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Department of Botany and Plant Pathology, Colorado State University, Fort Collins

GROWTH REGULATORS AND FLOWERING

III. ANTIMETABOLITES*

By

WALTER T. COLLINS**, FRANK B. SALISBURY and CLEON W. ROSS

With 5 Figures in the Text

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It seems clear that transformation of the vegetative bud of cocklebur (*Xanthium pennsylvanicum* WALL.) to the reproductive condition occurs in response to a hormone which is synthesized in the leaf starting about 8 hours after the beginning of darkness (e.g. SALISBURY 1961, 1963). The present paper describes the results of one indirect approach used to gain insight into the physiology and biochemistry of flowering hormone synthesis, translocation, and action at the bud. Antimetabolites which are known to inhibit specific metabolic steps in other systems are applied. If flowering is inhibited, the suspected step is implicated in a preliminary way in the flowering process. The participation of this suspected step can be further tested by attempts to reverse the effects of the antimetabolite by simultaneous application of a suspected corresponding metabolite or the product of the blocked step. The time of effectiveness of the antimetabolite can also be determined.

A related approach using conventional growth regulators has shown that measurement of the critical 8 hour 20 minute dark period (critical night) is metabolically different from the subsequent synthesis of flowering hormone, and that ATP synthesis is an essential part of hormone synthesis (SALISBURY 1957). Thus cobaltous ion inhibits flowering only when applied during time measurement, while 2,4-dinitrophenol inhibits during hormone synthesis. Auxins inhibit during the translocation period, and 2,2-dichloropropionic acid, 2,4-dichlorophenoxyacetic acid, and maleic hydrazide inhibit development of the floral bud.

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** Present address: WALTER T. COLLINS, Forestry Science Laboratories, 1221 S. Main Street, Moscow, Idaho., U.S.A.

5-Fluorourcil (5-FU) inhibits flowering when applied during the inductive dark period, and its inhibitory effects can be reversed by simultaneous application of orotic acid (SALISBURY and BONNER 1960). Considering the amount of material applied per plant, 5-FU was much more effective when applied to the bud than when applied to the leaf being induced. 5-FU has been shown by BONNER and ZEEVAART (1962) to inhibit RNA synthesis in the bud, and it was postulated that this was the mechanism of inhibition of photoperiodic induction.

The present experiments summarize work with a number of antimetabolites. In all cases, compounds which inhibit flowering (no promoting compounds were found) were tested as to their time of effectiveness, and attempts were made to reverse the inhibitory effect by simultaneous application of a suspected metabolite. Relative effectiveness of bud or leaf application was also tested. Preliminary reports on some of these observations have already been published (COLLINS and SALISBURY 1960, SALISBURY 1961).

Materials and methods

The procedures used in these experiments have all been previously described (SALISBURY 1957, 1963). Cocklebur plants, *Xanthium pennsylvanicum* WALL., were grown from burs kindly supplied by Dr. JAMES BONNER at the California Institute of Technology. They were maintained in the vegetative condition by extending the normal daylength to about 20 hours with supplementary incandescent light of approximately 50 ft-c. Plants were prepared before an experiment by sorting them according to the size of the half-expanded leaf and then removing all the leaves below this one and the one above it. Chemicals were applied by momentarily dipping the leaf or tip, or the leaf and tip together, into solutions containing the compound and 2 drops of Tween 20 per 100 ml. All solutions were at a pH between 4 and 8. Control plants were dipped in distilled water containing the wetting agent. Dihydrocholesterol and estradiol were dissolved in 50 per cent ethanol. Application of chemicals during the single inductive dark period (16 hours except in critical night experiments) was accomplished using a weak green safelight. At least 10 plants were used for each treatment. The flowering response was measured 9 to 11 days after induction by classification of the buds according to the stage system of SALISBURY (1955).

Four kinds of experiments were performed: *A. Concentration.* Immediately prior to the inductive dark period, plants were dipped in various concentrations of the compound in question. In some instances, only the bud or only the leaf was dipped; usually both were dipped. *B. Time of effectiveness.* Plants were dipped in an effective concentration of the compound in question at different times before, during, and after the 16 hour inductive dark period. *C. Critical night.* To test an effect upon time measurement, plants were treated with an effective concentration of the compound just before dark periods of various lengths. *D. Reversal.* Two concentration series of a suspected metabolite were prepared. One of these contained the antimetabolite in question at an effective concentration. Plants were treated with these solutions just prior to a 16 hour dark period.

All chemicals were obtained commercially except for chloramphenicol and 5-bromo-3-isopropyl-6-methyl uracil, which were gifts of Parke-Davis and Company, and Dupont de Nemours and Company, respectively.

Results

The majority of compounds tested in a concentration series did not inhibit flowering. These are shown in Table 1. Included are various purine, pyrimidine, and amino acid antimetabolites, and several miscel-

Table 1. *Ineffective compounds*

Compound	Number of trials	Highest concentration tried
Purine or pyrimidine antimetabolites		
2-Amino-4-methyl pyrimidine	1	0.01 Molar
8-Azaguine	2	0.01 (suspension)
6-Azathymine	3	0.05
Barbituric acid	1	0.01
5-Bromouracil	2	0.005
Amino acid antimetabolites		
Allyl glycine	1	0.01 Molar
Canavanine	1	0.01
B-2-Thienyl alanine	1	0.01
Indole	1	0.01
B-2-Thienyl serine	2	0.01
D-Leucine	1	0.01
D-Alanine[1]	3	0.04
DL-α-Methyl glutamic acid	1	0.01
DL-Methionine sulfoxide	1	0.01
Miscellaneous		
3-Acetyl pyridine	1	0.01 Molar
2-Chloro-4-amino benzoic acid	1	0.01
Desoxypyridoxine	1	0.01
Desthiobiotin	1	0.01
Dihydrocholesterol[1]	1	0.01
Estradiol	1	0.003
DL-β-Phenyllactic acid	1	0.01
Pyridine-3-sulfonic acid	1	0.01
Sulfanilamide	1	0.01
Chloramphenicol[1]	2	0.005

[1] Caused minor injury (chlorosis or necrosis) to the leaves.

laneous compounds. Inhibitory compounds are listed in Table 2, and the effects of various concentrations are indicated in Fig. 1. In Table 2 compounds are arranged according to their effective time of action, based on results from time of application studies summarized in Fig. 2. Their influence on the length of the critical night and inhibition when applied to leaf or bud separately are also indicated in Table 2. If the chemical did not reduce the flowering response when added only to the bud (at a concentration 50 per cent inhibitory when applied to the leaf) it is listed as being effective on the leaf. However, in certain cases a higher concentration applied to the buds did cause significant inhibition. Results obtained upon attempts to reverse the inhibitory effects by adding certain metabolites are summarized in Table 3. Fig. 3 shows representative results of these experiments.

Most compounds retarded flowering only if applied before the end of the inductive dark period. This group includes certain amino acid and

Table 2. *Summary data pertaining to inhibitory compounds*

Compound	Conc. 50 % inhibitory (molar)	Minimum conc. injuring leaves	Nature of injury	Critical night	Leaf or bud
Effective during induction					
Anti-amino acids					
1. DL-Ethionine (2)[1]	0.005 (2)	0.003	Leaf wrinkling, necrotic edges	Extends (2)	Either (4)
2. DL-p-Fluorophenylalanine (2)	0.02 (5)	0.02	Occasional necrotic spots	No effect (1)	Either (2)
Purine or pyrimidine antimetabolites (1)					
3. Benzimidazole	0.02 (3)		none	No effect (1)	Leaf (2)
4. 2,6-Diaminopurine sulfate (1)	0.005 (3)	0.01	New leaves wrinkled	No effect (2)	Either (1)
Miscellaneous					
5. α-Picolinic acid (3)	0.01 (1)	0.03	Wrinkling, necrotic edges	Extends (2)	Leaf (3)
6. Quercetin (1)	0.005 (1)		none	No effect (1)	Leaf (1)
Effective during induction and translocation					
Pyrimidine antimetabolites					
7. 6-Azauracil (2)	0.0003 (5)	0.0003	Young leaves chlorotic	No effect (1)	Leaf (6)
8. 2-Thiouracil (3)	0.005 (3)	0.002	Young leaves chlorotic	No effect (1)	Either (3)
9. 5-Bromo-3-isopropyl-6-methyl uracil (2)	0.0003 (3)	0.0001	Leaf edges chlorotic	No effect (1)	Leaf (3)
Anti-amino acid					
10. DL-α-Methyl methionine (1)	0.05 (2)	0.03	Young leaves misshapen		Leaf (1)
Effective at all times					
11. Thioproline (1)	0.05 (3)	0.04	Young leaves misshapen		Either (2)

[1] Numbers in parentheses indicate number of times experiment was performed.

purine or pyrimidine antimetabolites: ethionine, p-fluorophenylalanine, 2,6-diaminopurine, and benzimidazole. The inhibition caused by ethionine was overcome by methionine. The inhibition due to p-fluorophenylalanine was reversed by phenylalanine. Both benzimidazole and

2,6-diaminopurine effects were partly or entirely overcome by uracil or orotic acid, but although experiments have been repeated a number of times, results with benzimidazole remain unsatisfying.

Three pyrimidine analogs, 6-azauracil, 2-thiouracil, and 5-bromo-3-isopropyl-6-methyl uracil (BIMU), inhibited during induction and during the translocation period. Azauracil (0.003 M) in six of eight experiments failed to cause any significant reduction in floral stage when added only to the bud, even though chlorosis and slight malformation of the young leaves resulted. The effect of azauracil was not reversed by cytosine, uracil, or orotic acid, but was partially overcome by uridine (Table 3). Thiouracil inhibition was largely reversed by simultaneous application of uracil or orotic acid. Application of thiouracil to the leaf alone was much more inhibitory than was bud application. Contrary to 5-FU and azauracil, thiouracil and BIMU were seldom translocated

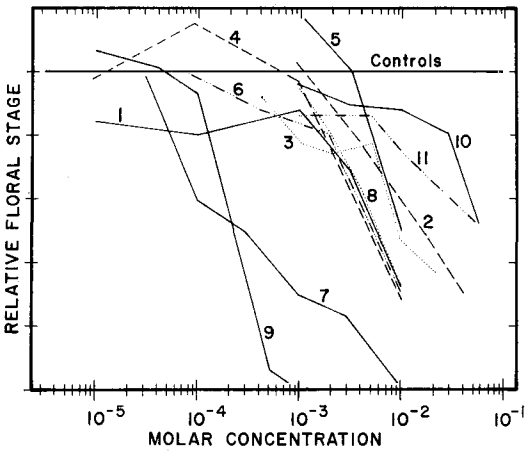

Fig. 1. Relative floral stage as a function of concentration of antimetabolite applied before a single inductive dark period. Numbers refer to the compounds of Table 2. Data of the individual experiments have been adjusted so that the controls of all experiments are at the same level. Curves as shown are drawn through actual experimental data, although variability is such that curves differ in detail when experiments are repeated

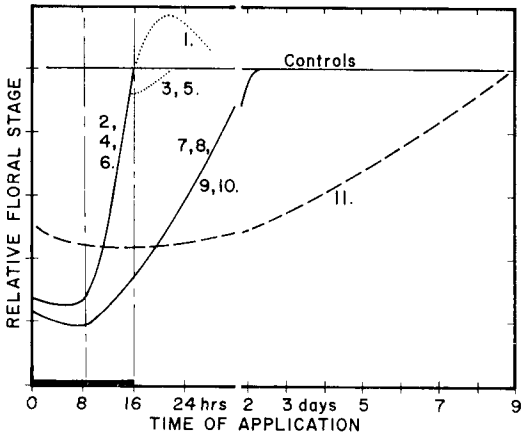

Fig. 2. Relative floral stage (nine days after induction) as a function of time when the antimetabolite is applied. Numbers refer to compounds of Table 2. Although the curves have been generalized, they lie very close to the original data. The level at time zero will, of course, depend upon concentration. The dotted lines represent deviations (of doubtful significance) in the original data for compounds *1*, *3*, and *5*. Aside from these deviations, the curves for these compounds are the same as for compounds *2*, *4*, and *6*, which fail to inhibit flowering when applied after the end of the inductive dark period

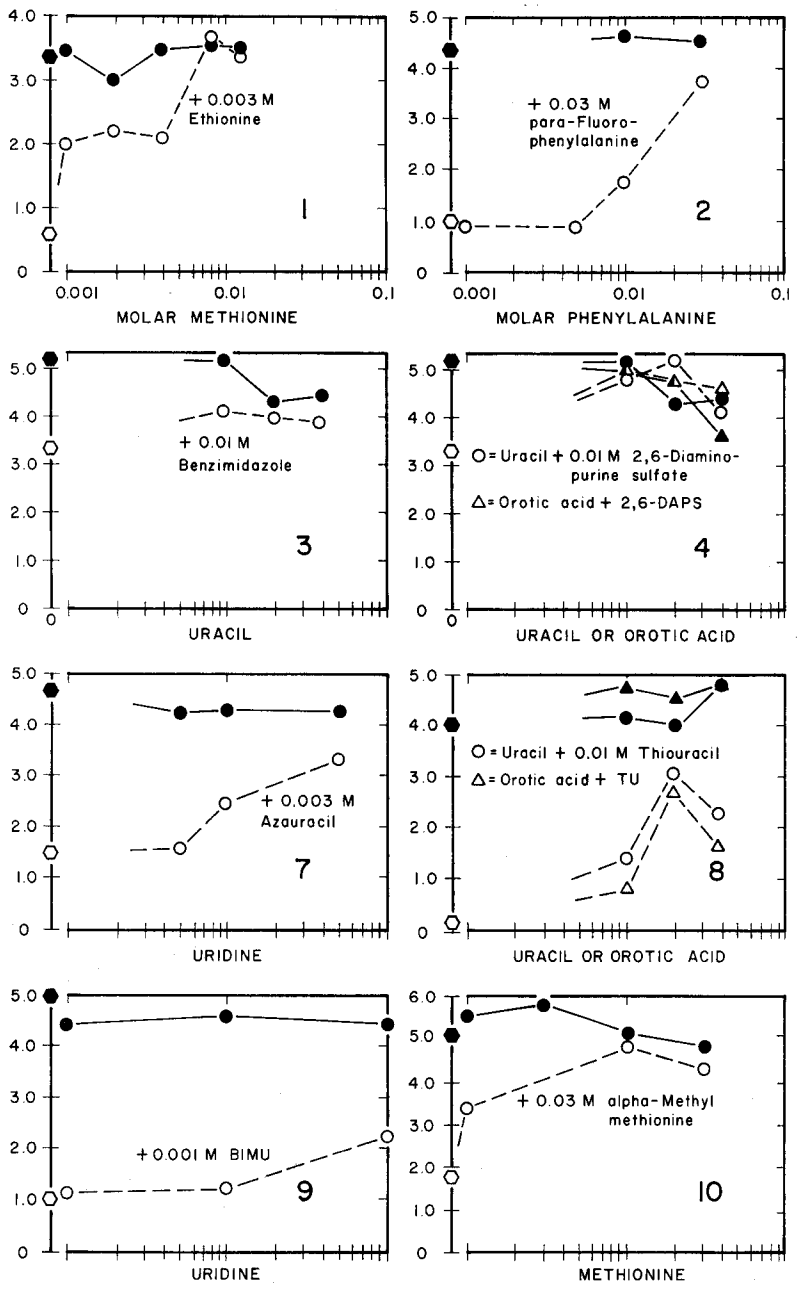

Fig. 3. Representative experiments showing floral stage (ordinates) as influenced by various concentrations of a metabolite applied alone (solid lines and solid points) or in the presence of the antimetabolite (broken lines and open points). Six-sided figures on the ordinates represent zero concentration of metabolite (solid figures: water alone; open figures: antimetabolite alone). Large numbers refer to compounds of Table 2 and 3

from the leaf to the bud in amounts sufficient to cause injury to the young developing leaves. BIMU was not effective when applied to the bud, even though it was translocated from the bud in amounts sufficient to cause chlorosis in the induced leaf. BIMU inhibition could not be reversed by uracil or orotic acid but was partially overcome by uridine.

Contrary to the other two amino acid antimetabolites, alpha-methyl methionine inhibited even during the translocation period. Its effect was almost entirely overcome by methionine. Only ethionine and alpha picolinic acid increased the length of the critical night (Fig. 4).

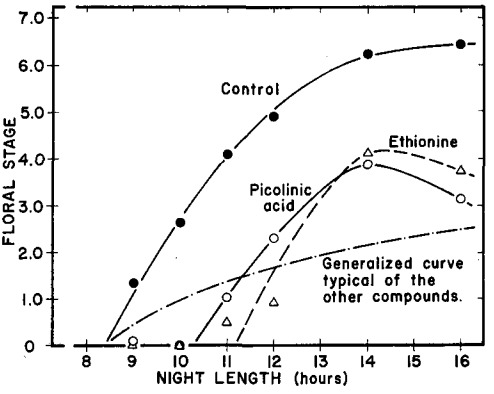

Fig. 4. Flowering response to various night lengths for control plants and for plants treated with various compounds just before a single dark period. Points show results of an actual experiment with picolinic acid and ethionine; the generalized curve summarizes experiments with the other nine compounds

Table 3. *Attempts to reverse antimetabolite inhibitions of flowering in cocklebur by various organic compounds*

Compound (Numbered to correspond with Table 2)	Ineffective reversing compounds	Effective reversing compounds	Extent of reversal %
1. DL-Ethionine		DL-Methionine (3; 0.016 M)[1]	100
2. DL-p-Fluoro-phenylalanine		DL-Phenylalanine (3; 0.03 M)	80—100
3. Benzimidazole	Orotic acid (2; 0.04 M[2])	Uracil (3; 0.04 M)	50—100
4. 2,6-Diaminopurine sulfate	Adenosine (1; 0.01 M)	Uracil (2; 0.04 M[2])	100
	Guanine (1; 0.01 M[2])	Orotic acid (2; 0.04 M)	100
7. 6-Azauracil	Uracil (1; 0.05 M)	Uridine (2; 0.05 M)	50
	Orotic acid (1; 0.05 M)		
	Cytosine (1; 0.05 M[2])		
8. 2-Thiouracil	Uracil (1; 0.04 M)	Uracil (3; 0.04 M)	60
	Orotic acid (2; 0.04 M)	Orotic acid (1; 0.04 M)	60
9. 5-Bromo-3-iso-propyl-6-Methyl uracil	Uracil (2; 0.01 M)		
	Orotic acid (2; 0.01 M)	Uridine (2; 0.05 M)	30
10. DL-α-Methyl methionine		DL-Methionine (1; 0.03 M)	90
11. Thioproline	L-Proline (4; 0.05 M)		

[1] Numbers in parentheses indicate number of trials and highest concentration used.

[2] At these concentrations orotic acid, uracil, cytosine, and guanine were added as suspensions.

However, extension of the critical night by a compound does not prove that it affects timing. Hormone synthesis may be inhibited in such a way that no hormone is produced during the first one or two hours after the critical night. Also, hormone synthesis may be reduced to a certain *percentage* as long as the inhibiting compound is present. These possibilities can be tested by light interruptions during an inductive dark period, using control plants and plants treated with the chemical (SALISBURY 1959). Such an experiment was performed with the two compounds which clearly extended the critical night, and results are shown in Fig. 5. Cobaltous ion was used as a control because of its known effect upon time measurement. Neither ethionine nor picolinic acid had any influence on time measurement.

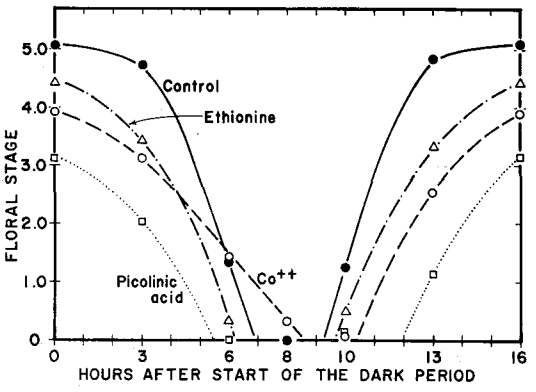

Fig. 5. Effects upon subsequent flowering of a red light interruption (one minute, four 300 Watt flood lamps about 40 cm above the plants, red plexiglas and 10 cm water filter) given at various times during a 16 hour inductive dark period to control plants and to plants previously treated with 0.01 M Ethionine or Picolinic acid or with 0.003 M $CoCl_2$

Discussion

1. Concentration experiments. The 16 hour inductive dark period is nearly optimal for promotion of flowering by a single cycle. The present experiments are thus not ideal for measuring a promotion of flowering, and none of the compounds has given any consistent promotive effect. As indicated in Table 1, most of the compounds tried have also failed to inhibit flowering at the concentrations used. It is possible that many of these compounds failed to penetrate into the plant. Except for dihydrocholesterol, chloramphenicol, and D-alanine none of them caused any vegetative injury. Thus no positive conclusions can be drawn based upon such negative evidence. It would, however, be difficult to understand how these compounds would fail to penetrate while other closely related compounds are effective inhibitors of flowering.

The concentration curves of Fig. 1 seem to fall easily into two main groups: those compounds which are effective at relatively low concentrations (6-azauracil and BIMU) and those which are only effective at

higher concentrations (the other compounds). Further division of the second group also seems possible, but it would not be as easy, even though there is almost a 10 fold range of effectiveness of these compounds.

2. Anti-amino acids effective during the dark period. Of the compounds effective during the dark period, none seem to have any effect upon time measurement. Ethionine, which might have had such an effect was eliminated by the results shown in Fig. 5. Thus we might suspect all these compounds of influencing the synthesis of flowering hormone, especially if we can show that their inhibitory action takes place in the leaf. The anti-amino acids are especially interesting in this respect, since they may implicate the participation of protein synthesis as a part of flowering hormone syntheses. These compounds are at most only slightly effective when applied to the bud. Reversal of the inhibitions of ethionine, alpha-methyl methionine, and p-fluoro-phenylalanine by their corresponding amino acids does show that these amino acids do take part in the flowering process. Whether the effect is in the bud or leaf, or both, is being further investigated. Failure of a number of anti-amino acid compounds to influence flowering should probably also be considered here (Table 1). Do these failures mean that only certain amino acids take part in hormone synthesis? Is the flowering hormone a peptide requiring only a few amino acids? Or is the participation of certain amino acids in flowering hormone synthesis only indirect? The failure of chloramphenicol, a well-known inhibitor of protein synthesis, to inhibit flowering is not understood.

3. Benzimidazole and 2,6-diamino purine effective during induction. Since the inhibition caused by these compounds was restricted almost entirely to the dark period, it is suspected that they probably interfere with hormone synthesis. Reversal of their effect with uracil or orotic acid suggests an interference with pyrimidine rather than purine metabolism. Neither adenosine nor guanine overcame the effect of 2,6-diaminopurine.

Benzimadazole was not inhibitory when added to the bud alone, while diaminopurine was effective either on the leaf or the bud. Further experiments are required to definitely establish their sites of inhibition.

4. Miscellaneous compounds effective during induction. The compounds alpha-picolinic acid and quercetin caused clear cut inhibitions of flowering, and their time of effectiveness was restricted to the dark period. They might prove to be valuable tools for understanding the biochemistry of flowering hormone synthesis, but at present we are hindered by our lack of information about their biochemical modes of action. Alpha-picolinic acid may interfere with pyridine nucleotide reactions. The effects of quercetin in our plant system can hardly be surmised.

5. Pyrimidine antimetabolites effective during induction and translocation periods. The effect of these compounds seems to be localized in the leaf, and the times of their effectiveness suggest that they may interfere with hormone translocation and perhaps synthesis. The partial or complete success in reversing some of these compounds with uridine, orotic acid, or uracil argues for the participation of pyrimidine metabolism in these phases of the flowering process. The inability of orotic acid to overcome azauracil inhibition may be due to the fact that, at least in yeast and animal tumors, a primary mechanism of action was an inhibition of the enzyme orotidylic acid decarboxylase (HANDSCHUMACHER and WELCH 1960). Uridine, which partially reversed the flowering inhibition, is probably converted to uridylic acid directly by a kinase using adenosine triphosphate (LIEBERMAN, KORNBERG, and SIMMS 1955). In this case the inhibited orotidylate decarboxylase step would be bypassed.

Thiouracil has also been reported to inhibit flowering in *Streptocarpus wendlandii* (HESS 1959), *Cannabis sativa* (HESLOP-HARRISON 1960), and *Pharbitis nil* (MARUSHIGE and MARUSHIGE 1962). In none of these cases was the site of inhibition definitely established. In *Xanthium*, thiouracil was more inhibitory when applied to the leaf than to the bud, but in this case the plant actually received more chemical because the surface area of the leaf was greater than that of the bud. Unpublished experiments with C^{14}-labeled 2-thiouracil have shown that it moved from the leaf to the bud and also in the reverse direction. The same was true for 6-azauracil.

Thus, although thiouracil, azauracil, and BIMU all inhibited flowering at least partially because of their ability to interfere with nucleic acid synthesis, further studies are essential to determine the sites of action. The inability of 5-bromouracil and 6-azathymine, considered to be thymine antimetabolites (see HANDSCHUMACHER and WELCH 1960), to influence flowering is consistent with conclusions of BONNER and ZEEVAART (1962) that DNA multiplication is not essential for floral induction of Xanthium.

6. Thioproline effective during development of the flower. This compound appears to exert a rather non-specific effect, as evidenced by its inhibition even after the translocation period. The fact that it inhibited flowering even when added to the bud alone suggests that it may simply retard development of the floral bud. The failure of L-proline to reverse its action may indicate that it acts on a process other than protein synthesis.

7. Effects of the compounds upon vegetative growth. Results with the compounds discussed in this paper, as well as others (SALISBURY 1957), seem to imply that the nature of vegetative growth differs in some

fundamental way from that of reproductive growth. Marked effects upon vegetative growth were observed with no effect upon development of the floral bud. This was true for dihydrocholesterol, chloramphenicol, and D-alanine applied at any time and for many other compounds applied after their time of effectiveness in inhibition of flowering (e.g. all of the 11 compounds reported here except for benzimidazole, quercetin, and thioproline). Both azauracil and alpha-picolinate applied to the buds caused injury to the very young leaves but had almost no effect on flowering. In many instances, vegetative damage with no influence on flowering can be observed by reversing the floral inhibition of the antimetabolite by simultaneous application of the corresponding metabolite. In all of the cases reported in Table 3 except one, effects upon vegetative growth were not reversed by the metabolite. The exception was p-fluorophenylalanine, the vegetative effects of which are completely reversed by phenylalanine. Failure in most cases may be due to a rapid metabolic removal of the metabolite, while the antimetabolite remains in the cells long enough to cause vegetative injury after the high concentration of the metabolite has been reduced. At any rate, it seems clear that interference with the biochemical processes required for normal leaf growth may have little apparent effect upon the growth and development of the floral bud. However, thioproline will influence growth of this bud, as will 2,4-dichlorophenoxyacetic acid, 2,2-dichloropropionic acid, and maleic hydrazide (SALISBURY 1957).

8. The biochemistry of the flowering process. Final understanding of the biochemistry of floral induction will have to depend upon *in vitro* studies and perhaps extraction of the flowering hormone. The experiments reported in this paper, however, provide a few suggestions. Synthesis of flowering hormone is apparently dependent upon the presence of adenosine triphosphate. It may also be related in some way to the synthesis of proteins or peptides and to the synthesis of nucleic acids (Table 2). The flowering hormone is thought to be labile to applied auxins so long as it is in the leaf (SALISBURY 1961), and it also seems to be dependent upon ribonucleic acid metabolism during this period. Its slow rate of translocation has long been a problem. Is it translocated by being newly synthesized from cell to cell, by a sort of autocatalytic chain reaction, depending upon nucleic acid synthesis? Obviously there is much to learn about the biochemistry of flowering, but the experiments reported in this paper may give us some leads to follow.

Summary

1. Selected antimetabolites were applied at various times to *Xanthium pennsylvanicum* plants to determine some of the biochemical reactions

required for the flowering processes. Attempts were made to overcome the inhibitory effects of these antimetabolites by addition of certain metabolites.

2. Most compounds had no influence on flowering at the concentrations used. Inhibition was caused by ethionine, p-fluorophenylalanine, benzimidazole, 2,6-diaminopurine, alpha-picolinic acid, and quercetin only if they were applied during the inductive dark period. The effects caused by all compounds except picolinic acid and quercetin were reversed by certain amino acids or pryrimidines, suggesting that protein or nucleic acid synthesis is required during induction. Whether this synthesis occurs in the induced leaf or the receptive bud is not yet established.

3. Alpha-methyl methionine and the pyrimidine analogs 2-thiouracil, 6-azauracil, and 5-bromo-3-isopropyl-6-methyl uracil inhibited flowering when applied during the inductive period or during the time of hormone translocation. The effect of thiouracil was overcome by simultaneous addition of uracil or orotic acid, while uridine incompletely reversed the inhibitions of azauracil and 5-bromo-3-isopropyl-6-methyl uracil (BIMU). Alpha-methyl methionine inhibition was reversed by methionine. These compounds were more inhibitory when added only to the leaf, but the possibility that they block necessary reactions occurring in the bud has not been excluded.

4. Only ethionine and alpha-picolinic acid increased the length of the critical night. This effect did not occur as a result of an inhibition of timing reactions.

5. Of the antimetabolites studied, only thioproline retarded flowering even after induction and hormone translocation processes were completed. L-Proline did not reverse this effect. It is believed that thioproline effects were due to a rather nonspecific influence on development of the flower buds.

6. The vegetative injury caused by most of the compounds and their inhibitions of flowering were usually clearly separated in time. These and other results discussed suggest that different biochemical reactions occur during flower bud development than occur during normal vegetative development.

Zusammenfassung

1. *Xanthium pennsylvanicum* wurde zu unterschiedlichen Zeiten mit verschiedenen Stoffwechselgiften behandelt, um einige für den Blühvorgang erforderliche biochemische Reaktionen zu ermitteln. Es wurde versucht, die Hemmwirkung dieser Stoffwechselgifte durch zugesetzte Stoffwechselprodukte zu beseitigen.

2. Die meisten Verbindungen hatten bei den benutzten Konzentrationen keinen Einfluß auf das Blühen. Hemmung wurde nur durch Äthionin, p-Fluorophenyl-

alanin, Benzimidazol, 2.6-Diaminopurin, α-Picolinsäure und Quercetin verursacht, sofern diese Stoffe während der induktiven Dunkelperiode geboten wurden. Die Wirkungen aller Verbindungen mit Ausnahme von Picolinsäure und Quercetin konnten durch bestimmte Aminosäuren oder Pyrimidine aufgehoben werden. Daher liegt die Vermutung nahe, daß während der Induktion Eiweiß- oder Nucleinsäure-Synthese nötig ist. Ob diese Synthese im induzierten Blatt oder in der reagierenden Knospe stattfindet, läßt sich noch nicht klären.

3. α-Methyl-Methionin und die Pyrimidin-Analoge 2-Thiouracil, 6-Azauracil und 5-Bromo-3-isopropyl-6-methyl-Uracil verhinderten die Blütenbildung, wenn sie während der induktiven Periode oder während der Zeit der Hormon-Wanderung geboten wurden. Die Wirkung von Thiouracil wurde durch Zugabe von Uracil oder Orotsäure beseitigt, während Uridin die Hemmung von Azauracil und 5-Bromo-3-isopropyl-6-methyl-Uracil (BIMu) unvollständig aufhob. Hemmung durch α-Methyl-Methionin konnte durch Methionin behoben werden. Diese Verbindungen hemmten am meisten, wenn sie nur dem Blatt zugegeben wurden. Es kann aber nicht ausgeschlossen werden, daß sie notwendige Reaktionen in der Knospe hemmen.

4. Nur Äthionin und α-Picolinsäure verlängerten die kritische Dunkelperiode. Dieser Effekt ist offenbar nicht das Ergebnis einer Hemmung der Zeitmeßreaktion.

5. Von den untersuchten Stoffwechselgiften verzögerte nur Thioprolin das Blühen, auch noch nachdem die Induktion und die Hormonwanderung vollzogen waren. L-Prolin konnte diesen Effekt nicht beheben. Es wird angenommen, daß die Thioprolinwirkung auf einer ziemlich unspezifischen Beeinflussung der Blütenknospenentwicklung beruht.

6. Die durch die meisten Verbindungen bedingten vegetativen Schädigungen und die Blühhemmungen waren im allgemeinen deutlich voneinander getrennt. Diese und andere diskutierte Ergebnisse legen die Vermutung nahe, daß während der Blütenknospen-Entwicklung andere biochemische Reaktionen ablaufen als während der normalen vegetativen Entwicklung.

The authors appreciate the capable technical assistance of Mr. Kelsey Day, Mrs. Annette Hullinger, Mrs. Marsha Scoles, and Mrs. Janet Thomas.

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Prof. Dr. FRANK B. SALISBURY,
Colorado State University, Fort Collins, Colorado, USA