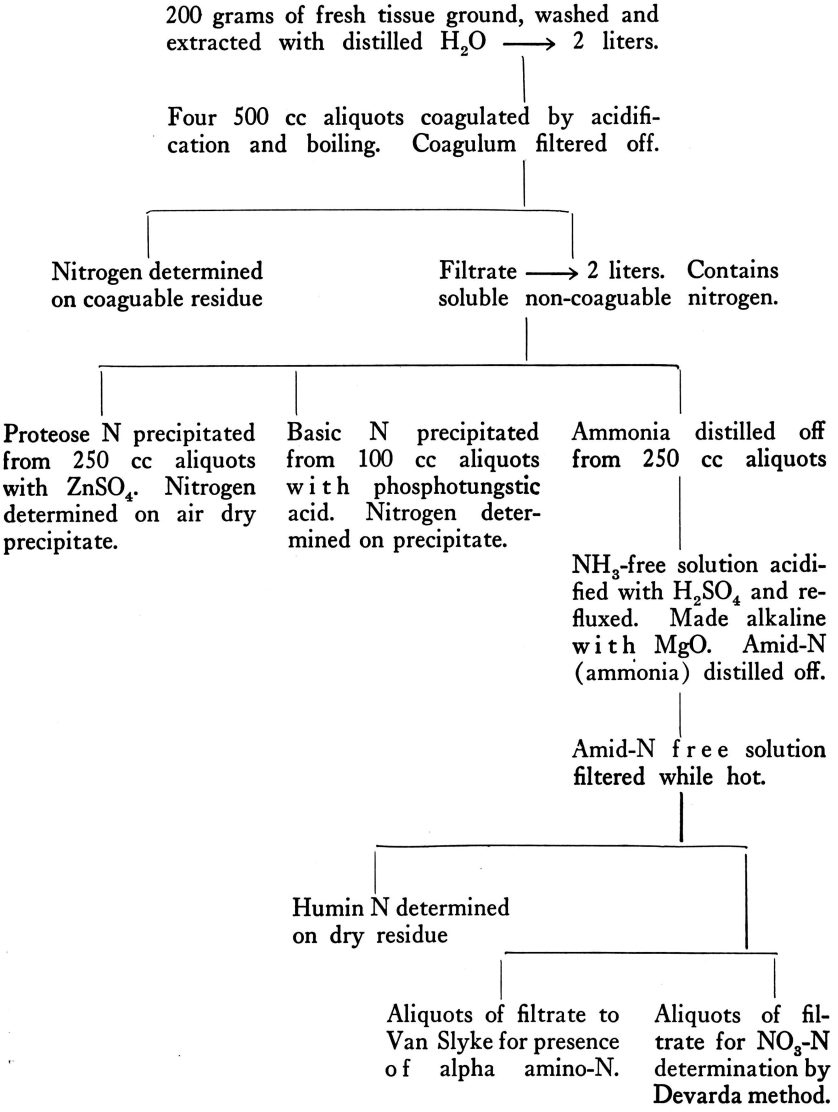
Amino N.—Aliquots from the humin nitrogen filtrate were made acid to litmus with glacial acetic acid and the alpha amino N determined by means of a Van Slyke apparatus, using a micro burette.

Nitrate N.—It was determined by the Devarda method on the humin nitrogen filtrate.

This method has been criticized by Phillips (160), who suggests that it may be employed satisfactorily if ammonia and amid nitrogen are removed before nitrate N is determined. This was done in the present procedure.

The following outline is presented for better visualization of the procedure adapted for fractionation of nitrogen compounds.

Schematic Outline of Fractionation of Nitrogen Compounds



Chloroplast Pigments

Duplicate and triplicate uniform samples of selected fresh plant tissue were chopped finely and ground with a little Na_2CO_3. When thoroughly disintegrated, about 75 cc of acetone was added and the grinding continued. The completely macerated tissue was

washed and filtered with acetone till the filtrate was colorless and then with ample amounts of ether.

Separation and purification of chlorophyll ($\alpha + \beta$), carotene and xanthophyll, the three chloroplast pigments taken into consideration, were conducted according to Willstätter and Stoll's method as modified by Schertz (199). The purified pigments were brought to volume and their quantities determined colorimetrically according to the procedures outlined by Schertz (196-198), using for comparison freshly prepared pure pigment solutions. The chloroplast pigment standards were of the following concentration: Chlorophyll—200 mgs. per liter, saponified with methyl alcoholic potash; carotene—100 mgs. per liter in petroleum ether; xanthophyll—100 mgs. per liter in absolute ethyl alcohol. Their purity was ascertained before each group of determinations by using the various concentration graphs prepared by Schertz.

Respiration

The apparatus of Heinicke and Hoffman (78) was employed for preliminary studies on the effects of photoperiod on respiration. Whole plants, sealed from soil in which they were growing, and properly enclosed in air-tight dark chambers, were used for this purpose.

While the determination of the amount of CO_2 released by the plant may not be acceptable by some physiologists as a satisfactory index of respiration, it gives at least a general idea of the approximate comparative rates of oxidation. The carbohydrates undoubtedly furnish the greatest proportion of material used in respiration, if they are present in sufficient amounts.

Enzymes

The enzymes studied in connection with this investigation were two related to oxidative processes—catalase (18) and peroxidase (19); two connected with carbohydrate metabolism—amylase and invertase; and reducase (79), which is thought to effect the reduction of nitrates in plants (49, 50).

Results obtained from the enzyme work will be the subject of a separate report, that will appear shortly.

PRESENTATION OF RESULTS

To make the factual evidence and the accompanying discussion as brief and readable as possible, only results from representative groups or series of plants are given herewith. In every instance the experiments reported have been duplicated and in many cases the records are in triplicate and quadruplicate. Nothing would seem to be gained and there is a distinct disadvantage in burdening the reader with a mass of quite similar data.

It will be evident forthwith that these studies deal primarily with the soybean, var. Biloxi. The growth and development of this plant under the various experimental treatments has been observed in detail and presented elsewhere (147). Other material has been used only in a supplementary way.

In considering the developmental physiology of the Biloxi soybean plants, the following facts must be kept in mind: (a) Induction of sexual reproduction of the short-day plants probably took place during the first 4-14 day period of growth of the seedlings, counting from the day of emergence above ground, although its inception may have been even earlier. Excepting for slightly lower stature and lighter color of foliage, both long- and short-day plants were quite similar in development during this time. (b) The earliest signs of initiation of the first potential and probably actual flower buds were observed in 14 day old plants and, of course, continued from then on. (c) The flowering stage was reached in about 30-45 days after germination, but on a few occasions this was delayed considerably in midwinter due to low temperature and cloudy weather.

To emphasize the physiologically "critical" time of induction of reproduction, the 7-14 day period has been shaded in the graphs.

The Growth Rate

When exposed to markedly different photoperiods most plants that are sensitive to the length of day are affected not only in respect to sexual reproduction but also as regards their vegetative development. Often enough, but by no means always, the stature of the plant is so altered that they are hardly recognizable as belonging to the same species or variety. The curtailment in stem elongation by a photoperiod, *photoperiodic inhibition,* is of quite common occurrence (60, 16, 17, 148). Examples are presented herewith of two types of influence of a relatively short day on stem elongation: (a) Complete inhibition of stem development resulting in a flat permanently vegetative rosette (Fig. 1) and (b) retardation of growth in height leading eventually to complete

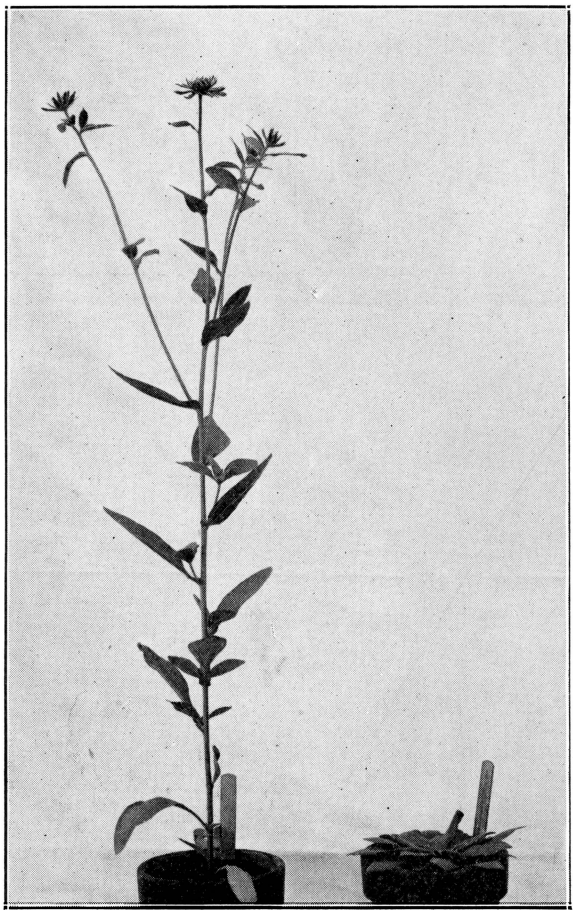


Fig. 1.—Relative growth in height of *Rudbeckia* plants of the same age. Left—plant exposed to a long day (14 hours). Right—plant exposed to short day (7 hours).

inhibition while the plant is comparatively young, followed by sexual reproduction (Fig. 2 and Table 1).

If measured in greater detail, there may be a slight more or less continuous increase in stature of the short-day soybean plants as a result of possible stimulation incident to synapsis and gametic union. This, however, is of no significant importance in the present discussion, since we are not dealing at present with a detailed consideration of various phases of physiology of sexual reproduction, but almost entirely with photoperiodism.

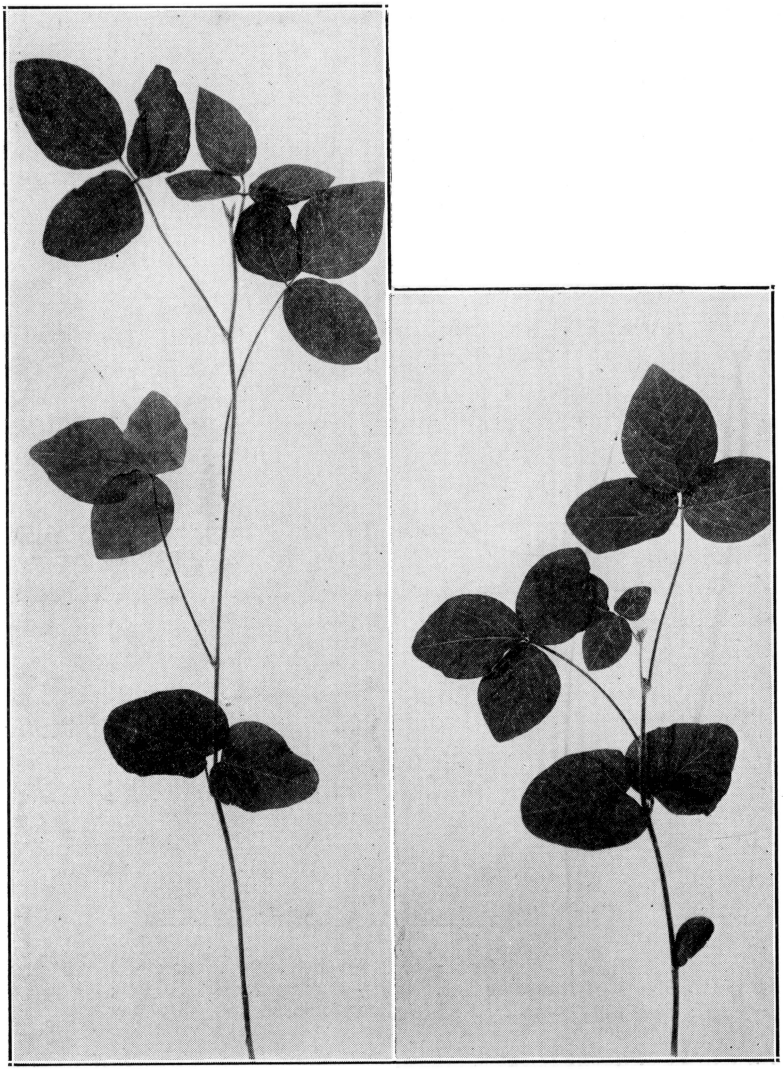


Fig. 2.—Relative growth in height of Biloxi soybean plants of the same age. Time of flower bud formation of the short-day plants. Left—plant exposed to a long day (14 hours). Right—plant exposed to a short day (7 hours).

That the curtailment in growth of the stem is not the result of development of flowers and fruits, as is the case of some other plants (136, 137, 144) can be demonstrated by disbudding and defloration experiments (17).

TABLE 1.—RATE OF STEM ELONGATION OF SOYBEAN PLANTS, VAR. BILOXI. HEIGHT IN CENTIMETERS.

Age, in Days	Long-day Plants	Short-day Plants
	(cm.)	(cm.)
2	4	4
6	10	10
12	18	17
20	25	20
27	33	21
33	42	21
40	48	21

Two groups of short-day plants were disbudded continuously for periods of 26 and 60 days respectively. In neither instance terminal growth could be induced, but the usual axillary shoots were produced, just like on normal fruiting short-day plants (147). There was a noticeable swelling of the nodes of the disbudded plants and their foliage was of an extremely dark green color.

Photoperiodic inhibition is a phenomenon that is distinctly separable from the effects of photoperiod on sexual reproduction (148). In the above examples it was associated either with vegetative type of development (*Rudbeckia*) or with reproduction (*Soja*). This subject will be discussed in greater detail in another publication.

Photoperiodic inhibition is of utmost importance in studies of the physiology of photoperiodism, with special reference to chemical composition of the plants. It stands in cause and effect relationship to the accretion and concentration of various substances in the organism.

Fresh and Dry Weights of Plants

At the time of full development of the reproductive organs (flowers) the percentage of dry matter is usually much higher in the short-day (reproductive) than in long-day (vegetative) soybeans of the same age, especially so in the stems (Table 2).

TABLE 2.—DRY MATTER IN SOYBEAN PLANTS.

	(In Percentage of Fresh Weight)		
	Peking	Tokyo	Biloxi
Vegetative:			
Stems	18.72	18.20	18.46
Leaves	21.62	19.01	18.35
Roots	11.63	11.90	9.43
Reproductive:			
Stems	27.86	27.90	25.30
Leaves	25.33	27.97	22.65
Roots	13.47	13.90	11.26

A better view of the effects of photoperiod on the relative development of various parts of the Biloxi soybean may be obtained by a study of total fresh and dry weights of plants of various ages. Table 3 shows that there was a continuous increase in weight of the stems of both short- and long-day plants. Naturally this increase was greater in the latter due to their larger stature. Augmentation in weight beginning with the twenty-seventh day of those under short-day exposure was due largely to the percentage increase in dry matter.

TABLE 3.—INCREASE IN FRESH AND DRY WEIGHT OF SOYBEAN PLANTS, VAR. BILOXI. IN GRAMS PER 100 PLANTS.

	Fresh Weight					
	Long-day Plants			Short-day Plants		
Age—Days	Stems	Leaves	Cotyledons	Stems	Leaves	Cotyledons
6	39.7	26.0	99.4	44.9	26.2	96.8
12	83.0	87.6	82.3	76.1	78.6	89.5
20	142.7	151.6	----	93.2	111.4	----
27a	205.0	345.0	----	116.0	266.0	----
33b	284.8	289.8	----	128.3	208.4	----
40c	368.5	249.0	----	145.3	173.5	----
	Dry Weight					
	Long-day Plants			Short-day Plants		
Age—Days	Stems	Leaves	Cotyledons	Stems	Leaves	Cotyledons
6	3.3	4.1	10.5	3.6	3.8	7.3
12	9.0	15.5	5.5	6.9	10.1	6.2
20	19.9	26.8	----	10.8	16.4	----
27a	30.6	60.2	----	17.9	44.9	----
33b	51.2	62.4	----	28.8	45.8	----
40c	76.6	60.8	----	39.8	49.2	----

a—Flower buds on short-day plants.
b—Flowers on short-day plants.
c—Flowers and small pods on short-day plants.

In both groups of plants the leaves reached maximal development approximately on the twenty-seventh day, thereafter their total weight either remained stationary (short-day group) or decreased (long-day group). As new leaves developed, the lowermost became senescent and abscissed in succession. Decreasing some in fresh weight and rapidly in dry weight, the cotyledons abscissed between the twelfth and twentieth day on practically all plants, but less so in the case of those exposed to a short photoperiod.

If the accumulation of dry matter of the two groups of plants is followed throughout their development, then the picture obtained is of still greater significance. It will be noted from Table 4 and Figure 3 that during the period of induction of reproduction ("critical" period), the relative amount (percentage) of dry matter in stems and leaves was considerably smaller in the short-day (repro-

TABLE 4.—CHANGES IN DRY WEIGHT DURING GROWTH OF SOYBEAN PLANTS, VAR. BILOXI.

Age—Days	Percentage of Dry Matter					
	Stems		Leaves		Cotyledons	
	Long-day	Short-day	Long-day	Short-day	Long-day	Short-day
6	8.2	7.9	15.8	14.3	10.6	7.6
12	10.9	9.1	17.7	12.8	6.7	6.9
20	13.9	11.6	17.7	14.8	----	----
27a	14.9	15.4	17.4	16.9	----	----
33b	18.0	22.5	21.5	23.8	----	----
40c	20.8	27.4	24.3	28.4	----	----

a—Flower buds on short-day plants.
b—Flowers on short-day plants.
c—Flowers and small pods on short-day plants.

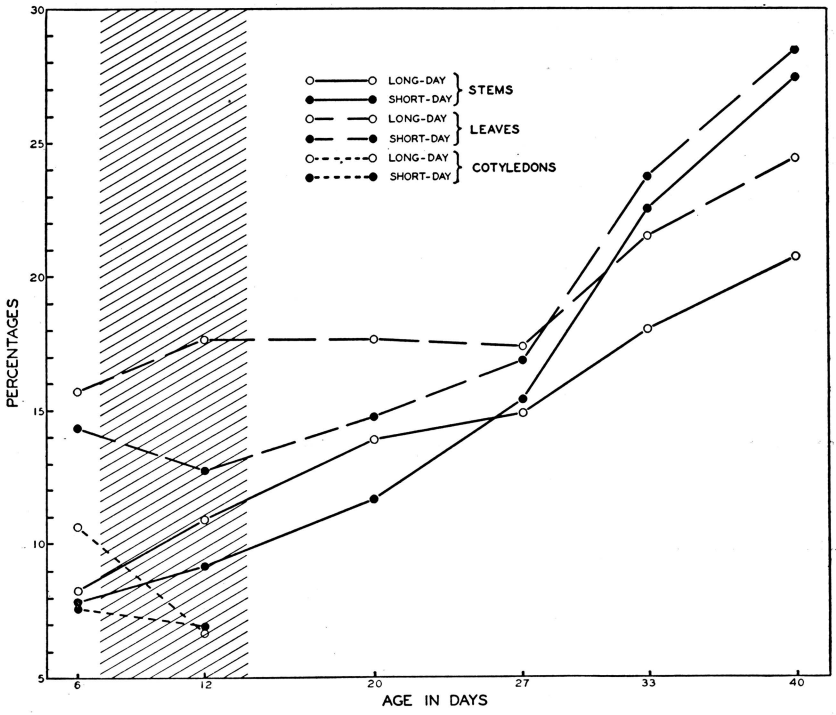


Fig. 3.—Effects of photoperiod on changes in dry weight of stems and leaves during growth of the Biloxi soybean plant. In percentages of fresh weight. Time of photoperiodic induction is shaded.

ductive) than in the long-day (vegetative) plants. Only at the time of flower bud development the concentration of various substances in the stems, and soon thereafter also in the leaves, of the short-day group begins to exceed that of the long-day ones.

This difference becomes marked at the time of full bloom and later, as has been pointed out already (Table 2).

That the accretion of dry matter stands in no direct relationship to flowering can be demonstrated with several plants, especially those that are not greatly inhibited in their vegetative development by a photoperiod, *Salvia* and *Cosmos*, for example.

Nitrogen and Carbohydrate Content

No soil nutrient substance is probably as effective as nitrogen in promoting growth and altering development, while the total dry weight of a plant is determined largely by the concentration of diverse types of carbohydrates, principally polysaccharides. Usually a large proportion of the latter substances—starch, hemicellulose, etc.—are storage reserves and hence are largely available for metabolic processes.

Total Nitrogen.—The total nitrogen content of stems, leaves and roots of three varieties of soybeans, at the time the short-day plants had fully developed flower buds, is presented in Table 5. It shows that the nitrogen concentration in the stems and roots was conspicuously higher in the short- than in the long-day plants of corresponding ages. The leaves seem to participate but slightly, if at all, in the accretion of this soil nutrient. Despite this increase in nitrogen, the short-day plants remained inhibited in growth (Table 1), due to the direct effect of the reduced photoperiod on stem elongation (148).

TABLE 5.—TOTAL NITROGEN IN THREE VARIETIES OF SOYBEAN PLANTS. (PERCENTAGES ON DRY WEIGHT BASIS).

	Peking	Tokyo	Biloxi
Vegetative:			
Stems	2.48	2.57	2.71
Leaves	4.14	4.63	5.39
Roots	2.82	2.92	2.95
Reproductive (in bloom):			
Stems	4.36	4.28	3.61
Leaves	4.91	4.68	5.40
Roots	3.18	3.47	3.82

Developmental changes in total nitrogen concentration in various parts of both short- and long-day plants are given in Tables 6 and 7. It will be observed (Table 6 and Figure 4) that, while there was a decrease with age in percentage of nitrogen in all parts, the total quantity per plant increased, due to a continuous absorption of N from the soil and a maintained function of nodule bacteria. The greater augmentation of nitrogen, and of many other

TABLE 6.—CHANGES IN TOTAL NITROGEN CONTENT OF SOYBEAN PLANTS, VAR. BILOXI.

Age—Days	Percentage on Dry Weight Basis					
	Stems		Leaves		Cotyledons	
2	7.51		----		9.21	
	Long	Short	Long	Short	Long	Short
6	8.15	8.96	8.17	8.93	10.43	9.90
12	6.44	7.52	6.40	7.79	7.34	9.21
20	3.73	4.87	5.82	6.80	----	----
27a*	3.33	4.02	5.24	5.74	----	----
33b	3.04	3.61	4.54	4.80	----	----
40c	2.75	3.13 (Pods 5.16)	3.82	4.20	----	----
80d	1.55	3.77 (Pods 4.90)	2.39	4.88	----	----

Age—Days	In Grams per 100 Plants					
	Stems		Leaves		Cotyledons	
2	.27		----		1.28	
	Long	Short	Long	Short	Long	Short
6	.27	.32	.33	.37	1.10	.72
12	.58	.52	.99	.78	.41	.57
20	.74	.53	1.56	1.12	----	----
27a*	1.02	.72	3.15	2.58	----	----
33b	1.56	1.04	2.83	2.24	----	----
40c	2.11	1.25	2.32	2.07	----	----

*See footnote under Table 4.
d—Pods of various sizes (up to 4 cm. long) on short-day plants.

substances, with the short photoperiod, of course, is the result of photoperiodic inhibition—curtailed growth and consequent reduction in utilization of nitrogen and other building materials for vegetative development.

That there was a greater reduction in nitrogen concentration of the stems of the reproductive (short-day) than the vegetative (long-day) plants is evident from Table 7. Naturally this decrease begins at the time of flower development and is accelerated when, in addition to flowers, the plants support also fruits, which have a high N content. The internodes and bases of the stems seem to release the stored nitrogen more readily than the nodes, whence to the seeds there is usually an ascending gradient of this element.

In general, the gross distribution of total nitrogen in the soybean plant seems to be as follows. From its storage organs, cotyledons, the seedlings draw it rapidly and completely for the development of the stem, roots and young leaves. As soon as the reserves in cotyledons are exhausted, they absciss. In the short-day plants this removal of organic food from the cotyledons may not be completed before inhibition of stem growth commences and hence they

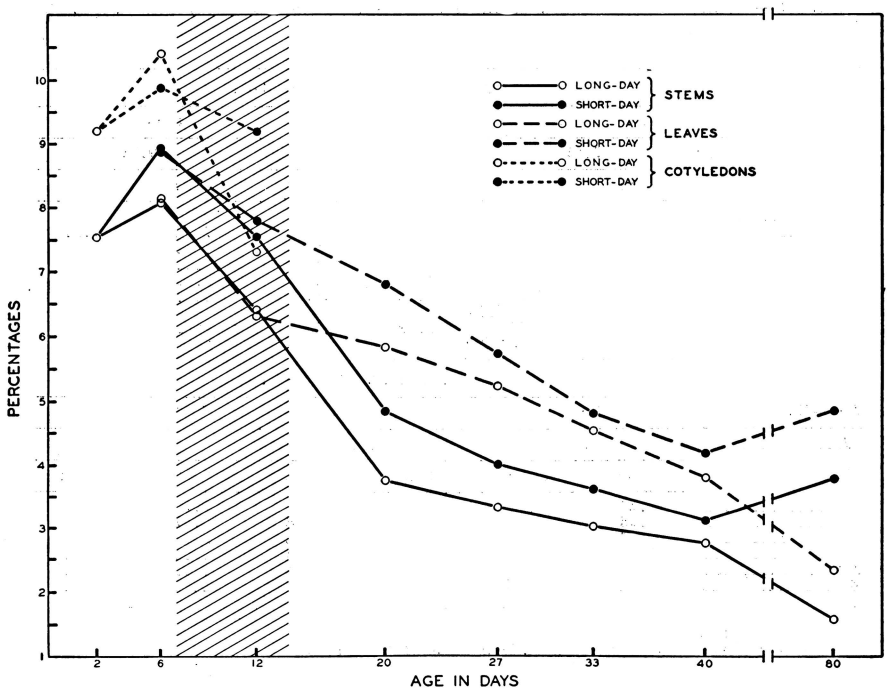


Fig. 4.—Effects of photoperiod on changes in total nitrogen content of soybean plants, var. Biloxi. In percentages of dry weight. The induction period is shaded.

TABLE 7.—NITROGEN CONCENTRATION IN VARIOUS PARTS OF STEMS OF SOYBEAN PLANTS, VAR. BILOXI.—PERCENTAGES ON DRY WEIGHT BASIS.

Stage in Development of Short-day Plants	Long-day Plants (Vegetative)			Short-day Plants (Reproductive)		
	Nodes and Tips	Internodes	Bases	Nodes and Tips	Internodes	Bases
Large flower buds (40 days old)	3.68	4.03	2.66	4.35	4.77	3.40
Flowers and young pods (49 and 52 days old)	3.36	3.39	2.23	3.92	3.17	2.81
Large pods (90 days old)	3.54	3.24	2.70	3.44	2.52 (Pods 4.30)	2.72

may persist for a long time assuming the function of leaves. Once the seedling is established, nitrogen is drawn from the soil and supplied by the root nodules. In vegetative plants it is utilized primarily for growth of the stem and development of leaves and roots. While in the short-day plants nitrogen is used for the same purpose

during the early period of growth, it begins to accumulate as soon as inhibition of stem elongation commences. As a result of the shortened photoperiod, the plant is so reduced in size that, after the initial period, very little nitrogen is required for its vegetative growth, and it is stored in large quantities. As soon as the reproductive organs begin to grow, nitrogen is used in increasing amounts for flower and fruit formation and growth of accessory tissues. Naturally other indispensable substances, especially the carbohydrates, are utilized likewise and in even larger quantities for reproduction.

Nitrogen Fractions.—Attempts have been made occasionally in research of this type to associate growth and development of plants with the relative accumulation of specific kinds or groups of nitrogen substances, especially in their relation to carbohydrate concentration (102-103, 149-150, 222, etc.). In almost all instances where plants had been subjected to such an analysis and interpretation, they were well advanced in their development, conspicuous flower buds, flowers and even fruit being present on those that had become "reproductive."

In order to learn how much and in what direction the nitrogen metabolism of whole plants and separately of the stems, leaves and roots may vary when they are exposed to definite photoperiods, *Cosmos, Salvia* and three varieties of soybeans were submitted to a detailed assay of certain N fractions. In all instances the short-day or "reproductive" plants had developed conspicuous flower buds, but were not in anthesis. The long-day or "vegetative" ones were grown in a similar environment, excepting of course for a different photoperiod, and were of the same age. The results are presented in Tables 8 and 9.

It is quite obvious from the records shown in Table 8 that there was a higher concentration of nitrogen in the reproductive plants. Judging from the greater proportion of soluble nitrogen, and of practically all determined groups of substances making up this portion of N, it would seem that the nitrogen metabolism was more active in the short-day plants in an early stage of sexual reproduction than in long-day plants of corresponding age. In light of our present knowledge, no particular nitrogen fraction can be assigned a specific dynamic role in the initiation and development of floral organs and associated tissues.

The carbohydrate content in this stage of development was either the same (*Cosmos*) in both groups of plants or higher (*Salvia* and *Soja*) in the "vegetative" ones. This undoubtedly was

TABLE 8.—NITROGEN FRACTIONS IN REPRODUCTIVE (SHORT-DAY) AND VEGETATIVE (LONG-DAY) COSMOS, SALVIA AND SOYBEAN PLANTS. PERCENTAGES ON DRY WEIGHT OF WHOLE PLANTS, EXCEPT ROOTS.

Kind of Nitrogen Fraction	Cosmos		Salvia		Soybean, var. Minsoy	
	Reproductive	Vegetative	Reproductive	Vegetative	Reproductive	Vegetative
Coaguable	1.756	1.273	2.04	1.24	2.91	2.51
Soluble	1.378	.881	1.25	.715	1.35	1.21
Unextracted	.138	.254	.33	.366	.24	.28
Protein	1.894	1.527	2.37	1.606	3.15	2.78
Proteose	.022	.013	.073	.067	.137	.11
Basic	.148	.023	.085	.005	.069	.062
Ammonia	.041	.020	.026	.019	.032	.028
Amid	.061	.025	.036	.003	.092	.057
Humin	.045	.021	.052	.045	.157	.185
a-Amino	.220	.086	.440	.360	.330	.33
Nitrate	.739	.527	.620	.091	.42	.30
Other Soluble N	.102	.166	.082	.125	.113	.136
Total N	3.272	2.408	3.62	2.321	4.50	3.99
Total Sugars	4.45	4.85	2.88	5.19	2.16	3.58
Starch	1.36	1.15	1.79	3.54	2.35	1.29
Hemicellulose	10.65	9.50	8.35	13.78	8.95	11.73
Total Carbohydrates	16.46	15.50	13.02	22.51	13.46	17.23

TABLE 9.—NITROGEN FRACTIONS IN STEMS AND LEAVES OF TWO VARIETIES OF SOYBEAN PLANTS THAT HAD BECOME REPRODUCTIVE UNDER A SHORT PHOTOPERIOD OR HAD REMAINED VEGETATIVE UNDER A LONG PHOTOPERIOD. PERCENTAGES ON DRY WEIGHT BASIS.

Kind of Nitrogen Fraction	Stems		Leaves		Roots	
	Reproductive (Short-day)	Vegetative (Long-day)	Reproductive (Short-day)	Vegetative (Long-day)	Reproductive (Short-day)	Vegetative (Long-day)
Biloxi—						
Coaguable	1.242	.593	2.76	2.85	1.297	.946
Soluble*	2.156	1.599	2.150	2.152	1.730	1.410
Unextracted	.315	.316	.490	.391	.417	.388
Protein	1.577	.908	3.250	3.241	1.714	1.334
Proteose	.107	.043	.100	.117	.082	.061
Basic	.314	.228	.414	.319	.315	.153
Ammonia	.089	.022	.043	.032	.050	.061
Amid	.517	.228	.218	.280	.350	.204
Humin	.109	.006	.270	.245	.070	.070
a-Amino	.844	.275	.470	.406	.462	.350
Nitrate	.176	.797	.635	.753	.401	.511
Total N	3.828	2.649	5.533	5.554	3.555	2.866
Peking—						
Coaguable	1.297	.698	2.985	2.790	----	----
Soluble*	2.605	1.537	1.598	1.098	----	----
Unextracted	.458	.265	.332	.262	----	----
Protein	1.755	.963	3.317	3.052	----	----
Proteose	.201	.031	.160	.094	----	----
Basic	.278	.152	.434	.320	----	----
Ammonia	.103	.026	.005	.024	----	----
Amid	.678	.258	.172	.072	----	----
Humin	.239	.020	.182	.093	----	----
a-Amino	.811	.318	.333	.128	----	----
Nitrate	.295	.732	.312	.367	----	----
Total N	4.483	2.704	5.059	4.296	----	----

*Sum of the determined soluble fractions.

due to the longer photosynthetic period to which they were exposed.

The analysis of whole plants naturally does not give as true a picture of their composition as the determination separately of the chemical state of the major structural parts. In Table 9 are given data to show the concentration of various nitrogen fractions in the stems, leaves and roots of two varieties of soybeans. The "reproductive" plants in this instance were again in an early stage of development of sexual organs and the "vegetative" plants of equal age.

An examination of this table will show that the most conspicuous and possibly the most significant feature is the relatively higher nitrogen concentration of the stems of short-day plants. Almost all the determined fractions show this increase, with the exception of nitrate nitrogen, which was present in smaller amounts. The somewhat lower nitrate content in the stems under a short day very probably was due to their more rapid utilization in synthesis of organic nitrogen compounds during the period of sexual reproduction. This increased nitrogen metabolism in the reproductive period has been observed before (137, 142, 144). While the process may be largely anabolic, a higher nitrogen catabolism is also evident. It is shown by the increased concentration of the proteose form of nitrogen. The lower leaves and the basal part of the stem may undergo senescence while the top portion of the plant is in an active state of development, in this case in the direction of rapid initiation and formation of large numbers of floral organs and accessory tissues (137).

The roots markedly, and the leaves to some extent also, exhibit an augmented nitrogen metabolism of plants subjected to short photoperiods and in an early stage of sexual reproduction.

Since the most conspicuous changes in nitrogen composition seem to occur in the stems of plants grown with definite photoperiods, and the reproductive organs (flowers, fruit) being in close juxtaposition to the stem and drawing upon the nitrogen substances therein, analyses have been made of fractional parts of this organ secured from several series of soybeans, var. Biloxi.

The plants used for this purpose consisted of triplicate groups and were 39, 41 and 41 days old and remarkably similar in their development, considering that no other factor, excepting the photoperiod, was kept under strict control. The short-day ones were 21-23 cm. high, with 4 or 5 nodes, including cotyledonary (Figure 2, right) and with a noticeably greener foliage in comparison to those exposed to a long day. Vegetative development

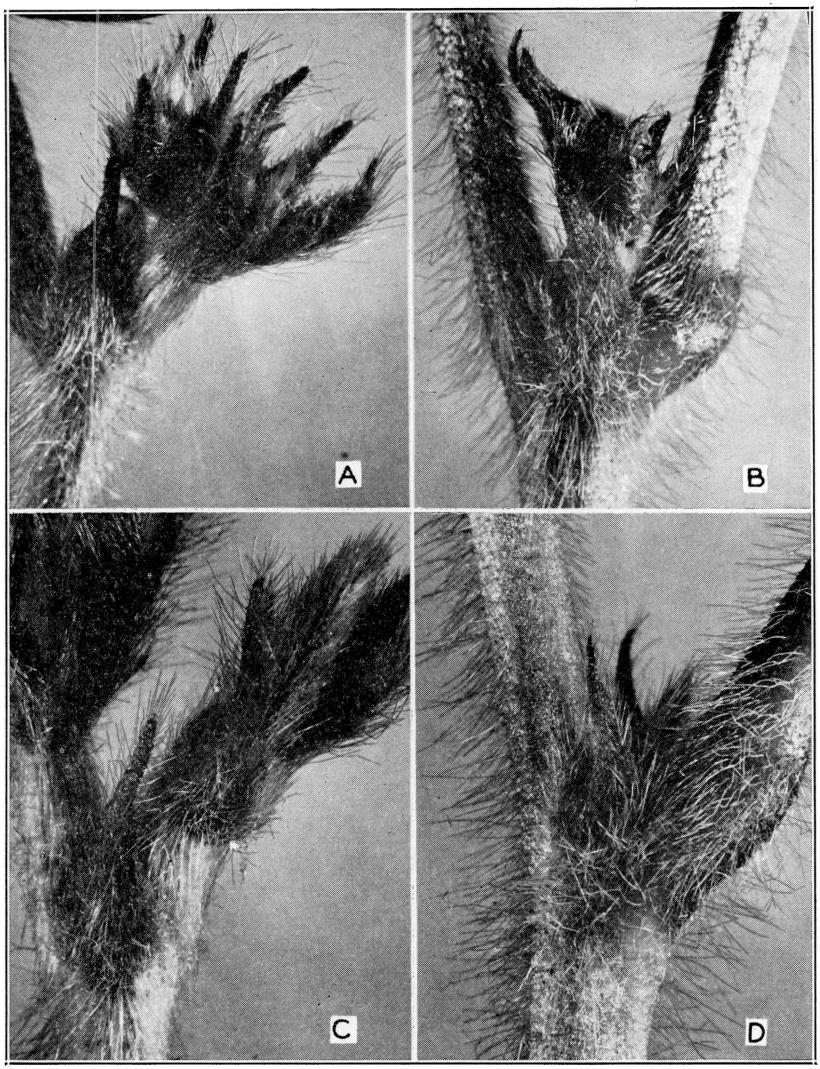


Fig. 5.—A closeup of plants of Figure 2, showing the development of terminal and axillary flower buds under a short photoperiod (A and B) and long photoperiod (C and D).

of these (short-day) plants had been inhibited for a period of about 3 weeks and flower buds were distinct at two topmost nodes and terminally (Figure 5, a and b). The corresponding long-day ones were 46-51 cm. high with 6 or 7 nodes, including cotyledonary

(Figure 2, left). Growth was continuing and there was no sign of formation of flower buds (Figure 5, c and d).

Several hundred plants were used in each case for chemical analysis. After the leaves had been cut off close to the nodes, the stem was partitioned into: *Tip*—terminal part nearest to dissected nodes, *nodes*—1.5 cm. long for short-day plants and 3.0 cm. for long-day plants, *internodes*—all tissues between nodes, and *base of stem*—basal part below point where last node was attached. In addition large numbers of stems were used for the removal of "two top nodes," each consisting of no more than ½-¾ cm. nodal tissue.

The results of these analyses are recorded in Tables 10 and 11. They are from two groups of plants, both were 41 days old and almost identical in general appearance, though grown at different times, one being cut on January 22, the other on March 16. The data are given mainly to show that two series of plants may differ considerably in the relative quantity of nitrogen present yet show the same general distribution of various determined fractions in the stems. The analyses given in Table 12 are of specimens 47 days old and in full bloom. They indicate that the same general distribution of nitrogen fractions existed in stems of plants in a more advanced stage of development.

The data in Tables 10 and 11 show that from the base of the stem, through internodes, nodes, to the tip there was an ascending gradient of total, coaguable, proteose, basic, ammonia and humin forms of nitrogen. Excepting for the stem base, a descending gradient in the same direction existed for nitrate, amino and amid forms. This was true for both the vegetative and reproductive plants. The soluble fractions, being a sum of all water soluble substances that have been taken into account, reflects the trend of those fractions that are present in the largest quantities. Should one interpret this situation as indicating that nitrogen in the form of nitrates, amino acids and amids are but the material present and available to the plant for the synthesis of proteins and protoplasm? In that sense not only nitrate-N but that of amino acids and amids should be considered still "non-assimilated."

In respect to availability a line is sometimes drawn between inorganic (nitrate) and organic (amino acid, etc.) forms of nitrogen, the latter being considered as already "assimilated." Evidence is accumulating, however, that in legumes amino acids are synthesized by the root nodules (our plants, of course, were inoculated) and translocated to other parts of the plant. They may be even excreted in large quantities from the nodules into the surrounding

TABLE 10.—NITROGEN FRACTIONS IN SOYBEAN STEMS, VAR. BILOXI (GROUP 1). SHORT-DAY PLANTS IN LATE FLOWER BUD STAGE AND LONG-DAY PLANTS OF CORRESPONDING AGE—41 DAYS.

	Total N	Coaguable	Soluble*	Proteose	Basic	Ammonia	Amid	Humin	*a*-Amino	Nitrate
	(Percentages on Fresh Weight Basis)									
Long-day (Vegetative)										
Tip of Stem	.634	.335	.246	.019	.036	.009	.028	.036	.087	.031
Two Top Nodes	.495	.139	.334	.006	.016	.004	.065	.010	.111	.122
All Nodes	.497	.084	.349	.012	.018	.005	.085	.011	.081	.137
Internodes	.509	.071	.373	.010	.016	.002	.094	.009	.101	.141
Base of Stem	.404	.056	.269	.008	.014	.002	.055	.010	.007	.171
Short-day (Reproductive)										
Tip of Stem	.917	.378	.536	.016	.030	.023	.172	.030	.202	.063
Two Top Nodes	.920	.252	.527	.007	.028	.019	.155	.026	.220	.072
All Nodes	.858	.162	.572	.016	.023	.009	.191	.026	.221	.086
Internodes	.975	.123	.687	.024	.027	.026	.228	.027	.243	.112
Base of Stem	.676	.095	.445	.010	.022	.012	.113	.013	.134	.141
	(Percentages on Dry Weight Basis)									
Long-day (Vegetative)										
Tip of Stem	4.22	2.231	1.647	.128	.239	.062	.189	.241	.582	.208
Two Top Nodes	3.96	1.155	2.689	.055	.137	.029	.540	.087	.825	1.016
All Nodes	3.82	.642	2.663	.092	.158	.039	.643	.081	.612	1.038
Internodes	3.91	.548	2.910	.074	.165	.019	.723	.068	.778	1.083
Base of Stem	2.44	.336	2.003	.050	.083	.013	.331	.059	.432	1.035
Short-day (Reproductive)										
Tip of Stem	4.93	2.098	2.734	.097	.167	.126	.663	.169	1.166	.346
Two Top Nodes	5.24	1.438	3.010	.047	.159	.109	.884	.148	1.253	.410
All Nodes	5.26	.997	3.517	.102	.142	.055	1.173	.157	1.359	.529
Internodes	5.34	.757	4.268	.149	.170	.160	1.417	.169	1.508	.695
Base of Stem	3.66	.514	2.356	.053	.119	.062	.614	.072	.672	.764

*Sum of the determined soluble fractions.

TABLE 11.—NITROGEN FRACTIONS IN SOYBEAN STEMS, VAR. BILOXI (GROUP 2). SHORT-DAY PLANTS IN LATE FLOWER BUD STAGE AND LONG-DAY PLANTS OF CORRESPONDING AGE—41 DAYS OLD.

	Total N	Coaguable	Soluble*	Proteose	Basic	Ammonia	Amid	Humin	*a*-Amino	Nitrate
	(Percentages on Fresh Weight Basis)									
Long-day Plants (Vegetative)										
Tip of Stem	.653	.432	.189	.024	.032	.005	.016	.033	.071	.008
Two Top Nodes	.434	.170	.244	.010	.018	.008	.044	.011	.105	.048
Nodes	.384	.096	.262	.013	.016	.004	.063	.012	.089	.065
Internodes	.442	.079	.304	.010	.018	.004	.085	.010	.111	.065
Base of Stem	.353	.075	.250	.010	.014	.005	.056	.007	.068	.090
Short-day Plants (Reproductive)										
Tip of Stem	.802	.414	.384	.015	.021	.006	.075	.041	.184	.041
Two Top Nodes	.917	.250	.632	.017	.018	.019	.198	.035	.294	.051
Nodes	.939	.181	.643	.014	.024	.016	.224	.029	.278	.058
Internodes	.950	.155	.679	.009	.004	.012	.250	.022	.309	.073
Base of Stem	.692	.127	.523	.012	.020	.013	.153	.021	.201	.103
	(Percentages on Dry Weight Basis)									
Long-day Plants (Vegetative)										
Tip of Stem	3.365	2.160	.956	.120	.170	.027	.078	.166	.355	.040
Two Top Nodes	3.225	1.118	2.047	.080	.153	.065	.371	.095	.880	.403
All Nodes	2.697	.646	1.771	.085	.111	.030	.422	.083	.602	.438
Internodes	2.845	.564	2.165	.069	.128	.028	.607	.072	.796	.465
Base of Stem	1.782	.346	1.148	.047	.065	.022	.254	.034	.312	.414
Short-day Plants (Reproductive)										
Tip of Stem	3.988	2.008	1.854	.071	.100	.027	.365	.202	.893	.197
Two Top Nodes	4.446	1.211	3.079	.083	.087	.094	.967	.172	1.427	.249
All Nodes	4.385	.845	3.004	.067	.112	.077	1.044	.135	1.297	.272
Internodes	4.457	.726	3.194	.041	.020	.058	1.175	.104	1.452	.344
Base of Stem	2.997	.548	2.262	.053	.087	.058	.662	.089	.869	.444

*Sum of the determined soluble fractions.

medium (Virtanen, *et al*). Then, too, since one of the primary products of symbiotic nitrogen fixation is aspartic acid (Virtanen and Laine), it is highly possible that asparagine and other amids (146) may be produced in the root system and moved to the stem in legumes, such as soybeans (See also Fred and Wilson).

The tremendous differences in chemical composition of various parts of the stem should make it evident that, in detailed analysis of nitrogen metabolism, it would seem to be highly desirable to take into consideration separately rather restricted portions of a plant. This, of course, is true of all major structural parts or organs. Referring to our present examples (Tables 10 and 11) it will be noted that the percentage of coaguable N, for example, may be 4, 5 or 6 times as high in the terminal as in the basal portion of the same stems. Very great differences in local concentration of other nitrogen fractions was also obtaining. Moreover, the quantity of tissue in the various portions will vary greatly, depending on the size of the plants. By grinding up and analyzing a whole plant or even a large part of the stem one may obtain an *average* composition that is quite non-existent and, therefore, the results are more difficult to interpret.

What differences, if any, are there between nitrogen metabolism of the vegetative (long-day) and reproductive (short-day) plants? In general, the stems of the reproductive ones are decidedly higher in almost all forms of nitrogen, excepting nitrates. This undoubtedly was due to their inhibited growth (photoperiodic inhibition) and the fact that the reproductive organs, which are of comparatively high nitrogen content, had not developed to a point where they would withdraw much nitrogen from the stem and other vegetative structures. The lower nitrate concentration in this part of short-day plants may be due either to the reduced absorption by the roots or to a more rapid transformation of nitrates to amino acids and other organic forms of nitrogen. The increased carbohydrate content of the short-day plants perhaps would tend to facilitate such a synthesis.

Attention should be called to the conspicuously lower relative concentration of nitrate nitrogen in the tips than elsewhere in the stem of the long-day plants. This may be explained on the simple and logical assumption that the terminal part of these plants is the region of greatest growth and development and therefore nitrates are utilized in very large quantities; hence the dilute state. The somewhat lessened concentration in the tips of stems of reproductive plants may be due to their use in formation of reproductive tissues. Flowers and fruits of the Biloxi variety of

TABLE 12.—NITROGEN FRACTIONS IN SOYBEAN STEMS, VAR. BILOXI. SHORT-DAY PLANTS IN FULL BLOOM AND LONG-DAY PLANTS OF CORRESPONDING AGE—47 DAYS OLD.

	Total N	Coaguable	Soluble*	Proteose	Basic	Ammonia	Amid	Humin	*a*-Amino	Nitrate
	(Percentages on Fresh Weight Basis)									
Long-day (Vegetative)										
Nodes	.527	.141	.340	.0069	.0416	.0064	.0518	.0120	.100	.095
Internodes	.524	.093	.423	.0044	.0534	.0067	.0788	.0107	.122	.124
Base of Stem	.590	.077	.466	.0055	.0608	.0060	.0812	.0130	.139	.137
Short-day (Reproductive)										
Nodes	.90	.278	.590	.0152	.036	.0200	.142	.0312	.211	.086
Internodes	1.08	.138	.802	.0052	.075	.0260	.189	.0240	.322	.103
Base of Stem	1.03	.122	.845	.0104	.131	.0224	.161	.0230	.306	.143
	(Percentages on Dry Weight Basis)									
Long-day (Vegetative)										
Nodes	3.96	.920	2.55	.052	.310	.048	.390	.089	.746	.714
Internodes	4.23	.717	3.23	.034	.413	.052	.610	.082	.945	.960
Base of Stem	3.31	.432	2.66	.031	.343	.034	.455	.073	.784	.770
Short-day (Reproductive)										
Nodes	4.50	1.190	3.02	.072	.180	.100	.708	.156	1.06	.430
Internodes	4.83	.616	3.98	.023	.333	.116	.843	.107	1.43	.458
Base of Stem	4.67	.535	3.88	.046	.596	.098	.710	.101	1.39	.468

*Direct determination.

soybeans are developed in largest aggregation in this region (147).

The apparently higher concentration of nitrates in the stems (Tables 10-13) and probably other parts (Table 9) of the vegetative long-day soybean plants precludes one to accept the idea expressed by some investigators that a short day (or long night) will tend to increase the nitrate content, because of marked curtailment of reducase activity. Moreover, by analyzing soybean plants of various ages or of particular cultural treatments, it is possible to show a higher nitrate content now in the short- and now in the long-day plants. Stems of Biloxi soybeans grown in a very rich soil, for example, were of higher nitrate content under short-day exposure (Table 13). Then, too, according to Eckerson (49) reducase activity, which is thought to be responsible for the reduction of nitrates, is affected markedly by other factors (soil nutrients, shading, etc.), which do not interfere greatly with photoperiodism.

TABLE 13.—NITRATE CONTENT OY SOYBEAN PLANTS GROWN IN VERY RICH SOIL. IN PERCENTAGES OF N ON DRY WEIGHT BASIS.

Stage of Development of Short-day Plants	Short-day (Reproductive)			Long-day (Vegetative)		
	Stems	Cotyledons	Leaves	Stems	Cotyledons	Leaves
Young plants, 20 days old	2.38	2.32	.812	1.83	2.12	.400
	Upper Stem		Lower Stem	Upper Stem		Lower Stem
Early flower bud stage	2.08		2.37	1.53		1.47
Flower buds and first flowers present	1.43		1.39	1.19		.82
Full bloom	.75		.76	.88		.66

TABLE 14.—RELATION OF SEXUAL REPRODUCTION TO NITRATE CONTENT IN STEMS OF SHORT-DAY SOYBEAN PLANTS, VAR. BILOXI.

Stage of Reproductive Development of Plants	Percentages on Fresh Weight Basis		Percentages on Dry Weight Basis	
	Upper Half of Stem	Lower Half of Stem	Upper Half of Stem	Lower Half of Stem
Flower buds, small	.084	.142	.65	.75
Flower buds, large	.082	.130	.48	.57
Flowering*	.073	.120	.40	.46
Small pods*	.067	.111	.32	.41
Medium size pods*	.055	.080	.28	.29

*Plants of the same age and collected on the same day.

During the period of active development of the reproductive organs (flowering and fruiting) the nitrate content of the upper stem is reduced conspicuously though the plants be kept through-

out this period in a shortened day. The decrease is greatest in the upper part of the stem where most of the reproductive tissue is developed. It takes place even in plants of unusually high nitrate content (Table 14). That this is not merely a phenomenon associated with increased age is indicated by the fact that samples collected on the same date from the same planting will show a decrease in nitrates with advance of reproductive development—from flowering to fruit setting and development of pods. The rapidly growing fruits draw upon the nitrogen supply, including nitrates, which of course have to be reduced before they can be utilized in synthesis of proteins. This reduction evidently can proceed in the soybean under a short-day exposure.

But assuming that there be a cause and effect relationship between the photoperiod, reducase activity, nitrate and carbohydrate accumulation and the developmental response of plants, one should be able to prove or disprove the validity of this idea by supplying plants exclusively with NH_3 and NO_3 ions as a source of nitrogen and subjecting them to a short and long photoperiod. The short-day plants receiving ample amounts of ammonia nitrogen should continue to grow in height and not accumulate organic reserves and possibly not become sexually reproductive, provided that there be a sufficient carbohydrate supply. We have shown elsewhere that, with normal temperature, the products of photosynthesis are present in large and often excessive amounts in the short-day soybean plants.

To provide a test, three series of soybean plants, var. Biloxi, were grown in quartz sand cultures and supplied throughout their life either with $Ca(NO_3)_2$, $(NH_4)_2SO_4$ or NH_4OH as source of nitrogen of the nutrient solution, as per procedure given elsewhere. Each group, of course, was subdivided into two parts for short- and long-day exposure.

Plants receiving NO_3 ions grew almost as well as in soil cultures, while those supplied with NH_3 were somewhat lighter green in color, especially the group given NH_4OH and the leaves had brown spots, mostly along the margins. This, however, in no way interfered with their response to the length of day. The results showed clearly that *irrespective of the form of nitrogen in the culture medium, all groups growing in a short photoperiod developed reproductive organs at the usual time, while those in a long photoperiod remained vegetative.* In this respect they behaved precisely the same as plants grown in soil. This would seem to point to the fact that any possible mechanism responsible for the reduction of

nitrates (NO_3 to NH_3), while operative in the plant when nitrate ions are present and used, probably has nothing to do with photoperiodism.

But were our NH_3 culture media and the plants growing in them really free of nitrates, despite the precautions taken? Bacterial action, leading to nitrification, probably was not entirely precluded. Tests made carefully and repeatedly with diphenylamine showed occasionally traces of presence of NO_3 in the sand and roots. In one or two cases there was a suggestion of nitrate reaction with this reagent also in the stems. Analyses of stems (whole) of plants, at flower bud stage, for ammonia and nitrate concentration are given in Table 15. Conspicuously greater

TABLE 15.—AMMONIA AND NITRATE CONTENT OF STEMS (WHOLE) OF SOYBEAN PLANTS, VAR. BILOXI, GROWN IN SAND CULTURES WITH NO_3 AND NH_3 AS SOURCE OF NITROGEN. PERCENTAGES ON FRESH WEIGHT BASIS.

Photoperiod and Source of Nitrogen	Ammonia N	Nitrate N
Long—$Ca(NO_3)_2$	.003	.013
Long—$(NH_4)_2SO_4$	.009	.002
Short—$Ca(NO_3)_2$	.004	.018
Short—$(NH_4)_2SO_4$	.012	.000
Short—NH_4OH	.006	.000

amounts of nitrate were present in the stems of plants receiving $Ca(NO_3)_2$ in the nutrient solution and of ammonia in those being provided with $(NH_4)_2SO_4$ or NH_4OH. It will be noted that some nitrate apparently was in the stems of long-day plants that were growing in the solution containing ammonium sulphate.

While convincing enough, the proof, therefore, cannot be conclusive until plants are grown absolutely without the presence of nitrates. This has been done by means of water cultures, the results of which will be reported in another publication. Therein will be presented also data on reducase determination. It may be said here that they preclude any possible direct causal connection between nitrate reduction (reducase activity) and inhibition of growth or induction of sexual reproduction by a photoperiod.

Carbohydrates.—The carbohydrate metabolism of plants as affected by the length of day is of general importance because of their preponderant use as building materials for the development of various organs of the plant. They are of specific interest in the present problem, since they have been posited by some investigators as participating in the creation of an internal environment in the plant which is supposed to be conducive to the initiation and maintenance of sexual reproduction.

TABLE 16.—EFFECTS OF PHOTOPERIOD ON CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI—IN PERCENTAGES OF FRESH WEIGHT.

Description of Plant Material	Red. Sugars		Total Sugars		Starch		Hemicellulose		Total Carbohydrates	
Plants 2 days old										
Stems	.266		.545		.616		3.05		4.477	
Cotyledons	.192		1.52		.067		3.25		5.029	
	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day
Plants 6 days old (2+4 days)										
Stems	.178	.036	.208	.186	.176	.086	1.370	.720	1.754	.992
Leaves	.050	.098	.320	.244	.132	.121	1.270	1.030	1.722	1.395
Cotyledons	.072	.045	.180	.190	.062	.000	1.168	.648	1.410	.838
Plants 12 days old (2+10 days)										
Stems	.095	.103	.204	.123	.129	.142	1.177	.664	1.510	.929
Leaves	.232	.082	.331	.156	.299	.211	1.504	1.975	2.134	2.342
Cotyledons	.114	.116	.146	.153	.062	.069	1.060	.987	1.268	1.209
Plants 20 days old (2+18 days)										
Stems	.152	.081	.238	.192	.162	.146	1.332	1.651	1.732	1.989
Leaves	.163	.113	.330	.162	.376	.148	2.338	1.495	3.044	1.805
Plants 27 days old (2+25 days)										
Stems	.096	.069	.173	.204	.052	.191	2.013	2.505	2.238	2.900
Leaves	.134	.135	.341	.426	.202	.144	2.200	1.906	2.743	2.476
Plants 33 days old (2+31 days)										
Stems	.288	.207	.628	.392	.584	2.205	3.011	3.265	4.223	5.862
Leaves	.368	.229	1.389	1.067	.979	.912	3.146	2.576	5.514	4.555
Plants 40 days old (2+38 days)										
Stems	.506	.584	.726	1.162	1.256	5.365	4.208	5.132	6.190	11.658
Leaves	.318	.329	1.402	1.230	2.568	3.560	3.823	4.467	7.793	9.257

TABLE 17.—EFFECTS OF PHOTOPERIOD ON CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI—IN PERCENTAGES OF DRY WEIGHT.

Description of Plant Material	Red. Sugars		Total Sugars		Starch		Hemicellulose		Total Carbohydrates	
Plants 2 days old										
Stems	1.54		3.16		3.57		17.68		25.95	
Cotyledons	.92		7.28		.32		15.58		24.10	
	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day
Plants 6 days old (2+4 days)										
Stems	2.16	.45	2.52	2.35	2.13	1.08	16.60	9.09	21.25	12.52
Leaves	.32	.68	2.03	1.70	.84	.84	8.06	7.18	10.93	9.72
Cotyledons	.68	.60	1.70	2.52	.58	.00	11.04	8.57	13.32	11.09
Plants 12 days old (2+10 days)										
Stems	.87	1.13	1.87	1.35	1.18	1.56	10.80	7.28	13.85	10.19
Leaves	1.31	.64	1.88	1.22	1.69	1.65	8.48	15.42	12.05	18.29
Cotyledons	1.70	1.69	2.17	2.22	.92	1.00	15.73	14.32	18.82	17.54
Plants 20 days old (2+18 days)										
Stems	1.09	.70	1.71	1.65	1.16	1.26	9.56	14.22	12.43	17.13
Leaves	.92	.76	1.87	1.09	2.13	1.00	13.23	10.08	17.23	12.17
Plants 27 days old (2+25 days)										
Stems	.64	.45	1.16	1.32	.35	1.24	13.47	16.24	14.98	18.80
Leaves	.77	.80	1.96	2.52	1.16	.85	12.64	11.28	15.76	14.65
Plants 33 days old (2+31 days)										
Stems	1.60	.92	3.49	1.74	3.25	9.80	16.75	14.52	23.45	26.06
Leaves	1.71	.96	6.45	4.47	4.55	3.82	14.62	10.80	25.62	19.09
Plants 40 days old (2+38 days)										
Stems	2.43	2.13	3.49	4.24	6.04	19.58	20.22	18.73	29.75	42.55
Leaves	1.31	1.16	5.77	4.33	10.56	12.54	15.73	15.73	32.06	32.60

TABLE 18.—EFFECTS OF PHOTOPERIOD ON CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI.—IN GRAMS PER 100 PLANTS.

Description of Plant Material	Red. Sugars		Total Sugars		Starch		Hemicellulose		Total Carbohydrates	
Plants 2 days old										
Stems	.056		.114		.129		.639		.882	
Cotyledons	.128		1.015		.045		2.173		3.233	
	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day	Short day
Plants 6 days old (2+4 days)										
Stems	.071	.016	.083	.084	.070	.038	.545	.323	.698	.445
Leaves	.013	.026	.084	.064	.034	.032	.330	.270	.448	.366
Cotyledons	.072	.044	.179	.185	.061	.000	1.161	.628	1.401	.813
Plants 12 days old (2+10 days)										
Stems	.079	.078	.169	.094	.107	.108	.976	.505	1.252	.707
Leaves	.204	.064	.290	.123	.262	.166	1.317	1.553	1.869	1.843
Cotyledons	.094	.104	.120	.137	.051	.062	.872	.885	1.043	1.084
Plants 20 days old (2+18 days)										
Stems	.217	.076	.340	.179	.231	.136	1.900	1.539	2.471	1.854
Leaves	.247	.125	.502	.179	.572	.164	3.545	1.655	4.619	1.998
Plants 27 days old (2+25 days)										
Stems	.196	.081	.355	.237	.107	.223	4.120	2.915	4.582	3.375
Leaves	.463	.359	1.180	1.132	.699	.382	7.600	5.070	9.479	6.584
Plants 33 days old (2+31 days)										
Stems	.820	.265	1.787	.502	1.665	2.830	8.580	4.190	12.032	7.522
Leaves	1.067	.248	4.020	1.155	2.838	.987	9.120	2.790	15.978	4.923
Plants 40 days old (2+38 days)										
Stems	1.862	.849	2.673	1.690	4.625	7.800	15.500	7.465	22.798	16.955
Leaves	.796	.571	3.508	2.131	6.420	6.176	9.570	7.740	19.498	16.041

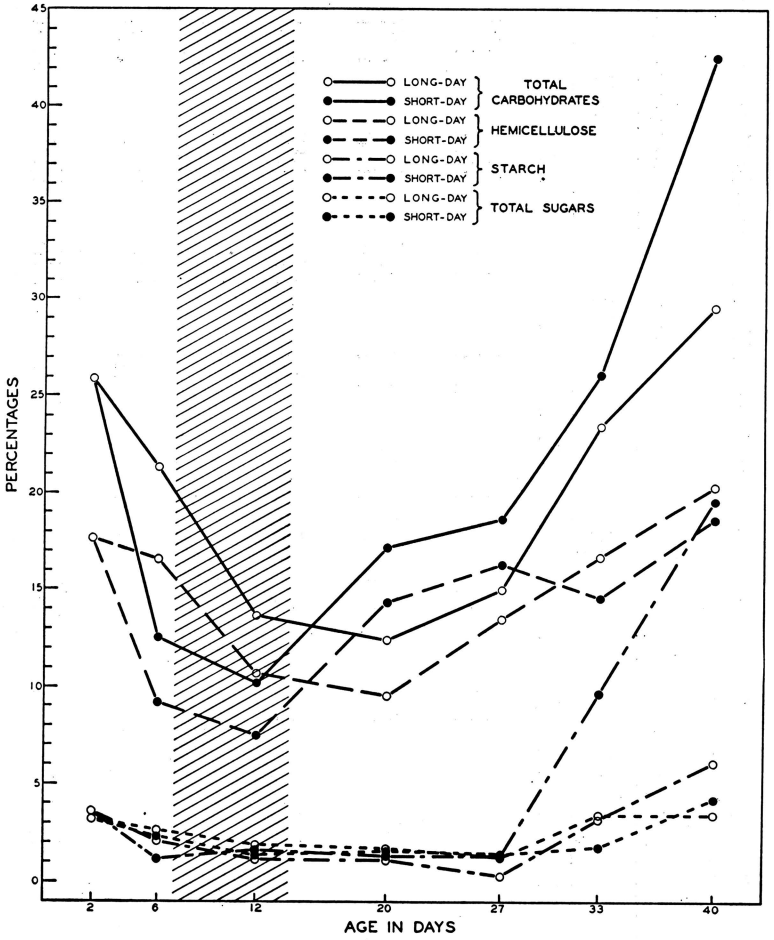


Fig. 6.—Relation of photoperiod to carbohydrate concentration of stems of soybean plants, var. Biloxi. In percentages of dry weight. Induction period shaded.

In Tables 16-18 and Figures 6 and 7 are presented data showing the carbohydrate contents of long- and short-day soybean plants of various ages. When the seedlings were but 2 days old the largest quantity of all available carbohydrates was in the cotyledons, whence they were translocated to the roots and to the stems, which had a relatively high concentration of sugars and starch. After the plants had been exposed for 4 days to definite photoperiods there was a still further decrease in carbohydrates in the cotyledons and an augmentation in the stems and newly developed

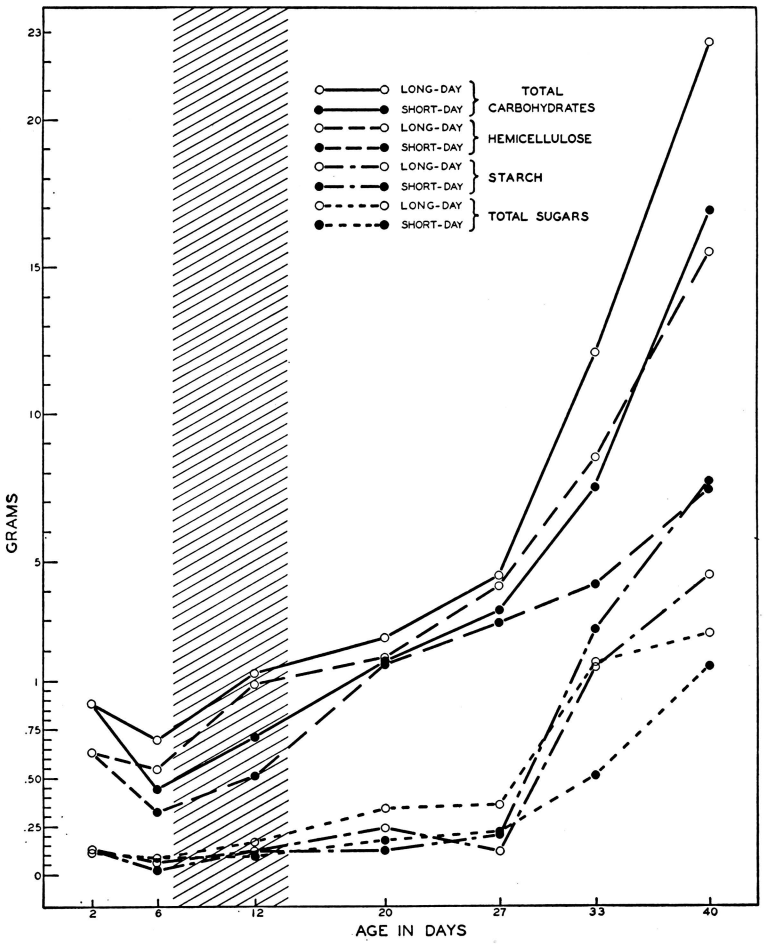


Fig. 7.—Relation of photoperiod to carbohydrate content in stems of soybean plants, var. Biloxi. In grams per 100 plants. Induction period shaded.

leaves. Because of the reduced light period, the short-day plants are now lower than the long-day ones in both starch and hemicellulose content. On the twelfth day, while the plants of both groups differed but little in size and appearance (Table 3), and sexual reproduction was either fully or at least partly induced, the total carbohydrate content of the stems of short-day plants was still lower, on the basis of both percentage and total amount. Many of the cotyledons that had not abscissed and were used for analysis, were functioning as leaves in the synthesis of carbohydrates. Be-

ginning with the twentieth day, when 18 days of experimental treatment had been given and as a result growth was inhibited under the 7 hour light period, the total carbohydrate content of the stems of short-day plants was higher in comparison to stems of plants exposed to a long day. Thenceforward the products of photosynthesis accumulated and were stored primarily as starch in ever increasing quantities. When, for example, the short-day plants were 33 days old and in bloom, the starch content (on fresh weight basis) in the stems was 2.205 per cent as against .584 per cent in the long-day plants (Table 16).

Despite their much smaller size, the quantity (grams) of starch present was markedly greater in the stems of short-day than long-day plants 27 days old or older (Table 18 and Figure 7). With increasing age the stems under a short photoperiod became packed with this storage carbohydrate, which sometimes made up over 20 per cent of its total dry weight. First mostly nitrogen and then the reserve carbohydrates apparently are utilized finally in the production of seeds and associated tissues.

Even if we assume an increased photosynthetic efficiency under a shortened day length (111), which has not been proven (207, 208), the very rapid storage of carbohydrates in the main structural and storage organs of these plants is the result of inhibited growth in height by the reduced light period, and photoperiodic inhibition is entirely independent of the effects of photoperiod on sexual reproduction (148 and unpublished records).

During the period of induction of reproduction and early initiation of flower buds (3rd-15th day) of the Biloxi soybean plant, there was no noticeable increase in carbohydrates in the stems of short-day (subsequently reproductive) plants. The data presented in Table 19 are from the upper portion of the stem of plants

TABLE 19.—CONCENTRATION OF CARBOHYDRATES AND TOTAL NITROGEN IN THE UPPER PART OF STEMS OF YOUNG SOYBEAN PLANTS, VAR. BILOXI.

	In Percentage of Fresh Weight				
	Red. Sugars	Total Sugars	Starch	Hemi-cellulose	Total N
Plants 12 days old—					
Long-day	.110	.168	.154	.876	.582
Short-day	.088	.104	.154	.696	.660
	In Percentage of Dry Weight				
	Red. Sugars	Total Sugars	Starch	Hemi-cellulose	Total N.
Plants 12 days old—					
Long-day	.87	1.33	1.22	6.95	4.62
Short-day	.71	.84	1.24	5.60	5.31

12 days old. This region contained almost all of the actual and potential nodes. Naturally the reproductive organs are in close proximity to the nodes and draw upon the substances therein.

Carbohydrate-Nitrogen Relationships (Ratios)

In attempts to correlate biochemical with developmental changes in plants the concept of the relationship of carbohydrates to nitrogen, in their influence on vegetative growth and sexual reproduction, has frequently been used as a guiding principle (102, 103, 150, 80, 88, 222, etc.). The main and possibly leading idea in such an interpretation of plant behavior has been that "a changing morphological expression is the external evidence of a changed or changing chemical composition." To certain carbohydrates and various nitrogen substances and to their balance (C/N ratio) has been given a predominant role in this method of explanation of plant development. The data secured in the present biochemical analyses of various groups of plants permits one to estimate the carbohydrate-nitrogen relationship (by calculating various C/N ratios) during certain stages of development of short-day (reproductive) and long-day (vegetative) plants. It is the belief of the writer that nothing can be gained by a continuous use of a generalized statement of a "carbohydrate-nitrogen relationship" without specific estimates of the quantities of each of the principal types of substances considered and of their ratios. This in full cognizance of the fact that a ratio between the two groups of substances will have its greatest possible metabolic significance only when one of them will act as a limiting factor. This, however, is obtaining only under rather extreme conditions of metabolism, which rarely occurs when plants are grown in an average greenhouse environment, and the experimental treatment is not extreme.

When short- and long-day soybean plants, var. Biloxi, are subjected to a gross analysis (such as is frequently made by investigators) for total, presumably available carbohydrates and total nitrogen, the following general relationships (ratios) may be obtained (Table 20).

TABLE 20.—C/N RATIOS IN SOYBEAN PLANTS, VAR. BILOXI.

Description of Plant Material	Short-day	Long-day
Seedlings—whole plants (Height 7-8 cm. First real leaf out)-----	1.14	1.68
Immature plants—stems only (Height 20 cm. 3 nodes. No Flower buds on short-day plants)----------	3.43	3.59
Mature plants—stems only (Height, short, 23 cm. long, 33 cm. Large flower buds on short-day plants. Growth inhibited) --	6.50	4.15

These results would appear to indicate that as the short-day plants become reproductive, there is a greater relative accumulation of carbohydrates than of nitrogen in their stems. It does not tell us, however, whether the two phenomena are connected or stand in a cause and effect relationship, or whether it is a mere coincidence. Analyses in greater detail and at closer intervals during the development of the plants seem to be required for a more satisfactory interpretation of such data.

In Table 21 and Figure 8 are shown various calculated C/N ratios and the trend in their changes when stems of Biloxi soybean plants in definite stages of development are taken into account. At the age of 12 days, when photoperiodic induction of reproduction very probably was completed, the carbohydrate-nitrogen ratio of stems of the short-day plants was smaller than that of the vegetative long-day ones. This difference most likely

TABLE 21.—CARBOHYDRATE-NITROGEN RELATIONSHIPS (RATIOS) IN STEMS OF SOYBEAN PLANTS, VAR. BILOXI, WHEN GROWN UNDER CERTAIN PHOTOPERIODS.

Description of Plant Material	Sugar N	Starch N	Sugar and Starch N	Total C-h* N
Plants 2 days old				
Before exposure to photoperiod. Whole stem	.42	.48	.90	3.25
Plants 6 days old (2+4 days)				
Long-day, height 10 cm. Whole stem	.31	.26	.57	2.61
Short-day, height 10 cm. Whole stem (Beginning of induction of reproduction.)	.26	.12	.38	1.40
Plants 12 days old (2+10 days)				
Long-day, height 17 cm. Whole stem	.29	.18	.47	2.15
Short-day, height 17 cm. Whole stem (Induction probably completed.)	.18	.21	.39	1.36
Plants 20 days old (2+18 days)				
Long-day, height 24 cm. Whole stem	.46	.31	.77	3.33
Short-day, height 20 cm. Whole stem (Primordia formed, but no visible flower buds. Growth inhibited.)	.14	.26	.40	3.34
Plants 27 days old (2+25 days)				
Long-day, height 32 cm. Whole stem	.35	.10	.45	4.50
Short-day, height 21 cm. Whole stem (Flower buds distinct.)	.34	.30	.64	4.68
Plants 33 days old (2+31 days)				
Long-day, height 41 cm. Whole stem	1.17	1.07	2.24	7.72
Short-day, height 23 cm. Whole stem (Buds, flowers and small pods.)	.47	2.72	3.17	7.22
Plants 40 days old (2+38 days)				
Long-day, height 47 cm. Whole stem	1.27	2.20	3.47	10.82
Short-day, height 24 cm. Whole stem (Buds, flowers and pods up to 4 cm.)	1.35	6.25	7.60	13.60

*C-h in this and subsequent tables refers to total carbohydrates or the sum of total sugars, starch and hemicellulose.

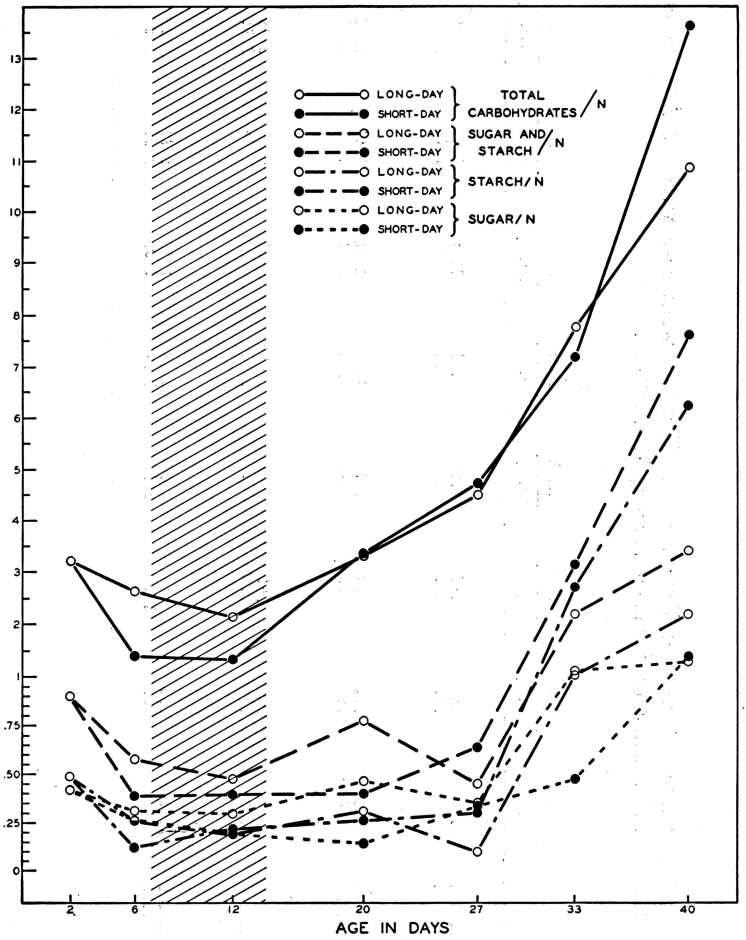


Fig. 8.—Carbohydrate-nitrogen ratios, in relation to photoperiodism, in stems of soybean plants, var. Biloxi. Induction period shaded.

was caused by reduced photosynthesis under the shortened day and no curtailment in growth as yet. Only on the twentieth day, when inhibition in vegetative extension of the short-day plants had begun, the total carbohydrate (C-h)*—nitrogen ratio was the same in both groups of plants. This was due almost entirely to a marked increase in hemicellulose content (143) in the stems under a relatively short photoperiod (Table 17). There were no

*C-h is used an an abbreviation for total determined carbohydrates (Total sugars starch + hemicellulose).

TABLE 22.—EFFECTS OF PHOTOPERIOD ON CARBOHYDRATE-NITROGEN RELATIONSHIP (RATIO), WITH AND WITHOUT ACCOUNTING FOR NITRATE N, IN WHOLE AND UPPER PART OF STEM OF SOYBEAN PLANTS, VAR. BILOXI.

Description of Plant Material	Sugar / N	Sugar / $N\text{-}NO_3$	Starch / N	Starch / $N\text{-}NO_3$	Sugar and Starch / N	Sugar and Starch / $N\text{-}NO_3$	C-h / N	C-h / $N\text{-}NO_3$
Plants 20 days old								
(Whole stems)								
Long-day	.29	.42	.43	.63	.72	1.04	3.67	5.34
Short-day	.05	.08	.20	.30	.26	.38	2.25	3.28
(Flower bud primordia formed)								
Plants 30 days old								
(Upper part of stems)								
Long-day	.50	.74	.87	1.28	1.37	2.02	5.42	8.02
Short-day	.27	.45	.56	.93	.82	1.37	4.07	6.77
(Early flower bud stage)								
Plants 37 days old								
(Upper part of stems)								
Long-day	.60	.88	.81	1.18	1.41	2.05	6.02	8.75
Short-day	.28	.39	.86	1.23	1.14	1.63	5.94	7.76
(Flower buds and flowers present)								
Plants 45 days old								
(Upper part of stems)								
Long-day	.54	.74	.63	.85	1.18	1.59	6.73	9.09
Short-day	.43	.53	1.25	1.52	1.69	2.05	7.18	8.48
(Full bloom)								

TABLE 23.—CARBOHYDRATE-NITROGEN RELATIONSHIPS (RATIOS) IN STEMS OF SOYBEAN PLANTS, VAR. BILOXI.

Description of Plant Material	Percentages on Dry Weight Basis		Sugar	Sugar	Starch	Starch	Sugar and Starch	Sugar and Starch	C-h	C-h
	Sugars	Starch	Sol. N	N	Sol. N	N	Sol. N	N	Sol. N	N
Upper portion of stem										
Plants 12 days old										
Long-day	1.33	1.22	.47	.29	.43	.26	.91	.55	3.37	2.06
Short-day	.84	1.24	.22	.16	.32	.23	.53	.39	1.97	1.45
(Induction probably completed)										
Whole stem										
Plants 41 days old										
Long-day	3.03	2.55	1.87	1.32	1.58	1.11	3.45	2.42	8.82	6.22
Short-day	3.52	11.25	1.31	.97	4.20	3.10	5.50	4.06	8.75	6.46
(Late flower bud stage)										
Plants 47 days old										
Long-day	.88	1.40	.31	.23	.49	.37	.83	.59	5.45	3.92
Short-day	2.81	11.42	.78	.60	3.73	2.45	4.76	3.05	9.67	6.29
(Full bloom)										

visible flower buds on the short-day plants at this time, but floral primordia had been formed already (147). As thenceforward inhibition in stem elongation under the reduced light period became more or less permanent, carbohydrates accumulated rapidly, were stored in the form of starch and the C-h/N ratio of the stems of reproductive plants increased. *It is quite evident, therefore, that the initiation of sexual reproduction preceded the accumulation of carbohydrates and the increased C-h/N ratio.*

But the objection may be raised by some that the nitrate-N fraction was included in total N in the above calculations. Being of inorganic form and highly oxidized, it is supposed to be metabolically "neutral." While it was shown that nitrates are readily assimilated and utilized, either in the whole or in certain parts of the soybean plant under both short and long photoperiods, still C-h/N ratios were calculated with and without consideration of the nitrates (Table 22). These records came from duplicate series of plants grown in rich soil, and hence high in nitrate nitrogen content, but whose developmental states in response to the photoperiod were in every way typical. It will be observed that the relative differences between long- and short-day plants in respect to various C-h/N ratios is quite the same, whether nitrate-N is or is not included in the calculations.

With total soluble nitrogen considered in the calculations, stems of reproductive (short-day) soybean plants in various stages of development and those of vegetative (long-day) ones of corresponding ages exhibited the following C-h/N ratios (Table 23). Here, too, the comparable differences are not significantly altered by the use of soluble instead of total nitrogen in calculation of the ratios. The tremendous increase in starch content in the stems of short-day plants, as a direct result of photoperiodic inhibition, of course, increased conspicuously the C-h/N ratio, whenever in the calculation starch is used either entirely or as part of the carbohydrate fraction. Similarly, a more detailed grouping of certain nitrogen fractions, and their use in calculation of the C-h/N ratios, does not give results that are any more significant, different as the figures may be.

"Switchover" Effects

Another approach in the study of effects of photoperiod on plant metabolism is by means "switchover" of plants in certain stages of development from one exposure to another. This has been done on several occasions with not a few series of plants during the progress of this investigation.

TABLE 24.—"SWITCHOVER" EFFECTS ON NITROGEN AND CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI. GROWN IN LATE SPRING. WEATHER CLEAR AND WARM.

Description of Plant Material	(Percentages on Dry Weight Basis)												
	Total N	Coagulable N	Ammonia N	Amid N	α-Amino N	Nitrate N	Sugars	Starch	Hemicellulose	Total C-h	Starch / N	Sugars and Starch / N	C-h / N
Long-day plants													
(18 days old)													
Height 22 cm.													
Upper stem	3.55	1.196	.038	.457	.709	.531	2.86	2.29	9.35	14.50	.65	1.17	4.08
Lower stem	2.16	.496	.023	.277	.412	.575	3.18	.59	9.76	13.53	.27	1.74	6.26
Short-to-long													
(9+9 days old)													
Height 20 cm. No flower buds.													
Upper stem	3.80	1.182	.029	.442	.797	.509	3.26	1.18	7.23	11.67	.31	1.15	3.07
Lower stem	2.60	.571	.023	.385	.614	.611	3.18	.81	10.79	14.78	.31	1.54	5.68
Short-day plants													
(18 days old)													
Height 17 cm. No flower buds.													
Upper stem	5.34	1.496	.022	.802	1.400	1.298	.88	.58	6.02	7.48	.11	.27	1.40
Lower stem	3.85	.797	.065	.524	.813	1.373	.69	.66	8.76	10.11	.17	.35	2.62
Long-to-short													
(9+9 days old)													
Height 19 cm. No flower buds.													
Upper stem	4.59	1.309	.029	.375	.992	1.152	.88	.44	5.90	7.22	.10	.29	1.57
Lower stem	2.43	.508	.033	.143	.477	.998	.58	.36	10.87	11.81	.15	.39	4.86

TABLE 25.—"SWITCHOVER" EFFECTS ON NITROGEN AND CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI. GROWN IN MIDWINTER. COOL AND CLOUDY.

Description of Plant Material	(Percentages of Nitrogen on Fresh Weight, of Carbohydrates on Dry Weight Basis)											
	Total N	Coagulable N	Amid N	*a*-Amino N	Nitrate N	Sugars	Starch	Hemicellulose	Total C	Starch N	Sugar and Starch N	C-h/N
Plants 13 days old												
(Basic group)												
Long-day, 15 cm. Whole stem	.721	....	....	....	....	2.15	2.13	13.95	18.23	.29	.59	2.52
Short-day, 13 cm. Whole stem	.756	....	....	....	....	2.25	2.20	12.25	16.70	.27	.55	2.07
(Inhibition of growth begins.)												
Plants 30 days old												
(13+17 days switchover)												
Long-day, 40 cm. Upper stem	.463	.115	.047	.127	.097	.80	1.70	14.70	17.20	.31	.45	3.10
Short-to-long, 31 cm. Upper stem	.499	.124	.042	.143	.091	.95	1.90	16.30	19.15	.33	.49	3.32
(No flower buds. Growth resumed.)												
Short-day, 19 cm. Upper stem	.715	.206	.123	.212	.122	3.01	4.05	20.07	27.13	.80	1.39	5.34
(Flower buds initiated. Growth inhibited.)												
Long-to-short, 22 cm. Upper stem	.672	.130	.079	.188	.122	1.45	1.95	16.80	20.20	.33	.58	3.48
(No flower buds. Growth inhibited.)												
Plants 64 days old												
(13+51 days switchover)												
Long-day, 70 cm. Upper stem	.525	....	....	....	....	1.55	1.59	21.20	24.34	.44	.88	6.80
Short-to-long, 60 cm. Upper stem	.520	....	....	....	....	1.47	1.35	20.30	23.15	.38	.79	6.49
(Flower buds abscissed. Good growth)												
Short-day, 21 cm. Upper stem	1.065	....	....	....	....	5.62	14.18	17.65	37.45	3.60	5.04	9.54
(Abundant flower buds. Growth inhibited.)												
Long-to-short, 26 cm. Upper stem	.800	....	....	....	....	4.20	16.20	15.00	35.40	5.62	7.08	12.30
(Abundant flower buds. Growth inhibited.)												

TABLE 26.—"SWITCHOVER" EFFECTS ON NITROGEN AND CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI, IN ADVANCED STATE OF DEVELOPMENT. GROWN IN EARLY SPRING. EXTREMELY CLOUDY WEATHER.

Description of Plant Material	(Percentages on Dry Weight Basis)												
	Total N	Coagulable N	Ammonia N	Amid N	α-Amino N	Nitrate N	Sugars	Starch	Hemicellulose	Total C-h	Starch N	Sugars and Starch N	C-h N
Plants 21 days old (Rather spindling in growth. Basic group.)													
Long-day, height 22 cm.													
Upper stem	5.32	....	....	....	....	....	1.98	2.25	18.43	22.66	.42	.80	5.26
Lower stem	4.05	....	....	....	....	....	1.26	1.49	22.00	24.75	.37	.68	6.13
Short-day, height 15 cm. (Growth abridged.)													
Upper stem	5.37	....	....	....	....	....	1.23	1.50	17.60	20.33	.28	.51	3.78
Lower stem	5.15	....	....	....	....	....	1.60	1.75	17.03	20.38	.34	.65	3.96
Plants 45 days old (21+24 days switchover.)													
Long-day, height 52 cm.													
Upper stem	3.97	1.092	.184	.562	1.073	.308	1.95	2.08	17.56	21.59	.52	1.02	5.44
Lower stem	2.53	.632	.069	.480	.761	.340	.58	.43	20.65	21.66	.17	.40	8.58
Short to long, height 41 cm.[1]													
Upper stem	4.44	1.288	.066	.743	1.260	.571	2.20	2.30	17.60	22.10	.52	1.01	4.99
Lower stem	2.94	.612	.077	.516	.775	.578	.87	1.22	19.65	21.74	.41	.71	7.40
Short-day, height 24 cm. (Growth inhibited. Flower buds conspicuous.)													
Upper stem	4.24	1.169	.134	.885	1.021	.235	4.55	8.25	17.20	30.00	1.95	3.02	7.07
Lower stem	3.21	.648	.214	.590	.772	.184	3.50	7.83	18.66	29.99	2.44	3.53	9.33
Long to short, height 33 cm.[2]													
Upper stem	4.69	.998	.082	1.111	1.256	.493	4.15	2.80	16.67	23.62	.60	1.48	5.04
Lower stem	2.85	.587	.057	.507	.841	.509	3.35	2.65	19.42	25.42	.93	2.10	8.93

1. Solitary flowers in axils of lower trifoliate leaves on a few plants, which abscissed after anthesis (Fig. 9). About ⅓ of the stem has grown since the date of switchover.

2. Terminal growth apparently ceased. Plants in early stage of profuse flower bud development.

The effects of a reversed photoperiod on nitrogen carbohydrate metabolism of several typical groups of Biloxi soybeans are presented in Tables 24-26. The first consisted of a series grown in late spring, when the weather was unusually clear and warm. When 9 days old, a desirable number of plants were subjected to a photoperiod opposite to that which they had received up to this time. Naturally an ample number were left as checks. After 9 days of this treatment, all were cut and subjected to a detailed chemical analysis. The results show (Table 24) that, though there was still a lag, particularly in the lower half of the stem, the nitrogen and carbohydrate concentration, after a 9 days' switchover, was now quite typical of that usually obtained under a similar but continuous exposure to the particular photoperiod. In this respect the "short-to-long" day plants had in general the same composition as the "long-day" ones and the "long-to-short" plants were "chemically" quite similar to "short-day" ones of the same age. A 9 day switchover seems to have had a more noticeable effect on the S→L than L→S groups. Possibly there was a greater metabolic stability in the long- than the short-day plants. In general a 9 day exposure to a definite photoperiod appears to be sufficient to alter completely and markedly the chemical composition of a young soybean plant, the change being most complete in the upper part of the stem and most marked in the content of sugar, starch and the more soluble forms of nitrogen. The nitrate concentration in the upper half of the stem was noticeably smaller in the long-day and short-to-long day groups than in others of this series, which most probably was due to their more rapid utilization in nitrogen metabolism coincident with the greater growth of these plants. Similarly their sugar content was higher, because of the longer hours of photosynthesis under a continuous (18 day) or a recent (9 day) exposure to a long photoperiod.

At the time of switchover the above groups of plants were 9 days old, counting from the day of emergence above ground, and therefore sexual reproduction presumably was not fully induced under the short photoperiod. What chemical changes would possibly take place when somewhat older plants were exposed to an altered length of day? In order to obtain some information on this point, a part of a very uniform and large group of plants was switched over when they were 13 days old and analyzed respectively after 17 and 51 days of this treatment. As the Biloxi variety would seem to require a minimum period of 10 days for photoperiodic induction under a short-day (60, 147) at least some of

the plants probably had become "physiologically" reproductive after 13 days' exposure.

The description of all material considered and the results of chemical analysis will be found in Table 25. An inspection of the data will make it evident that after 17 days of switchover there was a decided orientation in chemical composition of the various groups in accordance with the new exposure. As on the previous occasion (Table 24) the short→long plants made a more rapid physiological adjustment to the new light regime than the long→ short ones. The quickest and most complete change seems to have taken place with the most labile forms of nitrogen and carbohydrates—the nitrate, amino acid, sugar and starch content.

It should be emphasized once more, here, that differences in chemical composition of the stems are subject to the rate of growth (long- and short-to-long day) or growth inhibition (short- and long-to-short day) of plants within the various groups. And at this comparatively early stage of development of soybeans, growth and organic food reserves, while correlated between themselves, appear to be quite independent of the initiation and development of tissues associated with sexual reproduction. Developmental changes may arise, however, that are more complex and somewhat difficult to interpret, as may be illustrated by the following specific case.

The short-to-long day plants, while resuming growth and having no visible flower buds after 17 days of switchover, evidently had been induced to become reproductive, to some extent at least, during the preliminary exposure for 13 days to a short photoperiod. For subsequently a few flower buds appeared slowly, all of which abscissed, though, by the time these plants were 64 days old. The highly interesting question now arises, whether the destruction of these flower buds was due to a direct effect of the long photoperiod or was brought about indirectly as a result of reduced supply of organic nutrients, presumably carbohydrates or possibly also certain indispensable nitrogenous substances. When these plants were either 30 or 64 days old the sugar, starch, and total nitrogen content was almost precisely the same as in long-day plants of the same ages and the various C-h/N ratios were nearly the same. Can we consider the rather hypothetical explanation that during the ontogenic changes in the life of a plant the photoperiod can control not only the speed and amount of development at any particular stage, but that a preceding stage, because of excessive development, may destroy a subsequent one, if it is resumed again (11, 119, 126, 209). In this particular case,

the reproductive organs perhaps were destroyed (abscissed) because of resumption of vegetative growth, which is controlled by the photoperiod.

To study the effects of switchover at a still more advanced age, a group of plants 21 days old was subjected to this treatment. Because of very cloudy weather subsequent to seeding, these plants, which were of the above chronological age, had very likely a physiological age of 16 to 17 days. In height they were slightly above normal and somewhat spindling in appearance.

Chemical analyses of plants on the date of switchover and 24 days later are presented in Table 26. Evidently when the treatment began the short-day plants, though curtailed in growth, had not had time to accumulate carbohydrates. This is a quite typical condition. Twenty-four days later, when they had been in an inhibited state of growth, these plants had large reserves of starch, a very high sugar content, and conspicuous flower buds. The long-day plants had approximately the same carbohydrate composition as 24 days earlier. The total nitrogen content of both (control) groups of plants naturally had altered during this period, but the short-day ones always showed a higher percentage.

As in the preceding experiment, so in this one the short-to-long day plants developed solitary flowers in the axils of the lower trifoliate leaves (Figure 9), all of them abscissing, however, after anthesis. At this time they were growing in height rapidly. In other words they had become typical long-day vegetative plants. This is also shown by their metabolic state in respect to carbohydrate and nitrogen content.

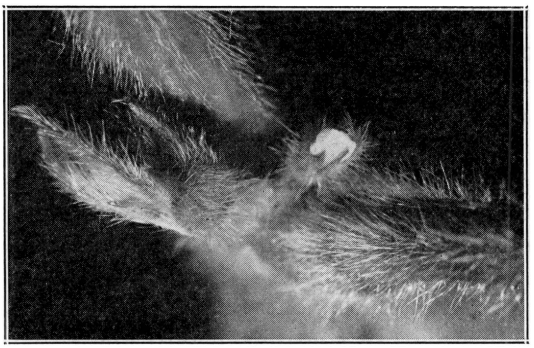


Fig. 9.—Showing transitory flower in axil of short-to-long day soybean plant, var. Biloxi. Such flowers abscissed without setting fruit.

After the 24 day exposure, the long-to-short day plants had ceased to grow in height and were in an early stage of profuse flower bud development. While the chemical composition of these plants had not yet attained the state of typical short-day specimens, this was being approached rapidly.

When comparatively old plants were switched over to the opposite photoperiod, it was far more difficult to alter their chemical composition and developmental states. At the time the experiment was begun, the short-day plants were approaching anthesis and the long-day ones were of a corresponding age, 34 days old. The respective average heights of these plants were 22 and 41 cm. Though in a 7 hour photoperiod, terminal growth of the L→S plants seemed to continue, the stems had become more lignified and the color of the foliage had changed to a lighter green tinge which is more or less typical under short-day exposure before flower buds appear. The S→L plants showed a continuous flower development under the increased light during the observed period.

After 14 days of switchover treatment, no significant changes in carbohydrate or nitrogen content were brought about in either group of plants. The L→S ones remained relatively low in nitrogen and carbohydrate content and the S→L high in these two groups of organic substances. If the treatment were prolonged, then undoubtedly more conspicuous alterations would have been brought about in these plants.

In general the "switchover effects," as shown by differences in development and more or less typical changes in carbohydrate and nitrogen metabolism, are more conspicuous and definite during the early period of growth than in more mature stages, when the plants are approaching the time of flowering. This would seem to point to the fact that the photoperiod influences the organism most easily while it is still young. The effect, once being brought about, is difficult to neutralize or alter.

Chloroplast Pigments

In addition to the effects on rate of growth and time of sexual reproduction, the photoperiod influences quite conspicuously the greenness of foliage. This has been observed in several species (*Salvia, Cosmos,* etc.) but especially in the soybean (*Soja max*), var. Biloxi. When exposed to a short (7 hour) day, for instance, the plants are lighter in color early in their development, but soon match with the long-day ones and, during the period of reproduction, become intensely green. Since color of the foliage is due primarily to chloroplast pigments, a survey has been made of the

concentration of chlorophyll ($\alpha + \beta$) carotene and xanthophyll in various parts of certain plants, but mainly in leaves and nodes of soybeans.

Determinations made on leaves of *Cosmos, Salvia,* and *Soja,* at the time when the short-day plants were in full bloom, show that approximately the same concentration of chlorophyll was present under both exposures, but specimens that had changed from vegetative development to the reproductive state and were flowering had an increased carotene and xanthophyll content (Table 27).

TABLE 27.—CHLOROPLAST PIGMENTS IN LEAVES OF SHORT- AND LONG-DAY PLANTS.

	Milligrams in 10-gram Sample of Leaves		
	Chlorophyll (a and β)	Carotene	Xanthophyll
Cosmos			
Vegetative (long-day)	20.0	0.95	1.50
Reproductive (short-day)	20.0	1.17	1.85
Salvia			
Vegetative (long-day)	25.8	1.85	2.50
Reproductive (short-day)	25.0	2.07	2.80
Soja			
Vegetative (long-day)	22.7	1.10	1.52
Reproductive (short-day)	22.1	1.49	2.00

Although considerable variations may exist, more or less typical changes in chlorophyll content of leaves of Biloxi soybean plants were observed. (Table 28). The greatest increase in depth of color and consequently in chlorophyll concentration was exhibited by short-day plants when the fruit was setting and thereafter (31). This was particularly intensified as a result of early and continuous removal of the reproductive organs (disbudding and defloration).

TABLE 28.—CHLOROPHYLL CONTENT OF LEAVES OF SOYBEAN PLANTS, VAR. BILOXI.

	(Milligrams in 10-gram sample) Stage of Development of Short-day Plants			
	Flower Buds	Full Bloom	Young Pods	Pods 2.5-5.0 cm.
Short-day	20.0	23.0	26.7	32.0
Long-day	21.5	22.4	23.4	29.2

The concentration of the two carotinoid pigments seems to reach a maximum at the time of flowering and then decreases (Table 29). This suggests the possibility that they may have

TABLE 29.—CAROTENE AND XANTHOPHYLL CONTENT OF SOYBEAN LEAVES, VAR. BILOXI.

	Milligrams in 10-gram Sample			
Vegetative (long-day) plants				
Carotene	0.32 →	0.46 →	0.65 →	0.76
Xanthophyll	1.1 →	1.96 →	1.57 →	1.25
Reproductive (short-day) plants				Flowering
Carotene	0.35 →	0.43 →	1.26 →	1.07
Xanthophyll	1.1 →	2.1 →	2.44 →	1.77

TABLE 30.—CAROTENE CONTENT OF LEAVES, NODES AND REPRODUCTIVE ORGANS OF THE SOYBEAN, VAR. BILOXI. (MILLIGRAMS PER 10 GRAM SAMPLE).

	Reproductive (Short-day) Plants			Vegetative (Long-day) Plants
	Flowering	With Young Pods	With Large Pods	
Leaves	1.42 →	1.47 →	1.51	1.20
Nodes	.122 →	.106 →	.097	.066
Buds and Flowers	.166	----	----	----
Young Fruit	----	.158	----	----
Large Fruit	----	----	.080	----

TABLE 31.—CHANGES IN CAROTENE AND XANTHOPHYLL CONCENTRATION IN NODES OF STEMS OF SOYBEAN PLANTS, VAR. BILOXI. (MILLIGRAMS PER 10 GRAM SAMPLE).

	Stage of Development of Short-day Plants				
	Flower Buds	Flowering		Pods Developing	
Carotene					
Short-day Plants	.05 →	.15 →	.12 / .07 →	.09 / .05 →	(Without pods) / (With pods)
Long-day Plants (Vegetative)	.07 →	.08 →	.05 →	.07	
Xanthophyll					
Short-day Plants	.10 →	.21 →	.19 / .11 →	.13 / .08 →	(Without pods) / (With pods)
Long-day Plants (Vegetative)	.15 →	.17 →	.14 →	.12	

something to do with sexual reproduction (145). Consequently a more detailed study was undertaken of the distribution of carotene and xanthophyll, especially in the short-day (reproductive) plants.

In Table 30 are presented data on the carotene content of leaves, nodes and reproductive organs and in Table 31 the carotene and xanthophyll concentration in nodes of both reproductive

and vegetative plants. The decrease in both carotene and xanthophyll content of the nodes, especially in presence of fruits and seeds (pods), would seem to suggest that either the carotinoids are translocated from the nodes to the reproductive organs or else are changed into some other compound, possibly vitamin A, which may have been moved into the developing seeds. The relatively high carotene content of the buds, flowers and young fruit would seem to support such an assumption.

Our attempts to determine quantitatively the presence of vitamin A in certain tissues has been unsuccessful, because of lack of satisfactory methods for the determination of very small quantities.

Respiration

One of the direct ways of measuring the metabolism of a plant is by determining the CO_2 output per unit time and weight of tissues involved. This is a rough estimate of the respiration rate —the oxidation of carbohydrates and other substances with consequent release of energy.

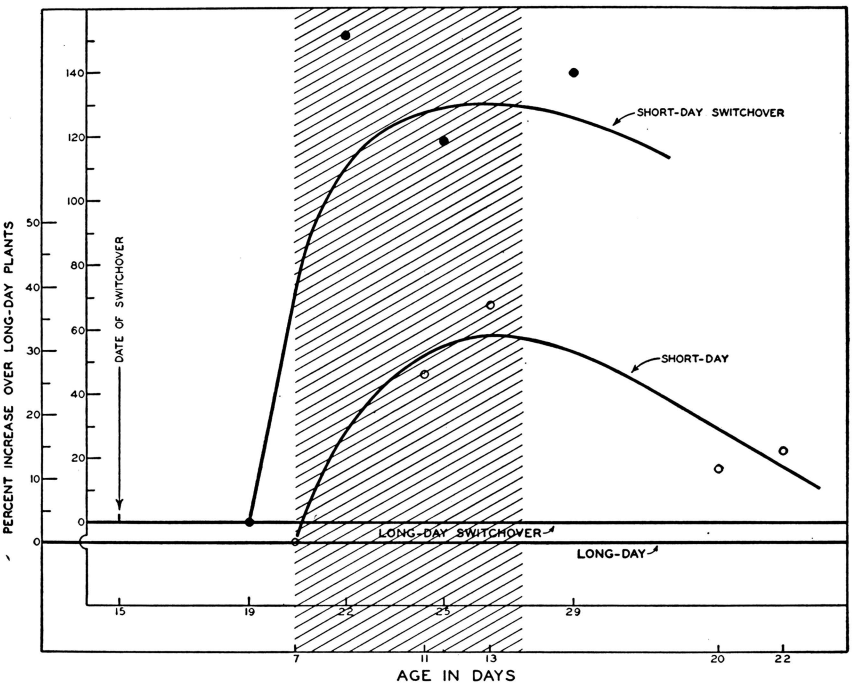


Fig. 10.—Effects of photoperiod on respiration (CO_2 output) of soybean plants, var. Biloxi. Induction period shaded.

When the soybean plant, var. Biloxi, is exposed to a short (7 hour) day, and the environment is otherwise optimal for its development, the CO_2 given off may show a marked increase over long-day (control) plants, which may reach a maximum between the seventh and fifteenth day (Figure 10). This, it will be remembered, is the approximate period of photoperiodic induction of sexual reproduction of these plants. The time of this increase in respiration may be greatly delayed, however, if the temperature is low (midwinter) and the plants develop slowly. In fact, it may occur after twice as long a period and the rise may be more gradual. At the same time there is a corresponding retardation in development and function of the sexual organs.

Additional evidence of an increased respiration has been obtained when plants were switched over from a long to a short photoperiod. In fact, the rise in CO_2 output may be even more marked and may occur sooner (Figure 10).

Invertase activity seems to parallel quite consistently, and peroxidase activity to some extent, the respiration rate.

Enzymes

The relation of photoperiodism to the activity of catalase, peroxidase, invertase, amylase and reducase has been studied extensively in connection with this investigation. It will be the subject of a separate report on "Photoperiodism and Enzyme Activity in the Soybean Plant."

Tests with "Plant Hormones"

In one of their papers, Hitchcock and Zimmerman (82) report that the flowering of Turkish tobacco was hastened by application to the soil in which they grew of certain plant hormones—indole butyric, indole propionic and phenyl acetic acids. To test the possible effects on sexual reproduction these "growth substances" were used on tobacco, tomato, but particularly on soybean plants, both in soil and sand cultures.

Indole acetic, indole propionic and phenyl acetic acids were dissolved in a few drops of 95% ethyl alcohol and given in various quantities of 5 to 45 milligrams, in 100 ml. of water, per plant. The solution was poured uniformly on the surface of soil or quartz sand cultures of the experimental material. The application usually commenced when the plants were in early seedling stage, were given at intervals of 2-7 days, and were continued over a sufficiently long period—till the control plants were in full bloom. In addition several groups of soybean plants received periodic treatments with indole acetic acid in lanoline paste (1:500), which was applied either on the stem, cotyledons, or leaves.

In no instance was there a hastening in appearance of the flower buds or the time of anthesis of the treated plants. The results, therefore, were completely negative. Naturally there was the usual effect of bending of the stem and proliferation of tissues when the growth substances were applied to the plants directly or to the roots through the sand. This response (bending) was frequently observed even when the "hormones" passed through soil into the plants.

DISCUSSION

While the presented experimental data have been analyzed and commented upon already in the preceding section of this report, a general discussion of some of the salient features of the photoperiodism problem should not be out of place.

In the response of a plant like the soybean, var. Biloxi, to a photoperiod conducive to sexual reproduction (short day), two highly important developmental phases must be recognized and emphasized: (1) *Photoperiodic induction* of reproduction, which occurs in the seedling during the first 4-14 days and (2) *photoperiodic inhibition* or curtailment of growth in height, most striking soon after induction is completed. These two phenomena seem to be of fundamental significance in a biochemical assay of the physiology of photoperiodism.

The effects of length of day on initiation of the reproductive state can be best studied at the time when it occurs, despite the fact that it may take place over an extremely short period in the early development of the plant. Unfortunately this has been done by investigators but rarely and only in a cursory way. Attempts have been made in this investigation to ascertain from the early seedling stage the comparative physiological states, of subsequently reproductive and vegetative plants, by means of a study of their comparative chemical composition. Beginning with plants 2 days old, analyses have been continued at close intervals through the time of inception of reproduction, flower bud formation, flowering and fruit setting. This prolonged period in the life of the plant overlaps and is characterized also by inhibition in growth of the main stem, which has a most marked effect on its metabolism. More specifically, the concentration of nitrogen and carbohydrate substances, during the period of development and function of the reproductive organs, is due almost entirely to photoperiodic inhibition of vegetation and seems to be affected but slightly by the physiology of sexual reproduction. Only in their later stages the reproductive processes assume a predominant role in the general metabolism.

Photoperiodic induction may occur surprisingly early in the development of a plant, in fact, it may be initiated to some extent with two exposures to an appropriate length of day. Though possible, it is difficult to see how a single treatment might commence it. Moreover, a preliminary period of adjustment, brief though it may be, to the new physiological state may also be required, especially in the case when more mature long-day plants are moved to a short-day exposure.

At present we are almost totally ignorant as to the essential mechanism of induction. Circumstantial evidence seems to point to the following antecedent features of it. Only a small quantity (3-5 foot candles) of light seems to be sufficient to bring about induction and all wave lengths of the visible spectrum (300-800$\mu\mu$) appear to have an influence, though the greatest effect evidently comes from the red rays, less so from the blue and very little from green. The leaves are the main organs of reception of the photoperiod, whence the effect is transmitted up or down the plant, but primarily to the apex of the stem. But what is it that is produced in the leaves and how does the length of day bring about this stimulus? Is induction brought about during the light period (photoperiod) only or has the dark period something to do with it also? Is the process entirely "catalytic" or "inhibitory," or has it an "inhibition phase"? What happens to the meristematic tissue at the growing point, the terminal and subterminal apical cells, that changes their activity from production of vegetative organs to the initiation of floral primordia? What is the minimal number of exposures to a definite photoperiod that are necessary for the complete initiation of reproduction in various plants and to what extent is this light regime required for the continuation of flower development and function? None of these and many other fundamental questions have been answered as yet and hence we know very little about how photoperiodism fosters sexual reproduction and nothing at all concerning how it affects the vegetative development of plants.

The concept that a specific hormone-like substance is produced in the leaves as a result of the exposure to suitable length of day seems to be gaining ground. "Florigen" and other names have been proposed for the hypothetical hormone. This idea is but a modernized version of an old suggestion advanced by Sachs in 1865; namely, that flower producing substances are elaborated by the leaves and moved to certain regions of the stem where the buds are initiated. In light of the newer knowledge of plant physiology, this is now highly probable. The requirement of a

minimum number of days or of a certain "dosage" for complete initiation and growth of the reproductive organs suggests this possibility, as do also certain treatments resulting in the formation of incomplete or partly vegetative flowers. There seems to be a quantitative aspect in the production, distribution and concentration of this "flower producing hormone." It may have a stimulating effect also upon the whole organism as is evidenced by the increased rate of respiration (CO_2 output) of the short-day plants, which appears to reach a peak at the approximate time of completion of induction.

The nutritional state of the plant does not seem to influence the action of the hormone in initiation of flower buds, since it may be produced under widely varying conditions of soil nutrient supply and storage of carbohydrates and other food reserves. The amount of the necessary catalyst required for the production of floral primordia most probably is very small indeed. Therefore one may expect that the necessary material for its formation and function may be obtaining even in a young seedling. During the time of photoperiodic induction our short-day Biloxi plants were usually somewhat higher in nitrogen content but considerably lower in carbohydrates and total dry weight and gave upon analysis a lower C-h/N ratio than vegetative long-day plants of a corresponding age.

In this connection the following experimental results may be of some interest also. No retardation in the formation of flower buds was obtained when cotyledons were removed from short-day Biloxi seedlings as early as 5 days after emergence, though vegetative development was reduced. When leaves were cut continuously, as soon as they appeared, but cotyledons left intact, flower bud production was weak, irregular and absent in some parts of the plant. Under such treatment the cotyledons were large, very green and evidently undertook to a considerable extent the functions of leaves. Mutilations of this sort, resulting in very weak development of the experimental plants and greatly reduced synthesis of organic substances, still permitted the initiation of flower buds.

Further development of the reproductive organs and their normal function and the successful setting and growth of fruits and seeds is, however, very much dependent upon the physiological condition of the plant, especially the supply of various nitrogen and carbohydrate substances. Despite an otherwise favorable external environment, including a proper length of day, a plant can not become successfully (visibly) reproductive unless organic food

reserves are available in ample quantities. One of the aspects of this requirement may be the existence in the structural parts or storage organs of a certain proportionality between available nitrogen and carbohydrates, the quantitative relationship of which may be expressed by a C-h/N ratio. Without ample nutrition flower buds may absciss, the flowers will not develop normally and there will be a poor set of fruit and seeds. In general then, while the initiation of sexual reproduction may be the function of a special catalyst, the number of flowers successfully formed and the amount of fruit produced would seem to depend upon an abundant supply of the products of synthesis of nitrogen and carbohydrate substances. Consequently in the production of fruit crops the carbohydrate-nitrogen relationship concept probably is of real significance.

Though the present evidence seems to point to the fact that in the soybean the reduced light period (7-hour day) effects independently induction of reproduction and inhibition of vegetation, there may be perchance a connection between the two phenomena. It is possible that a special substance may be produced by the floral primordia which inhibits stem elongation. Reduction in growth of the short-day plants could be detected already when the seedlings were 12 days old. This, it will be noted, was the end period of induction. Assuming such a mechanism, then growth may be inhibited during the following stages of sexual reproduction of plants: (1) At the time of initiation of floral primordia (*Soja*), (2) when plants are in full bloom (*Zea, Triticum,* etc.) and (3) during the period of fruit and seed development (*Lycopersicon, Cucumis,* etc.).

The most interesting question arises as to the general physiological significance of growth reduction in its relationship to sexual reproduction. Plants that become highly reproductive, form a large crop of fruits and seeds, would seem to require a very considerable supply of available substances of diverse type, chiefly organic products containing nitrogen and carbon. Since these substances are used also for the production of vegetative tissues, though not necessarily in the same proportion, one would expect a great use for setting into operation of a mechanism in the plant that will lead to curtailment of vegetative growth and diversion, storage and supply of the requisite food materials for the growth of embryos and accessory tissues. That this negative correlation between vegetation and reproduction should be and is controlled by the developing embryos would seem to be logical, that it is brought about by external factors, such as length

of day, temperature, etc., is more unusual, that it may be set into operation during a very early phase of the reproductive period with, as it were, an expectancy of future demand, is extraordinary.

As a direct result of photoperiodic inhibition, the following physiological changes take place in the Biloxi soybean plant: (1) Marked increase in dry weight, (2) augmentation of the products of synthesis of nitrogen substances, (3) rapid accumulation of the storage forms of carbohydrates, especially starch and, as a result, (4) the establishment of a higher proportionality between carbohydrates and nitrogen, which may be translated into a greater C-h/N ratio. While these changes in the general metabolism of the plant probably have nothing to do with initiation of flower buds, they very likely promote the proper development and successful function of the reproductive organs.

A conspicuous feature of the short-day plants is the marked increase in chlorophyll concentration of the leaves beginning at the time of full bloom. This is particularly intensified when plants are disbudded continuously. The amount of carotene and xanthophyll in the foliage seems to reach a maximum during the period of flowering. The real significance of these striking changes in the content of chloroplast pigments is unknown at present. Carotene and xanthophyll may have some metabolic function in sexual reproduction of plants. This particular phase of the subject matter has been discussed by the writer elsewhere (145).

SUMMARY

1. Photoperiodism expresses itself in various degrees through changes in development of both the vegetative and reproductive organs of plants. It may result in more or less complete inhibition of stem development (*Rudbeckia*), partial curtailment of growth in height (*Soja*), or less marked effect on the vegetative organs (*Cosmos, Salvia*). Sexual reproduction may be prevented or fostered by the photoperiod. The two effects, on vegetation and reproduction, appear to be independent of each other. They seem to be brought about *directly* by the length of day. The present study and discussion deals primarily with the physiology of photoperiodism of the soybean plant, var. Biloxi.

2. When exposed to a 7-hour day growth in height of this plant is retarded after 10 days and may cease completely in another 10 days. Under a 14-hour day vegetative development is continuous and no flower buds are formed. The short photoperiod only permits sexual reproduction.

3. There is greater accumulation (percentage increase) of dry matter in the reproductive (short-day) than the vegetative (long-day) plants. It begins as soon as photoperiodic inhibition has taken place and is the result of suppressed growth in height.

4. A relatively higher nitrogen metabolism and N concentration is maintained by the short-day plants, especially in early stages of sexual reproduction. The quantity and completeness of removal of nitrogen from the vegetative organs (N catabolism) depends on the amount of fruits or seeds produced.

From the base of the stem, through internodes, nodes, to the tip, there is an ascending gradient in total, coaguable, proteose, basic, ammonia and humin forms of nitrogen in both vegetative and reproductive soybean plants. A descending gradient in the same direction exists for nitrate, amino and amid forms. Considering our present knowledge of nitrogen metabolism in sexual reproduction, no specific dynamic function can be ascribed to any particular form or group of nitrogen substances in the initiation of floral organs.

Stems of reproductive Biloxi plants were higher in all determined forms of N, excepting nitrates. A conspicuously lower nitrate concentration existed in the tips than elsewhere in the stem of long-day plants due to its greater utilization in synthesis of proteins coincident with the rapid terminal extension of these plants. With age nitrates are used in the short-day plants in

ever-increasing quantities for the development of flowers and fruits.

5. Soybean plants, grown in quartz sand and receiving either NO_3 or NH_3 ions, developed equally well and became sexually reproductive at the usual time, when exposed to a short photoperiod, irrespective of the form of nitrogen in the culture medium. Any possible mechanism (reducase activity), while operative when nitrate ions are present, does not seem to have any significant differential function in photoperiodism.

6. The carbohydrate concentration was relatively lower in short- than in long-day soybeans during the first 15-16 days—the period of induction of reproduction and early initiation of flower buds. As soon as growth was inhibited, the products of photosynthesis accumulated and were stored in increasing quantities. The carbohydrate reserve was utilized eventually for the production of seeds. Its striking augmentation, primarily as starch, in the short-day plants seems to be entirely independent of the effects of photoperiod on sexual reproduction. Though produced in large quantities in the long photoperiod, carbohydrates were utilized rapidly for growth and were stored but slowly in the vegetative plants.

7. When Biloxi soybeans, given a 7-hour photoperiod, were 12 days old and photoperiodic induction of reproduction was probably completed, analyses of stems of these plants gave smaller carbohydrate-nitrogen (C-h/N) ratios than stems of vegetative long-day plants of a corresponding age. Subsequent to photoperiodic inhibition the C-h/N ratio increased and at the time of full bloom and thereafter frequently higher values were obtained for the short-day (reproductive) than for the long-day (vegetative) plants. Since the initiation of sexual reproduction preceded the relatively greater accumulation of carbohydrates than of nitrogen, resulting in an increased C-h/N ratio, these two features cannot be placed in a cause and effect relationship. When various carbohydrate and nitrogen fractions, either singly or in combination, were used in the calculation of the "C/N ratios," results were obtained that were of no greater significance in this problem.

8. A "switchover" of young soybean plants from a short to a long light period, and *vice versa,* resulted in 9-10 days in an almost complete alteration of nitrogen and carbohydrate concentration, which was typical of that obtaining under a similar but continuous exposure to the new length of day. In 17 days the

orientation was complete physiologically and morphologically. A 9 or 17 day switchover had a more noticeable effect on the short→long than long→short groups of plants and the change was more complete in the upper part of the stem than elsewhere. Induction of reproduction was destroyed by moving 13 and 16 day old Biloxi soybeans to a long photoperiod. The few flower buds that appeared abscissed. When older, more mature plants were moved to the opposite photoperiod, it was more difficult to change their chemical composition and developmental states.

9. When exposed to either a 7 or 14 hour day, leaves of *Cosmos, Salvia* and *Soja* plants had approximately the same specific chlorophyll ($\alpha + \beta$) concentration at the time the short-day plants were in full bloom but were higher in carotene and xanthophyll content. The concentration of the two carotinoid pigments seems to reach a maximum in the reproductive soybean plants at the time of flowering and then decreases. Their translocation seems to be from the leaves through the nodes to the reproductive organs. Buds, flowers and young fruit tissues have a relatively high carotene content.

10. The respiration rate (CO_2 output) of short-day Biloxi plants is higher than that of long-day ones, reaching, under favorable environmental conditions, a maximum after 7-15 days—the approximate time of photoperiodic induction. A similar increase occurred when plants were moved from a long to a short photoperiod.

11. Growth-promoting substances (hormones)—indole acetic, indole propionic and phenyl acetic acids—do not seem to affect sexual reproduction in plants. Whether these substances were applied directly to the plants or to the roots through soil or sand, no change was brought about by the various treatments in respect to the time of appearance of flower buds or in their subsequent development.

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