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F. B. MUMFORD, *Director*

Photoperiodism and Enzyme Activity in the Soybean Plant

AUBREY D. HIBBARD

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ABSTRACT

Changes in activity of the enzymes, catalase, peroxidase, invertase, amylase, and reducase, were followed in soybean (var. Biloxi) plants when grown under a seven-hour day (favorable to reproductive development) and a fourteen-hour day (favorable to vegetative growth) from germination until the plants under the shorter light period had produced flowers.

The activities of most of the enzymes studied were depressed in the beginning under the short-day treatment. Later, there was an increase under this treatment which continued as long as the plants were exposed to day-light periods of different lengths.

Invertase and peroxidase may begin to increase in activity as soon as five days after the treatments are started. Catalase is depressed for 15 to 20 days, and then becomes more active in the leaves of the reproductive plants. The depression of catalase is greatest in the tip of the stem. Changes in enzyme activity in the tip of the stem are similar to those observed in the leaves but occur several days later.

Amylase and reducase do not seem to be affected by the length of the daily light period. All changes in enzyme activity observed appear to be more closely correlated with changes in the growth response of the plant than with its reproductive development.

Photoperiodism and Enzyme Activity in the Soybean Plant*

AUBREY D. HIBBARD

Many cultivated plants will not produce flowers until they have received a daylight period of a definite duration for a certain number of days. Although the environmental conditions necessary for reproduction are well known, the way in which this response is brought about is unknown. At present all attempts to explain the mechanism of the reproductive response of plants to the length of day have been incomplete or else confused with some other simultaneous reaction to the photoperiod, especially its effect on vegetative development. No external observations of the plants can give any definite clue to the possible chain of events resulting from the stimulus set up by the light period. There is some evidence that the length of the dark period is the causative factor, since plants under short periods of both light and darkness behave as though exposed to a long day. A clear picture of the way in which the reproductive state is brought about by the length of the photoperiod is to be had only by making observations upon the internal metabolism of the plant. The present study is a phase of such an investigation.

The most direct method of securing information on the ability of the tissues and cells to bring about material changes is through a study of the protoplasmic and cellular enzymes which are responsible for the intensity with which the various phases of activity are carried on in the plant. A study of the activity of the various enzymes occurring in the tissues should throw some light on the dynamics of the biochemical changes observed in the various organs. It is not enough to observe that certain changes have taken place or that they will take place; the ability of the plant to catalyze these changes and the rate at which they can be carried out should be known.

The object of this investigation was to determine the activity of some common oxidizing and hydrolyzing enzymes in a typical short-day plant growing under conditions of long and short photoperiods. There is the possibility that the degree of activity of certain cell catalysts may be correlated either with morphological changes brought about by the length of day, the induction of reproduction, the growth rate or the accumulation of organic reserves.

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An attempt was made to make a detailed study of the changes which occur in the tips and leaves while the plant is developing from seed to flowering under a seven-hour and fourteen-hour day. The day lengths were so adjusted that the plants under the short day passed into the reproductive condition as rapidly as possible, while those under the long day would remain in the vegetative condition indefinitely.

The enzymes studied in this investigation were catalase, peroxidase, amylase, invertase and reducase. This group includes two oxidizing enzymes, catalase and peroxidase, which have been extensively studied in relation to oxidative processes in the plant. In spite of the meager evidence, their activity is generally accepted as an indication of the rate at which active metabolism is being carried on. The facility with which a plant can mobilize and transport carbohydrates is regulated by the activities of invertase and amylase. Under the short photoperiod there is a definite modification in the carbohydrate metabolism as shown by the accumulations of starch and other reserves in these plants. Reducase was studied in connection with nitrate metabolism. Its significance as an oxidizing enzyme should not be overlooked.

REVIEW OF LITERATURE

When plants become reproductive under a short day, there is an accompanying increase in the amounts of reserve organic materials, such as starches, sugars, and nitrogenous substances (37) (46)*. Several attempts have been made to correlate such accumulations of plastic materials with the reproductive condition of the plant (18), (24). It appears that this is only a coincident phenomenon, since no causal relationships have been conclusively established. It is also interesting to note (26) (31) that some of the short-day plants contain larger amounts of chlorophyll and are evidently more efficient, since they can in some instances produce a greater total dry weight per plant under a shortened day. At the same time their respiration per unit weight, as measured by carbon dioxide, seems to be more intense (45). Although differences in the concentration of reserve materials cannot be linked in a causal way with the reproductive response, there are evidently some common factors which may be influenced by the length of the light exposure. These materials may in turn modify the type of growth, as well as the way in which the products of photosynthesis are used.

*Numerals refer to Literature Cited, Page 46.

According to the concept of phasic development, derived from studies of photoperiodic induction and vernalization, (1) (31) the plant acquires the ability to make certain changes at some later stage in its development. These are not brought about until conditions in its growth become favorable for their expression. The plant has been so modified that it may catalyze certain reactions or chains of reactions which are not carried out until the materials or the internal environment for these processes have accumulated.

Catalase has probably been investigated more thoroughly than any other plant enzyme. Most of the studies have dealt with the factors affecting its activity *in vitro*. The conditions affecting the activity of this enzyme in the plant have not been investigated to any extent.

The work of Appleman (2) indicated that catalase activity and plant respiration could be closely correlated. His paper stimulated much investigation from that point of view. However, it was found later that although these two indicators of plant metabolism were generally associated, there were exceptions to be found in the case of germinating seeds (41), yellowing leaves (39), and ripening fruits (40).

The exact role of peroxidase in cellular oxidations is obscure. It is highly probable that it aids in the transfer of oxygen from peroxides to the respiratory pigments.

Very little is known concerning the environmental factors affecting invertase and amylase activity in the plant. It is the general opinion that they assist in the transformation of sugar and starch, when the need arises.

The recent work (38) (47) on the oxidizing enzymes (dehydrogenases and oxydases) has made any investigations concerning the nitrate reducing power of tissue extracts of doubtful value. The evidence seems to indicate that the nitrate functions as an oxygen donor for one of the dehydrase systems present. The plant uses the oxygen from the nitrate instead of molecular oxygen in the chain of reactions set up by dehydrogenation of glucose or other respirable substrates.

The first studies on the relation of enzymes to the photoperiodic response were made by Knott in 1927 (26). Working with celery and spinach, he showed that there were no differences in the catalase activity of the leaves of reproductive and vegetative plants. He was also unable to correlate the pH of the juice with the catalase activity of these leaves. In the tips of the stems of the spinach there was a difference in the catalase content which was significant. The reproductive plants growing under long day

conditions were about one-fifth less active than the purely vegetative plants under the short day. There was also a close agreement between respiration, as measured by carbon dioxide production, and catalase activity; both decreased as the plants became reproductive. Celery plants just starting to produce seed stalks showed decreased catalase and respirational activities with increasing length of the stem. When the spinach plants were made to change from a vegetative to a reproductive state by shifting them from a short to a long day the catalase activity decreased. If on the other hand, a plant which had started to enter the reproductive state were forced back into the vegetative states by returning it to the short-day treatment the catalase activity increased to its former level. In another study (27) the catalase activity was measured shortly after the vegetative plants under the short day had been shifted to the long-day treatment; the catalase activity immediately increased under the long light period. This should eventually lead to a decrease in activity of those plants, as in the first experiments, which were made 42 days after the treatments were started.

Cajlachjan and Alexandrova (9) could detect no differences in the catalase activity in the leaves from treatments with short and long photoperiods in millet and soybeans. The peroxidase content of the leaves of the soybean was higher under short-day conditions, when the growth processes are retarded. In millet, another short-day plant, the leaves showed a higher peroxidase content under the long day. An exposure to 6 short days lowered the activity of this enzyme in a long-day plant, while 6 long days raised it in a short-day plant. They concluded that the oxidizing enzymes behave differently in different species of plants because of the differences in chemical composition.

A shortening of the day brings about an increase in the activity of the oxidizing enzymes, catalase and peroxidase, in the common bean and chrysanthemum according to the work of Krassinsky *et al.* (29). They investigated the activities of these enzymes in the leaves and fruits. They could detect no differences in the leaves of the Cineraria which is indifferent in its reproduction response to the photoperiod. Bean plants which had been induced to flower and later removed to the long day still showed a difference in the activities of enzymes due to photoperiodic induction. A shortening of the day in the bean and chrysanthemum provoked changes in the activity of amylase and saccharase (invertase), but the direction of these changes was different in the different plants. They were of the opinion that the activity of the hydrolyzing enzymes under

the influence of a shortened day depended apparently on the individual peculiarities of the plants and their enzymatic systems.

Demskowskii (1) also found a higher peroxidase activity in the leaves of short-day soybean plants.

A possible explanation of the photoperiodic response of certain plants has been suggested by the work of Eckerson (16). She found that under a short day the juice of the Biloxi soybean reduced nitrates at about one to five per cent of the rate shown under the long day treatment. This suggests that the inhibition of growth in the short-day plants is due to a lack of nitrogen, in the reduced form, which is necessary for the manufacture of new protoplasm required in the upward growth of the stem. Therefore, since nitrates cannot be reduced, the plant fails to grow. Nitrogen, in the form of nitrates, and the products of photosynthesis accumulate. This is in accord with other experimental data upon the carbohydrate-nitrogen metabolism of the plant (37) (18).

According to Eckerson (16), the reducase activity of the plant extract is affected by many factors which fail to modify the response of the plant to the photoperiod. A deficiency of phosphorous or potassium in the nutrient media may cause a great decrease in the nitrate reducing power of the plant. These treatments do not affect the response of the plant to the photoperiod. Shading so as to cut off a considerable portion of the total light reaching the plant causes a decrease in the nitrate reducing activity. Several cloudy days in succession bring about the same response; but have little effect on the photoperiodic response. A plant like the tomato which is indifferent in flowering response to the length of day shows a great change in reducase activity when the photoperiod is changed.

Few investigators have concerned themselves with the relation of the enzymatic content of the plant to the type of growth. Heinicke (22) working with bearing and non-bearing apple trees found a correlation between the catalase activity and the type of growth. The vegetative buds were much higher in catalase than fruit buds during the dormant season. The bark from limbs that had fruited was more active than that from limbs that had not. The bark in all cases from bearing apple trees was lower than that of non-bearing trees. Knott and Anthony (28) have shown that blossom buds at the time of differentiation have a greater catalase activity than vegetative buds. These differences may be present for some time prior to any differences which can be detected morphologically.

In summarizing the work on soybeans it can be said that, under short-day conditions there is an increase of peroxidase in the leaves, a decrease in the reducase and no differences are to be found in the catalase activity. None of this work has established any definite connection between the enzymatic activity and the reproductive phase of plant development. It is of interest to note that in the work of Knott (26) there was a decrease of catalase in the organs which were changing to the reproductive condition, whereas, Krassinsky *et al* found an increase in the same structure on a different plant as it approached the reproductive state. Investigators have almost invariably used material from plants that were already reproductive. It is difficult to see how enzymes are to be linked in a causal way with reproductive responses when studies are made after the plant has reacted morphologically.

Most enzymes have their optimum activity within a very narrow pH range. No interpretation of the activity of an enzyme in the plant can be given unless something is known about the active acidity of the medium in which the enzyme is functioning. It was observed by Garner and Allard (18) that there was a considerable difference in the active acidity of plants growing under different photoperiods. This was most pronounced in the region of the stem nearest the growing point, which was more acid than the rest of the plant. Under the short day the acidity was uniform throughout. The most striking results were obtained by suddenly shifting plants growing under a long day to the short-day treatment. After a lapse of two or three days there was a sharp drop in the hydrogen-ion concentration of the sap of the top most leaf. This lasted only a few days, when it again returned to the level of the plants which had grown continuously under the long photoperiod.

Zimmerman and Hitchcock (49) measured the pH of the stems and leaves of the dahlia under different lengths of day. They found that there was practically no difference in the acidity of the expressed juice of the leaves of the plants under long and short-day treatments. The pH varied slightly from 5.4 during the entire life of the plants.

Knott (26) made measurements of the hydrogen-ion concentration of leaves from spinach plants under increasing lengths of day, but could establish no relationship between the acidity of the leaves and the reproductive stage of the plant.

Measurements made by Krassinsky *et al.* (29) upon leaves and fruit showed no relationship to the stage of development in the garden bean and chrysanthemum.

MATERIALS AND METHODS

In order to investigate the variations in enzymatic activity relative to the photoperiodic response the following materials and methods were used.

Plant Material

The Biloxi variety of soybean was selected, since during the last six years this plant has been used in intensive studies at this station upon the biochemical and morphological changes occurring under short and long photoperiods (35).

This variety of soybean has many characteristics which make it very desirable for use in this type of work. These are well known since it has been used in many studies on photoperiodism (35) (16) (18) (37). Its response to the photoperiod is probably better understood than that of any other plant. It is a typical short-day plant. Vegetative growth continues for an indefinite length of time if the daily light period is over thirteen hours. When the light period is shortened as little as one hour, flowering will begin in about forty days. Further shortening of the photoperiod will decrease the time for the appearance of flowers. The minimum time for the appearance of flower buds is 25 days. A day shorter than eleven hours will not hasten the response. A short day of seven hours seems to delay somewhat the appearance of flowers (44). Plants exposed in a satisfactory temperature to a length of day suitable for flowering for a period of ten to twelve days will, when removed to a long day, proceed to develop flowers as if they had been continuously under the short photoperiod. Vegetative growth is soon resumed. The plant continues to grow vegetatively and to produce flowers at the same time.

It has been pointed out that temperature determines the length of time for the soybean to react to a photoperiod suitable for flowering (13) (20) (44). If the average daily temperature is below 77°F. there is a delay in the time of flowering. The optimum seems to be around 83°F.

Plant Culture

The methods used in growing the plants have been described in detail by Murneek (36). Briefly, the procedure was to grow the plants under standard greenhouse conditions. The planting was done directly into boxes three feet long, twelve inches wide, and ten inches deep. The plants were allowed to grow in these until they were used in the enzyme determinations. At the start of the treatments they were thinned to thirty plants per box. There was a gradual thinning due to sampling so that the plants were never crowded. The photoperiod was lengthened when necessary

by supplementary illumination with mazda lamps so adjusted as to give an intensity of 250 foot candles at the soil surface. The boxes intended for the short-day treatment were placed on trucks which were rolled into a ventilated dark room at night. The long-day exposure was from sunrise to sunset under natural light and from then until 9 p. m. with artificial light. The short-day exposure was from 8 a. m. to 3 p. m. under natural light.

The daytime temperature varied from 70° to 80° F. An effort was made to keep the night temperature above 65°F., though this was not possible during the coldest periods of winter. It was essential that each series of plants be as nearly alike as possible. During the winter months when it was more difficult to maintain the temperature, some trouble was experienced in securing uniform stands. If there were many cloudy days in succession during this period the plants under the short day showed an abnormal increased growth in height. The appearance of floral organs was also delayed. If a lot did not behave in a typical manner, it was discarded. No plants were grown during the extremely warm summer months.

The seedlings appeared, generally, at the end of 4-7 days after planting. As soon as the cotyledons had shed their seed coats, the separate treatments were started. Under the short-day exposure the leaves were lighter green than those of long-day plants for about the first eighteen or twenty days; afterwards, they became darker green in color. The long-day plants grew in height at a constant rate during the entire period of treatment. In the short-day groups the growth was at the same rate during the first few days but soon began to fall off, so that by the twentieth day growth in height had practically ceased. The stems of the long-day plants were thicker, more succulent, and contained more lignified tissues. They also had a greater number of nodes, longer internodes and leaf petioles, and larger leaves.

Collection of Samples

For enzyme determinations the plants were cut before daylight. They were immediately fractionated, weighed and frozen. Each lot was carefully selected for uniformity, all abnormal ones being discarded. A sample of leaves consisted of similar parts from leaves of the same age. Only the leaf blade was used. Leaves showing any sign of injury or any abnormality were rejected. An effort was made to secure leaflets of the same size but there was always some variation. For a four gram sample the central leaflets from approximately fifteen leaves were used.

For the tip sample the first two-centimeter portion of the tip was used. All young leaves that could be grasped with the fingers were removed. A four gram sample consisted of the tips from about thirty-five plants.

In the soybean plant the first leaves expanding from the central axis are the cotyledons. The next pair of leaves are simple and opposite. All other leaves produced are trifoliate and alternate. For the purpose of this study the trifoliate leaves were numbered in the order of their appearance, the oldest being called the first leaf.

Immediately after the samples were weighed, they were put into labeled paper bags and placed in a mechanical refrigerator where the temperature of -14°C. caused rapid freezing. The time from cutting to freezing required about one and one-half hours. The samples were analyzed for enzyme activity at convenient intervals. Preliminary trials showed that there were no detectable changes in the enzymatic activity for a period of at least six weeks, if the material was kept frozen.

Hydrogen-ion Determination

The material was collected at the same time as that used in enzyme determinations. After the plants had been divided into stem and leaf samples they were frozen rapidly in a mechanical refrigerator. They were held in this condition until transferred to the metal cylinder of a Carver laboratory press. The sap was expressed as the tissue melted. A pressure of 10,000 lbs. per sq. in. was exerted on the press plate during the extraction. As soon as the juice had reached room temperature the pH was measured in a quinhydrone electrode vessel.

In the present investigation the pH of the leaf and stem sap was measured, but owing to the limited amount of material available the work of Garner and Allard (18) on "top most leaf" could not be repeated.

Enzyme Extraction

The frozen tissue was ground with a small amount of washed quartz sand and 3 cc. of an extraction solution, made by mixing equal parts of glycerol and phosphate buffer, pH 7.0 (3). Grinding was continued for ten minutes or until the paste was homogenous in appearance. This was mixed with thirty-three cubic centimeters of the glycerol extraction solution. Each cubic centimeter of this suspension was equivalent to one-tenth gram of fresh tissue. The enzyme solutions were kept in small flasks in a pan filled with cracked ice until the determinations were made. The

TABLE 1.—THE EFFECT OF CENTRIFUGING ENZYME EXTRACTS UPON CATALASE ACTIVITY.

Sample	Method of extracting	Catalase activity per gram fresh weight
1	Ground 10 minutes, suspended in glycerol-buffer mixture	.36
2	Same as above	.37
2	Ground 10 minutes, suspended in mixture, centrifuged 5 min., supernatant liquid used	.21
2	Same as above but resuspended by shaking	.37

interval of time between grinding and measurement of enzyme activity was never longer than eight hours.

This is a modification of the extraction method described by Balls and Hale (3). Their procedure called for centrifuging the suspension after grinding to remove fragments of cells and vascular tissue. It was found that centrifuging, even at moderate speeds, brought about a great reduction in the catalase activity of the supernatant liquid. This could be regained by resuspending the material thrown down by the centrifuge as shown in Table 1.

Catalase and Peroxidase Determination

The methods used were those described by Balls and Hale (3) (4), with the following modifications. Their precautions to remove dissolved gases by boiling the reaction mixture in vacuum were not observed. No activator for catalase was used. The course of reaction for soybean peroxidase seemed to be monomolecular rather than linear. All results are expressed as the activity constant for one gram of fresh material.

Invertase Determination

A modification of the method of Cattle (10) was used. A mixture of 25 cc. of 4 per cent sucrose plus 25 cc. of phosphate buffer, pH 6, plus 2 cc. of the enzyme solution was incubated under toluene in stoppered 125 cc. Erlenmyer flasks for 18 hours at 35°C. Twenty-five cc. of this mixture were pipetted into 125 cc. beakers and heated on a hot plate to boiling. This was filtered and washed into 100 cc. volumetric flasks.

An alternate method of clearing (42) with sodium tungstate was found to be just as effective in stopping enzyme action and removing interfering substances and had the added advantage of being much more rapid. In this procedure the 25 cc. portion of the digest was pipetted into a 100 cc. volumetric centrifuge tube. One cubic centimeter of 15 per cent sodium tungstate and five

drops of .04 per cent thymol blue indicator were added at once. Enough concentrated sulphuric acid was added from a small dropping pipette to change the indicator to a red tint and one drop in excess. The tubes were made up to volume, the contents mixed thoroughly and centrifuged for five minutes.

A 50 cc. aliquot of the solution cleared by either of the above methods was taken for the sugar determinations. The amount of reducing substances present was estimated by the procedure of Shaffer and Hartmann (43). The difference between the amounts of reducing sugars present before and after incubation was taken as a measure of the invertase activity of the enzyme preparation. Results were expressed as the number of cubic centimeters of tenth normal thiosulphate required to titrate the reducing sugars produced by one gram of fresh material under the conditions described.

Amylase Determination

The method used was a modification of that described by Denny (12). The reaction mixture consisted of 25 cc. of 2 per cent soluble starch, plus 25 cc. of phthalate buffer, pH 5, plus 2 cc. of the enzyme solution in 125 cc. Erlenmyer flasks. Ten drops of toluene were added as a preservative. The tightly stoppered flasks were incubated at 35°C. for 18 hours.

Then 25 cc. of this mixture were pipetted into a 125 cc. beaker and brought to boiling, to stop enzyme action and coagulate the protein-like materials present. This was filtered and washed while hot into a 100 cc. volumetric flask. A 50 cc. aliquot was taken for sugar determination.

The sugar was estimated by the Shaffer and Hartmann procedure. The difference between the number of cubic centimeters of tenth normal thiosulphate required to titrate a similar amount before incubation and that required to titrate a similar amount after incubation was regarded as a measure of the amylase activity. Results are expressed as the number of cubic centimeters of tenth normal thiosulphate required to titrate the reducing sugar produced by one gram of fresh tissue under these conditions.

Reducase Determination

The author's modification (23) of the Eckerson (15) method for determining nitrate reducase was used in all cases where the reducing action of the juice was determined. The concentrations recommended are too dilute for some tissue. An equivalent of one gram of fresh material to 20 cc. of cleared solution seems to be about right for soybean leaf material. If the reducing activity is

low the time should be extended to forty hours. It makes little difference whether the flasks are stoppered or not, provided a sufficient quantity of toluene has been added to keep the reaction mixture sterile.

RESULTS

Experiment 1

A preliminary planting was made to secure information on the catalase activity of plants that had been treated under the different photoperiods until a definite response was evident. At the time of taking the first sample, the short-day plants were in the reproductive stage of development. They were dwarfed, dark green in color and had many small flower buds about the tip. The long photoperiod, on the other hand, had prevented the development of floral organs. These plants had many light green leaves and were growing in height at a rapid rate. They were already 13 cm. taller than the short-day group. The last sample was taken 45 days after the beginning of the treatments. The short-day plants were then in full bloom and only 8 cm. taller than when the first sample was taken. The other lot was 40 cm. taller than these and still vegetative. No floral tissues of any sort were present.

TABLE 2.—CATALASE ACTIVITY IN REPRODUCTIVE (SHORT-DAY) AND VEGETATIVE (LONG-DAY) SOYBEAN PLANTS.

Date Sampled	Days Treated	Tissue Examined	Catalase activity per gram of fresh weight	
			Short-day	Long-day
Oct. 18	22	Leaves	.75	.70
Oct. 24	28	Nodes	.03	
		Leaves	.85	.75
Oct. 31	35	Nodes	.05	.01
		Leaves	.67	.50
Nov. 6	41	Tips	.11	.14
		Nodes	.08	.09
		Leaves	.89	.44

The catalase activity of the leaves of the short-day plants was higher at each of the four times when samples were taken. The data for the nodes was incomplete but showed no consistent tendencies. The one tip sample had a much lower catalase activity under the short photoperiod.

Experiment 2

These plants were grown during the winter months. The cool house during this period caused slow growth. These plants were not strictly typical of the other lots. The short-day group was

somewhat spindling during the first part of the growing period, but by the end of the experiment had assumed the external characteristics of typical short-day plants, though no buds were visible when the last sample was taken.

The records from this crop are not as significant as those from the later ones; but do show that the catalase in the tips of the vegetative, long-day plants is much more active than in the plants whose growth has been inhibited by the short photoperiod. Although the plants were somewhat abnormal, they showed the same relationship of catalase to the photoperiod as found in all of the other lots. After the treatments began, the catalase activity of the tip of the long-day plant became more active and held this relative position regardless of the modifying factors which tended to affect the activity of this enzyme in the plant. The variations in the successive samples were so great that it was difficult to secure a comparable measurement. There was, however, a definite indication that the catalase activity of the leaf tissue was slightly greater in the long-day plants.

TABLE 3.—THE ACTIVITY OF CATALASE AND PEROXIDASE PER GRAM OF FRESH TISSUE IN THE TIPS AND LEAVES OF SOYBEAN PLANTS FROM EXP. 2.

Date Sampled	Days Treated	Tissue Examined	Catalase Act. per gm.		Peroxidase Act. per gm.	
			Short-d	Long-d	Short-d	Long-d
Jan. 18	0	Stem tip	.11	.11	.50	.50
		Leaves	.15	.15	.30	.30
Jan. 25	7	Stem tip	.06	---	.75	---
		Leaves	.26	.33	1.11	.80
Feb. 2	14	Stem tip	.04	.07	.70	.60
		Leaves	.33	.42	1.60	1.40
Feb. 7	19	Stem tip	.06	.10	.74	.73
		Leaves	.43	.40	2.00	1.22
Feb. 10	22	Stem tip	.07	.10	1.50	.90
		Leaves	.42	.50	1.09	.90

It is well to stress the fact here that there is a general tendency for the catalase activity of a given leaf to decrease with age. When the leaf is very young the activity is low. There is a rapid increase as the leaf expands and finally, a gradual decline as the leaf ages. In making comparative measurements of the activity of this enzyme in leaf tissue, the samples must be very carefully selected to secure leaves of the same age. Although the tissues are of the same chronological age, there is still the problem of physiological age, in respect to enzyme activity. Gross observations suggest that

the leaves of the long-day plants age more rapidly than those exposed to a short day. Evidence for this can be seen in the early shedding of cotyledons by the long-day plants. This variety of soybeans sheds its cotyledons much less frequently when grown under a seven-hour day. After allowing for differences due to the rate of aging, there is still an increase in catalase activity in the leaf as a direct result of the short photoperiod.

The catalase activity of the leaves was many times greater than that of the tips, regardless of the treatment given the plant. (Table 3). This fact has been previously observed by Knott (26), Bunzell (5), and Heinicke (22). On the contrary, there were no such wide differences in the peroxidase activities of the tips and leaves. This is another instance in which peroxidase and catalase activity cannot be correlated (40). It can be seen from the data of later experiments that these two enzymes do not follow each other during the daily changes in the plant. The fluctuations are probably the result of varying environmental factors, growth rate, or stage of development. In the light of these facts, it is difficult to correlate the functions of these two enzymes in the plant.

The peroxidase activity of the leaves and tips was greater in the short-day plants for each determination from this experiment.

Experiment 3

Plants used for this experiment were placed under the different photoperiods as soon as they appeared above the soil. The first sample was taken twelve days later. The sampling was done so that data on the enzymatic activities of the plants during the stage of growth midway between the first two experiments could be obtained. An effort was made to substantiate the findings from the preceding experiments. The last sample was taken when the plants were thirty-four days old. In this crop and all later ones the plants were allowed to grow until the short-day group had produced flower buds. The lot was normal in every respect and typical of the majority of the plants used in these studies. When the last sample was taken the long-day plants were growing vegetatively and were much taller. The short-day plants had almost ceased growth and flower buds had formed around the tips and in the leaf axils.

The data from this crop (Table 4) shows again that the catalase activity in the tip of the long-day plant was greater throughout the experiment. The leaves of the short-day plants on the other hand, possessed the greater catalase activity. Although both lots showed a gradual decrease in the activity of this enzyme during

TABLE 4.—CATALASE AND PEROXIDASE ACTIVITY OF SOYBEAN PLANTS OF EXP. 3; EXPRESSED AS THE CATALITIC POWER OF ONE GRAM OF FRESH TISSUE.

Date Sample	Days Treated	Tissue Examined	Catalase Act. per gm.		Peroxidase Act. per gm.	
			Short-d	Long-d	Short-d	Long-d
Feb. 13	12	Stem tip	.12	.15	1.21	.90
		Leaves	.41	.34	1.50	1.00
Feb. 21	20	Stem tip	.10	.18	1.00	.61
		Leaves	.85	.75	1.70	1.50
Feb. 28	27	Stem tip	.07	.12	1.10	.80
		Leaves	.62	.37	1.30	1.00
Mar. 6	34	Stem tip	.08	.13	.74	.43
		Leaves	.54	.35	.83	.78

TABLE 5.—CATALASE AND PEROXIDASE ACTIVITY IN SOYBEAN PLANTS USED IN EXPERIMENT 4.

Date Sampled	Days Treated	Tissue Examined	Catalase Act. per gm.		Peroxidase Act. per gm.	
			Short-d	Long-d	Short-d	Long-d
Apr. 16	3	Leaves	1.16	1.29	1.60	1.40
Apr. 20	7	Leaves	.28	.40	.82	.75
Apr. 24	11	Stem tip	.06	.13	---	---
		Leaves	.38	.45	.85	.77
Apr. 29	16	Stem tip	.07	.14	---	---
		Leaves	.40	.51	1.46	1.20
May 4	21	Stem tip	.24	.16	---	---
		Leaves	.38	.69	.87	.81
May 8	25	Stem tip	.06	.13	---	---
		Leaves	.53	.43	.83	.60

the life of the plant, the change was much more pronounced in the long day.

Here again, the peroxidase activity of both tips and leaves was greater in the short-day plants.

Experiments 4 and 5

These plants were grown with the intention of duplicating the work of the earlier experiments, if possible, under the favorable growing conditions of late spring. It had become evident by this time that extreme care had to be used if comparative samples were to be secured. When the enzymatic activities of certain tissues or parts are to be compared, the tissues under examination must be of the same age. It was thought at that time that a shortening of the interval between successive samplings would give a more complete picture of the differences between the two treatments.

TABLE 6.—CATALASE AND PEROXIDASE ACTIVITY IN SOYBEAN PLANTS OF EXPERIMENT 5.

Date Sampled	Days Treated	Tissue Examined	Catalase Act. per gm.		Peroxidase Act. per gm.	
			Short-d	Long-d	Short-d	Long-d
May 1	4	Stem tip	.13	.13	---	---
		Leaves	.60	.83	1.29	1.05
May 4	7	Stem tip	.13	.15	1.39	1.36
		Leaves	.28	.41	.79	.66
May 8	11	Stem tip	.12	.15	1.65	1.07
		Leaves	.26	.35	.51	.45
May 12	15	Stem tip	.01	.07	.73	1.04
		Leaves	.42	.56	1.09	.94
May 16	19	Stem tip	.01	.09	.98	1.16
		Leaves	.14	.19	.66	.67
May 20	23	Stem tip	.05	.11	1.28	1.39
		Leaves	.33	.67	1.09	1.06
May 24	27	Stem tip	.07	.15	2.00	1.15
		Leaves	.49	.54	1.14	.67
May 28	31	Stem tip	.13	.11	1.82	1.14
		Leaves	1.01	1.01	----	----

The data (Tables 5 and 6) support those obtained from the other crops. During the early stages of growth the catalase content of the leaves under the long-day treatment was much higher but gradually declined until the activity in the leaves from the two treatments was almost the same. The catalase activity in the tips was about twice as great in the long day group.

The peroxidase activity was much higher in all tissues of the short-day plants. There was an unexplainable change in the peroxidase activity of the tips of the plants in experiment 5 (Table 6). Results from previous crops showed that the peroxidase activity was higher in the short-day tips. At the beginning the trend was in the same direction but between the eleventh and fourteenth days some unknown influence caused a lowering of the activity of this enzyme. The plants did not resume the normal trend until after the twentieth day. In most of the leaf samples peroxidase was more active in the group receiving the short light exposure.

Experiment 6

This experiment was planned so as to avoid some of the difficulties encountered in the previous trials. In addition to repeating the work already done, an effort was made to follow the enzymatic changes occurring in a given tissue during the course of the treatments. These tissues had to be as nearly alike as possible and of the same age. The first and second trifoliate leaves and the tip of the stem were the parts used. In addition to catalase and peroxi-

dase, the changes in invertase and amylase were also followed. In order to secure mature trifoliate leaves at the beginning of the experiment, the plants were grown for ten days under the long day.

In all of these experiments the long day is understood to be the control condition. Individuals of this plant remain in a vegetative condition and continue to grow in height as long as they are held under this treatment. However, when they are changed to the short day, there is an immediate slowing down in the rate of growth, together with other morphological changes (35). Although probably not a related condition, the change from the vegetative to the reproductive condition is the most striking reaction to the shortening of the photoperiod. The length of the previous long-day exposure does not affect the rate at which the plant reacts to the short photoperiod. Under a seven hour exposure, and satisfactory temperature, this plant will produce visible flower buds in less than 25 days regardless of the previous treatment.

This crop was grown during the period from Sept. 1 to Oct. 7. The lot was normal in appearance, in spite of the extremely high temperatures which prevailed during the first part of the period. Samples were taken at intervals during the experiment when it was thought that important changes in the reproductive metabolism might be occurring.

It is interesting to compare the enzyme activities occurring in different tissues at the same time. The data from the long-day plants show the typical changes in enzyme activity which occur with the aging of the tissues. (Fig. 1).

The catalase activity of the leaf goes through a maximum period of activity and then declines. The tip appears to gradually increase in catalase activity. Peroxidase activity seems to follow a similar course, although the changes are much slower. The decline in activity seems to follow a similar course, though it is not as rapid, and the peak is not reached as soon. The behavior of invertase is different in that its activity declines from the beginning of the period of measurement. This decrease in activity is greater in the leaves than in the tips. The slight changes shown by amylase strikingly resemble those exhibited by peroxidase. These changes explain, in part, the variations in enzyme activity of leaf samples when taken without considering the age of the tissue. Even when this precaution is taken there will be a considerable fluctuation in successive readings. These differences are caused, largely, by variations in the environment of the plant. Errors due to sampling should not be more than 10 per cent.

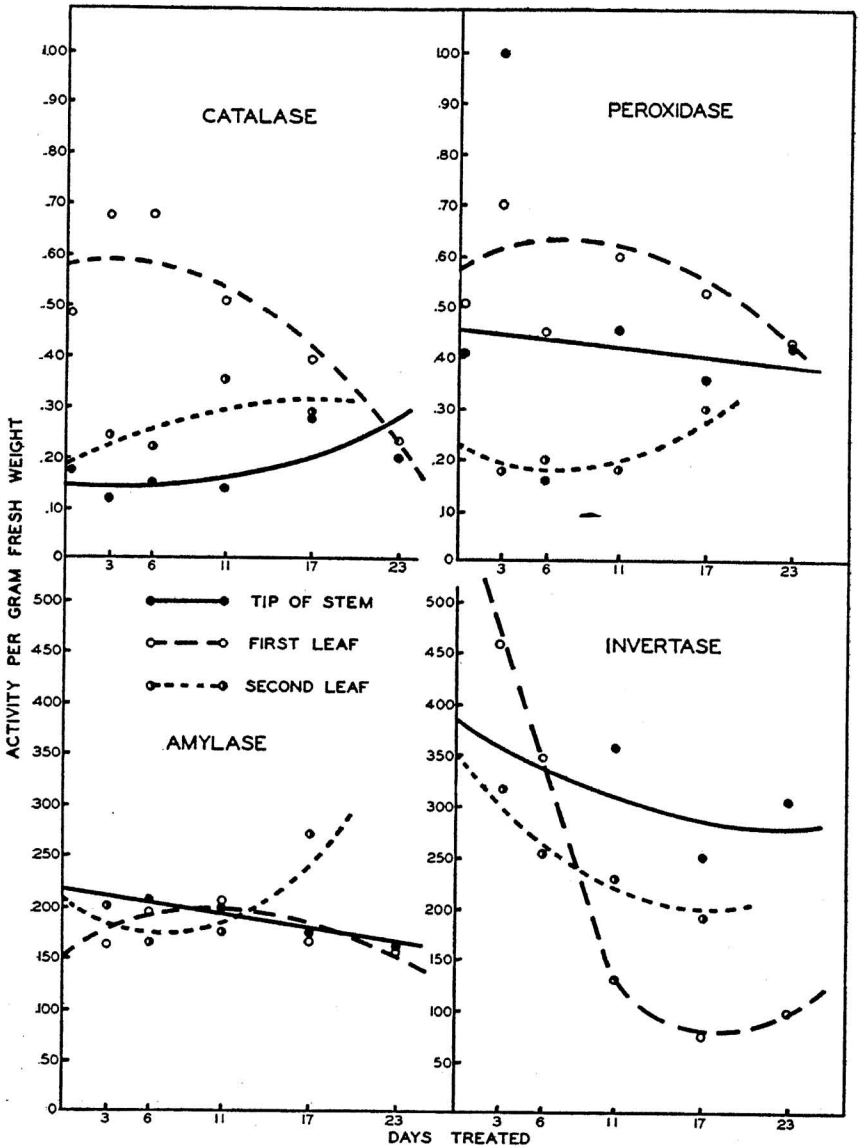


FIGURE 1. CHANGES IN ENZYME ACTIVITY IN TIPS AND LEAVES OF VEGETATIVE PLANTS WITH INCREASING AGE.

Figure 2 shows the variations which occur between successive samplings when plants grown under the two photoperiods are compared. From this it is evident that very little significance can be attached to data of this sort unless the observations are paired.

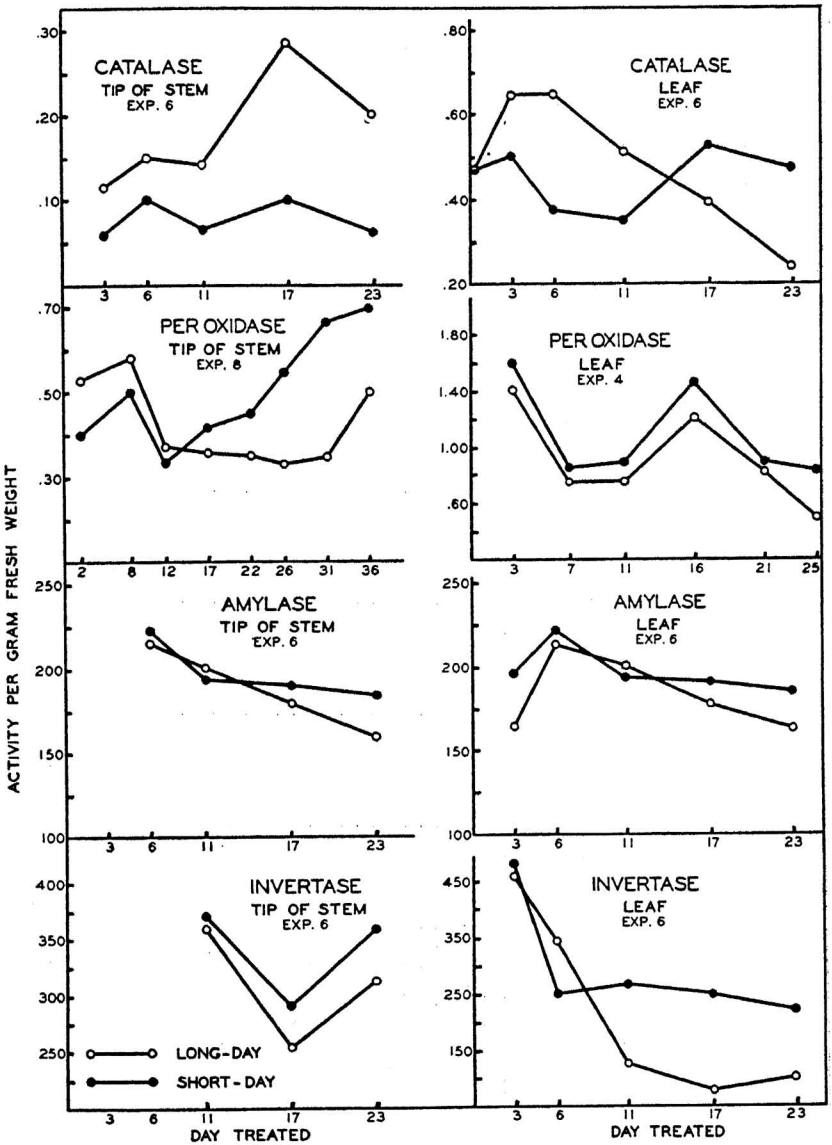


FIGURE 2. ACTUAL CHANGES IN ENZYMATIC ACTIVITY UNDER SHORT AND LONG PHOTOPERIODS.

The records from this experiment confirm those from earlier trials. (Table 7). The increased activity of peroxidase in the short-day plants is not as great as was shown by most of the other crops. Invertase indicated a similar increased activity under

TABLE 7.—CHANGES IN ENZYMATIC ACTIVITY OF THE TIPS AND LEAVES FROM PLANTS IN EXPERIMENT 6.

Date Sampled	Days Treated	Tissue Examined	Activity per gram fresh weight							
			Catalase		Peroxidase		Amylase		Invertase	
			Short day	Long day	Short day	Long day	Short day	Long day	Short day	Long day
Sept. 14	0	Stem tip	.18	.17	.45	.41	---	---	---	---
		Leaf 1	.48	.48	.50	.51	---	---	---	---
Sept. 17	3	Stem tip	.06	.12	.83	1.00	---	---	---	---
		Leaf 1	.50	.67	.55	.70	197	164	472	460
		Leaf 2	.28	.24	.18	.18	192	202	380	322
Sept. 20	6	Stem tip	.10	.15	.19	.16	224	215	---	---
		Leaf 1	.38	.67	.44	.45	186	194	250	349
		Leaf 2	.17	.22	.20	.20	194	164	204	252
Sept. 25	11	Stem tip	.07	.14	.23	.45	196	200	373	362
		Leaf 1	.35	.51	.55	.60	188	204	262	134
		Leaf 2	.35	.35	.26	.18	193	176	338	235
Oct. 1	17	Stem tip	.10	.28	.36	.36	192	176	290	252
		Leaf 1	.52	.39	.56	.53	188	172	250	76
		Leaf 2	.31	.29	.46	.30	226	270	462	194
Oct. 7	23	Stem tip	.07	.20	.51	.42	188	166	360	314
		Leaf 1	.48	.23	.45	.42	190	166	238	100

the short-day condition. This enzyme exhibits the most rapid response to the photoperiod. From a few days after the treatments were started until the end of the experiment, the activity of invertase steadily increased under the short photoperiod. Amylase, however, did not exhibit any change under the different lengths of day.

Catalase appears to be very sensitive to changes in the photoperiod. It is less active in the leaves of short-day plants during the first fifteen or twenty days. Afterwards, the activity increases so that after twenty-five days the leaves of the short-day plants are more active. This brings into agreement former observations which showed a lower activity in the leaves when the samples were taken early in the treatments, but if sampling were delayed until after the twentieth day the catalase activity was higher.

In this series also, the catalase activity of the tip of the short-day plants was about one-half that of the long-day plants. The activity had fallen to this level very early in the experiment and remained lower throughout. There was a slight tendency for the catalase in the tips of the long-day plants to become more active as the plants grew older.

The invertase activity was higher in the plants grown under the short light period. Soon after the experiment started this enzyme became more active under the short photoperiod and tended to increase as the plants grew older. The activities of invertase and peroxidase increased in a similar way. The changes in invertase preceded those occurring in peroxidase.

The response produced by moving plants after they had grown for eleven days under one photoperiod to the opposite treatment is shown in Table 8. When the plants that had grown under the long photoperiod for twenty-five days were shifted to the short day, there was at first a decrease in enzyme activity. This was soon followed by a corresponding reactivation, so that by the twelfth day after the change, the activities of all leaf enzymes, except peroxidase, were greater than in the plants continuously under the long-day treatment. The peroxidase was beginning to recover from the depressing effect resulting from the shortened light exposure.

The moving from the short to the long photoperiod brought about a tendency for the activity of the enzymes to decrease, although these changes were not as pronounced as when the plants were moved to the short photoperiod.

The pH of the juice was measured at the same time the samples were taken for enzyme determination. The active acidity of

TABLE 8.—EFFECT ON ENZYME ACTIVITY PRODUCED BY MOVING PLANTS FROM ONE PHOTOPERIOD TO THE OTHER.

Date Sampled	Days Treated	Tissue Examined	Activity per gram fresh weight							
			Catalase		Peroxidase		Amylase		Invertase	
			Moved	Long	Moved	Long	Moved	Long	Moved	Long
Oct. 1	11 short, 6 long	Stem tip	.04	.28	.10	.36	132	176	224	252
		Leaf 1	---	.39	---	.53	152	172	188	76
		Leaf 2	.30	.29	.52	.30	228	270	392	194
Oct. 7	11 short, 12 long	Stem tip	.18	.20	.18	.42	198	166	160	314
		Leaf 1	.72	.23	.33	.42	242	166	266	100
		Leaf 2	.45	---	.50	---	---	---	336	---
Oct. 1	11 long, 6 short	Stem tip	.23	.28	---	.36	118	176	218	252
		Leaf 1	---	.39	.17	.53	160	172	96	76
		Leaf 2	.37	.29	.36	.30	206	270	270	194
Oct. 7	11 long, 12 short	Stem tip	.16	.20	.46	.42	190	166	290	314
		Leaf 1	.45	.23	.34	.42	226	166	158	100
		Leaf 2	.34	---	.49	---	---	---	210	---

the juice from the leaves was constant. It varied but slightly from 6.2 during the entire period of sampling. There was a uniform difference of .2 to .3 of a pH between the stem samples from the different treatments. The data for all hydrogen-ion determinations made in this study are shown in Table 11.

Experiment 7

These plants were grown to study in detail the effects produced by suddenly changing the photoperiod. The light treatments were started as soon as the plants had come through the soil and continued for twenty days. At the end of this time the treatments were reversed, so that the lot which had been receiving the short day was now under the fourteen hour exposure. In spite of the unfavorable photoperiod these plants came into flower at the same time as normal short-day plants and continued to flower until the end of the experiment, although the stems began to elongate at the rate characteristic of the long photoperiod.

TABLE 9.—CHANGES IN ENZYMATIC ACTIVITY PRODUCED BY REVERSING THE TREATMENTS AFTER TWENTY DAYS.

Date Sampled	Days Treated	Tissue Used	Catalase Act. per gm.		Peroxidase Act. per gm.		Invertase Act. per gm.	
			Short day	Long day	Short day	Long day	Short day	Long day
Oct. 21	15	Tip	.03	.05	.25	.19	300	272
		Leaf 1	.40	.44	.40	.23	438	376
Oct. 26	*20	Tip	.03	.07	.42	.36	312	318
		Leaf 1	.32	.39	.40	.35	414	282
		Leaf 2	---	.38	---	.38	404	336
*The treatments were reversed after this sample was taken.								
Oct. 29	23	Tip	.08	.20	.42	.35	414	378
		Leaf 1	.34	.35	.62	.34	550	296
		Leaf 2	.41	.47	.37	.30	516	374
Nov. 1	26	Tip	.12	.13	.49	.48	308	296
		Leaf 1	.39	.31	.66	.61	414	234
		Leaf 2	.52	.34	.51	.43	412	300
Nov. 4	29	Tip	.09	.08	.21	.26	406	380
		Leaf 1	.32	.28	.39	.63	388	310
		Leaf 2	.43	.28	.33	.37	358	358
Nov. 7	32	Tip	.09	.06	.43	.45	360	334
		Leaf 1	.43	.40	.35	.45	300	262
		Leaf 2	.42	.37	.19	.28	294	352

Samples were collected on the fifteenth and twentieth days after the beginning of the treatments and every third day after the treatments were reversed. The changes in activity of peroxidase, invertase and catalase are shown in Table 9. These data are expressed graphically in Figure 3.

On the fifteenth and twentieth days these plants were typical of the other lots in appearance and enzyme activity. The peroxi-

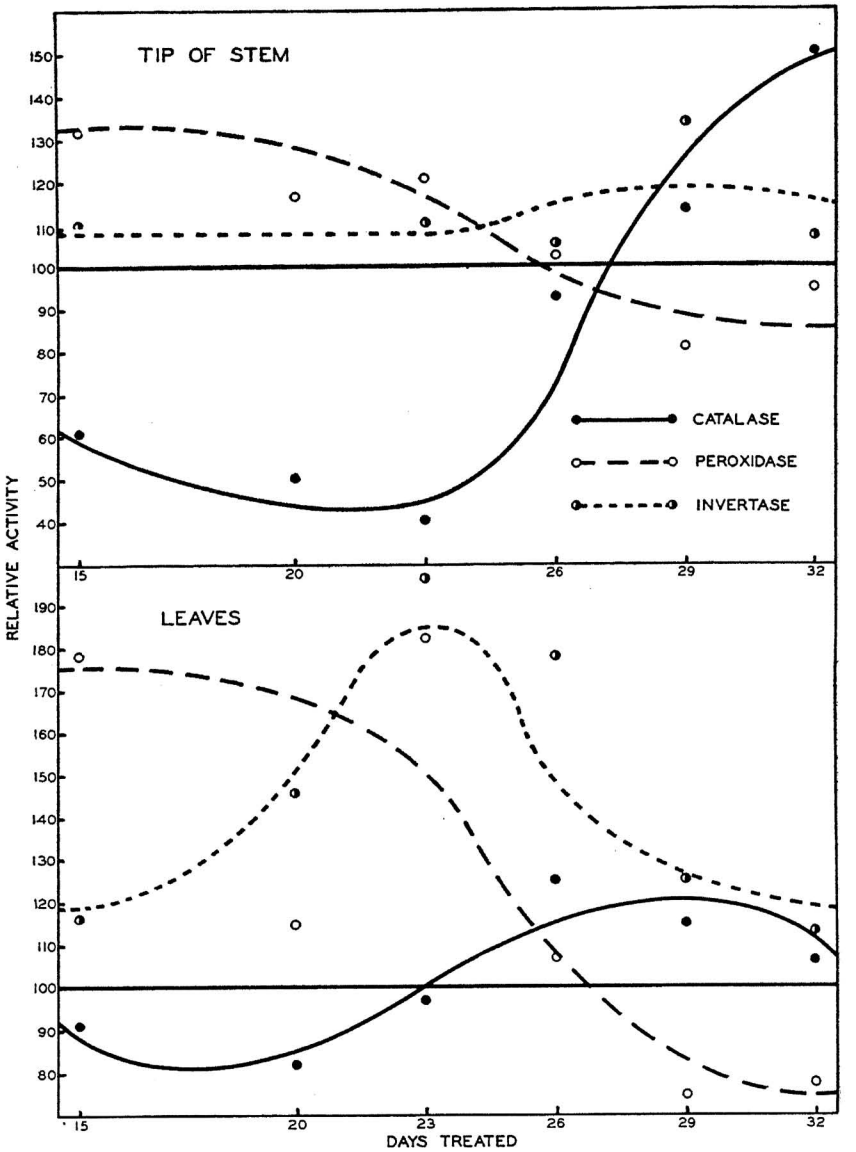


FIGURE 3. EFFECT OF REVERSING PHOTOPERIODS AFTER BEING TREATED FOR TWENTY DAYS. THE CURVES SHOW RELATIVE ACTIVITY OF ORIGINAL SHORT-DAY PLANTS EXPRESSED AS PERCENT OF ORIGINAL LONG-DAY.

dase and invertase were more active in the tissues of the short-day plants. Catalase was slightly lower in the leaves and much lower in the tips. Immediately after the plants were shifted, there was no appreciable influence on the direction of change in activity except for a slight depression in the plants moved to the short-day exposure. After this, a rapid change set in so that by the ninth day after the shift the relative activities of these enzymes had become almost completely reversed. The short-day plants under the influence of the long day had the same relative enzymatic activity as the long-day plants before they were shifted. These plants, however, proceeded with their reproductive development although they resumed growth. It seems that the changes in the relative enzymatic activities can probably be correlated more closely with the type of growth which the plants are making than with their stage of reproductive development.

The changes in the active acidity of the cell sap in the stems of these plants was highest in the short-day plants before the change in the photoperiods. Afterwards, there was no appreciable difference in the pH value of the juice. The active acidity of the leaves remained constant throughout the treatments.

Experiment 8

The object of this experiment was to study in detail the tip portion of the plant, since the data on this part was incomplete. Treatments were started as soon as the plants appeared above the soil. The samples were taken until the plants were thirty-six days old. These plants were normal in every respect, although grown during a period of the year when greenhouse temperatures are likely to be below the optimum. The enzymatic activity of the tip was typical of all the former lots. The data from these measurements are shown in Table 10. The divergene in the changes in catalase, as well as in peroxidase and invertase, are brought out

TABLE 10.—CHANGES IN ACTIVITY OF ENZYMES IN THE TIPS OF SOYBEAN PLANTS IN EXPERIMENT 8.

Date Sampled	Days Treated	Catalase Act. per gm.		Peroxidase Act. per gm.		Invertase Act. per gm.	
		Short day	Long day	Short day	Long day	Short day	Long day
Jan. 5	2	.11	.10	.40	.54	374	384
Jan. 11	8	.23	.25	.50	.57	304	272
Jan. 15	12	.08	.10	.34	.36	272	252
Jan. 20	17	.06	.12	.42	.38	294	272
Jan. 25	22	.07	.17	.45	.36	261	237
Jan. 29	26	.10	.17	.55	.34	586	398
Feb. 3	31	.07	.10	.60	.35	424	320
Feb. 8	36	.15	.21	.85	.50	233	167

clearly. During the first twenty days there was very little difference in the activities of invertase. After this period it increased greatly. Peroxidase was depressed in the short-day group at first, but about the fifteenth day equaled the long-day plants and for the rest of the period it steadily increased in activity. The activity of catalase was much lower in the tips of the short-day plants from the very beginning. There was a gradual decrease until a minimum was reached on the twentieth day. After this time there was a slight increase but it was never more than 70 per cent as active as in the tips of the long-day plants.

The active acidity of the juice of the stems was measured, but there was no appreciable difference under the two treatments. (Table 11).

TABLE 11.—THE EFFECT OF CHANGING THE PHOTOPERIOD UPON THE HYDROGEN-ION CONCENTRATION OF THE STEMS AND LEAVES.

Method of Treatment	Exposed Days	Tissue Used	Acidity of juice in pH	
			Short day	Long day
Grown continuously under the same exposure	2	Stem	5.90	5.80
	8	Stem	5.80	5.80
	12	Stem	5.70	5.70
	17	Stem	5.90	6.00
	22	Stem	5.70	5.68
	26	Stem	5.78	5.78
	31	Stem	5.78	6.00
	36	Stem	6.15	5.98
All plants grown for fourteen days under the long photoperiod, then one-half were given the short day	3	Leaves	6.25	6.27
	6	Stem	5.87	6.05
		Leaves	6.30	6.30
	11	Stem	5.75	6.05
		Leaves	6.05	6.13
	17	Stem	5.72	6.00
		Leaves	6.07	6.30
	23	Stem	5.88	6.20
		Leaves	6.30	6.35
Plants were given treatments indicated until twenty days old, then exposures were reversed	15	Stem	5.60	5.80
		Leaves	6.10	6.15
	20	Stem	5.70	5.90
		Leaves	6.25	6.05
	23	Stem	6.00	6.00
		Leaves	6.30	6.25
	26	Stem	6.05	6.08
		Leaves	6.23	6.27
	29	Stem	6.05	6.05
		Leaves	6.20	6.15

Experiments on Reducase

The work of Eckerson (16) has suggested that the ability of the plant to reduce nitrates is impaired under the short day. A series of experiments was planned to determine if the nitrate reducing power of the plant extracts could be connected in a causal way with the reproductive response of the plants.

In the first experiments, the nitrate reducing power of plants growing under the different treatments was determined. The reducase activity of the leaves and tips of the plants from crop 8 was measured. The results as shown in Table 12, are not entirely conclusive. It seems that most of the time the nitrate reducing power of the tissues of the short-day plants is much lower. At other times there is practically no difference in the activity. The leaves generally showed a greater activity than the tips. The reactivity was often so low in the stem apex that it was almost impossible to secure a reading.

TABLE 12.—CHANGES IN THE RELATIVE REDUCASE ACTIVITY IN TIPS AND LEAVES OF SHORT-DAY PLANTS.

Date Sampled	Days Treated	Tissue Examined	Relative reducase activity per cent of long-day, fresh weight basis	
			Crop 8	Crop 9
Jan. 5	2	Stem tip	83	--
		Leaves	109	--
Jan. 11	8	Stem tip	100	--
		Leaves	50	--
Mar. 3	8	Stem tip	--	94
		Leaves	--	98
Mar. 6	11	Stem tip	--	53
		Leaves	--	72
Jan. 15	12	Leaves	100	--
Mar. 9	14	Stem tip	--	90
		Leaves	--	118
Jan. 20	17	Leaves	42	--
Mar. 13	18	Stem tip	--	115
		Leaves	--	142
Mar. 16	21	Stem tip	--	85
		Leaves	--	69
Jan. 25	22	Leaves	46	--
Mar. 20	25	Stem tip	--	85
		Leaves	--	79
29	26	Leaves	118	--
Feb. 3	32	Leaves	25	--
Feb. 8	37	Leaves	144	--

Mean of both crops 80.5 ±4.2.

This experiment was repeated with crop 9, but there again the same changes in activity of this enzyme showed no definite trend.

It is commonly accepted that most crop plants can make normal growth if their supply of nitrogen is limited to the ammonium ion. (33). Leguminous plants, in addition, thrive upon nitrogen fixed by symbiotic bacteria. The work of Hopkins (25) shows that plants of the Manchu variety of soybeans make typical growth responses to the different photoperiods when securing their entire supply of nitrogen through nodule bacteria.

The problem was to determine if Biloxi soybean plants when grown in the absence of nitrate nitrogen would continue to grow vegetatively under the long-day treatment as they do when grown in a soil well supplied with nitrates. Does the lower nitrate reducing power of the short-day plants so modify the ratio of carbohydrates to metabolized nitrogen that the plant can no longer grow in a vegetative manner and must, as a result, become reproductive?

Two methods were used to determine if nitrate nitrogen were essential for continued vegetative growth of the soybean plant. The first was to grow inoculated plants in a sand culture fed with a nitrogen free nutrient solution. These cultures were set up in four-gallon earthenware crocks. Six inoculated seedlings were set in each vessel. After the plants had become established the tops of the containers were covered with waxed cloth to prevent the entrance of foreign nitrogenous materials. The plants were fed weekly with Hiltner's (48) nitrogen free nutrient solution. They were flushed weekly with tap water. The plants grew rapidly from the start. They were similar in height to plants the same age in soil but were somewhat lighter in color. When they were three weeks old, the long-day plants showed evidences of acute nitrogen starvation. The leaves were pale yellow and showed many necrotic areas. The short-day plants, while showing signs of nitrogen deficiency, were not as severely affected.

The plants were sampled when 34 days old. The two lots were typical of plants grown under similar treatments in soil cultures. The short-day group had developed many flower buds and stem elongation was almost inhibited. The leaves were dark green in color. The long-day plants were growing strongly and were beginning to acquire a more healthy color due, no doubt, to the greater activity of the nodular bacteria in supplying nitrogen. Nodules were very numerous upon the older roots in both lots.

Microchemical tests failed to show the presence of nitrates in any tissues in either short or long-day plants. Tests were made upon the leaves, petioles, tip of stem, nodes, internodes and roots. In every case the nitrate test was negative. Plants of the same age that had grown in soil showed a large amount of nitrate present in all parts. The greatest nitrate accumulation was in the stems of the short-day plants. The nitrate concentration in the pith of the long-day plants was very low. All tests were made with diphenylamine reagent.

A comparison of the heights of the plants grown in sand with those from the soil cultures showed a very slight difference. The short-day plants from the sand cultures were 10.1 cm. compared with 10.3 cm. for the soil. The difference for the long-day was slightly greater; the sand culture plants measured 30.4 cm. tall while those in soil had reached only 31.6 cm. Although the nutritive conditions at the beginning of the growing period had been more favorable in the soil cultures, there was an insignificant increase in height. In other characteristics the soil culture plants were much superior. They had thicker stems and nodes, larger leaves of a darker color and were free from disease.

TABLE 13.—NITRATE REDUCING POWER OF JUICE FROM SHORT- AND LONG-DAY PLANTS RECEIVING NITROGEN IN DIFFERENT FORMS.

Source of Nitrogen	Milligrams of nitrite nitrogen produced in forty hours by one gram of leaf tissue	
	Short-day	Long-day
Soil (nitrate)	.0020	.0030
Nodule bacteria	.0017	.0034
Ammonium sulphate	.0320*	.0222*

*These figures are higher due to the greater amount of sunshine in late spring.

In the second method water cultures were used. These plants were given Hiltner's nutrient solution with the nitrogen being supplied through ammonium sulphate. By renewing the solutions twice a week the nitrifying bacteria were inhibited so that no nitrate could be detected in the solutions at any time. The pH varied between 6.0 and 5.6 during the experiment.

Samples were collected when the plants were 23 days old. Flower buds could just be seen on the short-day plants. These plants were dwarfed and growth in general was inhibited. Under both treatments the plants in the water cultures were far superior to those the same age growing in soil. The long-day plants were vegetative.

Microchemical examination of all parts of both short- and long-day plants gave negative tests for the presence of nitrates.

DISCUSSION

It is unfortunate that most enzyme and chemical studies in relation to photoperiodism have been made upon material from plants which had already reached the reproductive condition. Any differences in enzyme activity which are to be associated in a causal way with the orientation of the reproductive state must be detectable before the reproductive organs can be observed morphologically.

Murneek and Gomez (36) have shown that differentiation in the Biloxi soybean can be detected very clearly microscopically fourteen days after the beginning of the short-day treatments. Any differences in the activity of those enzymes which are catalyzing the flowering response should be evident during the first fourteen days. Changes observed later are either a direct result of differences in the amounts of light received, or an effect resulting from the physiological condition of growth or reproduction induced by the photoperiod. The reproductive development of a plant may affect the activities of the various enzymes, since these catalysts are needed to carry on the specialized processes incident to the development of reproductive organs and to their functions.

There is also the possibility that even though the enzymes are influenced directly by the photoperiod they may, in turn, catalize transformations connected with some other response to the length of day other than reproductive activity. The inhibition of growth by the short photoperiods, photoperiodic inhibition, no doubt is the result of certain enzymatic changes, and is in itself the cause of still different activities in other catalysts.

It is highly probable that enzyme activity is only one factor in a chain of reactions from the stimulus set up in the plant by the photoperiod to the first definite indication of a response as observed in the differentiation of flower parts.

Reports of observations on the effect of the photoperiod upon catalase activity are conflicting. Knott (26) and Cajlachjan (9) were unable to detect any differences in the catalase activity of the leaves. On the other hand, Krassinsky *et al.* (29) found that the photoperiod had a marked influence upon the activity of this enzyme. The present data show that any relationship between catalase and the length of the photoperiod may be found if measurements are made at only one or two times during the latter part of the experiment.

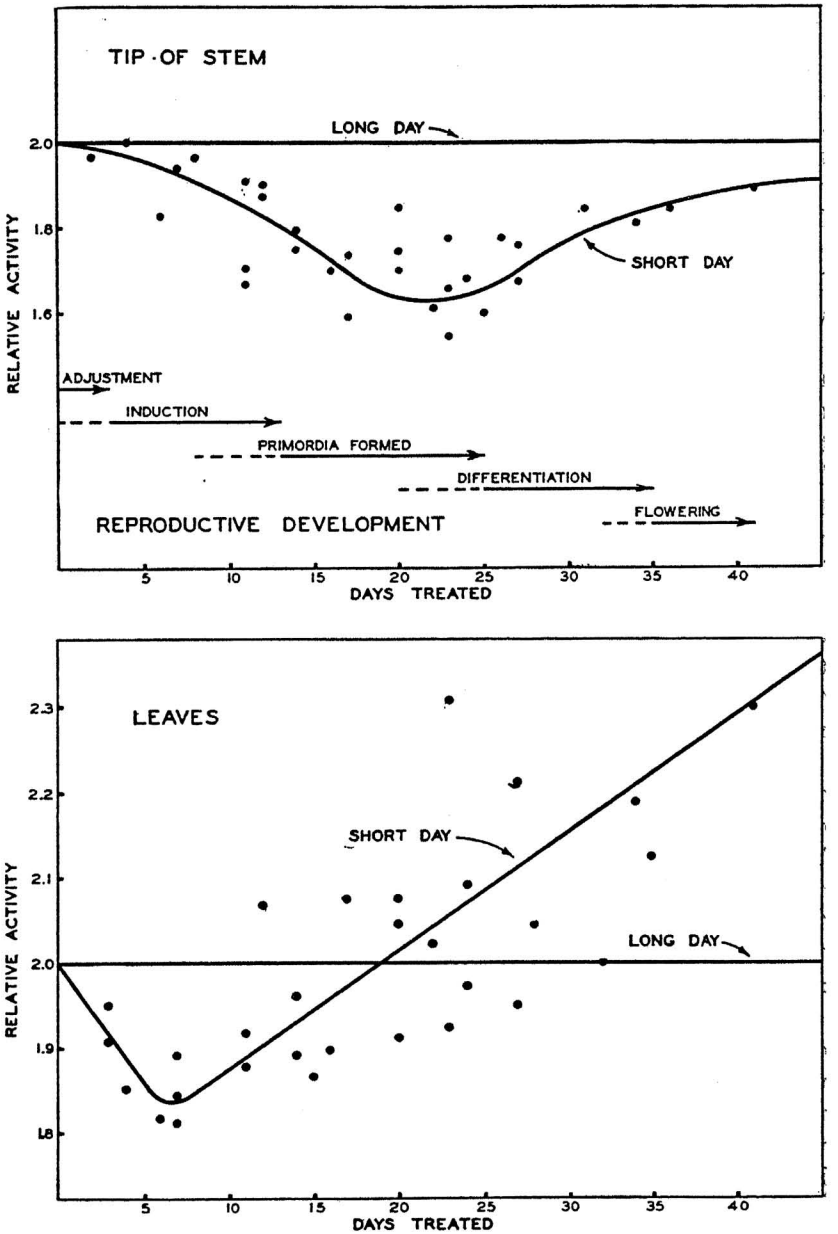


FIGURE 4. RELATIVE CATALASE ACTIVITY IN SHORT-DAY PLANTS EXPRESSED AS LOG PERCENT OF LONG-DAY.

Figure 4 is a summation curve of all the catalase measurements made during the various experiments. The points show the activity of catalase in the tips and leaves of the short-day plants relative to that of long-day tissues of the same age. These points are the results of paired determinations from successive samplings. Where several points are plotted for a single day they represent samples from as many different crops. The line of averages is drawn through the means of the points grouped in five day intervals. This curve represents the relative change in the short-day plant when the activity of long-day plants is held constant.

In the leaves of the short-day plants there was in every experiment a lower relative catalase activity during the first part of the experimental period. The low activity persisted until about the tenth day. At that time some factor began to operate which brought about an increase in this enzyme. By the eighteenth day there were equal catalase activities in the leaves under both treatments. If other plants behave in a manner similar to the soybean, it would not be impossible for an investigator to fail to find any appreciable difference in the catalase activity in the leaves. This comparative trend in catalase is the same, regardless of the age of the plant when the short-day treatment is started. It is not due to a difference in the age of the plants, but is more or less a direct result of the short light period.

Any attempts to definitely explain the significance of the comparative catalase activity is quite futile, since the exact role of catalase in plant metabolism is unknown. It has been observed by Burge and Burge (6) that an increase in light results in a corresponding increase in catalase activity. The lower concentration of this enzyme might be explained in this way. The response to the length of day may at first be gradual and then become more pronounced as additional doses of the treatment are given.

The great increase in catalase as well as that in the other enzymes in the short-day plants is difficult to explain. The growth rate is much lower, therefore, it would appear that there is less need for a high metabolic rate. According to Child (11), the metabolic rate of most organisms usually declines to a certain level before the reproductive phase can be expressed. The theory of Lysenko (32) supports this assumption in that reproduction is not expressed until the plant has aged sufficiently, although the stimulation may have been received at some previous time. The accumulation of a large reserve of readily respirable materials should not mean a higher rate of their oxidation unless a definite need exists in the plant for the energy released in this manner.

But in spite of this stagnation of growth, these enzymes, which many believe to be indicators of the intensity of respiration or vital processes, increase in activity.

The relationship of catalase activity and the reproductive responses to the photoperiod is not definite. While it is true that the catalase activity begins to increase about the end of the period of photoperodic induction, this is not conclusive evidence that the two phenomena are related. It is probable that the stagnation of growth from the short photoperiod may be caused indirectly by the higher catalase activity. On the other hand, the inhibition of growth may itself be the cause for the accumulation of catalase.

When short-day plants are moved to a long-day after the completion of the period of photoperiodic induction, the enzymatic activity tends to return to the condition typical in the long-day plants, while flowering is continued. It is conceivable that the low level of catalase may be necessary for the initiation of the reproductive process and not for its continued expression. This enzyme could very well serve as an oxidizing catalyst which destroys the substance necessary for the development of floral organs. When the activity declined to a certain level, those substances necessary for flower production could accumulate in quantities sufficient to stimulate reproductive development.

The tip portion in a reproductive soybean plant contains many differentiating flower buds. Catalase activity in the plant apex is strongly inhibited by the short-day. For the first twenty days there is a gradual decrease in the relative activity until it is about 50 per cent that of the vegetative tip. About this time the catalase begins to increase so that when flower buds appear it is about 75 per cent as active as the long-day tip. The decreased activity can very well be explained by the decreased growth of the plant in height. Since this growth depends upon the activity of the tip, any condition existing in this portion may have some relation to growth. During the early part of the treatment, growth rate and catalase activity decrease. (Compare Figures 4 and 5). The late increase in catalase activity can be associated with the increased activity brought about by the speeding up of processes due to differentiation and maturation of the floral organs. It should be noted that the increase in activity begins prior to the time any flower buds are visible. This agrees with the work of Knott and Anthony, (28) in which they found that there was an increase in catalase activity prior to the time when fruit bud differentiation could be observed microscopically.

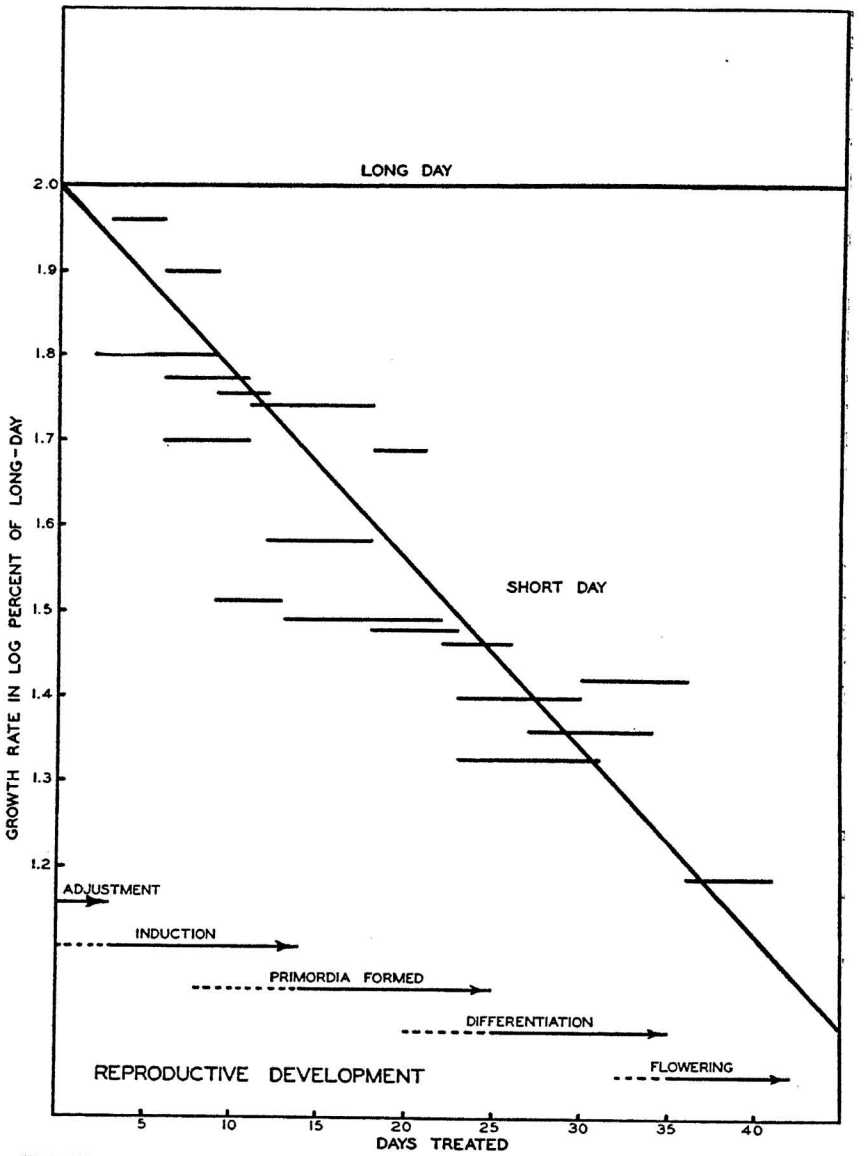
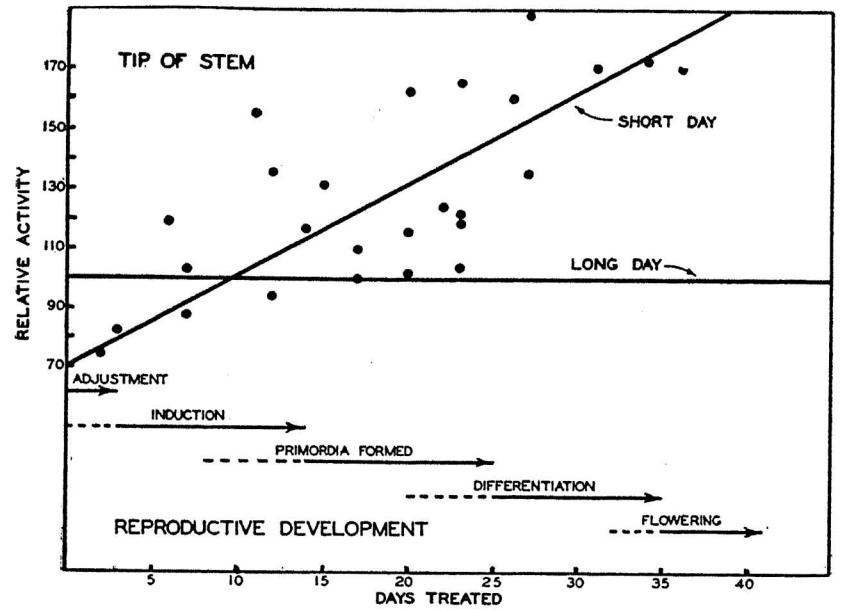


FIGURE 5. CHANGE IN RATE OF GROWTH IN HEIGHT OF SHORT-DAY PLANTS FROM PHOTOPERIODIC INHIBITION.

The relative changes in peroxidase activity of short-day plants can not be compared with those observed for catalase. (Fig. 6). From this and other observations it seems probable that there is



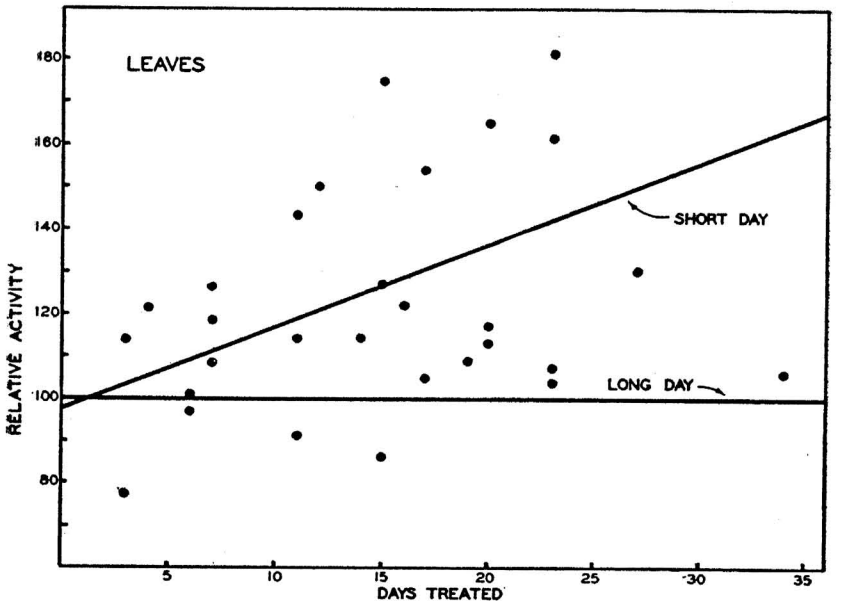


FIGURE 6. RELATIVE PEROXIDASE ACTIVITY OF SHORT-DAY PLANTS EXPRESSED AS PERCENT OF LONG-DAY.

very little relationship between the activities of these two enzymes. Their functions in plant metabolism seem to be wholly unrelated.

For the first few days, peroxidase activity may be slightly lower in the short-day plant. This is evidently a transient condition while the plant is adjusting to the short photoperiod. The slight inhibition could very well be brought about by differences in the amounts of light received by the two lots. After the first slight lowering, there is a gradual increase in peroxidase activity. The data from comparative peroxidase determinations do not follow as narrow a range as those from catalase, but nevertheless show that peroxidase is more active in the short-day plants, and also that there is a decided tendency for the relative activity to increase as the treatments are continued. (Fig. 6). The activity in the tip follows in general that shown by the leaves. Here there is a greater inhibition during the period of adjustment, but the subsequent increase is much more rapid. In all enzymes which showed any response to the length of day, the response in the tip, while being similar to that of the leaves, appeared from five to ten days later. According to Cajlachjan (8) and Lubimenko (30), the stimulus set up by the photoperiod is received by the leaves and later transmitted to the meristems from which the reproductive organs arise. Whether or not the leaves are the sole receptors of the stimulus, the fact remains that the enzymes in the leaves are definitely influenced by the photoperiod.

Invertase shows a behavior similar to peroxidase. (Compare Figs. 6 and 7). The increased activities of these enzymes parallel to some extent increasing carbon dioxide production by the short-day plants (35). This is a strong indication that the type of respiratory mechanism is modified by the photoperiod. The problem of respiration and photoperiodism has never been investigated save for a few measurements on the rate of carbon dioxide elimination.

Invertase showed a high sensitivity to the photoperiod, whereas the activity of amylase was unaffected. (Fig. 8). This suggests that the ease of starch transformation is not a factor in the photoperiodic response.

The data on reducase are not as definite and clear-cut as those obtained for the other enzymes studied. It is probable that nitrate reducase will not be classed with the true enzymes after the kinetics of its reaction rate have been worked out. A determination of its activity may be only an estimation of the quantity of nitrate reducing substances present in the plant tissue.

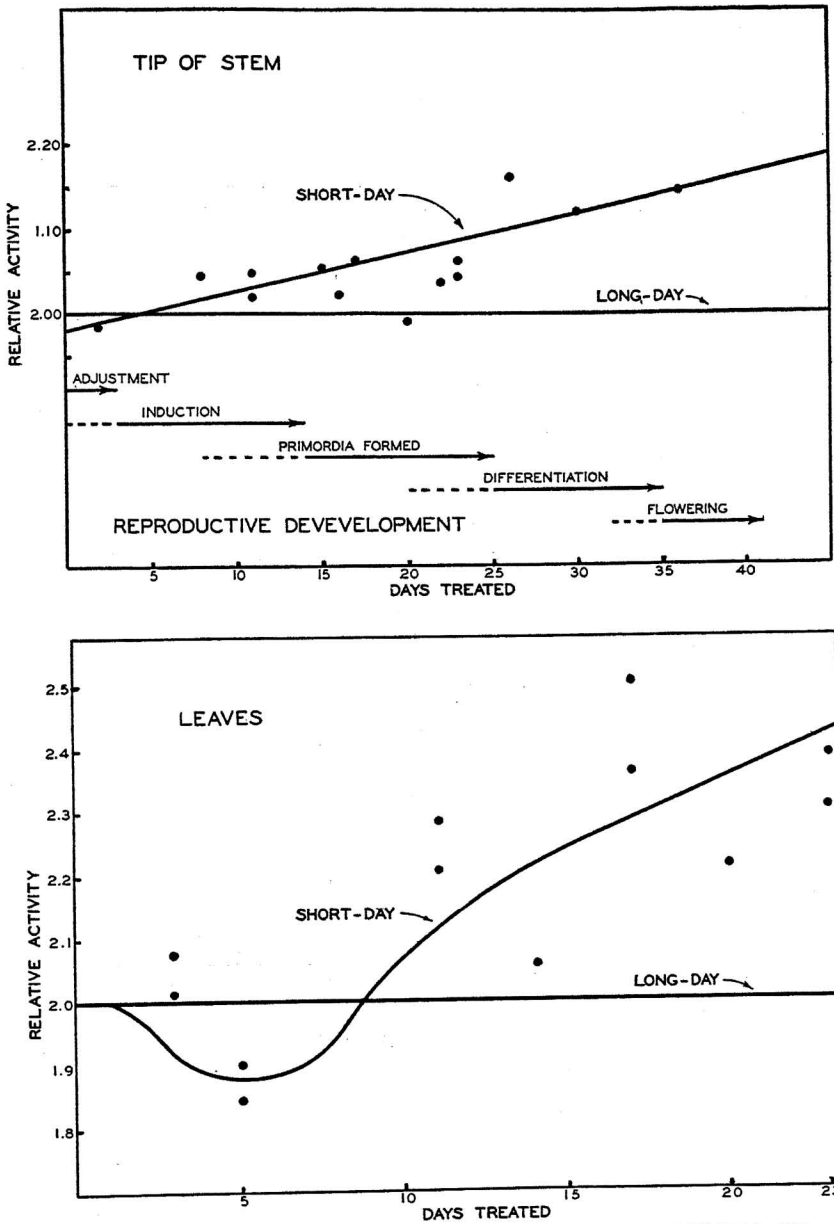
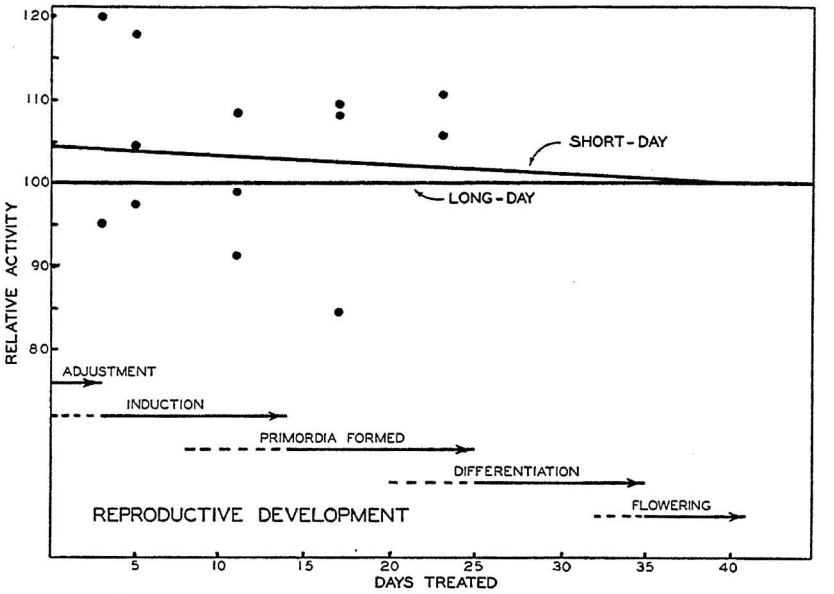


FIGURE 7. CHANGES IN RELATIVE INVERTASE ACTIVITY OF SHORT-DAY PLANT EXPRESSED AS LOG. PERCENT OF LONG-DAY



AMYLASE ACTIVITY IN SHORT-DAY PLANT RELATIVE TO THAT OF LONG-DAY PLANT EXPRESSED AS PERCENT OF LONG-DAY.

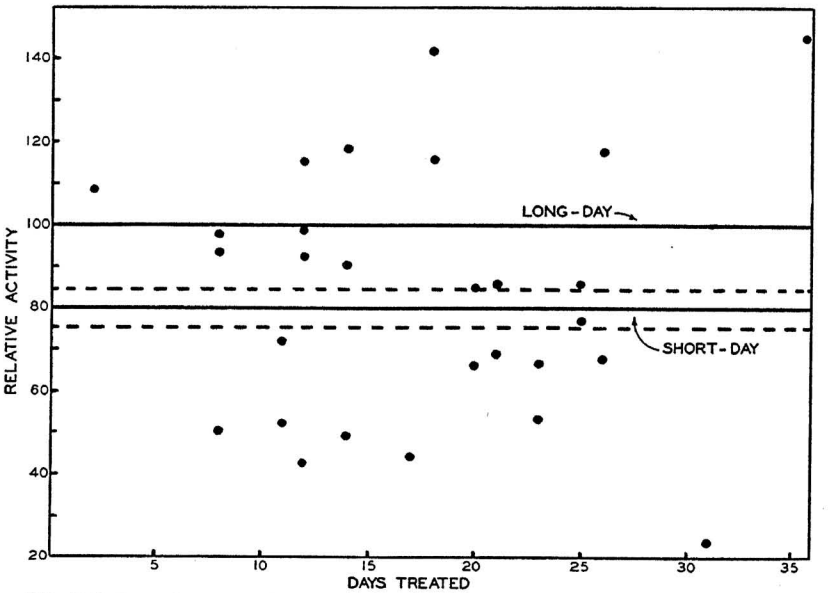


FIGURE 8. REDUCASE ACTIVITY OF SHORT-DAY PLANTS EXPRESSED AS PERCENT OF LONG-DAY.

When averaged, the data show that the reducase content of the short-day plants is about 80 per cent that of the other treatment. The tip showed the same relative activity as the leaves, although most of the activity is in the leaf tissue.

Even when no nitrate can be detected in the plant, the ability of the tissues to reduce this substance is not impaired. (Table 13 and Fig. 8). The photoperiod has a greater influence in determining the nitrate reducing power than the presence of nitrate itself. It is a common belief that a plant produces an enzyme according to the theory of need. If this were true, a plant grown in the absence of nitrate should lose the ability to reduce this substance. The data presented here show that the presence or absence of a substance does not influence directly the production of the catalyst necessary for its transformation.

Only those enzyme changes observed during the period of photoperiodic induction can have any possible direct effect upon the orientation of the developmental process. In the Biloxi soybean the induction is completed in the axils nearest the apex some time between the eighth and fourteenth days after the beginning of the treatments. At the end of this period the invertase and peroxidase contents are relatively higher in the leaves and tips of the short-day plants. The catalase content in the same tissue is lower, while amylase has remained unchanged. Reducase is likely to be lower although its behavior seems to be influenced more by other environmental factors than the photoperiod. The inactivation of this enzyme is probably due to the differences in the amount of light which the plants receive.

The present work would have been more conclusive if the differences between the light treatments had been made as small as possible with respect to intensity and length of exposure. Some of the changes in the activities, observed early in the treatments, are no doubt due directly to the respective quantities of light received by the plants.

If the functions of catalase and peroxidase in plant metabolism were known, a more exacting interpretation could be given.

The data presented in this paper show definitely that the activities of certain hydrolyzing and oxidizing enzymes are influenced by the length of the photoperiod. The relationship of these changes to the reproductive response of the plant is obscure. Nothing can be definitely stated regarding the position which these enzymes occupy in the reproductive processes until more information is available.

To determine whether or not the enzymatic changes observed in the soybean are associated with the reproductive response instead of photoperiodic inhibition, the changes in the various catalysts must be followed in a long-day plant. In this type of plant, growth and reproduction are both inhibited by the short-day. To make the study conclusive the changes should also be measured in a plant which is indifferent to the length of the photoperiod in both growth and reproduction.

The determinations made in this study show only that the length of the photoperiod has a definite influence on the biochemical catalysts in the plant cell. This effect on the enzymatic activity can be detected prior to any change which it is possible to detect by chemical or morphological examination.

The entire problem of the influence of light on reproduction was well summarized by Burkholder (7) when he stated that, "Photomorphosis in plants appears to rest upon both qualitative and quantitative aspects of the physico-chemical situation. Synthesis of special compounds by photochemical reactions, differential catalysis of physiological processes and correlation of plants by electrical potentials . . . suggest a variety of possible ways by which light may contribute to growth and differentiation of the plant body."

SUMMARY

1. In the leaves of short-day soybean plants, the catalase activity is at first inhibited. It begins to increase about the tenth day, becomes equal to the long-day plants near the eighteenth day, and is increasingly greater thereafter.

2. Catalase activity in the tip of the short-day plant is inhibited from the first. Maximal depression in activity is reached about the twentieth day. Afterwards there is a slight increase, but the activity is always less than in the tips of the long-day plants.

3. Peroxidase is more active in both the tips and leaves of the short-day plants. This difference, while small at first, steadily increases as the treatments are continued.

4. Invertase shows a tendency to increase in the short-day plants similar to the trend exhibited by peroxidase.

5. In the soybean plant amylase is unaffected by the lengths of the photoperiod used in this study.

6. Reducase was partially inhibited by the short-day, but showed no definite change as the plants responded to the photoperiod.

7. Plants grown in the absence of nitrates showed reducase activities similar to plants well supplied with this form of nitrogen.

8. If nitrogen metabolism is affected by the photoperiod so that this substance is a limiting factor in growth, some phase other than nitrate reduction must be preventing its utilization.

9. The activities of catalase and peroxidase change in an unlike manner in response to the length of day.

10. The changes in enzymatic activity can be observed as early as five days after the beginning of the treatments. These differences can be detected prior to any morphological or chemical changes.

11. The trends in relative enzymatic activities can probably be correlated more closely with the type of growth which the plants are making than with their stage of reproductive development.

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