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Xanthium strumarium L.: Extraction and Assay of Floral Promotive Principles and Additional Investigations into Inhibition of Flowering

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XANTHIUM STRUMARIUM L.: EXTRACTION AND ASSAY OF

FLORAL PROMOTIVE PRINCIPLES AND ADDITIONAL

INVESTIGATIONS INTO INHIBITION

OF FLOWERING

by

David D. Gibby

A dissertation submitted in partial fulfillment of
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

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Approved:

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Major Professor   Committee Member

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UTAH STATE UNIVERSITY
Logan, Utah

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ABSTRACT

Xanthium strumarium L.: Extraction and Assay of

Floral Promotive Principles and Additional

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Of Flowering

by

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Utah State University, 1972

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New techniques in extraction and assay of florigenic substances
were tested. Initial response to the buffer-PVP extracts proved
promising, but after several successful experiments, results could
not be duplicated. Possible reasons for the failure are discussed.

A long-day inhibitory effect, demonstrated earlier by Gibby and
Salisbury (25), was further investigated. Experiments with ^{14}C-labled
assimilates disproved the hypothesis that the inhibition is a result of
assimilate translocation. Other experiments pointed to the probability
of phytochrome involvement as well as protein synthesis as part of the
inhibitory process. The nature of inhibition was ascribed to a
localized inhibitory condition, probably a relatively immobile substance.

(57 pages)

INTRODUCTION

The study of the physiology of flowering can be most fascinating
and enlightening--but at the same time it can be most puzzling. Often
experimental results appear straight-forward on the surface, but upon
close examination and comparison with other related experiments it
becomes apparent that several explanations of the data are possible.
As a result, many authorities disagree in the interpretation of much of
the mountain of data accumulated during the past fifty years since the
photoperiodic control of flowering was demonstrated. Even though most
study into the physiology of flowering has been concentrated on about
20 species, much experimentation using many other species has been done.
Part of the difficulty in interpretation has come about in an attempt
to apply the results of experiments with one species to another or to
the flowering process as a whole.

Another handicap of flowering research and interpretation is that
flowering itself is at present the only measure of the effect of a given
treatment. The separation of the many factors and requisites of flowering
is often difficult or impossible. Several examples of this will be
apparent in the discussions to follow.

Flowering in response to photoperiod is a result of at least two
major processes: the events taking place in the leaf during an inductive
photoperiod (induction), and those occurring at the stem apex that
cause the morphological change committing the bud to a state of flowering
(evocation) (20). Most research has concentrated on the former processes
rather than the latter. It is sometimes difficult to say with certainty

that a given treatment's effect is restricted to one process or the other, even though they occur in different locations and are separated in time. The flowering that is actually observed is a function of both processes.

Light quantity (intensity times duration), light quality (color), light duration (often independent of intensity), temperature, nutrition, and plant or leaf age exert major influences on a plant's flowering (66). These are also complicated by interactions and timing effects.

Despite these difficulties and the differences of opinion among flowering physiologists, much is known and understood about the flowering process. Portions of the present knowledge have been extremely valuable to horticulturists and have enabled them to control the flowering of many plants by environmental control, breeding, and in some cases, the application of plant-growth regulators. Still, a complete understanding of the flowering process awaits resolution. This understanding could easily revolutionize modern agriculture.

This review is by no means comprehensive. Considerable selection of literature for review was done. For a comprehensive review of flowering literature, the reader is referred to reviews by Lang (46), Salisbury (67), Carr (6), Chailakhyan (10), Searle (72), and Zeevaart (86), and to books by Salisbury (66), Hillman (37), and a treatise edited by Evans (20).

The purpose of this study was to continue investigations with photoperiodic inhibition of flowering in cocklebur and to investigate methods of extraction and assay of floral promotive principles.

CHAPTER I

REVIEW OF LITERATURE

Hormonal Control of Flowering

In 1953, A. W. Naylor said, "After thirty years of effort to determine the nature of the photoperiodic stimulus, we still do not understand it. This is a vexing situation and a continuing challenge" (58, p. 157). Now nearly twenty years later the same statement can be repeated. Early experimenters noted that photoperiod often regulated tuberization (61). This suggested that the above-ground parts perceived the day-length stimulus and that a chemical signal evoked the change below the ground. Experiments were subsequently designed to determine if the levels responded to photoperiod in flowering. Various parts of a plant were covered for a portion of the day, providing short-day conditions, while the remainder of the plant was maintained under long-day conditions. It was found that covering the leaves of short-day plants results in flowering; of long-day plants, inhibition of flowering. (Covering the rest of a long-day plant, leafing the leaf exposed to long-day conditions, results in flowering.) This experiment was first per-formed by Knott (42) and subsequently by others (4, 7, 32, 33). As a result of these and other experiments, Chailakhyan (7) in 1937 advocated the existance of a floral hormone and named it florigen.

A second evidence for a flowering hormone was equally impressive. A plant under non-inductive conditions (receptor plant) could be made to flower when grafted to a plant that had previously been induced to flower (donor plant). This was first done by Kuijper and Wiersum (44)

in Holland and by Chailakhyan (12) in Russia. It was even possible to graft plants of different response types together. Chailakyan (7) induced flowering in the short-day plant, _Helianthus tuberosus_, by grafting to _H_. annus. Lang (46) provides a table of 44 grafting experiments among several plant species between 1936 and 1962. Experiments of this type are still being done. In 1970, Wellensick (83) reported that a leaf from a flowering _Xanthium_ plant can cause flowering in a _Silene ameria_ receptor and that the _S. ameria_ receptor can, after a period of time, act as a donor to other _S. ameria_ receptors. It is also interesting that the grafts of _Xanthium_ to _Silene_ form no vascular connections.

In the early 1950's three groups of investigators (41, 64, 75) independently developed a technique for studying movement of the flowering stimulus, or at least its slowest-moving component, from an induced _Xanthium_ leaf. Plants were defoliated at various times following a single inductive dark period, and the level of flowering was subsequently measured. This was then a third strong evidence for the florigen concept. The argument was that a substance must be present if its rate of translocation could be measured. This type of experiment can only be performed suitably with plants that are induced to flower with a single inductive dark period, in the case of short-day plants, or a single inductive light period in the case of long-day plants. The rate of translocation of the promotive factor, or its slowest moving component, is much slower than that of the assimilate stream in which it is thought to move and is affected by light and temperature (68).

A fourth evidence in favor of the florigen concept is that of florigenic extracts. Since Chailakhyan (7) proposed the name florigen, many workers have attempted to extract and isolate it from induced or flowering plants. Until recently, all attempts were unsuccessful or could not be duplicated. Roberts (62, 63) has reported successful extraction methods, but his work has not been confirmed by other laboratories, and he finally claimed only that his substances would increase flowering once it had been photoperiodically induced. In 1961, Lincoln et al. (49) produced an extract from lyophilized tissue of flowering Xanthium plants that evoked development of floral buds on test plants maintained under long-day conditions. Although these buds developed only to an early floral stage, the results were clear-cut and reproducible. After some purification, it was reported that the active principle possessed the partitioning properties of carboxylic acid (48, 55). Lincoln (48) and his co-workers suggested the name florigenic acid for the active principle.

Carr (6) reviewed floral promotive extracts confirming the work of Lincoln's group and reported the ability of GA_3 to increase the flowering response when added to the extract. Biswas, Paul, and Henderson (2) have examined sterol components in extracts from flowering Xanthium plants. Some fractions are reported to have evoked floral buds in Xanthium and chrysanthemum. Hodson and Hamner (38) recently substantiated the ability of gibberillic acid to increase flowering when combined with extracts from induced Xanthium plants. In addition to using vegetative cocklebur plants to test their acetone extracts, Lemna (duckweed) plants were floated on a solution containing the extract. Although the duckweed plants flowered in response to the

extract from induced plants and not to a comparable extract from vegetative plants, we should note that duckweed is affected in its flowering by a number of non-specific materials including ascorbic acid and copper ions (38, 40, 59).

The concept of florigen as it was originally developed specifies one substance that specifically evokes flowering, that is made in the leaves and that is common to all higher plants. The above-mentioned evidence in favor of this concept has seemed so strong that we can read, "The existance of florigen is so obvious from physiological experiments that its isolation and identification seem long overdue." (See 20, p. 458)

There are, however, strong arguments for proceeding beyond the simplicity of the florigen concept. These are summarized by Evans (20) and given here in brief form.

No specific organ-forming inducer has yet been identified in plants, but the known plant hormones have a broad spectrum of action. They also interact, and a balance of plant hormones controls many responses such as shoot formation, apical dominance, differentiation, abscission, etc. (84, 35, 23, 15).

Daylength controls or influences many plant responses in addition to flowering, including germination, tuberization, induction and breaking of dormancy, abscission, etc. (20). The formation of subterranean tubers in response to daylength must, of course, also involve translocatable substances. Lettuce seeds implanted in the petioles of Xanthium and covered with aluminum foil germinated to a far greater degree when the plants were under short-day conditions than when they were under long days (56). Could each of these processes be controlled

by the same substance, or is the plant generating a specific hormone for each process in response to daylength? The best possibility is that several substances interact to control these processes.

There is evidence, even with plants requiring only one inductive cycle, that the evocation and development of flower buds is quantitative as well as qualitative in nature. Buds in cocklebur will develop faster given a 16-hour dark period than if given 10- to 12-hour dark periods, and additional inductive cycles cause an increase in the rate of development of floral buds. Evans (20) summarizes the effects of environment and growth regulators on plants that are sensitive to photoperiod. He concludes that the evocation of flowering can be caused, in a great many cases, by alternative pathways such as low or high temperatures or the application of growth regulators. Evans lists fifteen compounds that have been found to evoke flowering in various short-day plants and six compounds found to evoke flowering in various long-day plants. The fact that many different species and many different response types can be affected as mentioned above leads one to question the concept of a single specific flowering hormone present in all higher plants.

The above-mentioned arguments may partially account for the difficulty encountered in attempts to extract and isolate florigen. The successful extracts have all been quite crude. Perhaps the reason that further separation of components of the crude extract has resulted in loss of the extract's ability to promote flowering is that there is more than one active component. Two experiments can be interpreted in this manner. Salisbury and Bonner (69) found that 5-fluorouracil was inhibitory to flowering when applied to the bud before or during the inductive dark period, but not after. They felt that it inhibited some

preparatory reaction that takes place in the bud and occurs during the inductive dark period, or that there is more than one product of induction, and some fast-moving product of floral induction is inhibited at the stem apex. Biswas et al. (2) reports the inability of a crude chloroform extract of flowering chrysanthemum plants to cause flowering in Xanthium and Chrysanthemum. Three of eight fractions of the crude extract, however, caused a little flowering in Xanthium and two of eight in Chrysanthemum.

Excised buds of Perilla on White's medium respond like intact plants to photoperiod if small leaves are present (60). If these leaves are absent, rudimentary floral stages develop regardless of photoperiod, so Perilla apparently has the inherent capacity to flower. This suggests that a balance between promotive and inhibitory substances controls its flowering. Long-day leaves are particularly inhibitory when situated between short-day leaves and the bud in intact Perilla plants (87). Chailakhyan and Butenko (11) fed $^{14}CO_2$ to various single leaves on long or short days. Then after 24 hours they determined the pattern by labeled assimilates by autoradiography of the entire plant. They then correlated the translocation patterns with promotion and inhibition. Excellent correlation existed. Zeevaart (87) interprets these results to mean that:

> ... non-induced leaves do not produce specific flower-inhibiting substances, but whenever such leaves happen to be in close proximity to receptor buds, they are the chief suppliers of organic substances. Thus, they prevent the products of the short-day leaves from reaching shoots in significant amounts. (Zeevaart, 87, p. 45)

This statement can be questioned on two points. First, Perilla requires from 9 to 14 short days for complete induction (87). It may, therefore, be erroneous to assume that the translocation pattern of each inductive

cycle is the same. Some evidence suggests that the reactions of various cycles are different (17, 81). Second, even assuming that the products of the leaves of each inductive night do translocate in the same way and that long-day leaves situated between short-day leaves and the bud do prevent the organic substances of short-day leaves from reaching the bud, the existance of inhibitory substances is not excluded. Why is it not possible for both to occur? The work with excised buds mentioned above points to this likelihood.

Phaseolus vulgaris has a system that is suggestive of a balance between promotive and inhibitory factors in its flowering mechanism. Long-day leaves are inhibitory regardless of position, and short-day leaves are promotive regardless of position. Evocation of floral buds is independent of daylength, but floral buds abscise under long days and develop under short days (88).

Photoperiodic Inhibition

Many other experiments suggest a balance between promoter(s) and inhibitor(s) in flowering, or at least the participation of inhibitor(s) in flowering. Early experiments by Hamner and Bonner (32) on Xanthium plants with two branches were suggestive of an inhibitory action by long days. One branch, the donor, was maintained on short days while the other, the receptor, was maintained on long days. Mature leaf tissue on the receptor branch was very inhibitory to flowering. Flowering could be prevented if as much as half a mature leaf were present on the receptor. Because this inhibition is very weak except when the long-day tissue is situated between the short-day tissue and the bud, it has been assumed that the direction and nature of assimilate flow accounted for

this inhibition. This is essentially the same explanation of long-day
inhibition given for <u>Perilla</u> by Zeevaart (87) and mentioned above.
Lincoln et al. (50), using the same donor and receptor system as Hamner
and Bonner (32), demonstrated that the inhibition itself exhibited a
photoperiodic behavior. A critical day (of different length from that
of the promoter) was measurable for inhibition. Light breaks on the
receptor branch could also cause active inhibition and immature leaves
could also. These experiments are difficult to explain with an assimi-
late flow hypothesis. Gibby and Salisbury (25) defined a long-day
inhibitory effect on <u>Xanthium</u> flowering. In the basic experiments, the
basal half of a single leaf inhibits response of the tip half to short
day; and a long-day leaf inhibits response of a short-day leaf, providing
it is between the short-day leaf and a receptive bud. Chailakhyan (8)
earlier had reported similar work with <u>Perilla,</u> and Harder et al. (34)
obtained similar results with <u>Kalanchöe</u>. In addition, they showed that
flowering resulted when the apical half of a single leaf was induced,
providing the basal leaf tissue was trimmed off. Gibby and Salisbury
(25) explored the following five hypotheses to account for this
inhibition:

1. The tip half of the leaf is not capable of induction.

2. Long days produce an inhibitor that subsequently inhibits
   production of promoter on short days. (Such inhibition has
   been demonstrated by Schwabe and others; see below.)

3. The apparent inhibitory effects depend on florigen moving
   only with the assimilate stream. For example, long-day
   tissue may be acting as an assimilate source (photo-
   synthesizing) in such a manner that assimilate from the

short-day tissue cannot reach the bud (as argued by Zeevaart).
Alternatively, under some conditions (low light intensity),
the long-day tissue may be acting as a sink for the assimilate
produced by the short-day tissue

4. There is a translocatable inhibitor produced on long days.

5. A substance or condition inhibitory to flowering is produced
    on long days, and its effect is localized near the tissues in
    which it originates.

Tip tissue could produce promoter when basal tissue was removed,
eliminating number one. Long-day inhibition occurred following short-
day induction eliminating number two. Translocational effects seemed
unlikely for various reasons, and inhibition was found to be localized
and may be a condition or a relatively immobile substance. Studies of
critical dark period, light intensity, and interruption of a dark
period showed that when the leaf is not actively producing promotive
substances it is actively inhibitory. It was also found that iron
deficient tissue could cause long-day inhibition, though it could not
cause promotion.

Experiments by Evans (16, 18) are also not to be explained on the
basis of assimilate flow. In his experiments with Lolium, a long-day
plant, short-day leaves were inhibitory regardless of position. He
demonstrated the ability of Lolium to produce a floral stimulus in an
anaerobic nitrogen atmosphere, but the inhibitor is not produced in
such an atmosphere. I attempted this with cocklebur but failed to
observe the same effect (24). Evans (21) compared translocation of
assimilates in relation to that of inhibition and promotion and
concluded that inhibition could not be explained on that basis. In

another series of experiments, Evans (19) found that an endogenous rise

of abscisic acid occurred in <u>Lolium</u> under short days, and that abscisic

acid could inhibit floral evocation in <u>Lolium</u>. He suggested that the

short-day produced inhibitor in <u>Lolium</u> may be abscisic acid (then called

abscisin II).

Using seedings of <u>Pharbitis</u>, Imamura (39) showed that a dark treat-

ment given to one cotyledon is less effective when the other cotyledon

is in the light than when it is removed. The inhibiting effect is not

reduced if the illuminated cotyledon is removed at the end of the 16-

hour dark period. These results are difficult to understand except by

the production of a translocatable inhibitor of flowering.

Strong evidence for the production of inhibitors by plants under

non-inductive conditions is obtained by experiments such as those of

Schwabe (70, 71). He interspersed various numbers of long days among

inducing short days, first with <u>Kalanchöe</u> and later with several other

short-day plants that require several inductive cycles to flower. He

concluded that one long day nullified the effects of the following 1.5

to 2.2 short days, depending upon the species concerned. Schwabe

deduced from these results that an inhibitor is produced on long days

that acts on the subsequent inductive processes brought about by the

following short days. Long (54) had earlier found that alternating

short days with long days did not induce flowering in Biloxi soybeans

regardless of the number of short days given.

Sirohi and Hamner (74) extended the findings of Schwabe and Long

in Biloxi soybean. They found that long days interspersed between

short days began to be inhibitory when they exceeded 12 1/2 hours in

length and were completely inhibitory when longer than 14 hours, the

critical day-length. Coulter and Hamner (13) performed some rather complex experiments with soybean. Their conclusion supports those of Schwabe. They conclude that one long day seems to inhibit twice as much as a short day promotes. Hamner stated in a recent interview:

> All of the recent work with Biloxi soybean in the reviewer's laboratory has indicated that long days fail to induce flowering because of an active inhibitory effect and that this inhibition is produced by exposing the plants to light during unfavorable phases of an endogenous circadian rhythm. (Hamner, 31, p. 79)

Inhibition is not always the result of intercalated long days on short-day induction. Toky and Nanda (77) observed a promotive effect in Impatiens balsamina.

Chenopodium rubrum requires three inductive dark periods to flower. Kredule (43) found that Actinomycin D was inhibitory when applied during the first two inductive cycles, but it was promotive when applied during the third. It could even eliminate the need for a third cycle. One can speculate that this is due to the action of Actinomycin D on the synthesis of inhibitory substances.

Silene ameria L., a qualitative long-day plant, has an interesting type of inhibition. Plants can be induced to plower at 20°C on long days. If the temperature during the dark period is above 32°C the plants can be made to flower on short days (51, 82). This work was recently extended by Van de Vooren (78). He found that high temperatures given in the middle of the dark period would cause short-day induction. Van de Vooren suggests the following mechanism is Silene flowering:

1. A deblocking (anti-inhibitory) process in the light; temperature insensitive.

2. A blocking (inhibitory) process in darkness starting shortly after the onset of darkness, reaching a maximum after 6-7

hours and decreasing afterwards; temperature sensitive high temperature slowing it down.

In strawberry it is difficult to explain floral induction in any other way than the removal of an inhibitory substance. This is to say that plants always tend to flower, but evocation is apparently prevented due to production of inhibitory factors (30). Long photoperiods or a light break of several hours duration during the dark period promotes vegetative growth and inhibits formation of flowers (27, 28, 29). This stimulation of vegetative growth can pass between donor-receptor units composed of two adjacent runner plants of a runner chain. Inhibition of one plant under inductive conditions was possible by light breaks on the other plant or by keeping the other plant on long days. Tracer experiments showed that assimilates could move in either direction by controlling factors of assimilate supply and demand. In another experiment (28), the flowering of the mother plants under short days was advanced by partial defoliation of daughter plants under long days. Flowering was progressively advanced by the increasing severity of the defoliation. Many strawberry cultivars flower as a result of defoliation (76). This suggests that the removal of long-day leaves results in the removal of the source of long-day inhibitor.

Other plants have also flowered in response to defoliation, including the long-day plant, Hysocyamus niger, which is promoted by defoliation under short-day conditions (47); Chenopodium, a short-day plant, which flowers under long days if supplied with sugar; tomato, which will flower under non-inductive conditions if they are partially or completely defoliated after emerging from the seed with their cotyledons intact (14); Perilla (80); and Chrysanthemum (79).

Fratianne (22) allowed dodder to become attached to various long and short-day plants. He found that the dodder would flower when the host plants flowered or when the host plants were defoliated, but not when the host plants were left intact under non-inductive conditions. He also used dodder as a bridge between plants under inductive and non-inductive conditions. The induced plants were delayed in their flowering due to the connection to non-inducted plants. This would be easiest to explain on the basis of a balance between inhibitory and promotive factors.

Amos and Crowden (1) cite evidence of an inhibitor produced in the cotyledons of greenfeast peas. They demonstrate that vernalization nullifies this inhibitor and produces the same promotion as that caused by removal of the cotyledons.

Conclusion

Of the several conclusions that could be drawn from the foregoing review, a few seem paramount. Several substances probably participate in induction and evocation of flowering. This statement is not new and has been suggested by several workers, including Evans (20), Salisbury (67), Carr (6) and others. Carr states:

> When one considers the wide variety of substances which can in one plant or another induce flowering, it is surprising that we have clung for so long to the hypothesis that there is a unique substance responsible for initiation of flowering; this substance is made in leaves; and it is identical in all angiosperms ... .
> (Carr, 6, p. 311)

There is undoubtedly more than one endogenous promotive substance as well as more than one endogenous inhibitory substance. The several different types of inhibitory and promotive responses suggest this.

Inhibition, for example, is translocated in Lolium (16, 18, 19), straw-
berry (27, 28, 29), Pharbitis (39), and in Fratianne's experiments with
dodder (22), to name a few. In these cases a balance between inhibitory
or promotive substances at the stem apex could possibly control flower
evocation. Schwabe's (70, 71) experiments on the inhibitory action of
long days interspersed with short days showed an inhibitory action on
subsequent floral induction. Experiments such as those of Gibby and
Salisbury (25) are probably best understood by assuming that the pro-
moter(s) is intercepted on the way to the receptor bud.

Although there are obviously important differences in the mechan-
isms by which various plant species flower, there is most likely some
overlap, that is to say some common features that are shared by many or
perhaps most species. This is indicated by many grafting experiments
in which induced plants of one response type or species evoke flowering
in another response type or species under non-inductive conditions (46,
85). This would support a new hypothesis of Chailakhyan (10) that the
primary photoperiodic stimulus may consist of two complementary sub-
stances. One of these, thought to be gibberellin(s), is limiting under
short-day conditions, and thus limiting in long-day plants; the other,
called anthesin(s), is limiting under long-day conditions and thus
limiting in short-day plants. The two in proper balance is necessary
for floral evocation.

Until the biochemical mechanisms of flowering have been worked out,
flowering physiologists will be at a loss to form a completely coherent
hypothesis that will take into account all the complexities in the pro-
cesses of flower induction and evocation. Experiments such as those of
Bronchart et al. (5), Loewenbert (52), and Sherwood, Evans, and Ross (73),

where the nature of RNA and protein synthesis during induction and evocation are studied may contribute to this undertaking.

CHAPTER II

EXTRACTION AND ASSAY OF FLORAL PROMOTIVE PRINCIPLES

Introduction

From the foregoing discussion on the hormonal control of flowering,
several things are evident that have a bearing on extraction and assay
of floral promotive principles: First, promotive principles are synthe-
sized in the leaves of induced plants. Second, there is some evidence
that possibly more than one promotive factor is involved. Third, under
non-inductive conditions, factors inhibitory to flowering are present in
most plants.

In the ten years since the first successful and reproducible
extracts of promotive principles of flowering were reported in 1961 (49),
little progress has been made in finding the identity of these prin-
ciples. There is little doubt of their existence in the extracts,
since several laboratories have confirmed the floral evoking ability of
the crude extracts (6, 55). In personal communication with Hodson and
Hamner (38), who published the last paper dealing with florigen extracts,
it was mentioned that experiments with extracts often failed for no
apparent reason, and that work with florigenic extracts is generally
difficult. Several possible reasons for this are:

1. There is no good clue as to the kind(s) of compound(s) being
    sought.

2. The promotive factor(s) may be unstable outside the cell.
    Also, with the breaking of cell and organello membranes, other
    compounds (e.g., digestive enzymes, phenolics, etc.) may act on
    the promotive principle(s).

3. There is, as has been mentioned, some evidence that two or more compounds are involved. Extraction may result either in a loss of one necessary component or in a change in concentration of one or more components in relation to the others. A certain balance might be necessary.

4. The bioassay of promotive factors is difficult. The only measure as to the promotive ability of an extract is to apply it in some way to vegetative plants and measure direct promotion. Several problems are encountered at this point. First, as has been discussed, evidence points to the existence of inhibitors of flowering being present in vegetative plants. Promotive principles in the extract could be destroyed upon entering the assay plants. There are two possible ways of overcoming this inhibitory effect: overload the system with greater than normal amounts of promotive principles, or block inhibition by creating a neutral state in some way. This might be done chemically by finding some specific metabolic inhibitor that would block inhibition while not influencing evocation at the bud. Another method might be to induce the plants at a threshold level or by using iron deficient plants under short days. Iron deficient plants can produce the inhibitory effect but not the promotive principle(s) in Xanthium (25). A final method might be to apply the extract between the long-day tissue and the receptive bud.

Second, it is often difficult to get the extracted material back into the vegetative plants. Hodson (personal communication) stated that he places the vegetative plants under high intensity

lights to create water stress conditions and to condition the
plants to high water use. He states that this stress is neces-
sary for good uptake of the extract, which is applied in 10 ml
vials via a stem flap. This action, however, would create a
condition of maximum inhibition in the vegetative plants.

Third, timing may be important in the application of the
extracted materials. Chemicals applied to plants to determine
their effect upon the flowering process show vastly different
effects depending upon the phase of the plants in relation to
their flowering rhythm at the time of application (13, 64, 66).

Fourth, one has little idea of the concentration of the
promotive principle(s). This could easily be important.

In view of the above-mentioned difficulties it is not hard to under-
stand the slow progress that has been achieved in isolating floral promo-
tive principles. The results presented in this section are preliminary
and reflect these difficulties.

Materials and Methods

Salisbury's (66) methods of handling and evaluation of floral
response were used and will be discussed in the section on additional
investigations into long-day inhibitions of flowering in Xanthium.

In May of 1970 the author's major professor, Frank B. Salisbury,
visited Hamner and Hodson's laboratory to learn their extraction and
assay techniques with Xanthium. Upon his return the extraction methods
of Hodson and Hamner (38) were tried. Briefly, their method is as
follows: Five hundred plants are induced with three weeks of short-
day cycles. The youngest four leaves plus the stem tip are harvested

and frozen in liquid nitrogen. The frozen material is subsequently pulverized and introduced into a large, one-gallon Waring blender along with absolute acetone at a minus 10°C. The material is then blended and filtered using a Büchner funnel and vacuum. The residue is extracted a second and third time in 70 percent acetone, and filtered. The filtrates are combined and then stored 12 to 20 hours at a minus 10°C. The filtrate is then diluted with 0.1 N NH_4OH (3 parts filtrate, 1 part 0.1 N NH_4OH) and flash evaporated at 30°C in several steps. The result is a brown-colored tar-like substance that contains the active principle(s). This is redissolved in water (0.15 mg/100 ml water). The pH is adjusted to 6.5 to 7.5 with 0.1 M HCl, and 10 ml are introduced to vegetative plants in small vials using a stem flap located below the leaves. The assay plants are harvested, and the degree of flowering is determined after three weeks. In Experiment 4 an attempt was made to reduce possible inhibition of leaves situated between the extract and the receptive bud. The petioles of some of the leaves were burned with a small jet of flame. Leaves so treated remain healthy and turgid for some time and are not inhibitory (25).

An alternate extraction procedure was also tried by the author. Extracted plants were either induced plants, usually given three weeks of short-day treatment, or vegetative plants kept vegetative by extending the day length with florescent lamps. Plants were removed from their last inductive night (in one experiment only inductive night was used), and as rapidly as possible their youngest four leaves and apical bud were cut off and frozen in liquid nitrogen. The reason for speed at this point is understood from the results of Experiment 5. Generally, 40 plants were extracted each time. The frozen tissue was ground to a

fine powder using a 750 ml mortar and pestle. Care was taken to prevent any thaw during grinding. A slurry of Tris buffer pH 8.0 and high molecular weight insoluble polyvinylpyrrolidone (Polyclar AT) or PVP, 120 g per liter, was slowly added to the frozen powder. Polyvinylpyrrolidone was added to the buffer to precipitate plant phelols and thus prevent their interacting with the active principle(s). The slurry was forcibly strained through cheesecloth, and the filtrate was centrifuged at approximately 20,000 xg for 15 minutes. The supernatant was frozen or applied to assay plants immediately. In some cases the supernatant was dialyzed against distilled water for 8 hours before being applied to the assay plants.

The assay plants were vegetative plants under long-day conditions as described above. They were generally trimmed to two mature leaves, the oldest leaves that had not begun their senescence. The stem flap was cut above these leaves (between the leaves and the receptive bud). Two ml of the extract were placed in a 2 1/2 ml vial, and the vial was secured on the stem with the stem flap in the vial. The stem and vial were wrapped with parafilm and sealed to prevent evaporation and drying of stem tissues. Floral stage was determined after three weeks. The amount of extract taken up by the plant varied, but was usually about 80 to 100 percent. Absorption time was about 24 hours or less. Any extract remaining in the vial after 24 hours generally remained in the vial until the end of the experiment.

Results and Discussion

The Hodson extraction procedure was tried on four occasions. The first was on July 21, 1970. In addition to the procedure outlined in

Materials and Methods, one treatment consisted of the extract from flowering plants plus 0.02 mg GA_3 per plant. None of the plants flowered in this experiment.

The next extraction was made on August 15, 1970 (Experiment 2-2). The assay differed in that the extract was diluted to a lesser degree, 0.3 mg extract/7 ml water and only 0.7 ml of diluted extract was applied to the assay plants. This is the same amount of crude extract per plant as the Hodson procedure. The extract was applied using small vials attached between the leaves and the bud. Half of the extracts contained GA_3 at 0.02 mg per plant. Fifteen assay plants were used per treatment. The results are given in Table 1, which indicates some flowering from one extract. This experiment was duplicated exactly with an extraction made on September 10, 1970. All plants were vegetative.

Induced plants for Experiment 2-4 were extracted on November 5, 1970. In this case, 120 g PVP was added to the tissue before it was blended. Otherwise the extraction procedure was the same as the Hodson procedure as outlined in Materials and Methods. The results of the assay, utilizing plants with burned petioles (see Materials and Methods) are given in Table 2. Again a low level of flowering is evident.

On December 10, 1970, plants were extracted after only one short-day treatment. In this experiment (Experiment 2-5) half of the induced plants were extracted immediately following the inductive dark period and half were extracted after four hours of exposure and sunlight. In each case half of each extract from induced plants was dialyzed. The buffer extraction technique was used. One notices from the results given in Table 3 that dialysis has no effect on activity, but that four

	Av. floral stage	% flowering
Extract A from vegetative plants	0	0
Extract A + GA$_3$	0	0
Extract B from flowering plants	1.53	29
Extract B + GA$_3$	0.71	28

Table 1. Ability of acetone extracts to cause flowering in vegetative plants. Extract applied between leaves and receptive bud on assay plants.

Treatment	% flowering	Av. floral stage
1. Petioles intact.	10	0.1
2. Petioles intact. GA_3 added to extract.	60	1.1
3. Section of petioles killed with a small jet of flame.	10	0.3
4. Section of petioles killed with a small jet of flame. GA_3 added to extract.	10	0.2
5. Untreated Controls	0	0.0

Table 2. Ability of PVP-acetone extracts to cause flowering in vegetative plants. Extract applied below the leaves.

Treatment	% flowering	Av. floral Stage
1. Plants extracted immediately following 16-hr. dark period.	40	2.0
2. Same as treatment 1, but extract dialyzed.	40	2.2
3. Plants extracted 4 hrs. after 16-hr. dark period.	0	0.0
4. Same as treatment 3, but extract dialyzed.	10	0.3
5. Vegetative control	0	0.0

Table 3. Ability of Tris buffer - PVP extracts to cause flowering in vegetative plants.

hours of sunlight rendered the extract from induced plants inactive. It is also important that a buffer extract successfully caused flowering in vegetative plants under non-inductive conditions. After the first success with a buffer extraction, attempts were made to change the techniques to try to better the level of flowering. Extracts were made at pH 7.0, 6.0, and 3.0 using a phosphate buffer. In addition, the extraction procedure was altered in some experiments so that the frozen and ground plant material was slowly added to the buffer PVP slurry rather than the reverse. The results of all these attempts were inconclusive. All changes in the original buffer extract resulted in extracts that failed to induce flowering. These attempts were all made during the spring between March and May in 1971. Two attempts were made in May 1971 and another on August 12 to repeat the original buffer extraction procedure, but they were also completely unsuccessful. In the experiment on August 12, half of the extract was separated and its volume was reduced by one-half with Lyphogel, which imbibes water and small molecular weight molecules. This was an attempt to concentrate the extract and to determine if the removal of low molecular weight substances would render the extract inactive. None of the plants flowered, however, so no conclusions could be drawn.

The reason for the many failures to reproduce the earlier successful experiments is not apparent, but two differences existed between the experiments. First, the plants used in the first series were much older (approximately 12-14 weeks old) than those used in the last attempts, which were 8 weeks old. Second, the later attempts were made in the spring and summer, while the first series was conducted (with one exception) during the winter. It has been noticed by the author

in earlier work that the inhibitory response to long days is weaker in older plants. Long-day tissue between short-day tissue and the receptor bud is usually completely inhibitory in young plants, preventing any flower evocation, but in older plants the inhibitory response, though still present, often does not result in complete supression of the promotive stimulus. It is also observed that cockleburs exhibit somewhat different growth in winter than in summer. Leaves are usually larger and leaf color darker.

CHAPTER III

ADDITIONAL INVESTIGATIONS INTO LONG-DAY INHIBITION

OF FLOWERING IN XANTHIUM[1]

In 1945, Chailakhyan (8) induced various parts of a single leaf of
Perilla, while other parts remained under various non-inductive condi-
tions. The basal half of the leaf under short days could strongly
induce flowering, regardless of the condition imposed on the tip half.
However, the tip half of the leaf was not capable of inducing flowering
when the basal half was under long-day conditions. Harder, Westphal,
and Behrens (34) extended this work with Kalanchöe. They showed that
flowering resulted when the apical leaf half was induced providing the
basal leaf tissue was trimmed off.

In an earlier publication, Gibby and Salisbury (25) defined a
long-day inhibitory effect on Xanthium flowering. There were two basic
experiments. In the first, approximately the same experiment was
conducted with Xanthium as Chailakhyan did with Perilla, and the same
results were noted, namely, an induced basal leaf half could cause
flowering nearly as well as the entire leaf even if the tip half was
maintained under long days. The tip half under short days could only
cause flowering when the basal leaf tissue under long days was removed.
In the second basic experiment a long-day leaf inhibited the response
of a short-day leaf provided it was between the short-day leaf and the
receptive bud.

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[1]To be submitted for publication with F. B. Salisbury and W. F. Campbell
as co-authors.

These two experiments led to the following five hypotheses to explain the inhibitory effect:

1. The tip half of the leaf cannot synthesize florigen.

2. A long-day inhibitor prevents subsequent florigen synthesis.

3. Long-day inhibition depends upon florigen moving only with the assimilate stream. Long-day tissue may be acting as an assimilate source (photosynthesizing) in such a manner that assimilate from short-day tissue cannot reach the bud. Alternatively, under some conditions (low light intensity), the long-day tissue may be acting as a sink for assimilate produced by short-day tissue.

4. There is a translocatable long-day inhibitor.

5. Long-day inhibition is localized and hence may be a condition or a relatively immobile substance which intercepts the promotive stimulus.

The first two hypotheses were clearly eliminated, since the tip induces flowering under all conditions when basal tissue is removed, and long-day treatment following induction is highly effective. Further, if both an inhibitor and a promoter are translocated to the bud where they compete for control of its development, it is not apparent why the long-day tissue must be situated between the short-day tissue and the bud to prevent evocation of flowering, making hypothesis 4 unlikely. Several experiments were conducted to determine if translocation (hypothesis 3) was responsible for the inhibition. Low light intensities well below the photosynthetic compensation point were highly effective for the long-day inhibition, making the third hypothesis suspect. To completely eliminate this explanation, however, direct studies of translocation using ^{14}C-labeled assimilated needed to be made. A portion of this paper reports these studies.

Studies have twice used labeled assimilates to study inhibition of flowering. Chailakhyan (11) studied inhibition of flowering of Perilla. In his experiments, conditions promoting transport of assimilates from induced leaves to the buds also promoted flowering; conditions that blocked this translocation were inhibitory. The conclusion that assimilate movement accounts for the observed inhibition is valid only if one assumes that the pattern of translocation is the same for each of the nine minimum inductive cycles necessary to evoke flowering in Perilla. This is because Chailakhyan's experiments covered translocation only during 24 hours after one inductive cycle. Other evidence points to a balance of inhibitory and promotive processes controlling flowering in Perilla (60).

Inhibition in the long-day plant, Lolium temulentum L. has been shown not to correlate with assimilate translocation (21).

Gibby and Salisbury (25) carried out further experiments to characterize the inhibitory effect. The inhibitory effect would not pass dead tissue, although the transpiration stream would. Inhibition was produced in iron-deficient tissue although florigen was not. Kinetic studies proved the inhibitory response to be exactly opposite to the kinetics of promotion, leading to the conclusion that when the leaf is not promotive it is actively inhibitory. This paper reports further characterization of the long-day inhibition.

Materials and Methods

Culture methods have been described (66). To summarize, plants of Xanthium strumarium L. (cocklebur) were germinated in sand, transplanted into 4-inch pots when they were about 2 inches tall, and kept

vegetative by extending daylength with fluorescent light. Plants were at least eight weeks old before use. The smallest leaf longer than 1 cm, measured at the midrib, was called leaf 1, the next largest, leaf 2, and so on. Plants were used when leaf 3 (maximally sensitive to induction) was between 6.9 and 8.5 cm long. In most experiments, all leaves except leaf 3 were removed 24 hours prior to the beginning of treatments. If plants were allowed to develop, flowering was scored after 9 days according to a system of stages (66).

Plants that were used to determine translocation of assimilates were spotted with 10 µl of either 36.7 mc/m mole glycine (2-^{14}C) 50 µc/ml, or 240 mc/m mole uniformly labeled glucose, 13.3 µc/ml. The former was dissolved in 0.01 NHCl and the latter in 50 percent ethanol. Prior to spotting, a circle of petroleum jelly was placed on the leaf with a suitably prepared syringe (needle filed blunt, filled with petroleum jelly from the back). The assimilate was spotted with this circle, and a round glass cover slip was placed over the spot so as to contact the petroleum jelly and seal the assimilate solution in a microchamber. This was done to prevent contamination and aid in uptake by preventing evaporation.

Eight hours after the end of the inductive night the plants that had been spotted were cut down and frozen between two blocks of dry ice. They were then mounted on cardboard and covered with Saran wrap. Prior to covering, several large crystals of silica gel were taped to the cardboard to aid desication of the plant material. Afterwards, the mounted plants were stacked until dry, usually 24-48 hours. The dried plants were placed against no-screen X-ray film in total darkness and placed inside exposure holders. The film was exposed for three weeks

and then developed. Control plants were allowed to develop, and floral stage was determined.

After the X-ray film was developed, counts were made on the stem tips of the spotted plants. An in-vial combustion method, similar to one developed by Gupta (26), was used to ash the stem tips as follows: The weighted tissue, which varied between 3.4 and 12.0 mg, was placed in a small paper cup. The paper was blackened with a black marking pen, and the paper cup and sample were mounted on a platinum coil stand and placed in the vial. The vial was flushed with oxygen, and combustion was triggered with a focused light beam from a projector. Just prior to flushing the vial with oxygen, 0.2 ml of phenethylamine was placed inside the vial to absorb $^{14}CO_2$ from the combustion. After the vials had completely cooled, the vials were opened momentarily to add 3 ml water and 10 ml of Aquasol liquid scintillator. The vials were counted in a Nuclear Chicago Uni·Lux II liquid scintillation spectrometer. Any data were rejected if counting efficiency was lower than 50 percent. Typical counting efficiency was 84.0 to 87.0 percent.

In those experiments where various compounds were screened to determine their effects on inhibition, either the entire leaf or basal half of the leaf was dipped in a solution of the compound to be tested prior to the inductive dark period. Solutions were of various concentrations and each contained one drop of wetting agent (Triton B-1956 modified phthalic glycerol alkyl) per 100 ml solution.

Results and Discussion

Assimilate translocation

In Experiment 3-1, treatments consisted of (a) plants given a
16-hour dark treatment on leaf 3, (b) plants in which the tip half of
leaf 3 was shaded with envelopes of black construction paper and the
basal half exposed to low intensity light (2 ft-c fluorescent and incan-
descent), (c) plants in which the tip of leaf 3 was shaded and the basal
half exposed to 4,000 ft-c fluorescent and incandescent light. Ten
plants of each treatment were allowed to develop to determine flowering
response and strength of inhibition (Figure 1). Metabolites were
spotted on the tops of leaf 3 of two plants in each treatment as follows:
Glycine before dark period, glycine after dark period, glucose before
dark period, and glucose after dark period. No difference in trans-
locational pattern could be detected in any of the treatments from the
autoradiograms. Figure 2 shows a typical autoradiogram.

Counts of the stem tips showed translocation to be almost identical
in the plants with the stem tip induced, basal half in low intensity
light, compared to those in which the entire leaf was induced. Trans-
location was significantly lower in the plants where the basal half of
the leaves were kept in high intensity light.

Two questions remained to be answered. First, is translocation of
assimilates to the bud still possible using a two-leaf system (second
basic experiment in Gibby and Salisbury (25). Second, is it possible
that some diffusion occurred during drying that would account for some
movement of assimilates? The latter was unlikely, because, excluding
the original spot of assimilate application, the apical meristem was by
far the darkest exposed area on the X-ray film.

	Av. Floral Stage	**Av. DPM/mg**
#3 leaf 16 hr. dark	7.30±1.13	257.6
Basal half #3 leaf in 2 ft-c continuos light, tip half 16 hr. dark	0.30±0.29	254.3
Basal half #3 leaf in 4,000 ft-c continuous light, tip half 16 hr. dark	0.00	168.5*

Fig. 1. Translocation of labeled assimilates spotted on the leaf tips under promotive and inhibitory conditions using one leaf per plant and two light intensities. *Significantly different at the 5% level.

Figure 2. Typical autoradiogram showing pattern of assimilate movement.

The first two treatments of Experiment 3-2 were identical to the first two treatments of Experiment 3-1. The third treatment consisted of a 16-hour dark treatment given leaf 3, and leaf 2 was left on the plant and exposed to low intensity light. Two plants were spotted on the tip of leaf 3 as before. Glucose was the only assimilate used. Prior to freezing the plants were mounted as before but dissected into small pieces to prevent any passive movement of assimilates. The control plants were allowed nine days to develop as before, and the results of their flowering and $^{14}CO_2$ translocation are given in Figure 3. Radioautograms in this case were identical to those of Experiment 3-1. In all cases, good translocation to the receptor bud was observed. It was also observed that some assimilate moved into leaf 2 when present. There was no significant difference observed in the counts between the three treatments.

The results of these two experiments clearly eliminate the hypothesis that inhibition is solely a result of assimilate movement.

Experiment 3-3 was an attempt to see if the assimilate in the tip half of the leaf would diffuse back into the basal half leaf tissue after having entered the midrib. If it did not, then one would have evidence that an inhibitor(s) was at least slightly mobile, moving at least to the midrib to affect the promotor(s). If it did diffuse back into the basal tissue, then a mobile compound would not be required. Leaves were spotted with glucose as in Experiment 3-1 and 3-7 before the dark period. Prior to spotting, the leaves were cut, except the midrib, to separate the basal half tissue from that of the tip. This had been previously shown to not affect the promotion where the entire leaf was shaded, nor the inhibition when only the tip was shaded, basal half in light (25, 34). The resulting pattern of translocation
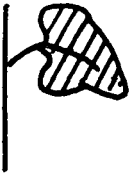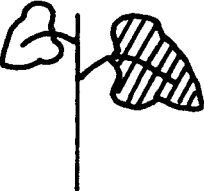
		Av. Floral Stage	**Av. DPM/mg**
	# 3 leaf 16 hr. dark.	4.30±0.92	143.5
	Basal half leaf 3 in 2 ft·c continuous light, tip half 16 hr. dark.	0.20±0.11	107.2
	Leaf 2 2 ft·c continuous light, leaf 3 16 hr. dark.	2.10±0.89	136.4

Figure 3. Labeled assimilate translocation under promotive and inhibitory conditions using one and two leaves per plant.

in the case where the entire leaf was induced and where the tip half
only was induced was the same. Both halves of the leaves showed them-
selves to be equally shaded. A mobile substance is therefore not
required to explain the observed inhibition.

Phytochrome control of inhibition

A light flash given Xanthium leaves in the middle of a long night
causes the leaves not only to be non-promotive but actively inhibitory.
Gibby and Salisbury (25) defined the kinetics of this light break and
determined that the critical night for blocking inhibition was the same
as the critical night for promotion. It remained to be determined if
phytochrome were the pigment responsible for the inhibitory response.
Experiment 3-4 is an attempt to determine this. Plants were given 16-hour
dark treatments on leaf 3 or the tip half of leaf 3, basal tissue removed.
In some treatments the dark treatment was interrupted at 8 hours by a
one minute red light flash from 3 very high output fluorescent lamps
and a red filter of 1/4-inch transluscent red plexiglass. This flash,
in some cases, was given only the basal half of the leaf with the tip
half uninterrupted in its long night. After the one-minute red
interruption, half of the irradiated plants were illuminated with red
light for 40 seconds using four incandescent flood lamps and a far-
red filter (special far-red transmitting plexiglass, 1/8 inch-FRF-700),
obtained from Westlake Plastics Co., Lenni Mills, Pennsylvania). The
treatments and results are given in Figure 4. Note the high level of
flowering in the uninterrupted plant in both the entire leaf (treatment 1)
and tip half (treatment 4). Note also the complete inhibition in the
first case after interruption (treatment 2) and near complete inhibition
in the second case (treatment 5). The far-red flash was capable of

Fig. 4. Phytochrome control of inhibitory process. Effect of red light flashes and red light flashes followed by far-red light flashes on various areas of leaves during a 16-hr. dark period.

partial reversion in both cases (treatments 3 and 6). Now observe the level of inhibition in the plants where only the basal half was interrupted, and note its reversion with far-red light (treatments 8 and 9). The red flash is clearly doing more than inhibiting the basal half of the leaf. Compare treatments 4 and 8. The tip half of the leaf received an uninterrupted 16 hours of darkness in both cases. The basal half in treatment 8 is actively inhibitory, and its inhibitory effect is partially reversed by far-red light (treatment 9). It is apparent, however, that the inhibitory response is less light sensitive than the promotive process, comparing treatment 8 with treatments 2 and 5. This compares favorably with threshold values of light needed to cause inhibition or prevent promotion responses, as previously determined by Gibby and Salisbury (25).

Screening experiments

Various compounds were screened to determine their ability to block the inhibitory effect. In each case controls consisted of: (a) plants with leaf 3 given 16 hours of dark and dipped in water plus wetting agent; (b) plants with the tip half of leaf 3 given 16 hours of dark while the basal half was in the light, basal half dipped in wetting agent. In all cases, the former flowered well with the latter being completely inhibited (see average floral stage of control, Figure 5). In the treatments, leaves were dipped in a solution containing the compound to be tested for effect (plus wetting agent). Plants were given light and dark treatments the same as controls. Figure 5 shows compounds tested, their concentrations, their average floral stages, and their effects on induction and inhibition. It will be noticed that of all compounds tested, most were at least somewhat inhibitory to flowering; see percent

Chemical	Induced portion of leaf	Concentration	Av. floral stage of controls	Av. floral stage	% flowering of promotive control
2,4-dinitro phenol		0.01 M in 30% EtOH	5.78±0.53	0.00	0
			0.11±0.07	0.00	0
		0.001 M	5.00±0.25	3.40±1.54	68
			0.00	0.00	0
Nicotinic acid		0.1 M	5.78±0.53	0.00	0
			0.11±0.07	0.00	0
GA$_3$		0.1%	5.15±0.37	--	-
			0.37±0.40	0.00	0
Ethionine		0.1 M	5.78±0.53	0.50±0.92	9
			0.11±0.07	1.75±0.46	34
		0.05	3.60±1.42	1.50±1.36	42
			0.00	0.20±0.36	6
Quercetin		0.1 M in 30% EtOH	5.00±0.25	4.00±1.03	80
			0.00	0.10±0.66	2
Rutin		0.1 M in 30% EtOH	5.00±0.25	3.20±1.16	64
			0.00	0.00	0
Maleic hydrazide		0.01 M	3.60±1.65	3.40±1.78	95
			0.00	0.10±0.21	3
α-Picolinic acid		0.1 M	3.60±1.65	0.40±0.81	11
			0.00	0.50±0.81	13
Naphthalene acetic acid		0.1% in 25% EtOH	4.50±0.55	0.00	0
			0.00	0.00	0
α-Naphthalene acetamide		0.1% in 25% EtOH	4.50±0.55	0.30±0.44	11
			0.00	0.00	0

Fig. 5. Effect of various compounds on promotion and inhibition of flowering. Leaves or basal halves of leaves dipped in solutions to determine effect.

flowering of promotive control (control with entire leaf induced). Only one was successful in blocking inhibition. This was DL Ethionine. The level of flowering was quite low, but it was clearly reproducable in two experiments.

These results are preliminary but do suggest direction for further research. In view of the weak inhibition blocking ability of ethionine, other amino acid antimetabolites should be tried. It is quite possible that protein synthesis is involved in the inhibitory process of Xanthium.

Summary Discussion and Conclusions

These experiments shed additional light on the nature of an inhibitory response defined by Gibby and Salisbury (25).

Experiments with ^{14}C-labeled assimilates clearly show the ability of at least some products of the induced leaf to by-pass long-day (inhibitory) tissue and reach the bud. Ability of a promoter to move with the assimilate stream must not be the only requirement. There is obviously some active inhibitory process involved, which intercepts the promotive principle of induction.

As pointed out, the inhibitory tissue, to be effective must be between the short-day tissue and the receptor bud. This points to a slow-moving inhibitor or some inhibitory condition in the long-day tissues. One is forced to visualize a double requirement for evocation of flowering:

1. Synthesis of promotive principle(s) during induction.

2. The blocking of inhibitory processes of blocking of synthesis of a slow-moving inhibitory compound.

The involvement of phytochrome in the inhibitory process is likely as a result of Experiment 3. One might say that it is possible to explain the results of treatments 7-9 on the basis of light inhibition of florigen synthesis and its reversal in the basal half of leaf 3 only, disregarding the tip. This seems quite unlikely considering the level of flowering that the tip demonstrated in the absence of basal tissue (treatment 4). An active inhibition is at least involved as a complicationto promotion, although its quantitative importance is difficult to determine.

The possibility of protein synthesis as a part of the inhibitory process seems a strong possibility in view of the preliminary screening experiments. Other antimetabolites of amino acids must be tested to affirm this hypothesis.

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