Plant Physiol. (1969) 44, 562–566

Inhibitory Effect of Carbohydrate on Flowering in Lemna perpusilla. I. Interaction of Sucrose with Calcium and Phosphate Ions[1]

Herbert B. Posner

Department of Biology, State University of New York, Binghamton, New York 13901

Received November 21, 1968.

Abstract. **Flowering in *Lemna perpusilla* 6746 grown on tenth-strength Hutner's medium under short days was inhibited by 30 mM sucrose, glucose or fructose, but not by mannitol. The inhibition by sucrose does not appear to be due to sucrose-induced acidification of the medium during growth, or to trace metal contaminants of the sugar. Inhibition was partially prevented by raising either Ca^{2+} or phosphate to levels used in half-strength medium. Possible mechanisms for these effects are discussed.**

The view stressed early in this century that substrates play an important regulatory role in plant growth and development has recently re-attracted attention (1, 2). In the case of flowering it has been suggested that unspecific substances such as sucrose might play a central role (8). It was therefore of particular interest to find that flowering in *Lemna perpusilla* 6746 and mutant strain 1073 grown on dilute Hutner's medium was inhibited by sucrose; the inhibition was prevented by growing the cultures on higher concentrations of the medium (14). These findings suggested 2 possible approaches to further study of the problem: one, to identify the component or components of Hutner's medium that overcome the inhibition by sucrose; the second, to identify those organic supplements that mimic the effect of sucrose.

The results given below, from studies based on the first approach, indicate that inhibition of flowering by sucrose is overcome by increasing the levels of either Ca^{2+} or phosphate.

Materials and Methods

Vegetative stock cultures, started with two 3-frond colonies of *Lemna perpusilla* strain 6746, were grown aseptically on about 50 ml of half-strength Hutner's medium (10) in 125 ml Erlenmeyer flasks plugged with cotton. Unless noted otherwise, the medium contained sucrose (30 mM). The growth conditions were continuous light (about 150 ft-c) from Sylvania cool-white fluorescent lamps, with the air temperature 24° to 26°.

Experimental cultures were started with single 3-frond colonies from stock cultures 7 to 9 days old, and were grown on 25 ml medium in 25 × 150 mm tubes capped with Morton stainless steel closures. The tubes were inclined at a 30° angle under flowering conditions (6): 8-hr photoperiod; about 300 ft-c from alternated cool- and warm-white Sylvania fluorescent tubes; air temperature 24° to 26° during the dark and 25° to 27° during the light periods.

The basal medium for experimental cultures was tenth-strength Hutner's medium which has the following composition. Macronutrients (mM): $Ca(NO_3)_2$-$4H_2O$, 0.15; K_2HPO_4, 0.23; NH_4NO_3, 0.25; $MgSO_4$-$7H_2O$, 0.20. Micronutrients (μM): $FeSO_4$-$7H_2O$, 9.0; $ZnSO_4$-$7H_2O$, 23.0; H_3BO_3, 23.0; Na_2MoO_4-$2H_2O$, 10.0; $CuSO_4$-$5H_2O$, 1.6; $Co(NO_3)_2$-$6H_2O$, 0.07; MnCl-$4H_2O$, 9.0. Chelator, Potassium form (mM): EDTA, 0.17 plus KOH, 0.36. Before autoclaving, the pH was adjusted to 6.5 with KOH. This slightly raised the total K^+ level. All media were made with reagent-grade chemicals in water produced by glass distilling deionized water from the general laboratory system.

Unless otherwise noted, sucrose-supplemented cultures were examined on day 6 and unsupplemented cultures on day 8, at which times their frond numbers were about equal. Fronds were counted under 8× magnification and then dissected under 20×. Flowering intensity when expressed as F1 % was determined as described elsewhere (6, 15). These references should be consulted for further details concerning medium composition and preparation, frond and flower morphology, and techniques.

Results

Cation-exchange Treatment of Sucrose. Before attempting to identify the component or components of Hutner's medium that overcome the sucrose-induced inhibition of flowering, experiments were done to determine whether the inhibition was due to a trace metal contaminant of the sucrose. The inhibitory effect of reagent grade sucrose (Fisher) was

[1] This work was supported by N.S.F. Grant GB-6559.

compared with that of sucrose treated with a cation exchange resin, Chelex (Bio Rad). An initial experiment indicated that such treatment reduces the inhibitory effect of sucrose. However, the reduction was observed only if the Chelex was not thoroughly washed before being used. Chelex appears to contain a water-soluble substance that overcomes the sucrose inhibition. Thoroughly washed Chelex did not reduce the inhibitory effect of sucrose. The day 6 Fl % values (6 cultures per group) were 0 for untreated and treated sucrose cultures, with a range of frond numbers of 29 to 42 and 32 to 43, respectively. The Fl % mean for minus sucrose controls (4 cultures) on day 8 was 44, with a range of frond numbers of 25 to 39.

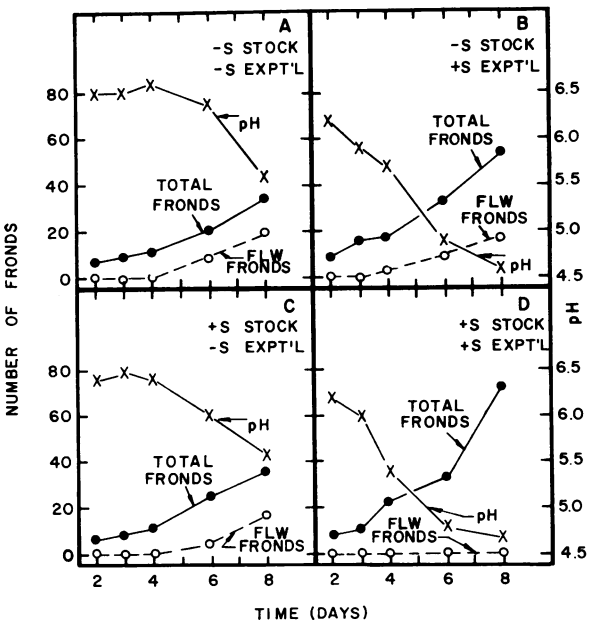

FIG. 1, A-D. Effects of sucrose on flowering, frond production, and pH. Stock cultures grown on half-strength Hutner's medium without or with sucrose (30 mM) under continuous cool-white fluorescent light (150 ft-c). Transferred to tenth-strength medium without or with sucrose (30 mM) under flowering conditions [8-hr photoperiod, cool- and warm-white fluorescent light (300-400 ft-c) at 24° to 27°]. Each point is mean of 3 values. Fig. 1, A) Stock, no sucrose; Experimental, no sucrose. Fig. 1, B) Stock, no sucrose; Experimental, plus sucrose. Fig. 1, C) Stock, plus sucrose; Experimental, no sucrose. Fig. 1, D) Stock, plus sucrose; Experimental, plus sucrose.

Acidification of the Medium. Another possibility, that inhibition of flowering by sucrose in dilute medium resulted from rapid acidification of the medium, was examined in an experiment similar to one reported previously (14). Vegetative stock cultures were grown with and without sucrose. From these, individual colonies were planted on tenth-strength medium with or without sucrose (30 mM), and placed under an 8-hr photoperiod. Replicate cultures were removed on days 2, 3, 4, 6, and 8; the fronds were counted and dissected, and the pH of the media measured (Fig. 1, A-D).

In the absence of sucrose in the experimental medium, there was little effect of sucrose in the stock cultures in terms of frond and flower production, as well as acidification (compare Fig. 1, A with 1, C). Confirming previous results (14), sucrose inhibited flowering on tenth-strength medium if the inocula were taken from stock cultures grown on sucrose-supplemented medium, but not if taken from unsupplemented stocks (compare Fig. 1, B with 1, D). Although sucrose increased the rate of acidification of the medium, comparison of Fig. 1, B with 1, D shows that about the same degree of acidification occurred regardless of the stock culture condition. Thus, decreased pH appears to be a symptom of growth with sucrose and not the cause of inhibited flowering.

Identification of Active Salts. Cultures were grown with or without sucrose (30 mM) on Hutner's medium modified as follows: A) all components at

Table I. *Effects of Increased Levels of Macro- and Micronutrients With and Without Sucrose on Flowering*

Light and temp as in Fig. 1. Stock cultures grown on half-strength Hutner's medium with 30 mM sucrose. Experimental cultures grown on various modifications of Hutner's medium with and without sucrose. Concentrations of micronutrients (including K-EDTA) and macronutrients expressed as fraction of full-strength Hutner's medium. Fl % and frond number values are means ± S. E. of 5 cultures.

Sucrose	Concn. of micro-nutrients	Concn. of macro-nutrients	Day 6 No. of fronds	Day 6 Fl %	Day 8 No. of fronds	Day 8 Fl %
mM						
	0.1	0.1	...	...	30.0 ± 1.0	33.6 ± 6.5
0	0.5	0.5	...	...	28.0 ± 2.4	32.0 ± 6.4
	0.1	0.5	...	...	33.8 ± 2.3	43.0 ± 4.3
	0.5	0.1		no growth		
	0.1	0.1	29.0 ± 1.3	5.8 ± 2.1	49.8 ± 3.2	0.0
	0.5	0.5	53.2 ± 4.2	41.3 ± 5.9	94.3 ± 3.5	47.5 ± 2.2
30	0.1	0.5	47.2 ± 4.3	57.0 ± 1.9	71.5 ± 3.1	50.5 ± 4.2
	0.5	0.1		no growth		

tenth-strength; B) all components at half-strength; C) micronutrients including K-EDTA at tenth-strength; macronutrients at half-strength; D) micronutrients including K-EDTA at half-strength; macronutrients at tenth-strength. The results are in table I.

Consistent with previous data, flowering was markedly inhibited by sucrose in tenth-strength medium, but not in half-strength. The inhibitory effect of sucrose was overcome by increasing the concentration of the macronutrients, indicating that this fraction contains an active component. However, since growth did not occur on the tenth-strength medium with the micronutrients and K-EDTA at half-strength, these results did not eliminate the possibility that the micronutrient fraction also contains an active component.

In order to prevent the toxic effect of Composition (D), K-EDTA was kept at tenth-strength regardless of the concentrations of the other fractions. This modification was incorporated in an experiment similar to the previous one. The results in table II show that in the absence of sucrose, flowering was unaffected by increasing the concentration of the macronutrients; increased levels of micronutrients slightly inhibited flowering. Flowering was inhibited on tenth-strength medium containing sucrose. Supplementing this medium with micronutrients had no effect, but raising the macronutrients to half-strength levels prevented the sucrose inhibition; since the mean number of fronds for both of these treatments did not differ, the higher number of flowering fronds produced in the macronutrient-supplemented medium was not merely due to a higher total number of fronds.

Identification of Active Macronutrients. Cultures were grown without and with sucrose (30 mM) in tenth-strength Hutner's medium with each of the macronutrients at half-strength. Controls included cultures with all macronutrients at either tenth-strength or half-strength.

The results, summarized in table III, confirm those obtained before: in the absence of sucrose, flowering was about the same with macronutrients at tenth- or half-strength; in the presence of sucrose, inhibition occurred on tenth-strength but not on half-strength. The results obtained with macronutrients tested individually are complicated by the inhibition of growth in cultures on tenth-strength medium with $MgSO_4$ at half-strength. However, sufficient growth occurred with sucrose to show that $MgSO_4$ did not overcome the inhibition of flowering. An increased concentration of NH_4NO_3 was also ineffective. In contrast, $Ca(NO_3)_2$ and to a greater extent K_2HPO_4 prevented the sucrose inhibition. As before, the presence of flowering fronds in cultures supplemented with these salts was not simply the result of a higher total number of fronds. This is indicated by the finding that on day 8, Fl %

Table II. *Effects of Increased Levels of Macronutrients and Micronutrients on Sucrose-Induced Inhibition of Flowering*

Light, temp, and stock conditions as in table I. Experimental cultures grown on tenth-strength medium without or with sucrose at 30 mM, and supplemented with micronutrients or macronutrients to half-strength medium levels. K-EDTA at tenth-strength levels in all cases. Minus sucrose cultures dissected on day 8; plus sucrose on day 6. Fl % and frond number values are means ± S.E. of 7 cultures.

Concn. of		— Sucrose		+ Sucrose	
micro-nutrients	macro-nutrients	No. of fronds	Fl %	No. of fronds	Fl %
mM					
0.1	0.1	33.1 ± 0.9	33.3 ± 2.6	39.7 ± 2.2	2.6 ± 1.7
0.1	0.5	39.6 ± 2.8	34.3 ± 3.3	49.0 ± 3.2	35.7 ± 2.2
0.5	0.1	32.0 ± 0.7	20.4 ± 0.9	49.8 ± 1.5	0.0

Table III. *Sucrose-Induced Inhibition of Flowering as Affected by Various Macronutrient Salts*

Growth conditions and sucrose supplementation as in table I. Salts added to bring level to that in half-strength medium. Plus sucrose cultures (7) dissected on day 6; minus sucrose cultures (5) on day 8, except for $MgSO_4$ which were dissected on day 10. Fl % and frond numbers are means ± S.E.

Salt added	— Sucrose		+ Sucrose	
	No. of fronds	Fl %	No. of fronds	Fl %
None	30.8 ± 2.0	25.0 ± 4.7	40.4 ± 2.3	0.0
$Ca(NO_3)_2$	36.8 ± 2.3	33.8 ± 4.2	54.9 ± 2.7	5.3 ± 2.0
K_2HPO_4	37.0 ± 4.1	37.6 ± 3.8	67.4 ± 3.5	22.6 ± 4.4
NH_4NO_3	37.6 ± 0.8	30.8 ± 1.5	49.3 ± 4.3	0.0
$MgSO_4$	18.4 ± 1.0	7.0 ± 4.9	35.3 ± 2.6	0.0
All 4	32.6 ± 2.0	35.4 ± 3.3	68.0 ± 2.6	44.9 ± 3.4

values for plus sucrose controls were still 0, even though their mean number of fronds was 84.

Identification of Active Ions. Those salts showing any activity were further studied to identify the active ions. In the first experiment of this type, K_2HPO_4, Na_2HPO_4, and KCl were tested to determine whether the activity exhibited by the K_2HPO_4 (see table III) was due to the K^+ or to the phosphate. The results (table IV) show that KCl, with a molar concentration of potassium equal to that of K_2HPO_4, was completely inactive, while Na_2HPO_4 with the same molarity of phosphate as the K_2HPO_4 completely prevented the sucrose inhibition.

The next experiment, designed to determine whether the activity shown by $Ca(NO_3)_2$ required both ions of the salt, compared $Ca(NO_3)_2$ with KNO_3 and $CaCl_2$. The results (table V) show that Ca^{2+} in the absence of additional $(NO_3)^-$ overcame the sucrose inhibition. Thus, either Ca^{2+} or phosphate prevented inhibition of flowering by sucrose.

Effects of Other Carbohydrates. It was of special interest to determine whether the inhibitory effect of sucrose was due to its action as an osmotic agent in the medium, or as a nutrient. Although this matter will be dealt with in greater detail in the second paper of this series, some pertinent results will be given here comparing the effect of sucrose with that of glucose, fructose and mannitol. The data, summarized in table VI, show that flowering was unaffected by mannitol, but was completely inhibited by the other carbohydrates.

Discussion

In the absence of exogenous carbohydrates, low levels of macronutrients that did not markedly reduce growth had no effect on flowering of *Lemna perpusilla* 6746; in the presence of sucrose, glucose or fructose, there was a marked reduction in flowering,

Table IV. *Sucrose-Induced Inhibition of Flowering as Affected by K^+ and Phosphate*

Growth conditions, sucrose supplementation, and dissection times as in table II. Fl % values are means ± S.E. of 4 cultures.

Salt added	Concn.	— Sucrose		+ Sucrose	
		No. of fronds	Fl %	No. of fronds	Fl %
	mM				
None	...	31.5 ± 1.3	33.3 ± 4.5	52.5 ± 4.5	0.0
KCl	1.84	32.5 ± 1.6	27.8 ± 3.1	41.5 ± 3.1	0.0
K_2HPO_4	0.92	34.8 ± 1.3	40.5 ± 2.7	68.8 ± 3.1	30.3 ± 1.4
Na_2HPO_4	0.92	36.0 ± 2.0	36.8 ± 2.1	74.8 ± 2.5	35.5 ± 2.9

Table V. *Sucrose-Induced Inhibition of Flowering as Affected by Ca^{2+} and $(NO_3)^-$ Added Individually or Together*

Growth conditions, sucrose supplementation, and dissection times as in table II. Fl % values are means ±S.E. of 6 cultures for —Sucrose and 7 cultures for +Sucrose.

Salt added	Concn.	— Sucrose		+ Sucrose	
		No. of fronds	Fl %	No. of fronds	Fl %
	mM				
None	...	34.0 ± 2.9	50.5 ± 2.3	40.4 ± 1.9	1.0 ± 1.0
KNO_3	1.2	34.6 ± 1.1	54.0 ± 4.1	44.0 ± 2.7	0.0
$Ca(NO_3)_2$	0.6	35.3 ± 2.0	46.5 ± 3.5	50.3 ± 3.1	24.3 ± 3.1
$CaCl_2$	0.6	37.0 ± 3.7	50.7 ± 1.9	48.4 ± 4.6	22.0 ± 4.3

Table VI. *Effects of Various Carbohydrates on Flowering*

Light, temp, and stock conditions as in table I. Experimental cultures tenth-strength Hutner's medium with supplements (30 mM) as noted. Fl % and frond number values are means ±S.E. of 4 cultures.

Supplement	Day 6		Day 8	
	No. of fronds	Fl %	No. of fronds	Fl %
None	16.8 ± 1.3	7.8 ± 0.9	26.5 ± 1.5	34.0 ± 1.8
Mannitol	17.8 ± 2.3	11.0 ± 5.2	26.0 ± 0.4	41.0 ± 1.9
Sucrose	28.3 ± 0.5	0.0	55.0 ± 1.4	0.0
Glucose	25.8 ± 0.3	0.0	36.0 ± 2.2	0.0
Fructose	18.0 ± 0.4	0.0	28.0 ± 1.4	0.0

whereas with mannitol flowering was unaffected. The inhibition of flowering by sucrose in low-macronutrient medium was prevented by increasing the levels of either Ca^{2+} or phosphate, but not by K^+, $(NO_3)^-$, $(NH_4)^+$, Mg^{2+}, or $(SO_4)^{2-}$. The effect of Ca^{2+} might have been due to an enhancement of the uptake or total accumulation of phosphate, as has been shown in other plant systems (13).

Hillman (9) has reported that Ca^{2+} and phosphate inhibit copper-induced long-day flowering of 6746 grown on a non-chelated medium, and has suggested that an inhibition of Cu^{2+} uptake might be involved. It is not possible at this time to implicate Cu^{2+} in the sucrose-Ca-phosphate interaction reported here, since the question as to whether Cu^{2+} is required for short-day flowering in 6746 has not been unequivocally resolved.

Another possibly related explanation for the interaction between sucrose and phosphate is that iron metabolism might be affected. Iron is required for photoperiodic induction of flowering in 6746 (7), and its uptake or activity can be affected by Ca^{2+} and phosphate (5).

Recent work implicating carbohydrates in a regulatory role in flowering includes the report that in *Chenopodium rubrum*, glucose, but not sucrose, either inhibits or stimulates flowering, depending on the time of feeding during a 72-hr dark period (3). This and other work has suggested that carbohydrates may be involved in circadian rhythms of flower induction (4). In this regard, Kandeler (11) has reported that sucrose, CO_2 and $(HCO_3)^-$ inhibit photoperiodically controlled flowering in *L. gibba*, and has suggested that the effect might be due to excessive consumption of ATP (12). A similar mechanism might be hypothesized for the sucrose-phosphate interaction reported here. It is clear, however, that interpretation of the results would be greatly aided by further identification and study of those substances that mimic the inhibitory effect of sucrose. Results bearing on this will be described in the second paper of this series.

Acknowledgments

The capable technical assistance of Miss Frances Kahanec and Mr. John Newby is greatly appreciated.

Literature Cited

1. ALLSOPP, A. 1965. The significance for development of water supply, osmotic relations, and nutrition. In: Encyclopedia of Plant Physiology. XV/1. W. Ruhland, ed. Springer-Verlag, Berlin.
2. CARR, D. J. 1966. Metabolic and hormonal regulation of growth and development. In: Trends in Plant Morphogenesis. Elizabeth G. Cutter, ed. John Wiley and Sons, Incorporated, New York.
3. CUMMING, B. G. 1967. Circadian rhythmic flowering responses in *Chenopodium rubrum*: effects of glucose and sucrose. Can. J. Botany 45: 2173–93.
4. CUMMING, B. G. AND E. WAGNER. 1968. Rhythmic processes in plants. Ann. Rev. Plant Physiol. 19: 381–416.
5. GAUCH, H. G. 1957. Mineral nutrition of plants. Ann. Rev. Plant Physiol. 8: 31–64.
6. HILLMAN, W. S. 1959. Experimental control of flowering in *Lemna*. I. General methods. Photoperiodism in *L. perpusilla* 6746. Am. J. Botany 46: 466–73.
7. HILLMAN, W. S. 1961. Photoperiodism, chelating agents, and flowering of *Lemna perpusilla* and *L. gibba* in aseptic cultures. In: Light and Life. W. D. McElroy and B. Glass, eds. John Hopkins Press, Baltimore, Maryland.
8. HILLMAN, W. S. 1962. The Physiology of Flowering. Holt, Rinehart, and Winston, New York.
9. HILLMAN, W. S. 1962. Experimental control of flowering in *Lemna*. IV. Inhibition of photoperiodic sensitivity by copper. Am. J. Botany 49: 892–97.
10. HUTNER, S. H. 1953. Comparative physiology of heterotrophic growth in plants. In: Growth and Differentiation in Plants. W. E. Loomis, ed. Iowa State College Press, Ames, Iowa.
11. KANDELER, R. 1967. Blühinduktion bei Lemnaceen. Biol. Rundschau. 6: 49–57.
12. KANDELER, R. 1967. The role of photophosphorylation in flower initiation of the long-day plant *Lemna gibba*. European Photobiol. Symp., Hvar, Yugoslavia, Book of Abstracts. p 45.
13. LEGGETT, J. E. 1968. Salt absorption by plants. Ann. Rev. Plant Physiol. 19: 333–46.
14. POSNER, H. B. 1967. Inhibitory effect of sucrose on flowering in *Lemna perpusilla* 6746 and mutant strain 1073. Plant Cell Physiol. 8: 535–39.
15. POSNER, H. B. 1967. Aquatic vascular plants. In: Methods in Developmental Biology. F. Wilt and N. Wessels, eds. Thomas Y. Crowell Company, New York.