Proc. Amer. Soc. Hort. Sci. 55: 247–253. 1950.
27. SCHAEFER, V. J. The formation of ice crystals in the laboratory and the atmosphere. Chem. Rev. 44: 291–320. 1949.
28. SCHOONOVER, W. R., BROOKS, F. A., and WALKER, H. B. Protection of orchards against frost. California Agr. Ext. Circ. No. 111. 1939.
29. SEEMANN, J. Über die Bedeutung der Unterkühlung für die Selektion frostresistenter Bohnenpflanzen. Züchter 14: 258–264. 1942.
30. TAMMANN, G. and BÜCHNER, A. Die lineare Kristallisationsgeschwindigkeit des Eises aus gewöhnlichem und schwerem Wasser. Z. anorg. Chem. allgem. Chem. 222: 12–16. 1935.
31. TURNBULL, D. and FISHER, J. C. Rate of nucleation in condensed systems. Jour. Chem. Phys. 17: 71–73. 1949.
32. TURNBULL, D. Kinetics of solidification of supercooled liquid mercury droplets. Jour. Chem. Phys. 20: 411–424. 1952.
33. ULLRICH, H. and MÄDE, A. Studien über die Ursachen den Frostresistenz. II. Untersuchungen über der Temperaturverlauf beim Gefrieren von Blättern und Vergleichsobjekten. Planta 31: 251–262. 1940.
34. VOIGTLÄNDER, H. Unterkühlung und Kältetod der Pflanzen. Beitr. Biol. Pflanz. 9: 359–414. 1909.
35. VOLMER, M. and WEBER, A. Keimbildung in übersättigten Gebilden. Z. physik. Chem. 119: 277–301. 1926.
36. VOLMER, M. Über Keimbildung und Keimwirkung als Spezialfälle der heterogenen Katalyse. Z. Elektrochem. 35: 555–561. 1929.
37. VOLMER, M. and FLOOD, H. Tröpfchenbildung in Dämpfen. Z. physik. Chem. A. 170: 273–285. 1934.
38. VOLMER, M. Kinetik der Phasenbildung. Steinkopff, Dresden-Leipzig. 1939.
39. WARTENBERG, H. Studien über den Kältetod der Pflanzen. Ernähr. Pflanze 34: 21–27. 1938.
40. WYLIE, R. G. The freezing of supercooled water in glass. Proc. Phys. Soc. (London) B 66: 241–254. 1953.
41. YOUNG, F. D. Frost and the prevention of frost damage. U. S. Dept. Agr. Farmers Bull. No. 1588. 1941.

THE RELATIVE SENSITIVITY OF XANTHIUM LEAVES OF DIFFERENT AGES TO PHOTOPERIODIC INDUCTION[1]

ABDUL-KARIM KHUDAIRI[2] AND KARL C. HAMNER
DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA

Lang (5), in his recent review, has discussed the literature dealing with the relative sensitivity to photoperiodic treatment of leaves of different ages. The work of Moskov (8, 9) with chrysanthemum, Borthwick and Parker (1) with Biloxi soybean, and Naylor (10) with Xanthium has indicated that the first expanded leaf showed the greatest sensitivity of those tested. Hamner and Bonner (3) in 1938, and others since, found that Xanthium plants defoliated except for the youngest leaves of less than 1 cm^2 area did not appear sensitive to short day treatment.

The tacit assumption has been that sensitivity to photoperiod increased as leaves expanded until the leaf reached full size and then gradually decreased as the leaf became older.

In most short day plants several photoinductive cycles are required to induce flowering. Xanthium is known to differ from these other short day plants since with this plant one short day is sufficient to bring about flower initiation. It is possible, therefore, to measure precisely the relative sensitivity of young expanding leaves of this plant. The results of certain experiments in this laboratory made it appear desirable to reexamine the photoperiodic sensitivity of Xanthium leaves of all ages.

MATERIAL AND METHODS

Burs from the species of cocklebur, *Xanthium pennsylvanicum*,[3] were collected from an area around Chicago, Illinois. These burs were soaked overnight and then planted in flats. After the seedlings were about two weeks old they were transplanted into four-inch clay pots or 10-oz. plastic cups as indicated in each experiment. From the time of planting until the time of experimentation the plants were kept in the greenhouse under long days of at least 18 hours of light, supplementing the natural daylight from sunset until 2:00 A.M. The plants were not used for experimentation until they had at least five fully expanded leaves.

After the plants received experimental treatment they were put back on long day for 3 weeks. They were then dissected to determine the presence of flower initiation and the size of the inflorescence. For the degree of magnitude of flowering in Xanthium an

[1] Received August 28, 1953.
[2] Present address: Botany Department, College of Arts and Science, Adhmia, Baghdad, Iraq.

[3] *Xanthium pen(n)sylvanicum* Wallr., synonym *X. saccharatum* Wallr., as defined in the Eighth Edition of Gray's Manual (1b). Specimens of the plant used in these studies have been filed at the U.C.L.A. Herbarium.

arbitrary scale was used to indicate the stage of flower initiation (11):

0—Vegetative condition.
1—Initiation of the terminal bud, which became dome shaped.
2—Formation of the floret primordia on the periphery of the flower head.
3—Florets initiated on most of the inflorescence.
4—The stage in which the terminal inflorescence was macroscopic.
5—A terminal head 1 mm in diameter.

For each 0.5 mm additional increase in diameter, the arbitrary scale was increased by one unit. To determine the average magnitude of flowering buds, the units of the scale were added and divided by the number of the plants used in the experiment.

Techniques different from those employed by Naylor (10) were used to measure the sensitivity of the leaves and their effectiveness in causing flower initiation. It has been shown that, while the critical dark period is 9.5 hours, the effectiveness of a dark period appears to increase quantitatively as the length of the dark period is increased up to at least 15 hours (11). The effectiveness of the leaf under investigation has been measured in terms of the rate of inflorescence and flower development subsequent to one short day treatment.

In each experiment several lots of 8 or 10 plants were used. On each plant all leaves except one were removed prior to experimental treatment. The position and the area of this leaf were taken as criteria of the age of the leaf. The area of the leaf was calculated by measuring the width and the length of each blade and considering the shape to be triangular. Because of the difficulty of obtaining leaves of exactly the same area, there was a small range of variation in the size among the leaves of a given area designation. The entire plant, including the single attached leaf, was then exposed to one or more short days. In some cases the single attached leaf was removed shortly after treatment, in other cases it was allowed to remain. In each experiment precise details are given of the defoliation techniques and of the length of the dark period and the time allowed for development under subsequent long day conditions. It should be pointed out that the values obtained for rates of development are comparable within an experiment but not between experiments.

In all experiments cited here, the term "flowering" is applied when the terminal bud of the plant has initiated flower primordia or further stages of flowering, i.e., has macroscopic flowers developing flower buds. For convenience of reference in the various experiments, the first expanded leaf is the one nearest to the tip and is the youngest. The leaves are numbered successively down the plant to the oldest leaf near the base. Plants kept continuously under long days were dissected from time to time during the course of the experiments and were always found to be vegetative.

Experimental Results

Sensitivity of the Expanded and Unexpanded Leaves: *Experiment 1:* A preliminary experiment was designed to reexamine in a quantitative manner the effect of age on sensitivity (of the leaf) to the photoperiodic stimulus. Six lots of 8 plants each were selected for treatment. The leaves of the first lot were left intact during the photoinduction period. Four lots were defoliated of all leaves except for a particular pair of alternate leaves. The uppermost pair of fully expanded leaves was retained in lot 2, and successive pairs of expanded leaves were retained in the successive lots. All expanded leaves were cut from the plants of the sixth lot and only very small unexpanded leaves were left on the plant. The leaves were allowed to remain on the plants for the duration of the experiment.

A 20 hour dark period was given to the plants in all 6 lots. Plants so treated were dissected 3 weeks after photoperiodic induction. The results (table I) showed that in lot 2, having only the first pair of expanded leaves, all plants flowered as did all of the non-defoliated plants in lot 1. In lot 3 (characterized by defoliation of all leaves but the third and fourth expanded leaves) only 3 out of 8 plants flowered. The older leaves were relatively ineffective in inducing flowering. An unexpected result was that the sixth lot, which was defoliated except for the very small expanding leaves, gave 4 flowering plants and 4 vegetative (50 % flowering). This latter observation led to the following experiments.

Table I

The Effect of Various Defoliation Treatments on the Response of Xanthium to One Short Day (20 Hours of Darkness). Eight Plants per Treatment

Lot No.	Expanded leaves left on plant	No. of plants flowering	Av. size of * flowering buds
1	All (intact plants)	8	5.6
2	First pair (uppermost)	8	4.2
3	Second pair	3	0.7
4	Third pair	4	1.5
5	Fourth pair	1	0.6
6	None expanded (few expanding) ...	4	1.5

* See text, Col. 1, this page, for the scale used to determine the average magnitude.

Experiment 2: A second experiment was set up to investigate further the sensitivity of the leaves of varying ages. Seven lots of 8 plants each were used. The first lot was defoliated so as to leave on each plant only one young expanding leaf of an area of 10 to 15 cm^2. The second, third, fourth, fifth, and sixth lots retained respectively the first, second, third, fourth, and fifth fully expanded leaf only (fig 1). The range in area of the expanded leaves was from 40 to 45 cm^2. The seventh lot was completely defoliated and used as the control. All 7 lots were given one short day with 15 hours of darkness. When they were

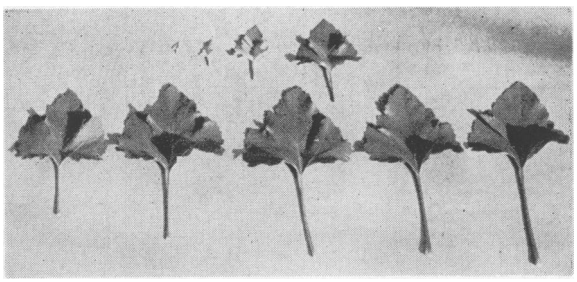

FIG. 1. Leaves at different ages taken from a vegetative Xanthium plant, arranged according to their position on the plant. Upper row, expanding leaves, from left to right: 0.5, 1.8, 9.0, 24.5 cm² in area.

Lower row, expanded leaves, from left to right: first (40 cm² in area), second, third, fourth and fifth (45 cm² in area). The oldest leaf is on the extreme right.

dissected 3 weeks after induction, the results showed that the expanding leaf gave 100 % flowering (fig 2). The first fully expanded leaf gave 87 % flowering, the second 62 %, and the third 12 %. The fourth and fifth fully expanded leaves did not cause a flowering response to the single short day. The completely defoliated control also failed to flower. Thus, the percentage of flowering plants decreased with the advance in leaf age (fig 2).

Experiment 3: Since the youngest leaf investigated in experiment 2 proved to be the most sensitive to the photoperiodic stimulus, a third experiment was designed to determine more precisely the size of the leaf which would give the maximum flowering response. Hamner and Bonner (3) tested the sensitivity of very young leaves, but in all cases the young leaves were removed, presumably soon after they received a photoinductive treatment.

Treatments were therefore included in which the induced leaf was removed from the plant soon after the inductive dark period. The results of Hamner and Bonner could also be interpreted to mean that, while young leaves may be induced, the stimulus may

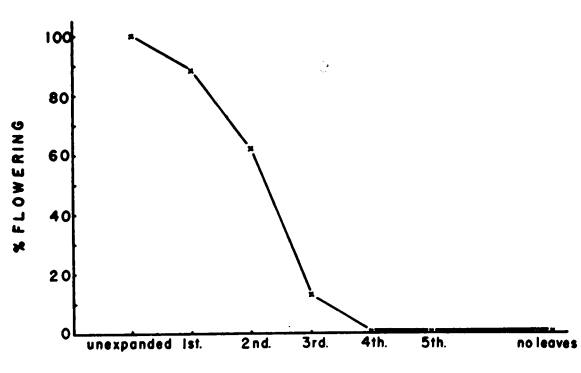

FIG. 2. Effect of age of leaf on percentage flowering. Plants were defoliated to a single leaf and exposed to one short day. The unexpanded leaf had an area of 10 to 15 cm² and the 1st to 5th leaf correspond respectively to those in figure 1.

not be translocated until the leaves expand further. Therefore comparisons were also made between plants in which the leaves developing from primordia subsequent to induction were allowed to remain on the plant, and treatments in which all leaves developing after induction were periodically removed. Each lot was defoliated except for one leaf. The leaves remaining on 4 lots of each group consisted of young expanding leaves of different sizes, ranging from the smallest leaves used (range 1 to 2 cm²) to leaves approximately two thirds fully expanded. The youngest fully expanded leaf (range 30 to 45 cm²) was retained on the fifth lot.

Four groups of plants were used, each consisting of 5 lots. Each lot consisted of 10 plants grown in

TABLE II

THE EFFECT OF VARIOUS DEFOLIATION TREATMENTS ON THE RESPONSE OF XANTHIUM TO ONE SHORT DAY (15 HOURS OF DARKNESS). TEN PLANTS PER TREATMENT

Lot No.	Area of treated leaf	Results of treatment: Treated leaf removed* and newly formed leaves: Removed*	Treated leaf removed* and newly formed leaves: Not removed	Treated leaf not removed and newly formed leaves: Removed*	Treated leaf not removed and newly formed leaves: Not removed
	cm²	*percent of plants flowering in each lot*			
1	1–2	0	10	0	0
2	3–5	0	20	70	90
3	8–15	20	20	100	100
4	18–25	20	20	100	100
5	30–45	10	0	50	50
		average stage of development of flowers			
1	1–2	0	0	0	0
2	3–5	0	0.3	3.7	2.8
3	8–15	0.6	0.4	5.6	5.2
4	18–25	0.4	0.6	4.6	5.0
5	30–45	0.2	0	1.8	3.3

* Treated leaves removed 2 hours after end of long dark period. Newly forming leaves removed before they reached maturity.

plastic cups. All 4 groups were given a single short day with 20 hours of darkness. The single intact leaf of each plant of one group was removed 2 hours after the end of the long dark period. The leaves of a second group were removed at the same time as those of the first but in addition the leaves subsequently formed were removed before reaching maturity. The treated leaves of a third group were left intact after induction as were those subsequently formed. In the fourth group the treated leaves were left intact and the new leaves which were formed following treatment were removed before reaching maturity.

The plants on which the induced leaf was allowed to remain flowered more markedly than those from which the induced leaf was removed. There was no apparent effect, however, on removal of the leaves which subsequently developed on the plants (table II).

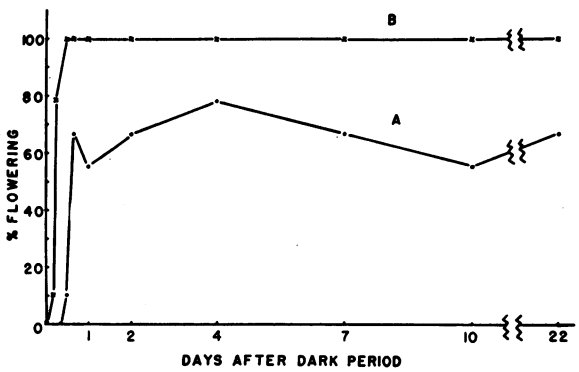

FIG. 3. Effect of time of removal of induced leaf on flowering response. All plants were defoliated except for a single leaf, exposed to one short day, and treated leaves removed at times indicated. Curve A represents responses in which the treated leaf was 3 to 5 cm² and for curve B the treated leaf was 7 to 15 cm².

The smallest leaf did not respond to a single short day. However the larger leaves all gave a high flowering response. The maximum flowering was with leaves one half to two thirds fully expanded. The first (youngest) fully expanded leaves gave a response less than those not fully expanded. Even when the leaves were removed 2 hours after the 20 hour dark period, the expanding leaves of intermediate size gave 20 % flowering. This finding led to a more precise investigation of transport of the flower promoting stimulus from the leaf.

Experiment 4: The next experiment was designed to determine the length of time required for the flowering stimulus to move out of the leaf. One hundred ninety-eight plants were divided into 2 groups. Plants in one group were defoliated to a single unexpanded leaf having a range in blade area of 3 to 5 cm², and those of the second group to a single unexpanded leaf of 7 to 15 cm². All plants were given one short day with 15 hours of darkness, then returned to long days. Each group was divided into 11 lots of 9 plants each. The attached leaf was defoliated in respective lots 0, 4, 8, 12, 16, or 24 hours, or 2, 4, 7, or 10 days, after the end of the long dark period. The last lot retained the leaf until the day of dissection, viz., 22 days after induction.

The results of this experiment are given in figure 3. The group of plants in which the treated leaf was the smaller (Curve A) exhibited a maximum response of 60 to 80 % flowering. This maximum response appeared to occur if the treated leaf was retained for 16 hours or more after the end of the long dark period. The group of plants in which the treated leaf was the larger (Curve B) gave 100 % flowering if the treated leaf was retained for 12 hours or longer following the long dark period. The curves for magnitude of flowering are not presented because they coincide with those for the percentage of flowering. Leaf area measurements showed a very slight increase within 12 to 16 hours after induction. Flowering in the plants with the smaller leaf never reached 100 % during the period of 3 weeks in which the experimental leaf became fully expanded even though other new leaves were formed and went through their development during this time.

This experiment indicates that the leaf age is critical for the formation of the stimulus only during the photoinductive period. It also demonstrates that the stimulus may move out of the leaves with great rapidity after the inductive dark period.

SENSITIVITY TO PHOTOPERIODIC INDUCTION OF BUDS OF DIFFERENT AGES: The response of small unexpanded leaves to short days was studied in experiment 5 using buds in different stages of development. The plants which were used in this experiment were about 6 weeks old and were grown in plastic cups. The entire top of the plants above the cotyledonary node was cut off, the cotyledons were removed and only one dormant cotyledonary bud was left on each plant. The bud began to grow immediately after this treatment and in this discussion its age is expressed as the number of days after trimming. The plants were divided into 6 groups of 24 plants each and 1, 4, 7, 10, 13, or 16 days later, plants of the respective groups were exposed to photoinductive treatment. Thus, the day after the plants had been trimmed down to one dormant bud, the first group of 24 plants was exposed to 16 hours of darkness. The next day the plants of this group were divided into 3 lots. One lot received only this one short day treatment. The second lot was given 2, and the third lot received 3 short days. All plants were then returned to long day conditions. Each successive group was treated in the same way after 4, 7, 10, 13, or 16 days had elapsed after the trimming. The plants of each lot were dissected 40 days after the short day treatment.

The results, presented in figure 4, show that the buds responded to one short day when they were 10 days old. When they were 16 days old they gave 100 % flowering. With more than one short day they responded faster. The relationship between the age of the bud, the number of short days of treatment,

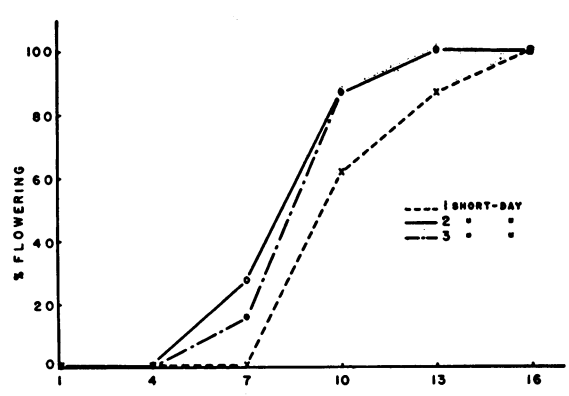

FIG. 4. Effect of photoperiodic induction on buds of different ages. Each short day consisted of 16 hours of darkness and 8 hours of daylight. Eight plants were used per treatment.

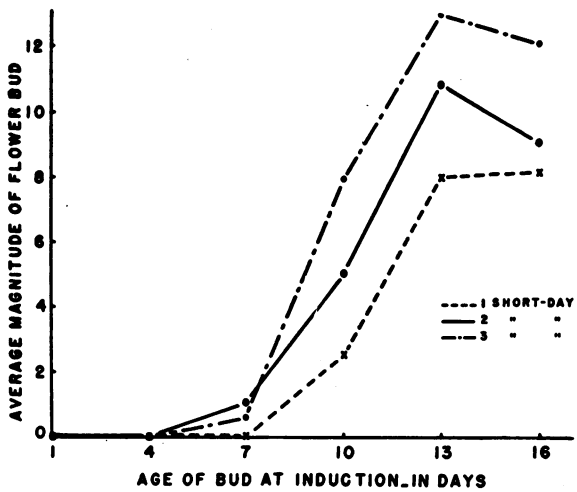

FIG. 5. Effect of photoperiodic induction on buds of different ages. Each short day consisted of 16 hours of darkness and 8 hours of daylight. Eight plants were used per treatment.

and the magnitude of the response are shown in figure 5. Maximum flowering was observed when the plants were treated for 3 days starting on the thirteenth day. There was no flowering when the buds were 1 or 4 days old when treated, but when they were 7 days old they showed 16 % or 28 % flowering when given 2 or 3 short days respectively.

In order to compare the flowering response to the stage of morphological development the length of the bud, number of visible leaves, the area of the visible leaves, and the number of leaf primordia were measured during the course of the bud development. At the age of 10 days when the percentage of flowering response was higher than 60 % (fig 4) the buds were 9 mm long (fig 6) with 3 to 4 visible leaves of an average total area of 0.22 cm^2 and 4 leaf primordia (fig 7). At the age of 13 days the flowering was 100 % and the buds were 14.3 mm long (fig 6), and there were 4 visible leaves of 1.12 cm^2 average total area (fig 7) and 4 leaf primordia. On the sixteenth day the branch was 27 mm long and there were 4 to 5 visible leaves of 2.26 cm^2 average total area, and 4 leaf primordia.

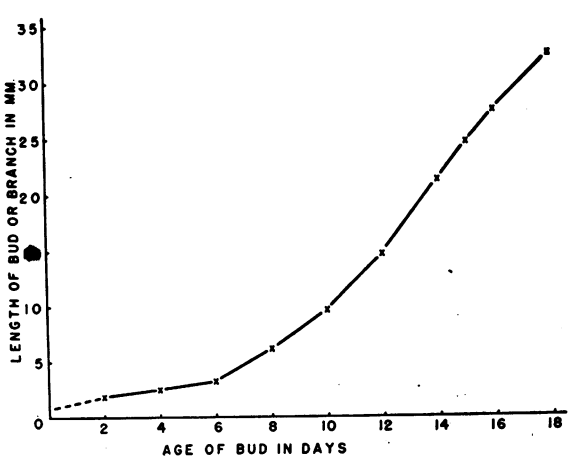

FIG. 6. Length of buds in experiment 5 (fig 4 and 5). Readings are average of 20 buds.

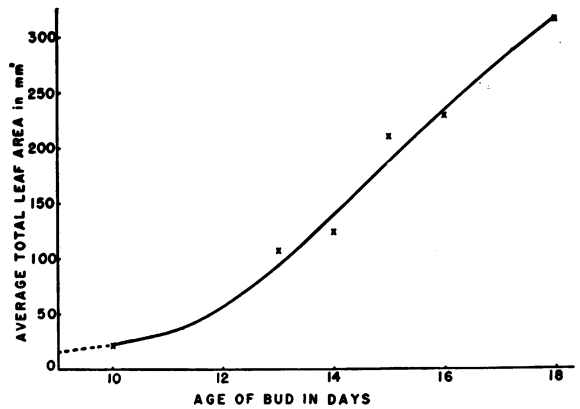

FIG. 7. Average total leaf area of buds in experiment 5 (fig 4 and 5). Each measurement was of buds on 5 plants.

The results indicate that a total area of 0.2 cm^2 is sufficient to cause response to 16 hours of darkness in a single photoinductive cycle. This is a very small amount of leaf tissue to respond to photoperiodic induction, and it seemed surprising when compared with previous work with young unexpanded leaves which had to have at least 3 to 5 cm^2 in order to respond.

The question arose whether the physiological age of the leaf was more significant than the area of the leaf in determining sensitivity to photoperiodic stimuli. An experiment was designed to determine the rate of growth of a leaf in a terminal bud. From Xanthium seedlings grown in pots, 5 plants were chosen having a leaf of the area 0.1 cm^2. Each leaf was measured daily for 12 days. Figure 8 shows that by the twelfth day the leaf reached full size. From

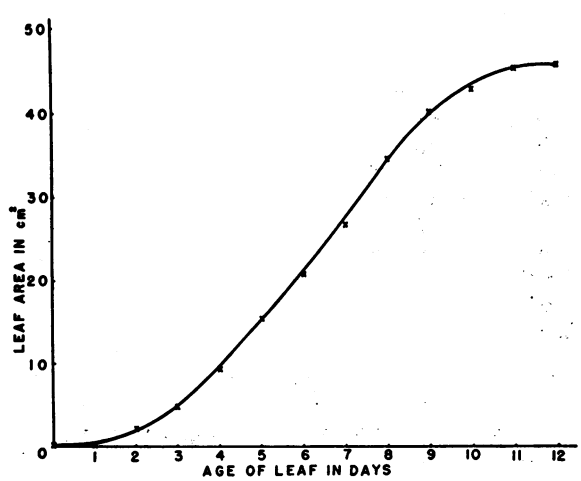

FIG. 8. Growth of untreated Xanthium leaf. A leaf of 0.1 cm^2 was chosen on each of 5 vegetative plants. Leaf blade area was measured daily until fully expanded.

this experiment it can be concluded that a small leaf (0.1 cm^2) reaches its greatest sensitivity after growing about 5 days. When the cotyledonary bud begins to grow the first few leaves never attain the size of subsequent leaves; but, even though they are small they respond photoperiodically in a manner similar to the larger leaves of the same age produced from the terminal bud.

Discussion

The sensitivity of Xanthium leaves to photoperiodic induction increases with aging of the expanding leaves reaching a maximum when they are half expanded. Sensitivity then decreases with increase of age of expanded leaves. Earlier workers believed that the youngest fully expanded leaf is the most sensitive to photoperiodic induction. Results reported here indicate that the half-expanded leaf is the most sensitive.

It is of importance to know which is the most sensitive leaf to the photoperiodic induction in order to study the photoperiodic changes in relation to biochemical changes. In order to follow the changes in leaves of a plant which has been induced it is important to choose a leaf which is photoperiodically sensitive. In preliminary experiments on auxin and growth inhibitor reported by Khudairi and Bonde (5) only the expanded leaves of Xanthium were taken for analyses. Results were inconsistent and the cause was not realized until the discovery of the relation between age of the leaf and its sensitivity to photoperiodic induction. Subsequently the leaves were chosen very carefully according to their age.

These experiments also make possible certain conclusions about the translocation of the stimulus from leaves to buds. It has been believed (4) that it takes about 4 days for the floral stimulus to move from the leaves through the stem to cause flowering in Xanthium. In these experiments it is shown by removing the induced leaves at different time intervals after the dark period of a short day that the stimulus begins to move out of the leaf in 2 to 4 hours after the end of a 15 hour period. This corresponds to results obtained by Raven (11) using intact plants. Two hypotheses may be made to explain the interval of time necessary after the dark period, for the stimulus to be produced and to move from the leaf in effective concentrations. First, it is possible that chemical reactions leading to formation of the stimulus have to take place in the leaf tissue after the long dark period and these may take appreciable time. Secondly, it may take time for the stimulus to move out of the leaf in effective amounts. It is possible that exposure to light after the dark period accelerates movement of the stimulus out of the leaves.

Dormant buds, which do not develop into branches normally, may be forced into growth by removing the top of the plant. The leaves on these buds become sensitive to short days after seven days growth. It is apparently not the leaf size which determines its sensitivity, but the physiological age of the leaf. Thus, the normally developing leaf in a terminal bud takes about 12 days to reach full size (45 cm^2 area), while it was found that the total leaf area of leaves of this age on newly developed buds was about 0.5 cm^2. Some workers (7, 8) claim that defoliated short-day plants may be induced to flower when given repeated favorable day lengths. It has been assumed, therefore, that the stem is slightly sensitive to short day. It may be that small axillary buds developed during the experimental period and produced small leaves. Such small leaves may have been overlooked by the experimenters, who would have assumed that they have no effect on flowering. Results obtained here with the newly developed buds indicate that small amounts of leaf tissue present on a defoliated plant should not be ignored during experimental treatment. These results seem to support the hypothesis that the flowering stimulus is formed only in green leaves.

Summary

1. Single leaves of different ages were left on Xanthium plants and given one short day of either 15 or 20 hours darkness. The age was determined by the position of the leaf on the plant and by the leaf area.

2. The youngest leaf blades (ranging in area 1 to 2 cm^2) on the plant gave no flowering response, but the response increased with the age of the expanding leaf, reaching a maximum in the leaves of an area approximately one half that of a fully expanded one. A reversal occurs at this point and the sensitivity decreases in the successively older ones.

3. When the induced single leaves were defoliated at different intervals after the end of the inductive period, it was found that with the most sensitive leaves, flowering could be detected when the leaves were removed within 4 hours after 15 hours of darkness, and 2 hours after a 20 hour dark period.

4. Plants with one dormant bud may be induced to flower when the buds are 7 days old and give 100 % flowering after 13 days.

Literature Cited

1. Borthwick, H. A. and Parker, M. W. Floral initiation of Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. Bot. Gaz. 101: 806–817. 1940.
1b. Fernald, M. L. Gray's Manual of Botany. 8th ed. P. 1473. American Book Co., New York. 1950.
2. Hamner, K. C. Interrelation of light and darkness in photoperiodic induction. Bot. Gaz. 101: 658–687. 1940.
3. Hamner, K. C. and Bonner, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100: 388–431. 1938.
4. Khudairi, A. K. and Bonde, E. K. Growth inhibitor and auxin in Xanthium leaves in relation to photoperiodism. Plant Physiol. (In press).
5. Lang, A. Physiology of flowering. Ann. Rev. Plant Physiol. 3: 265–306. 1952.
6. Liverman, J. Physiology and biochemistry of flowering. Doctoral thesis, California Institute of Technology, Pasadena. 1952.
7. Lona, F. La fioritura della brevidiurna a notte continua. Nuovo Giorn. Bot. Ital. 56: 479–515. 1949.
8. Moskov, B. S. The role of leaves in the photo-

periodic reaction of plants. Trudy Priklad. Bot. Genet. i Selektsii (Russian) A 17: 25–36. 1936.
9. Moskov, B. S. The photoperiodic reaction of leaves and possibility of its use in grafting. Trudy Priklad. Bot. Genet. i Selektsii (Russian) A 19: 107–126. 1937.
10. Naylor, A. W. Effect of nutrition and age upon rate of development of terminal staminate inflorescences of *Xanthium pennsylvanicum*. Bot. Gaz. 103: 342–353. 1941.
11. Raven, K., Lincoln, R. G., and Hamner, K. C. Unpublished data. 1951.

CARBOHYDRATE METABOLISM AND OXALIC ACID SYNTHESIS BY BOTRYTIS CINEREA [1,2]

ARTHUR C. GENTILE [3]
Department of Botany, University of Chicago, Chicago, Illinois

Most of the work on the metabolism of the fungi has dealt with the production of organic acids and other compounds formed via pathways which are, presumably, shunts from the main respiratory cycles (6, 14). Few attempts have been made to integrate these pathways into over-all metabolic systems. This study will follow the formation of oxalic acid, which appears to be a metabolic end product, from glucose through the respiratory system of *Botrytis cinerea* Pers.

Materials and Methods

In all experiments a strain of *Botrytis cinerea* (7) was grown at room temperature in 250-ml Erlenmeyer flasks containing 100 ml of the following medium: $NaNO_3$, 2.0 gm; KH_2PO_4, 1.5 gm; $MgSO_4 \cdot 7 H_2O$, 1.0 gm; Hoagland's A–Z trace element solution (9), 1.0 ml; glucose, 20.0 gm; H_2O to make 1 liter. Inoculum for flasks was provided by growing the fungus in Petri dishes on the above medium solidified with 2 % agar. Uniform discs were cut out with a cork borer 7 mm in diameter and each flask inoculated with 1 disc. Unless otherwise noted, the cultures were mechanically shaken during incubation (10 days). The pellets of mycelium which formed were then washed and shaken for 3 days in numerous changes of the above liquid medium without a carbon source. These pellets of starved mycelium were used in all respiratory and biosynthesis experiments (tables II to IV).

Oxalic acid was removed from culture filtrates essentially according to Pucher, Vickery, and Wakeman (12). The filtrates were acidified with H_2SO_4 and the oxalic acid precipitated with saturated $Ca(NO_3)_2$. One ml of the oxalic acid solution obtained was placed in a Warburg vessel and, at zero time, 0.5 ml of acid permanganate solution (8 % $KMnO_4$ in 7 *N* H_2SO_4) was tipped in. Under these conditions 2 moles of CO_2 were formed from each mole of oxalic acid. In numerous tests with known concentrations of oxalic acid, it was found possible to obtain an accuracy of about 5 % with this method. Of all the organic acids discussed in this paper, only citric interfered in this method. However, no trace of citric acid was found in any filtrates of this organism when they were analyzed chromatographically. Glucose was determined by the procedure of Folin and Malmros (5), nitrate by a phenoldisulfonic acid method (13), and organic acids, qualitatively, by the method of Lugg and Overell (8). All manometric studies were conducted at 26°C, pH 4.0, using standard Warburg techniques. The vessels contained 3.0 ml of the medium previously described, with added substrate, where indicated, and KOH with filter paper wicks were used in the center well.

Results

A 6-week time-course study was undertaken to investigate the pattern of oxalic acid formation and the physiological changes accompanying its synthesis. Each value in figure 1 represents the results of duplicate analyses for oxalic acid of combined filtrates and in addition the average mycelial weights of 6 standing cultures. The pH of the cultures rose slowly from 4.2 at the time of inoculation to 6.5 at the end of 6 weeks. Oxalic acid formation does not appear to accompany the growth of the fungus, since most of the oxalic acid was produced before the log phase of growth. There was only a slight increase in total oxalic acid during the second week, and it began to fall off slightly after this time. Oxalic acid synthesis apears to be concomitant with the rapid utilization of glucose. At the end of the 6 week period, the growth curves began to level off although only about 50 % of the carbohydrate and 30 % of the nitrate available in the medium had been utilized. Cessation of growth may have been due to production of "staling products" rather than to any limitation of available nutrient.

Figure 2 shows the rates of O_2 uptake of mats of mycelium of various ages, each value obtained from a random sample of segments of mats taken from several standing cultures. The segments were cut into small squares and shaken in Warburg vessels, each vessel containing about 20 mg dry weight of mycelium suspended in 3.0 ml of medium containing glucose (5×10^{-3} M). The Q_{O_2} reached a peak at 2 weeks and subsequently fell off rapidly, leveling off by

[1] Received September 10, 1953.
[2] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.
[3] Present address: Department of Botany, Duke University, Durham, N. C.