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Influence of Length of Day (Photoperiod) on Development of the Soybean Plant, var. Biloxi

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Independent of light intensity and quality, many plants respond in a most striking way to the duration of light exposure, or the length of day. This phenomenon was discovered and announced by Garner and Allard[4]† in 1920, who have named it *photoperiodism*.

Because of its scientific interest and economic importance, a large number of investigators have studied plants with respect to their development under various lengths of day (photoperiods). As a result of this work, it is now possible to group certain species and varieties into "short-day" plants, or those requiring for sexual reproduction a diurnal duration of light of 10 hours or less, and "long-day" plants with an optimal light requirement for reproduction above 10 hours.

Most of the late varieties of soybeans, for example, are typical short-day plants[5, 11]. When exposed to a length of day of 13-15 hours, such as exists during the growing season in the latitude of Columbia, Missouri, they will grow vegetatively but will not flower until the days become shorter. There are, of course, many other cultivated plants whose photoperiodic requirements are the reverse—they will become sexually reproductive only under a relatively long-day exposure to light. In addition to these two groups, there are so-called "neutral" plants, which do not react in this manner to the diurnal duration of light.

While considerable amount of work has been done on the physiological changes of plants coincident with their photoperiodic response, the mechanism of photoperiodism is still unknown. The present paper is an integral part of a study by the senior author of the effects of length of day on the soybean (*Soya max* (L) Piper). In turn, it is a phase of the general problem of "physiology of reproduction of higher plants."

The variety Biloxi was selected for most of the work because of its rapid growth and marked sensitiveness to relatively long and short photoperiods.[5]

Although our approach in this investigation has been mainly biochemical, it was found necessary to determine certain detailed structural changes in the experimental material. The morphology and

*This and other phases of the investigation have been supported in part by grants from the Committee on Effects of Radiation upon Living Organisms, National Research Council.
†Superscript numerals refer to "literature cited," page 28.

chemistry of soybean seeds has been studied intensively,[10] but very little seems to be known of the ontogenic development of the vegetative and floral parts of the soybean plant.

Specifically, the present study was prompted as a result of the need of determining exactly when the period of sexual reproduction begins in the short-day plants and how the floral primordia are initiated. Such information was required for the correlation of certain developmental stages with the biochemical changes in plants. This bulletin will be followed shortly by a second one giving a detailed account of the chemical investigations.

MATERIAL AND TECHNIQUE

The Biloxi variety of soybean was grown from October, 1933, to January, 1934, from seed obtained from a reliable commercial source under the general conditions of a standard greenhouse environment. The cultures were maintained under uniform nutrition and exposure, excepting for limited fluctuations in temperature and, of course, a definite photoperiod. Those exposed to a short day received 7 hours of daylight and will be referred to hereafter as "short-day plants." This was accomplished by keeping the planted boxes on steel trucks, which were moved into a large dark room specially built for this purpose in the greenhouse and forcefully ventilated (Fig. 2). The "long-day plants" were exposed to the full length of day, plus additional electric lighting with mazda bulbs in reflectors supplying about 250 foot-candles, to bring the light period to 14 hours (Fig. 1). With this treatment plants could be grown in repeatable series that were close duplicates in size, general appearance and detailed morphology, as will be described forthwith.

Material for the present study was collected at frequent intervals from plants 10 to 50 days old from the time of emergence of the seedlings. The modified Chamberlain's fluid (formol-acetic-alcohol) was used as killing and preserving agent. Following fixation, the axillary buds, except those at the terminal end of the stem, were isolated and sectioned separately. Since the primordia were arranged in parallel rows while still within the confine of the primary meristem, the material was sectioned vertically. This gave a typical picture of the arrangements of the bud primordia in their early stages of development. For gross morphological observation, both fresh material and frozen sections were used. Sections for histological study were cut six to eight microns in thickness. They were stained with one per cent Safranin-O in 50% ethyl alcohol and counterstained with Delafield's Haematoxylin.

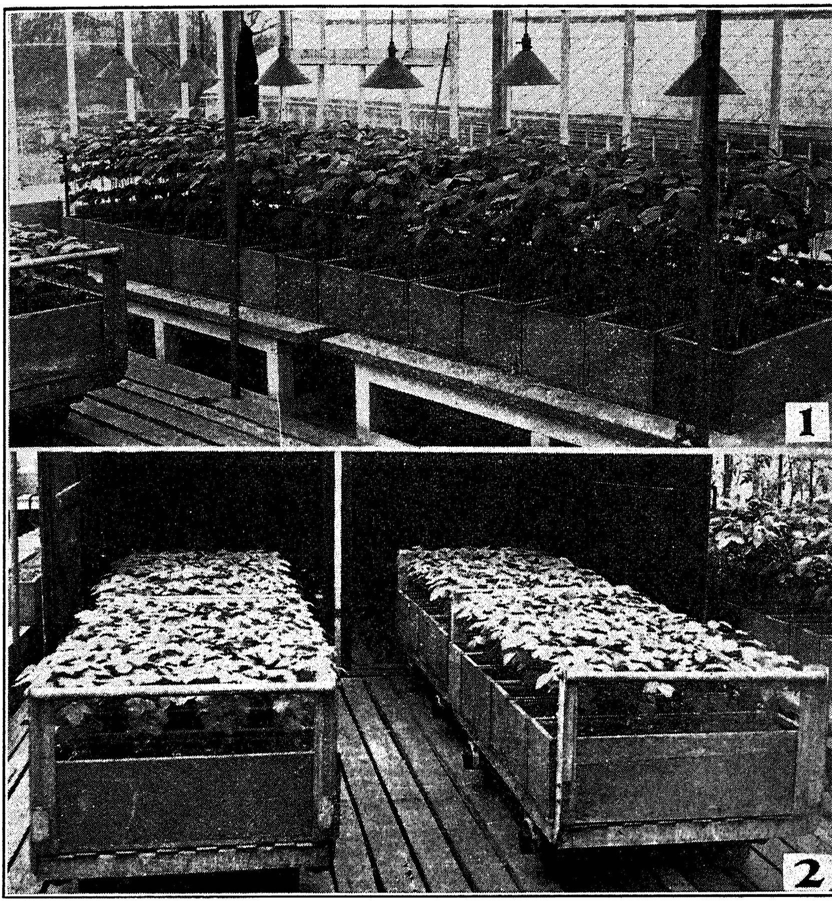

PLATE I

Fig. 1.—The long-day plants were exposed to full length of day and additional electric illumination to bring the total light period to 14 hours.

Fig. 2.—The short-day plants were grown in boxes placed on trucks, which were moved into a ventilated dark room, as required to shorten the light period to 7 hours.

EFFECTS OF LONG AND SHORT PHOTOPERIODS ON GROWTH AND GROSS MORPHOLOGY

The Biloxi variety of soybean is a short-day plant. Under a 7-hour exposure of light, it becomes sexually reproductive within a comparatively brief period of growth. Plants exposed to a relatively long day of 14 hours, on the other hand, remain strictly vegetative, flowering being delayed more or less indefinitely, since the "critical point" for reproduction has been passed.[3]

The time of appearance of flower buds on the short-day plants is determined to a considerable extent by temperature,[2] which, as was noted before, was not controlled. With a mean temperature of 80°F., for example, the period between germination and flowering is about 27-30 days.[5] Naturally flower buds are initiated and appear considerably earlier. The temperature of the greenhouse in which our plants were grown during the fall and winter seasons was from 70-75°F. during the day and approximately 5-10° lower at night. There was, therefore, a corresponding delay in flowering of the short-day plants. This, however, in no way interfered with the study, since these plants were typical in all other respects. Structurally they appeared very much the same as plants grown at higher temperatures. Moreover, it was desirable to study the morphology of plants raised in this particular environment, as similarly grown plants were used for biochemical analyses.

From the time of emergence (4-5 days after planting) up to 14 days of age, the seedlings exposed to long and short photoperiods showed no apparent difference in their external appearance. With advanced age, from 21-42 days, those of the long-day groups were much taller than short-day plants of corresponding age. Of course, they had also thicker but softer stems and larger leaves.

DESCRIPTION OF PLANTS 32 DAYS OLD

Long-day.—Height 30-38 cm. Terminal growth continuing. Seven to eight nodes, including cotyledonary. Stems quite flexible. Almost all cotyledons abscissed. In some instances slight marginal yellowing of juvenile leaves. No flower buds.

Short-day.—Height 15-18 cm. Terminal growth inhibited. Five nodes including cotyledonary. Stems fairly stiff. Most of the cotyledons still present and green. Flower buds in early stage of development.

DESCRIPTION OF PLANTS 42 DAYS OLD

Long-day.—Height 45-53 cm. Eleven to thirteen nodes. Stems slightly flexible. Cotyledons abscissed. Leaves large but of lighter green color than those of short-day plants of the same age. Juvenile leaves yellowing. No flower buds, but slight development of axillary buds, which are strictly vegetative. (Fig. 3).

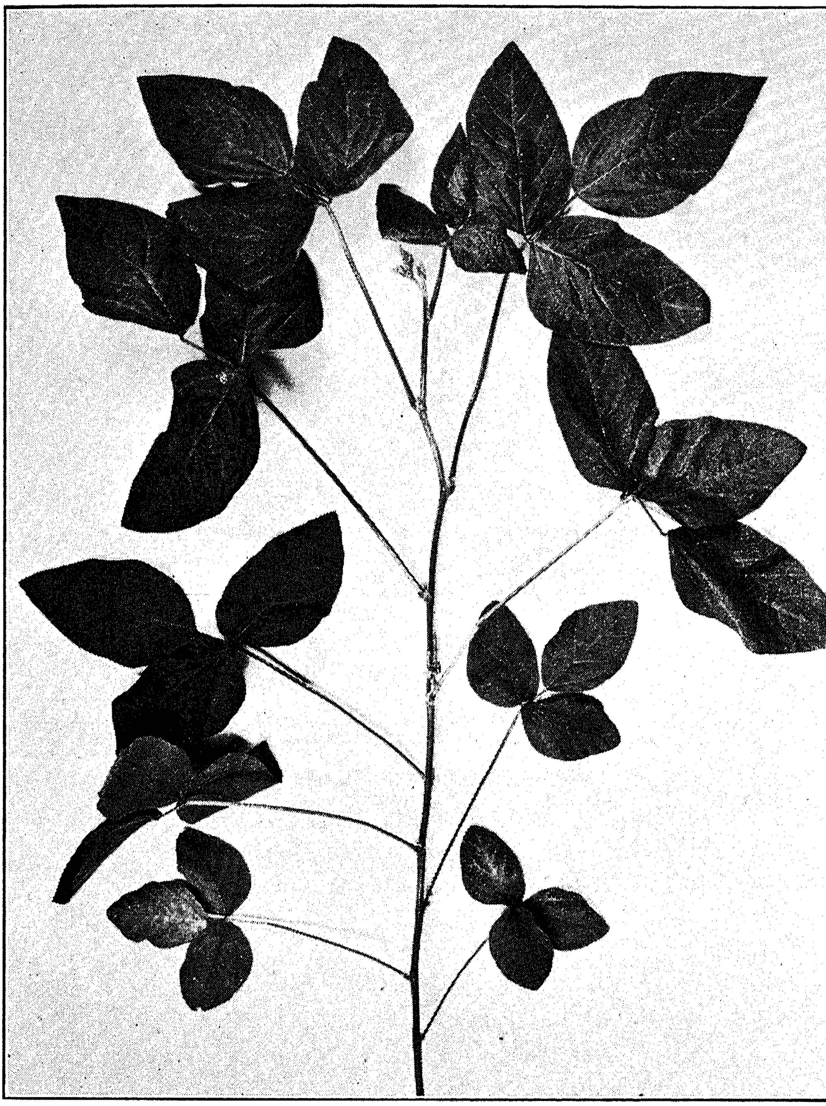

PLATE II

Fig. 3.—The main stem of a 42-day old Biloxi soybean plant grown in soil cultures in the greenhouse under a relatively long (14 hours) photoperiod (x¼)

Short-day.—Height 19-23 cm. Nine to ten nodes. Stems very stiff. Almost all cotyledons abscissed. Leaves, including juvenile, of deep green color. Many axillary and terminal flower buds and flowers. (Fig. 4).

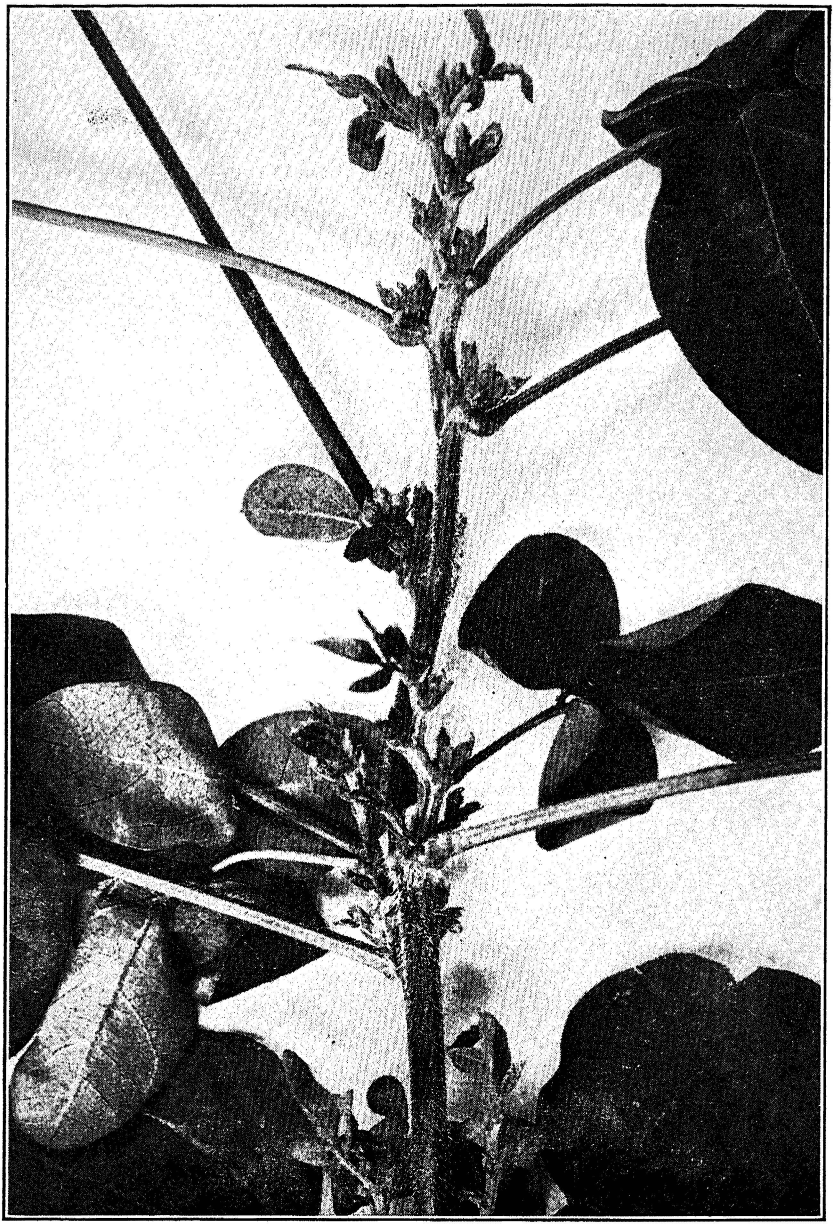

PLATE III

Fig. 4.—A portion of the main stem of a 42-day old Biloxi soybean plant grown in soil cultures and exposed to short photoperiod (7 hours). Some of the terminal leaves have been removed.

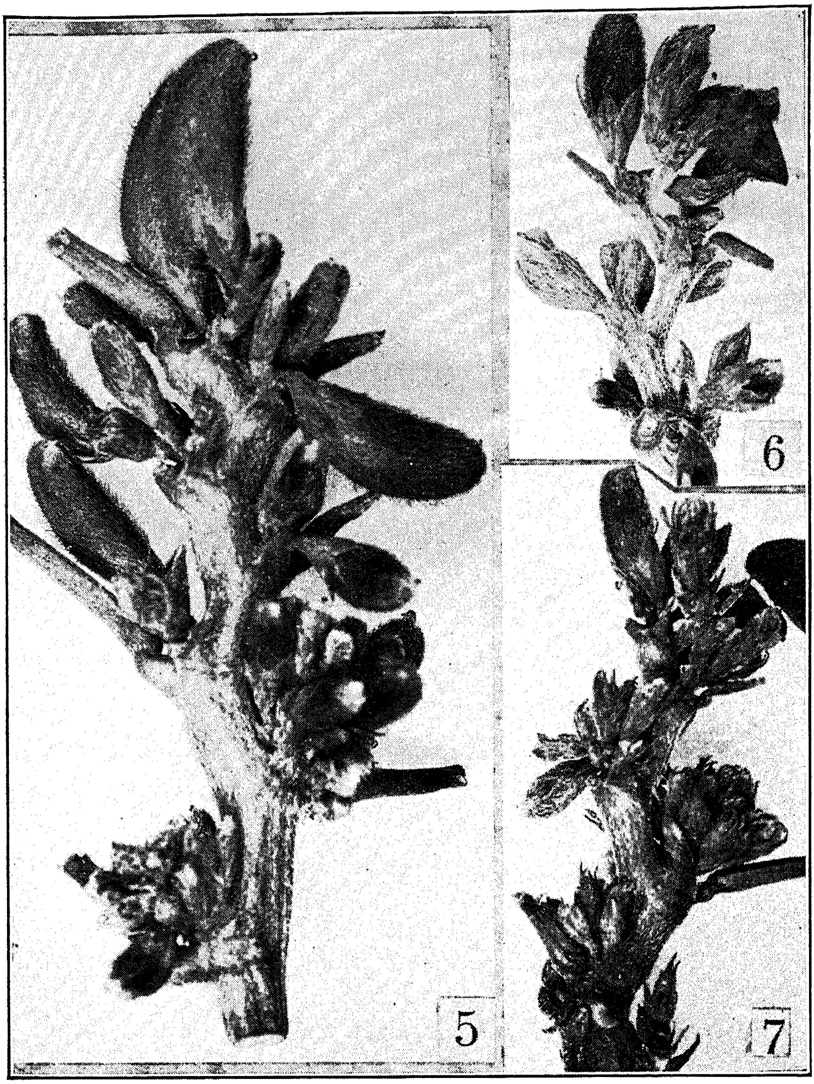

PLATE IV

Photographs of the terminal and axillary branches of a 50-day old plant exposed to a short photoperiod.

Fig. 5.—The terminal portion of a plant showing various stages of development of pods. Note that those near the tip are further advanced than the ones lower on the stem.

Fig. 6.—An axillary branch taken below the last axillary shown in Fig. 5. Note a similarity in the development of the buds with those shown in Fig. 5.

Fig. 7.—An axillary shoot below that shown in Fig. 6.

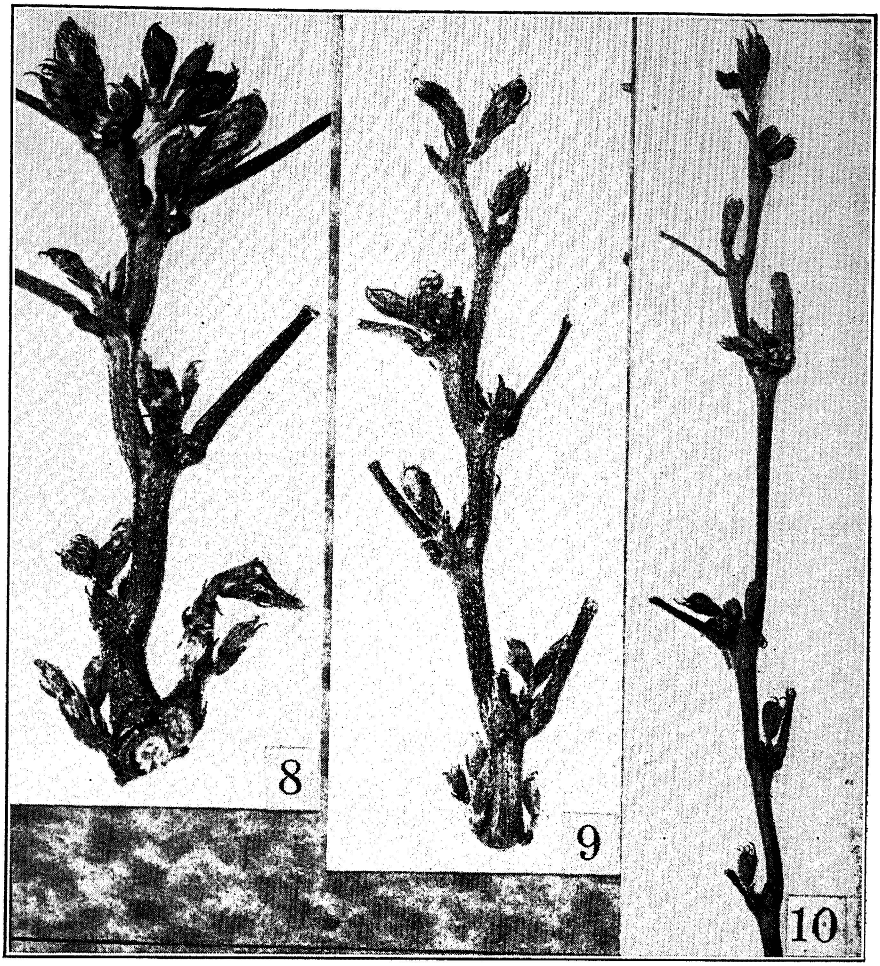

PLATE V

Photographs of the terminal and axillary branches of a 50-day old plant exposed to a short photoperiod.

Fig. 8.—An axillary shoot below that shown in Fig. 7. Note that the extent of vegetative growth is greater than in the above figures, and only one pod (right top) has formed.

Fig. 9.—An axillary shoot below that shown in Fig. 8. The reproductive organs are still largely in the bud stage.

Fig. 10.—The lowest axillary shoot usually grew more than the others. The reproductive organs are still in the bud stage.

At the immediate proximity of the promeristem of the main stem the flowers were borne in pairs, occasionally in threes. Those found at the lower axils usually occurred in threes, sometimes fours, but rarely eight or ten flowers were observed. This is illustrated with 50-day-old short-day plants in figures 5-10. In the Manchu variety of soybean Guard[7] reported that from five to sixteen flowers may be found at each axil. Piper and Morse[10] mention instances where as many as thirty-five flowers may be present in a single inflorescence.

Beside marked differences in size, a noticeable feature in the general appearance, of the two groups of plants was the change in color of the foliage. During the early stage of development of the short-day plants, their leaves were light green, which would seem to have been caused by the short-day exposure to light. Soon, however, the color changed to a deeper shade, and during the time of flowering and fruit development they were conspicuously darker green than long-day plants of the same age.[8]

COMPARISON OF THE DEVELOPMENT OF THE MAIN STEM AND AXILLARY BUDS OF LONG AND SHORT-DAY PLANTS

Long-day Plants.—During the early stage of development, the growth of the plants exposed to relatively long (14 hour) and short (7 hour) photoperiods were essentially the same. In other words, during the first fourteen to twenty-one days of age, on the average, a like number of leaves, internodes and axillary buds were found in both types of plants. With advanced age a rapid growth but only slight development of the axillary buds occurred in the long-day plants while in the short-day ones the rate of growth was markedly curtailed. Aside from variations in height and greenness of foliage, the conspicuous difference in the development between these two types of plants was in the extent and nature of growth of the axillary buds.

In detail the development of the main stems in the two groups of plants and the axillary shoots in the plants exposed to the long photoperiod was, excepting for size, identical since the very short axillaries were strictly vegetative. A description, therefore, of the development of the main stem of the plants exposed to a long photoperiod will be applicable to the main stem of the short-day plants and the axillary shoots of the long-day plants.

The 14-day-old plants consisted of cotyledons and a pair of juvenile leaves borne on a short internode above the cotyledons. At the terminal point of this internode, and completely protected by stipules, is the promeristem. On careful removal of the stipules, two leaf primordia arising alternately on the opposite plane of the promeristem were observed. In the axil of each primordial leaf an axillary bud had already emerged, at this time appearing as a rounded protuberance.

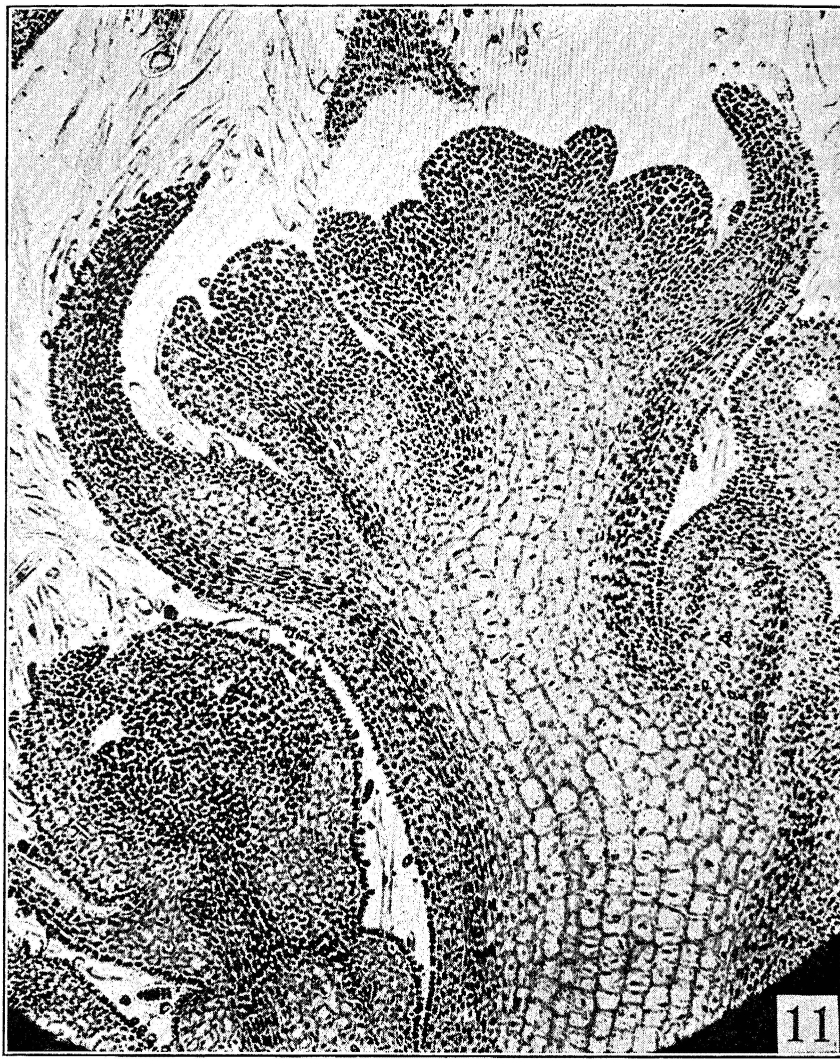

PLATE VI

Fig. 11.—A microphotograph of a sectioned terminal meristem of a 30-day old short-day plant, showing the degree of growth of internodes and the extent of development of the flower buds. (x116.)

The leaf primordia are conspicuously spaced by very short though distinct internodes.

The first axillary buds (immediately above the cotyledons) had already begun to differentiate into an axillary shoot, consisting of two rudimentary leaves markedly spaced by short internodes. At the axils of the lateral stipules a bud primordium had already emerged (Fig. 17).

On the 21st day, a new leaf primordium was observed at the promeristem of the main stem. Two more leaves were noted on the 25th and 28th days, and one on the 30th, 35th and 42nd day respectively. Occurring simultaneously with the appearance of each leaf primordium, was a corresponding differentiation of the axillary buds and the rapid growth of the internodes (Figs. 12, 18 to 20).

While the growth of the axillary shoots, in all respect, was similar to the main stem, the extent of their development varied markedly and according to the sequence of the appearance of the buds. Thus in older plants (42 days), growth of the axillary shoots may vary from a rounded protuberance in the axils of the juvenile leaves in the region of the primary meristem of the main stem to well developed though very short shoots, consisting of from one to ten leaves conspicuously spaced by as many internodes. At the axils of each leaf, bud primordia were present. Under conditions conducive to vegetative growth, these buds remained undeveloped almost indefinitely. However, on changing the exposure of the plants from a long to a short photoperiod, the axillary bud differentiated into flower buds within a reasonable time.

Short-day Plants.—As was mentioned previously, the development of the main stem of the short-day plants was in general similar to that of the long-day plants. In the former group, however, the elongation of the internodes was markedly curtailed, the plants appearing dwarfed (Figs. 4 and 11).

The leaves as well as the bud primordia were confined to the region of the short primary meristem. In long-day plants of a corresponding age the nodes bearing the leaf bud primordia were distinctly spaced apart. This is brought about by the growth of the internodes, which occurs almost simultaneously with the appearance of the leaf primordia and becomes more and more conspicuous with the advance in age of the plants (28 to 42 days). In the short-day plants of the same age, the region of the primary meristem was almost obliterated as a result of the greatly reduced internodes (Figs. 11 and 12). A description of the observations in the variation, type and extent of the development of the axillary buds of the short-day plants of various ages will show other marked differences in the morphology of the two types of plants.

In the 14-day-old plants, the oldest of the first axillary buds were still within the confine of the protective stipules. Examination, with careful removal of the stipules, revealed that the buds had already differentiated at this time into an axillary shoot, consisting of three

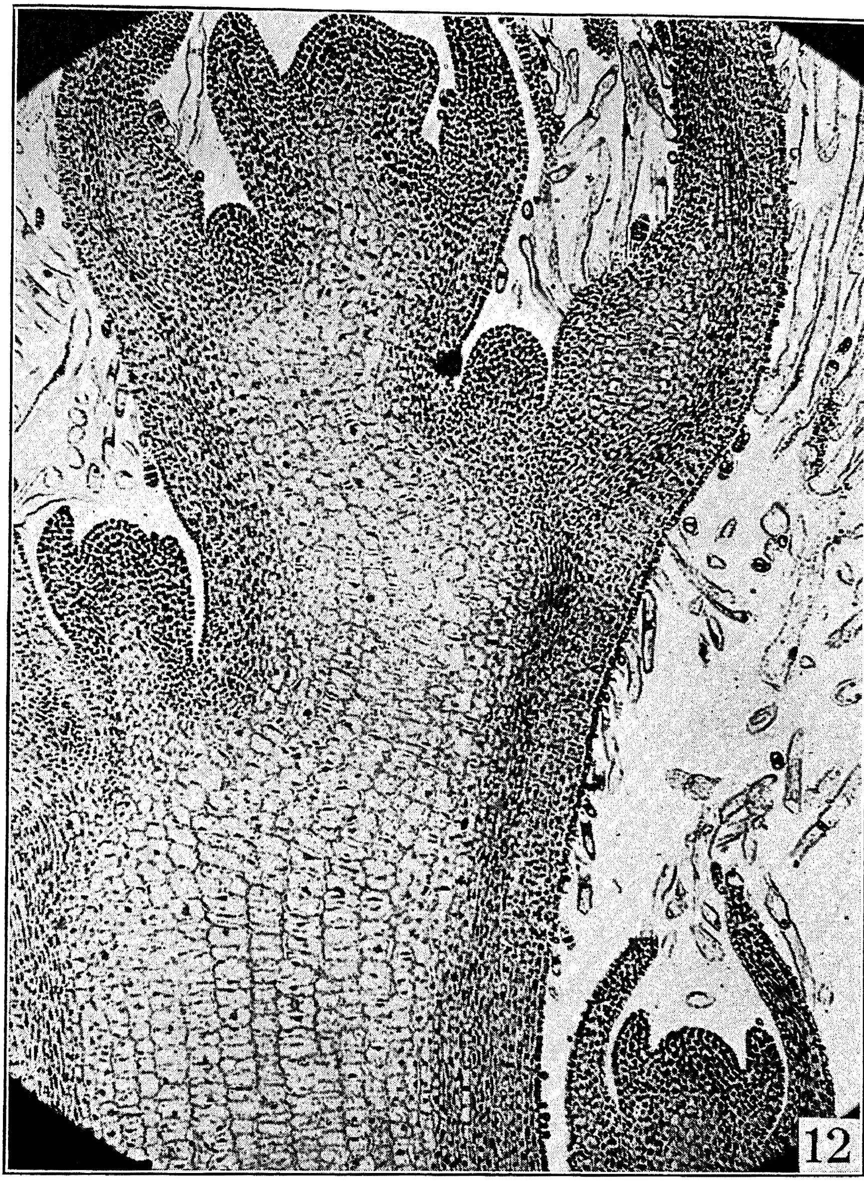

PLATE VII

Fig. 12.—A microphotograph of sectioned terminal meristem of a 30-day old long-day plant, showing the degree of growth of internodes and the extent of development of the axillary buds. (x116.)

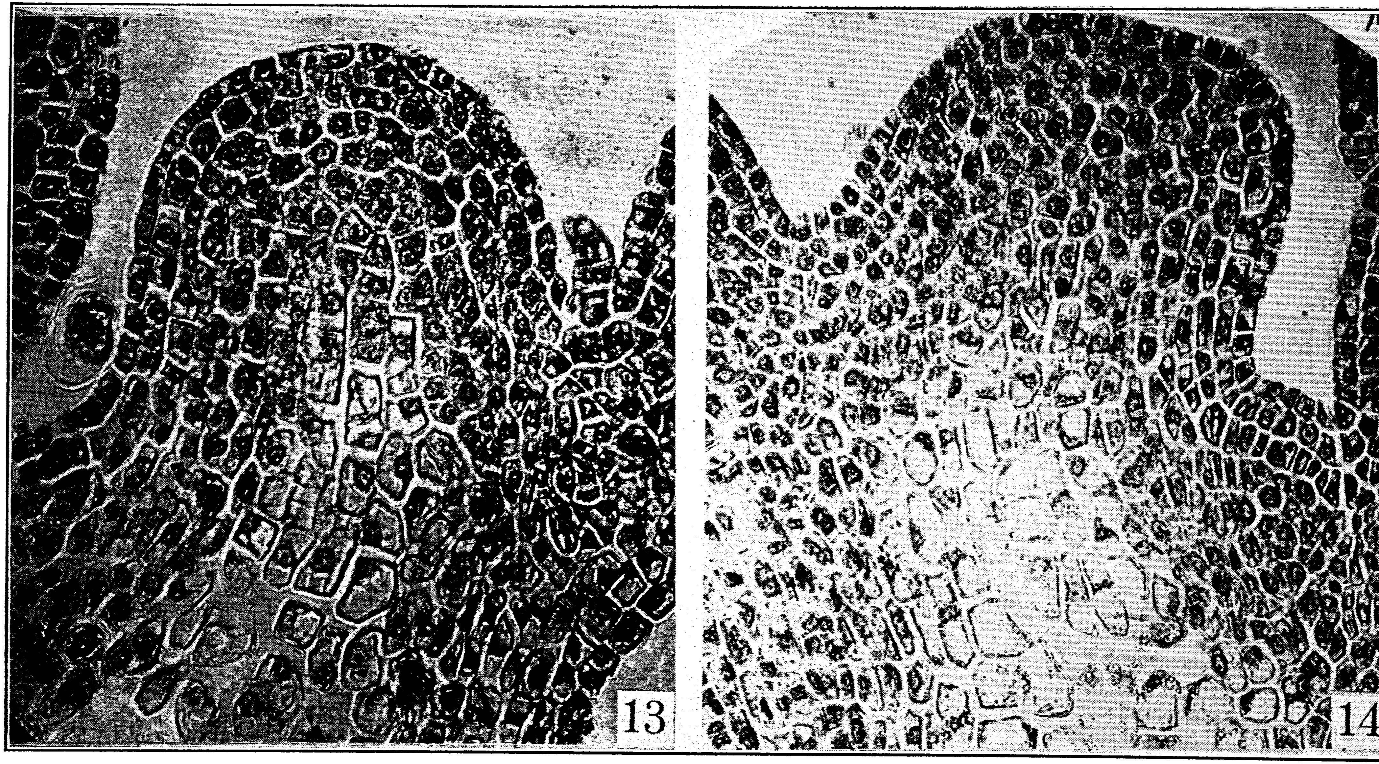

PLATE VIII

Fig. 13.—Microphotograph of sectioned apical promeristem of a 14-day old Biloxi soybean plant exposed to a long (14 hours) photoperiod (x120).

Fig. 14.—Microphotograph of sectioned apical promeristem of a 42-day old Biloxi soybean plant exposed to a long (14 hours) photoperiod (x120).

rudimentary leaves, distinctly spaced by short internodes. In the axils of the older leaf initials a bud was beginning to differentiate. *This should be considered as the earliest appearance of the first potential and probably actual flower bud.* If these plants had been exposed to a higher mean temperature, then undoubtedly there would have been a still earlier initiation of flower buds.

The second, third and fourth axillary buds in the region of primary meristem appeared as rounded protuberances in the axils of the rudimentary true leaves.

In 21-day old plants development extended slightly further than in the preceding stage. An examination of the axillary buds, however, showed a very marked increase in length of the shoots. In section, the first axillary shoot showed an early stage of development of the membranous sheath of the bract primordium. The second axillary bud had already commenced to develop into an axillary shoot, now consisting of three rudimentary leaves with its corresponding axillary buds. The bud primordia which arise in the axils of leaves of the second axillary shoot were much larger and further developed than the buds found in the first axillary shoot. All of them undoubtedly were flower buds. The third shoot developed vegetatively although there were indications of the presence of flower buds in the axils of the leaves. The fourth axillary at this stage was still purely vegetative. It consisted only of two leaves with no evidence of flower bud development. The fifth and sixth axillaries, still within the confine of the primary meristematic region, appeared as rounded protuberances in the axils of the rudimentary leaves.

On the 25th day, the first axillary shoot had markedly elongated. At this stage, the three leaves were well formed and their axillary buds had definitely differentiated into flower buds, as was indicated by the appearance in anterior plane of the promeristem of a membranous sheath of a bract. On the second and the third axillary shoots, floral organs (stamens, pistil, and petal primordia) in various stages of development were already distinguishable. Two flower buds were usually present on the fourth axillary. The oldest of these revealed the early stage of growth of the calyx, while the second still appeared as a rounded protuberance in the axil of the leaf. Immediately below the promeristem a slight evagination characteristic of the early differentiation of a bud was evident. The promeristem of the axillary shoot was considerably reduced. The fifth axillary bud often gave rise to a single flower. It was far more advanced in development than the buds on older axillary shoots, showing, in sections, two cycles of stamens, the petals and pistil. The sixth, seventh and eighth axillary

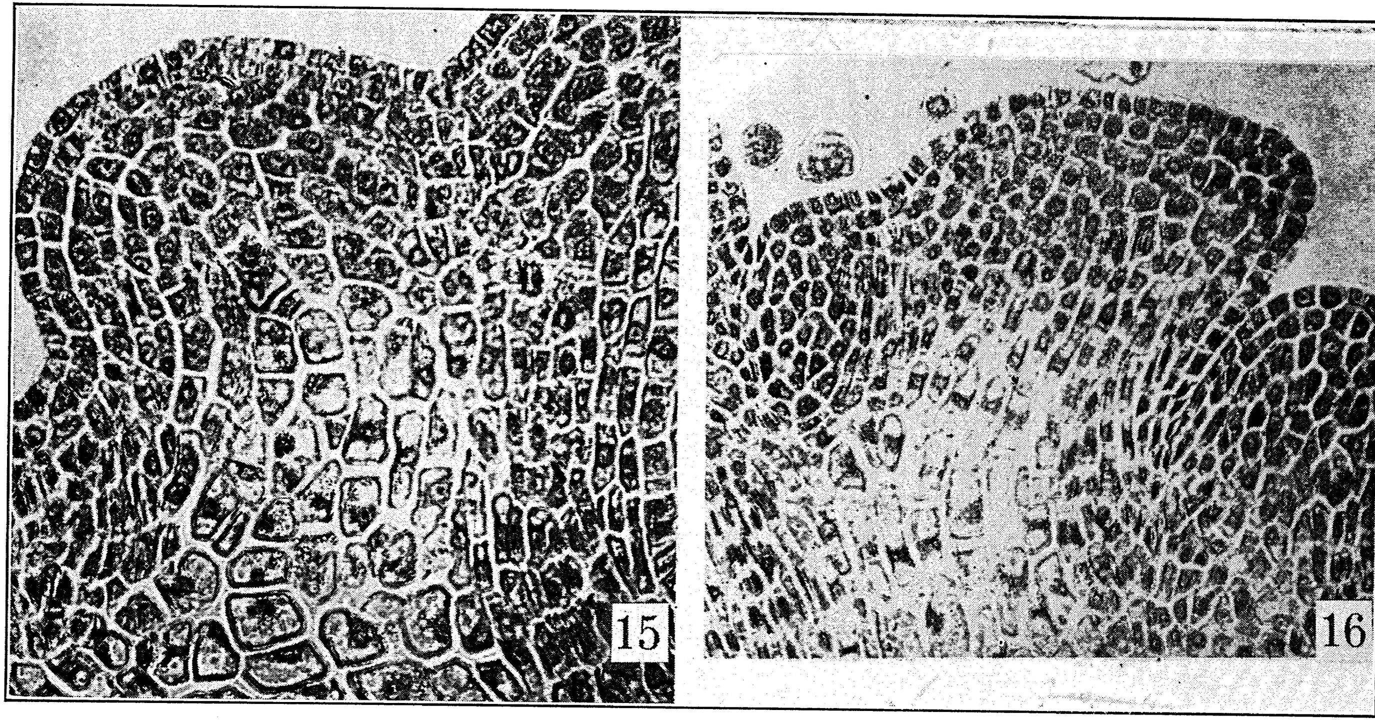

PLATE IX

Fig. 15.—Microphotograph of sectioned apical promeristem of a 14-day old Biloxi soybean plant exposed to a short (7 hours) photoperiod (x120).

Fig. 16.—Microphotograph of sectioned apical promeristem of a 42-day old Biloxi soybean plant exposed to a short (7 hours) photoperiod (x120).

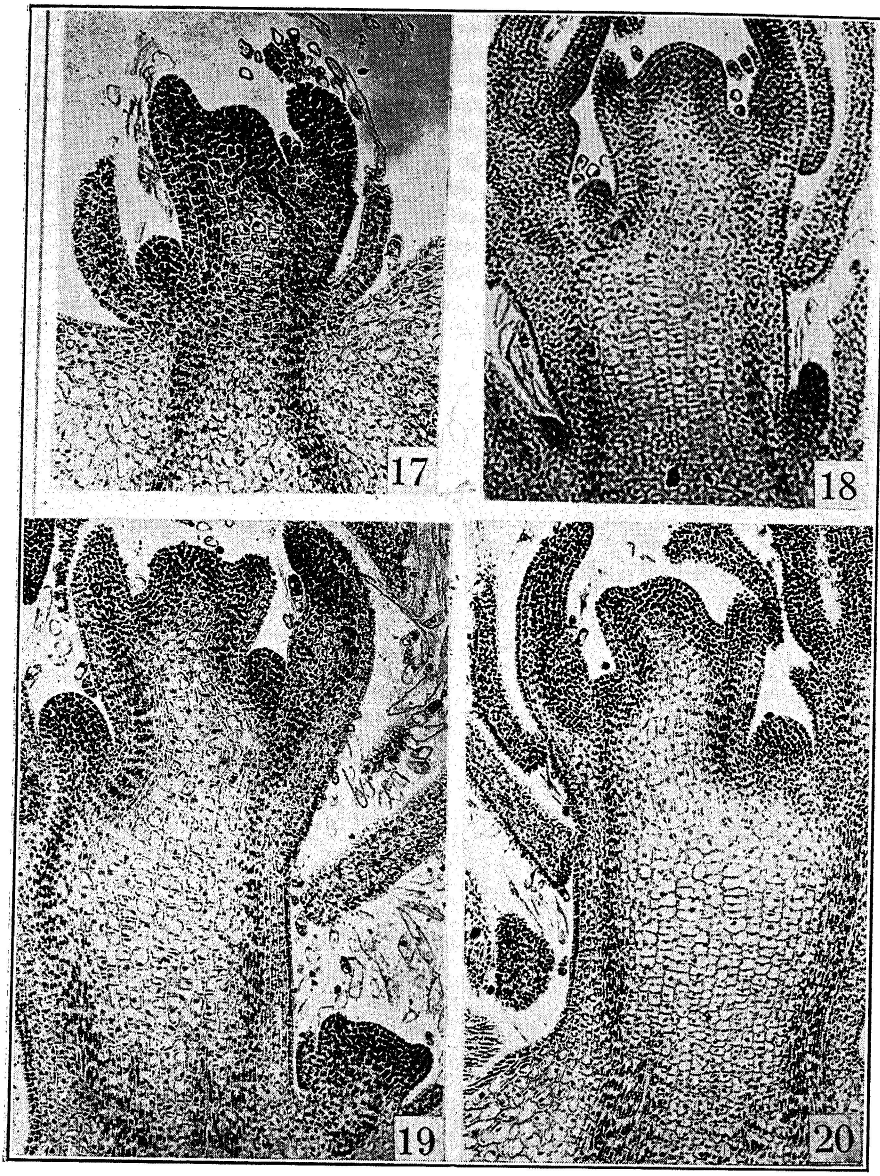

PLATE X

Microphotographs of sectioned axillary branches of Biloxi soybean plants exposed to a long photoperiod.

Fig. 17.—Section of the first axillary shoot of a 14-day old plant.
Fig. 18.—Section of the first axillary shoot of a 21-day old plant.
Fig. 19.—Section of the first axillary shoot of a 30-day old plant.
Fig. 20.—Section of the first axillary shoot of a 42-day old plant.

buds all indicated differentiation of flower buds. The ninth appeared as a protuberance in the axil of the undifferentiated leaf, while in the last primordial leaf axil, immediately below the promeristem, only a slight elevation of tissue suggestive of a primordial bud, was distinguishable. The promeristem of the main stem was reduced in size and distinctly more conical in shape. Furthermore, the size of the promeristem was reduced in comparison with promeristems of younger plants.

By the 28th and 30th days, the most noticeable changes were in the degree of differentiation of the flower buds. In the first or lowest axillary, the first flower buds, which in the preceding stage of development had only the bract, now showed primordia of various floral organs and further development of the calyx tube. In the second flower bud, the bract had emerged and the calyx lobe primordia were beginning to differentiate. The third appeared only as a rounded protuberance in the axil of the last embryonic leaf. No bud development was observed in the last leaf axil. The promeristem was greatly reduced in size.

Buds on the second to the fifth axillaries were now completely differentiated into floral organs, which could be seen with the unaided eye. Two to three flowers had formed in each axil. When two were present, both of them were found in the axils of separate leaves. A third flower may arise from either of the two secondary axillary buds. The sixth to the ninth flower buds were confined within the short primary meristematic region of the main stem. Due to the rapid growth and crowding of the flower buds, the exact position of each bud was rather difficult to determine. In all instances, the flower buds were borne singly, and only occasionally in pairs as a result of the differentiation of a secondary axillary bud into a flower bud. This bud seemed to have developed directly from the promeristem of the axillary branch. The terminal growing point was almost obliterated at this stage.

Except for further increase in growth and differentiation of the floral organs, the general morphological feature on the 35th day was similar to that of the 28- or 30-day-old plants. At this stage, a new leaf primordium developed at the promeristem of the main stem. The growth of the axillary promeristem was for the most part almost completely inhibited. In almost all cases it appeared at this time as a small rounded structure at the distal end of the axillaries and of the main stem. In some plants of the same age, the promeristem was still quite distinct, though considerably reduced in size. This was usually the case in specimens of an atypical development.

No further growth of the vegetative parts was observed when the plants were 42 days old. As in those 30 and 35 days old, there were present flowers in various stages of development, ranging from early emergence of the axillary buds to various degrees of differentiation of floral structures up to fully grown flowers and young seed pods. The promeristems of these plants varied somewhat in size and form. In general, they were markedly reduced in size with evidence of degenerative changes of the meristematic and apical cells. The flowers which appear in the terminal region of the main stem seem to arise directly from the differentiation of the promeristem and hence may be considered as terminal, in contrast to those that developed in the axils of the leaves of the axillary buds. For a given stage of development of the plant, as a rule, the flower buds which were found in the terminal region were, on the average, much further advanced than those found in the lower axillaries.

In 50-day old plants, seed pods of various sizes were present on the terminal portion of the main stem (Fig. 5). The type of development of the reproductive organs in the lower axillaries was similar to that on the main stem except that fruit setting and pod development were delayed.

The two or three lowest axillaries were somewhat abnormal. The reproductive organs, except in few instances, failed to develop beyond the flower bud stage (Figs. 9 and 10).

THE PROMERISTEM

It is apparent from the gross morphological and histological observations that a significant photoperiodic effect of the Biloxi soybean lies in the phenomenon of growth and differentiation. A possible explanation of the differences in development and in metabolism of the long- and short-day plant, may therefore be sought primarily in the changes of the promeristem where new organs or parts of organs were constantly formed.

A striking feature, but probably of little importance, was the relative difference in the size of the apical meristems of the short- and long-day plants of all stages studied, ranging in age from 14 to 42 days. In the long-day plants the promeristem was relatively larger than that of the short-day plants. This difference in size may be insignificant, since, as a rule, the long-day plants were much bigger and more vigorous than the short-day plants of similar ages.

Histological sections show that the shape of the promeristem of the short-day plant is distinctly conical (Fig. 15), while that of the long-day plants is somewhat cylindrical, the apical layer forming a plateau at the apex. (Fig. 13). In the series of long-day plants studied, the diminution in size of the promeristem was not observed until at a much later stage of development (42 days), when it began to approach the condition similar to the promeristem of the short-day plants in the early stages (14-21 days) of development (Fig. 14). In contrast to this condition, the promeristem of the short-day plants gradually diminished in size coincident with the succession of the appearance of the leaf buds, from the 14th to the 42nd day of development. By the time the flowers were well developed and those at the terminal end were crowded, the promeristem was already reduced to a flat layer of tissue. (Fig. 16).

The differentiation of the histogen takes place shortly after the emergence of the leaf primordium. In the long-day plants, the maturation of cells does not occur for some time after the embryonic tissues are laid down by the apical cells. In the short-day plants the entire terminal portion (primary meristem), for some distance, consists mainly of a small mass of uniform isodiametric cells. This histological difference in the promeristem of the short- and long-day plants became more conspicuous in later stages of development, ranging from 15 to 42 days. In general the promeristematic cells which build up the meristem of the short-day plants appeared to be larger but fewer in number than those of the long-day plants. Furthermore, mitotic figures were frequently seen in the apical region of the promeristem of the long-day plants while rarely, if at all, in short-day plants.

The axillary buds (shoots) presented features similar to those of the primary meristem of the main stem. Under favorable conditions for growth, the axillary buds may first undergo purely vegetable development, similar to the main stem, before they produce flower buds. Later in the development of the plant, the axillary buds formed into flower buds without much external evidence of vegetative growth. This was often observed in the case of older buds.

THE INTERNODES

As was previously mentioned, the internodes of the short-day plants were greatly reduced in size and, in the terminal or main meristematic region, may be completely obliterated. Histological observations seem to indicate that in these plants the differentiation and maturation of the tissues occur almost simultaneously, thus resulting in curtailment of growth of the internodes. In the long-day plants, on the other hand, the internodes are relatively elongated. Maturation of the tissues does not occur until some time after the maximum degree of differentiation is reached, permitting thereby the growth of various organs and their parts, as is indicated by the length and diameter of the internodes (Figs. 11 and 12)). In morphological features, nodes of the long-day plants varied but little from those of the short-day exposure. Only the central core (plerome) of the meristematic cells of the embryonic internodes had matured. The layer of embryonic tissue was made up of but a few cells, formed, of course, from the apical initials. The vascular strands in the primary meristematic region were still undifferentiated, while in the short-day plants of a corresponding age, the primary meristematic cells had already reached a stage of maximum differentiation and maturation into various tissues so that the vascular strands were already well defined.

THE AXILLARY BUDS

In the early stages of development, the axillary buds of the short and long-day plants were structurally identical. Subsequent to the progressive differentiation of the leaf primordium in the promeristem of the main stem, a rounded protuberance soon emerges in its axils. The further development of the bud depends upon or is influenced by the length of photoperiod to which the plant is exposed.

The Vegetative Buds.—The term "vegetative" is used as applying to the axillary buds, which did not give rise to reproductive organs. Such buds occurred in plants exposed to a relatively long photoperiod, although the buds formed during the early stage of growth of the plants exposed to a short photoperiod, ranging from 14 to 21 days, often could be put in this category. Usually the older axillary buds (first, second and occasionally the third), undergo vegetative development for some time before they become reproductive.

The first sign of a bud in the Biloxi soybean was the appearance of a knob-like protuberance at the axil of the developing leaf before it was completely differentiated. Early in its development, the bud is enveloped by a pair of stipules, which arise laterally at either side of the base of a developing leaf. Almost simultaneously with the emergence of the bud primordium, a pair of secondary stipules appears. As the axillary bud elongates and broadens somewhat, it leaves the secondary stipules at its basal region. In the axils of the secondary stipules, secondary bud primordia often arise. These buds usually remain rudimentary but under optimal conditions for reproduction, one or the other or both may differentiate into a mature flower. At the lateral plane of the anterior region (promeristem) of the bud, leaf primordia arise alternately on each side. When vegetative growth is rapid, three and sometimes four leaf primordia may be formed in the axillary buds, but the leaves, as well as the internodes, usually remain quite rudimentary. At times, however, the internodes may undergo a more rapid development, giving rise to short branches morphologically similar to the main stem (Figs. 17 to 20).

The Flower Buds.—The early ontogeny of the flower buds is identical to that of the vegetative buds. In fact, it is not until at a much later stage of development, that the flower or the vegetative bud can be distinguished.

The early axillary buds of either short- or long-day plants usually developed vegetatively in a manner described above. In the axils of the leaves of the axillary buds arise bud primordia. These buds are morphologically identical with the primary axillary buds in their early stage of growth. *Although being undistinguishable from similarly situated vegetative buds, these axillary buds of the short-day plants should be regarded as the "Anlagen" of the flowers (Figs. 21 and 23).* As the short-day plants advance in age (from 28 to 42 days), the axillary buds within the proximity of the terminal region usually differentiate directly into solitary flowers. The changes in development of the flowers are quite identical, no matter whether they arise directly from the primary axillary buds or from secondary buds formed in axils of leaves of the primary buds. The following description, therefore, will be in general applicable to the development of flower buds into their reproductive organs.

Simultaneously with the growth of the secondary axillary buds, the primordium of a membranous sheath of bracts arises anteriorly to the promeristem (Fig. 24). This appears to be the first indication of differentiation of the buds into flower primordia. Because of the rapid proliferation of the apical meristematic tissue, the position of the bract seems somewhat out of place. Before the bract reaches its full size, the anterior calyx lobe primordium appears. This is immediately followed by the growth of the lateral, and finally by the two obliquely posterior lobes. Early in its development the base of the calyx lobe broadens, effecting a shallow ridge or invagination, from which the floral organs differentiate. Subsequently to the broadening of the basal region, the calyx lobes merge and finally the whole basal region is pushed up as the calyx tube. The petal primordia appear next. They are found in positions alternating with the calyx lobes, and somewhat more distally on the floral axis (Figs. 25 and 26). On these primordia, the two keel petals developed first, followed by the two laterals and finally by the posterior lobes. The calyx tube envelopes the floral primordia and forms as it were a protective covering.

While the petal primordia are still in a rudimentary stage, the outer cycle of the stamens begins to appear. The sequence of development

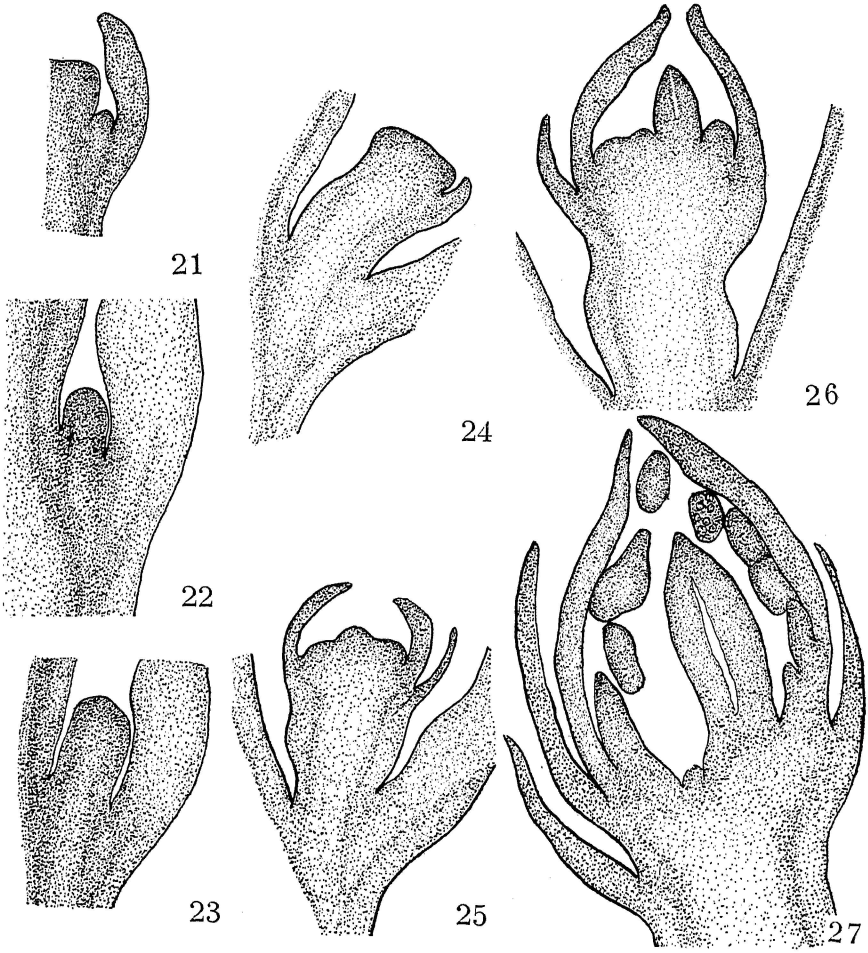

PLATE XI

Camera lucida drawings of various stages of development of the soybean flower.

Fig. 21.—The axillary bud, appearing as a rounded protuberance in axil of the embryonic leaf in a 14-day old short-day plant.

Fig. 22.—A corresponding bud of a 21-day old short-day plant.

Fig. 23.—A corresponding bud of a 25-day old short-day plant.

Fig. 24.—A corresponding bud of a 28-day old short-day plant. The bract and calyx primordium have already emerged.

Fig. 25.—A corresponding bud of a 30-day old short-day plant. The calyx tube completely envelops the floral primordia. The primordia of the petal and pistil have begun to emerge.

Fig. 26.—A corresponding bud of a 35-day old short-day plant. Further development of the calyx tube, pistil and petals. The two cycles of the stamens have appeared. Only one primordium of the inner cycle of the stamens is shown in the figure.

Fig. 27.—Further development of the flower of a 42-day old short-day plant, corresponding to that described above.

seems to be from the anterior to the posterior border of the receptacle, and immediately opposite the calyx lobes. The appearance of the last primordia of the outer cycle of stamens is followed at once by the growth of the primordia of the inner cycle, which appear in the same sequence of emergence as those of the outer cycle. In the early stage of development, the inner staminal cycle appears somewhat separated from the outer cycle, but soon they merge, due to the development of the basal tissue (Fig. 27).

The sequence of the appearance of the petals and of the calyx tube is in accord with the observation of Goebel[6] in the *Leguminosae*. Payer[9] has reported a similar order of development in *Trifolium ochroleucum* and Guard[7] in the Manchu variety of the soybean. The petal primordia grew comparatively slowly at first, but more rapidly at the onset of microsporogenesis. The calyx lobes were pushed apart as the flower emerged.

In its incipient stage, the pistil appears as a leaf-like carpel folded along its midrib, with its margins scarcely touching at the middle axis of the floral receptacle (Fig. 27). Rounded protuberances, which later develop into ovules, are produced alternately on the inside of these carpels before the margins coalesce. At first the pistil is somewhat more advanced in development than the other floral organs but later lags behind the stamens.

In *Trifolium pratense,* Westgate *et al*[12] and in *Melilotus alba* and *Melilotus officinalis,* Coe and Martin[1] reported a similar lagging behind the stamens. Unlike the *M. alba* and *M. officinalis,* in which the mycropyle of the ovules was facing the basal end of the ovary, the campylotropous ovules of the soybean were directed toward the distal end of the ovary. Usually two or three ovules, rarely four, were found in each ovary. The ovules developed simultaneously.

The Flower.—The perfect, polypetalous, zygomorphic flower of the soybean, var. Biloxi, is approximately six mm. in the distal diameter when fully developed. The corolla is composed of five petals, the posterior one being the largest, the two laterals intermediate, and the keels the smallest. Unlike many other legumes, the keel petals of the soybean flowers do not unite, although their margins touch each other.

The androecium consists of 10 diadelphous stamens. In the early development, all of these stamens are separate. Later, however, nine of the 10 filaments extend as a single unit, leaving the 10th filament free. At the time the flower opens, the single pistil is covered by unicellular and multicellular trichomes. The style is about two-thirds the length of the ovary and is curved toward the posterior stamens. The stigma is a knob-like structure which is usually receptive at the time of anthesis.

SUMMARY AND CONCLUSIONS

When the soybean plant, var. Biloxi, is exposed to a short (7 hour) and a relatively long (14 hour) photoperiod, it exhibits conspicuous differences in the rate of growth, time of sexual reproduction, and greenness of foliage. During the early stage of development the short-day plants are lighter in color but soon match with the long-day plants and, at the time of sexual reproduction, become intensely green.

The seedlings of both groups of plants, up to 14 days of age, were similar as regards the rate of growth and development of various organs. From approximately the 14th day on, the long-day plants began to grow faster than those exposed to a short photoperiod and were soon much taller, with thicker and softer stems and larger leaves. During the same period the short-day plants increased in height but little and appeared stunted. But they initiated and developed numerous flower buds on short axillary shoots. The region of the primary meristem was almost obliterated as a result of the greatly reduced internodes and hence some of the flower buds and flowers appeared terminal.

Histological observations of plants 21 days old and older indicated marked differences in development of the promeristem and internodes of the main stem and the axillaries. This would seem to have been effected as a result of exposure to different photoperiods. The promeristem of the short-day plants was distinctly conical in form, but of those of a long-day exposure, somewhat cylindrical, forming a plateau at the apex. A gradual but progressive diminution in size of the promeristem of the short-day plants was noted. This histological picture may be correlated with the marked curtailment of growth in height of these plants. By the 42-day stage, it appeared as a small flat structure at the distal end of the main stem or the axillaries. In the long-day plants decrease in size of the promeristem did not occur until at a very late stage of growth, when it seemed to approach the condition similar to that of the short-day plants during their early stage of growth (14 to 21 days).

Conspicuous histological differences were observed in the rate of formation and maturation of the tissues produced from the promeristem of plants of the two groups. In those of long-day exposure, maturation of the meristematic cells was markedly delayed, thus providing for initiation and further growth of leaf primordia and other organs. Differentiation of the histogen, however, took place soon after the appearance of the leaf initials. Both the promeristematic and meristematic cells of the short-day plants were larger in size but fewer in number than those of the long-day plants. Moreover, mitotic figures were seen but rarely among these cells. In general, the terminal meristematic region of plants exposed to a short photoperiod consisted of but a small mass of uniform tissue and the transition to the histogenic region was rather abrupt. The differentiating area in the meristem could be traced back to a few cell layers below the apical initials.

The development of the axillary buds of both groups of plants was similar up to the 21st day of growth. It consisted of a rudimentary shoot with 2-8 leaf initials and bud primordia in their axils. In the long-day plants it remained in this stage for considerable time. With very favorable conditions for vegetative growth, such as frequently exist during bright days in the spring, the axillary buds of the vegetative plants may grow into long shoots.

The earliest sign of the initiation of the first potential and possibly actual flower buds was in the 14-day old plants exposed to a short (7 hour) photoperiod. These buds appeared in the axils of the older leaf initials on the lowermost axillary shoots, while the latter were still in the bud stage. Unmistakable signs of initiation and development of flower buds were evident in short-day plants when 21 days old, from which time on they began to differentiate also in the terminal region of the main stem and the older axillaries. This was indicated by the appearance of the bract, which is a definite criterion for distinguishing the early differentiation of flower buds from vegetative buds.

On the 25th day of development, many flower buds had already reached a stage where the primordia of various floral organs (stamens, pistils, petals) and the early stage of growth of the calyx were distinguishable in histological sections. A week later many of them had attained a condition where differentiation of various parts of the flower could be detected with the unaided eye. On the 42nd day a large number of buds had reached their maximal size and there were flowers in anthesis. The long-day plants remained vegetative with no sign of reduction in growth and initiation of reproductive organs.

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