Plant Physiol. (1979) 63, 643–649
0032-0889/79/63/0643/07/$00.50/0

Indirect Action of Benzyladenine and Other Chemicals on Flowering of *Pharbitis nil* Chois

ACTION BY INTERFERENCE WITH ASSIMILATE TRANSLOCATION FROM INDUCED COTYLEDONS

Received for publication June 27, 1978 and in revised form October 23, 1978

YUKIYOSHI OGAWA[1] AND RODERICK W. KING
CSIRO, Division of Plant Industry, P. O. Box 1600, Canberra City, A.C.T., 2601, Australia

ABSTRACT

Benzyladenine (BA) brushed on the cotyledons of 4-day-old seedlings of *Pharbitis nil* Chois. markedly stimulates flowering. Greatest response is obtained for concentrations between 44 and 440 micromolar. The action of BA is on processes in the cotyledon as shown by the response to its site of application, to the dosage applied and to the requirement for its application prior to the dark period. There was little or no effect of BA treatment on either the time measurement processes of photoperiodic induction or on the generation of floral stimulus. Transport of photosynthetic assimilate from the cotyledons to the shoot apex was altered.

When only one of the pair of cotyledons was treated with BA it exported less ^{14}C-labeled assimilate to the shoot apex and there was a compensatory increase in assimilate outflow from the other cotyledon. When BA was applied to a cotyledon exposed to an inductive dark period, flowering was inhibited in association with the reduced export of assimilate. Conversely, when BA was applied to the noninduced cotyledon, flowering was promoted in association with an enhanced export of assimilate from the induced leaf. Clearly, cytokinins can have an indirect effect on photoperiodic induction by altering assimilate and, hence, floral stimulus translocation to the shoot apex.

Two other chemicals which were previously considered as specific inhibitors of processes of floral induction in the cotyledon [Tris(2-diethylaminoethyl)phosphate trihydrochloride (SK&F 7997-A_3) and cycloheximide] acted in the same manner as BA. Inhibitory effects of an illuminated cotyledon on flowering of *Pharbitis* were also shown to be mediated by interference of assimilate flow with transport of the stimulus for flowering.

It has been established beyond doubt that the leaf is the photoresponsive organ in photoperiodic induction of flowering (5, 21). However, little progress has been made either in unraveling the metabolic processes of floral induction in the leaf, or in identifying the factor(s) subsequently transported to the shoot apex where flowering is evoked. The effect on flowering of applying various chemicals to the leaf has offered one, although indirect, probe for examining the metabolic events of induction. Specificity of action in the leaf has been argued on three grounds: (*a*) that the time of sensitivity to application of the compound is specific for the timing of induction in the leaf; (*b*) that the compound is ineffective or markedly less effective at the apex; and (*c*) that the compound is not transported to the apex. Only when these three criteria are met is it valuable to consider further implications of the action of a particular compound in leaf tissue.

Aside from studies with photosynthetic inhibitors which presumably act on photosynthesis in the induced leaf (see literature cited in ref. 6), the only data which argue, compellingly, for leaf localization of effects on flowering are for applications of SK&F 7997-A_3,[2] ethylene, cycloheximide, and kinetin (21). All three criteria necessary for validating leaf-localized action have been met only for studies of suppression of flowering by SK&F 7997-A_3 (2) and by ethylene (17). The question of possible transport to the apex has not been examined adequately for responses either to cycloheximide (15) or kinetin (13). Also, it is useful to examine the dose response, not just the concentration response, on application of a compound either to the leaf or shoot tip, but such information is lacking for cycloheximide-induced inhibition of flowering in *Xanthium* (15) and for kinetin promotion of flowering of *Pharbitis* (13).

We have examined the question of the site and mechanism of cytokinin promotion of flowering of *Pharbitis* and show that cytokinins and some other chemicals affect leaf photosynthesis and/or assimilate distribution. There is apparently no direct effect of cytokinin on photoperiodic induction but possibly an indirect control over floral stimulus transport by way of effects on assimilate distribution.

MATERIALS AND METHODS

Growing Conditions. Seeds of *Pharbitis nil* Chois., strain Violet were treated with concentrated H_2SO_4 for 45 min and then washed in running water overnight at 30 C. The seeds were sown in a mixture of equal parts of Perlite plus Vermiculite in 12-cm-diameter pots. The seedlings were raised for 4 days at 29 C in light (200 $\mu E\ m^{-2}\ s^{-1}$, PAR) from fluorescent lamps and then placed in darkness at 27 C for various durations. Immediately before entering darkness all plants received a 10-min red irradiation to ensure phytochrome was in the far-red-absorbing form. In one series of experiments the seedlings were induced to flower by wrapping one of the pair of cotyledons in aluminum foil for 16 h. To minimize any possibility of light leaks interfering with the induction (11) the light intensity was reduced to 10 $\mu E\ m^{-2}\ s^{-1}$ PAR over the period of the wrapping treatment. Exposure of seedlings to this low intensity irradiation without wrapping a cotyledon did not induce flowering. To reduce the time taken to wrap the seedlings three people were employed but there were no statistically significant differences for flowering responses obtained between individuals. After darkness the seedlings were moved to continuous light at 19 to 20 C for 7 days and then at 25 C until dissected for measuring the flowering response 10 to 14 days later. Seedlings were watered twice a day, once with water and once with Hoagland nutrient solution.

[1] Present address: Faculty of Agriculture, Mie University, Tsu City, Mie Prefecture, Japan.

[2] Abbreviation: SK&F 7997-A_3: Tris(2-diethylaminoethyl)phosphate trihydrochloride.

Application of Chemicals. For treatments with BA, SK&F 7997-A_3, or cycloheximide, an aqueous solution (pH 7.0) with 0.05% (v/v) Tween 20 was applied to the cotyledons using a paint brush. On average this delivered 30 μl per cotyledon to give a total of 60 μl per plant if the whole plant was treated. A 5-μl drop was applied to the plumule using a microsyringe. Control plants were treated with water containing 0.05% (v/v) Tween 20. There were three or four pots in each treatment with six or seven seedlings per pot.

Distribution of ^{14}C-labeled Assimilate. The distal half of a cotyledon was exposed for 10 min in a Perspex assimilation chamber to $^{14}CO_2$ generated by adding 50% (v/v) lactic acid to $Ba^{14}CO_3$ (4 mg, 59 Ci mol^{-1}). The irradiance during $^{14}CO_2$ exposure was 360 μE m^{-2} s^{-1} PAR and this was then returned to 200 μE m^{-2} s^{-1} PAR. The initial CO_2 concentration was about 800 μl l^{-1}. To measure initial ^{14}C uptake and its subsequent export, 0.65-cm^2 discs were cut from one lobe of the treated cotyledon at 10 min and another disc was cut from the other lobe after 5 h. The discs were fixed with adhesive to aluminum planchets, dried at 70 C overnight and then ^{14}C activity determined using a Geiger-Muller counter. Apical buds were dissected under a stereoscopic microscope and comprised the apical meristem (0.3 mm in diameter) and the two youngest (<0.2 mm long) leaf primordia. The dissecting needle was rinsed in ethanol and wiped after each dissection. After drying at 70 C the apices were weighed individually on a Cahn electrobalance (Cahn Instrument Co., Paramount, Calif.), digested for 2 h at 50 C in 0.5 ml of NCS (Amersham/Searle), and ^{14}C activity counted in a liquid scintillation spectrometer. On average the dry weight of an apex was 4.6 ± 0.2 μg.

Photosynthesis. Exchange of CO_2 in light (500 μE m^{-2} s^{-1} PAR) was measured using an IR gas analyzer. Five pairs of cotyledons were enclosed in a Perspex assimilation chamber and air (about 320 μl l^{-1} CO_2) passed over the cotyledons at 0.08 liters s^{-1}. Leaf temperature was 25 C as determined using a copper-constantan thermocouple placed against the underside of the leaf. Cotyledon surface area was measured using an automatic area meter (type AAM-5; Hayashi Denko Co., Tokyo, Japan).

Cotyledon Dry Matter Export. To measure absolute rates of assimilate export, dry weight changes of the cotyledons as well as their rate of net CO_2 exchange were followed for 5 h at a light intensity of 500 μE m^{-2} s^{-1} PAR and a temperature of 25 C. Dry weight input was calculated from the rate of net CO_2 fixation by multiplying the mass of CO_2 fixed by 0.65 to convert from CO_2 to sucrose. The difference between the mass of sucrose formed photosynthetically and the change over 5 h in the weight of leaf discs (0.65-cm^2 diameter) gave a value for the calculation of cotyledon dry matter export.

RESULTS

Site and Timing of BA Promotion of Flowering. Early experiments of Ogawa (13) and Nakayama and co-workers (12) indicated that in *Pharbitis*, kinetin promoted a flower-inducing process in the cotyledons. We found that BA also promotes flowering when applied to the cotyledons of *Pharbitis* at the beginning of an inductive dark period (Fig. 1). The critical dark period may have been shortened by the BA treatment (Fig. 1), but it is difficult to distinguish such a response from a general stimulation of flowering. Normally the flowering response of the control plants was maximal in a 16-h dark period (about 8–10 flowers per plant). In some experiments conditions were not optimal and clear promotion of flowering by BA was evident in a 16- or 18-h dark period.

For promotion of flowering the optimal concentration of BA applied to the cotyledons at the beginning of the inductive dark period was between 44 and 440 μM (Fig. 2A). Applications of BA to the plumule were without effect on flowering (Fig. 2B) as were applications of a water plus Tween 20 solution to either the cotyledon or plumule. The total volume of solution applied to the cotyledons was 12 times that applied to the plumule (60 μl *versus*

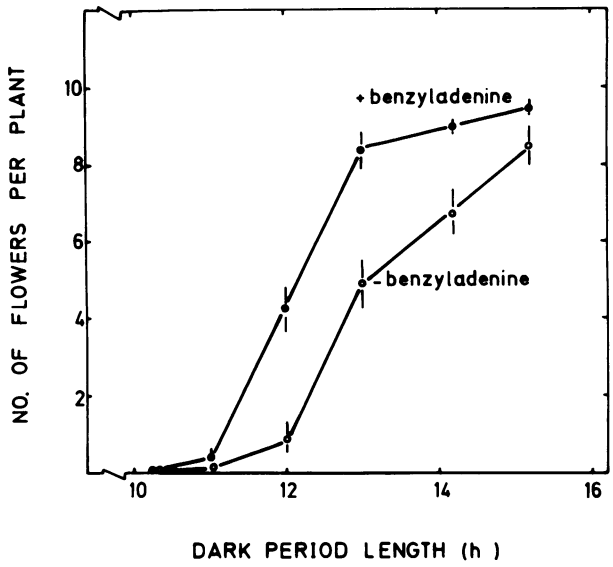

FIG. 1. Flowering response of *Pharbitis* to a single dark period of varied duration. Solutions of 0.44 mM BA (●) or water (○) were applied to both cotyledons 0.5 h before the dark period. Bars in this and subsequent figures are twice the standard error of the mean.

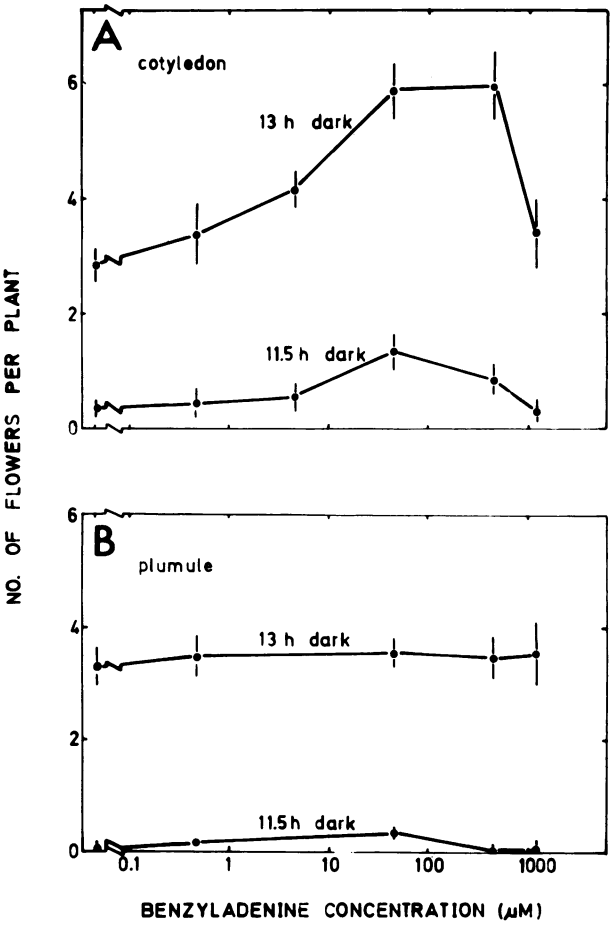

FIG. 2. Promotion of flowering of *Pharbitis* as a function of the concentration of BA applied to the cotyledons or plumule 0.5 h before the start of a single 11.5- or 13-h dark period. An average of 30 μl of solution was applied with a brush to each cotyledon and 5 μl was applied with a microsyringe to the plumule so that a 12× factor is required to establish equivalence of dosages per seedling.

5 μl). Even at an equal or greater total dosage, plumule applications of BA had no effect on flowering, *e.g.* 1.1 mM BA to plumule *versus* 44 μM to cotyledons. In further experiments, flowering was promoted significantly (5.1 ± 0.6 *versus* 3.3 ± 0.4 flowers per plant) when a total of 10 μl of BA (0.44 mM) was applied to the two cotyledons. A 5-μl drop of BA (1.1 mM) had no effect when applied to the plumule (3.6 ± 0.6 *versus* 3.3 ± 0.4 flowers per plant for BA-treated and control seedlings, respectively). In a preliminary study using ^{14}C-labeled BA there was little transport of radioactivity to the shoot apex in 24 h (0.01% of the total ^{14}C counts applied to the cotyledon).

For BA to promote flowering it had to be applied before the middle of darkness (Fig. 3). A similar result was obtained by Ogawa (13) in his experiments on kinetin application to seedlings of *Pharbitis*.

The promotive effects of BA on flowering of *Pharbitis* are long lived provided that application precedes the start of darkness. Promotion of flowering persisted when the start of the dark period was delayed until 5 days after BA application to the cotyledons. In the latter instance, only the higher concentrations of BA promote flowering (*e.g.* >44 μM) and the highest concentration (1.1 mM) was no longer inhibitory (*cf.* Fig. 2).

Mechanisms of BA Action on Flowering. From the results on its site of action it is clear that in promoting flowering BA acts in the leaf, not at the apex. Since BA is generally regarded as being immobile in plants when applied to leaves, and [^{14}C]BA showed little movement to the apex in *Pharbitis* seedlings, BA probably does not effect flowering by acting, indirectly, at some other site outside the cotyledon. Action in the cotyledon itself could be direct on inductive processes, or indirect on assimilate transport and, hence, on transport of the floral stimulus which is known to move with assimilate in *Pharbitis* (9).

To examine the question of BA effects on assimilate and stimulus transport, flowering was induced by darkening one cotyledon by wrapping it in aluminum foil whereas the other, noninduced cotyledon was held continuously in the light. Now the specificity of BA action for leaf induction, or for assimilate transport could be tested simply by applying BA either to the induced or noninduced cotyledon. In these experiments BA was applied at a concentration of 0.44 mM 2 h before one of the cotyledons was wrapped. In contrast to the promotive effects of BA shown previously, BA application to the induced cotyledon, alone, inhibited flowering compared with the flowering response of the water treated control (Fig. 4, A *versus* C). By contrast, BA application to the noninduced cotyledon promoted flowering (Fig. 4, B *versus* C). The nontreated, noninduced cotyledon also inhibited flowering when compared with the response of seedlings with the noninduced cotyledon removed (Fig. 4, D *versus* C). The flowering response of the water-treated control was the same no matter which cotyledon was treated. Similar responses to all of these were obtained in two further experiments.

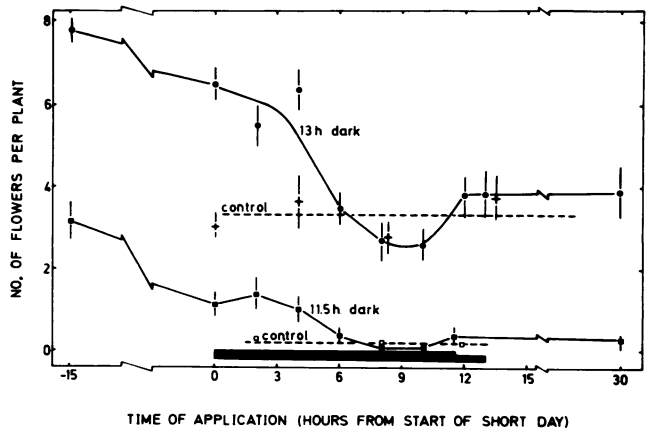

FIG. 3. Effect of time of 0.44 mM BA application to the cotyledons on the flowering response of *Pharbitis* to a dark period of 11.5 or 13 h. Water control treatments (+, □) at the different times in darkness indicate the lack of effect on flowering of working during darkness under the available green safelight. Timing of dark period indicated by darkened bar.

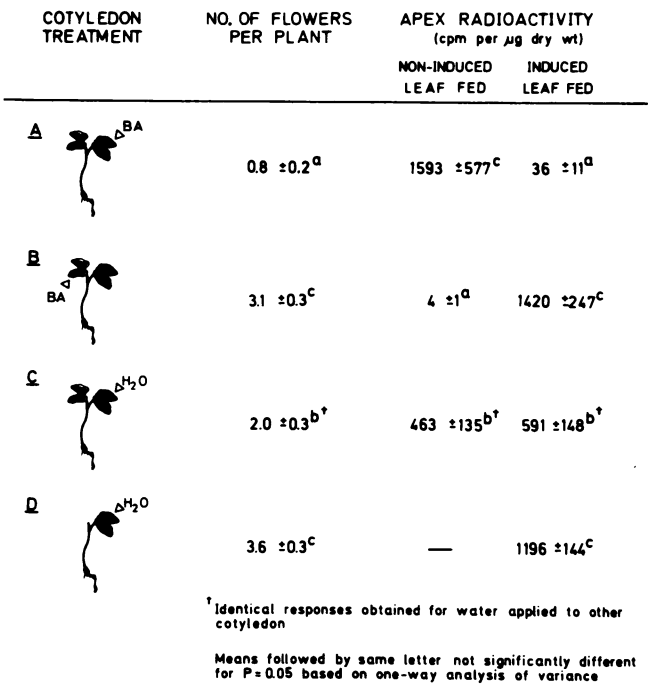

FIG. 4. Effect of site of application of 0.22 mM BA or water on the flowering response and import of ^{14}C-labeled assimilate into the shoot apex of *Pharbitis*. Induction was affected by darkening one cotyledon (blackened cotyledon) by wrapping it in aluminum foil for 16 h. The other, noninduced cotyledon was continuously illuminated (A, B, C) or removed at the start of induction (D). Solutions of BA or water were applied 2 h before induction either to the illuminated cotyledon or the darkened one. For separate groups of five seedlings either the induced or noninduced cotyledons were exposed to $^{14}CO_2$ for 10 min beginning 1 h after the end of the inductive treatment. ^{14}C activity at the shoot apex was determined 5 h later.

The significance of these data cannot be emphasized strongly enough. In contrast to the simple promotion of flowering when both cotyledons of darkened plants are treated with BA, it is clear that BA both promoted and inhibited flowering and in the induced cotyledon BA may actually inhibit (Fig. 4A) or promote (Figs. 1–3) flowering. Direct action of BA on induction seems unlikely and these data can be best understood in terms of BA effects on assimilate export as shown below.

Effects of BA on Assimilate Distribution. Using leaf ^{14}C-labeling techniques as outlined previously (1) and harvesting apices after 5 h, when their radioactivity should still be increasing linearly (1), we have found that the ability of BA to promote, or inhibit, flowering is directly related to the amount of assimilate reaching the shoot apex from the induced cotyledon (Fig. 4). The ability of a cotyledon to export labeled assimilate to the apex is reduced dramatically by BA treatment whether or not that cotyledon has been exposed to darkness (Fig. 4, A *cf.* B). The nontreated cotyledon takes over the major role as a source of assimilate for the apex and a similar compensatory increase in assimilate flow to the apex results when the noninduced cotyledon is removed prior to dark treatment of the induced cotyledon (Fig. 4, D *versus* C).

Despite the changes in ^{14}C counts at the apex following BA treatment of a cotyledon there was no significant effect of BA treatment on $^{14}CO_2$ fixation. The initial $^{14}CO_2$ uptake measured in a 0.65-cm^2 leaf disc, was similar for all treatments (Table I). Similar results for $^{14}CO_2$ uptake were obtained in a further experiment.

The summated ^{14}C counts arriving at the apex from both cotyledons fed separately was essentially constant despite widely divergent contributions from BA treated and nontreated cotyledons (Fig. 4) which suggests that ^{14}C movement to the apex reflects the movement of all assimilate. A reduction or increase in ^{14}C activity at the apex was associated with reduced or increased ^{14}C loss from the cotyledon (Table I). A preliminary examination of cotyledon dry matter export based on dry weight and net CO_2 exchange rates also indicated a reduction in assimilate export (13% less over a 5-h period) following BA application to one of a pair of cotyledons. This latter finding implies that the movement of ^{14}C counts is a valid indicator of transport of all assimilate.

The observation that differences in apical ^{14}C counts (Fig. 4) were greater than was cotyledon export of ^{14}C label (Table I) or plumule ^{14}C counts (not shown) is of some interest. Of the total ^{14}C radioactivity incorporated by the cotyledon only about 0.01% had appeared at the shoot apex after 5 h. The apex is 0.03% of the dry weight of the plumule (4.6 μg *versus* 12.8 mg, respectively) yet it received more of the ^{14}C label (0.08% of plumule cpm per μg dry weight). Clearly, inference about changes at the shoot apex must be based on the apex alone.

When flowering was induced by exposing the whole seedling to darkness (as distinct from the wrapping treatments above, Fig. 4) promotion of flowering by BA was again associated with increased import of ^{14}C label into the shoot apex. The results shown in Table II are for three replicate experiments. Treatment of the cotyledons with BA 2 h before a 16-h dark period resulted in a consistent and significant ($P < 0.01$) enhancement of ^{14}C import into the apex following a $^{14}CO_2$ exposure after darkness. There were unavoidable differences between experiments in $^{14}CO_2$ uptake (not shown) but there was no significant interaction between total ^{14}C activity and the BA enhancement of ^{14}C import. There was no significant effect of BA treatment on $^{14}CO_2$ fixation within an experiment (Table III). Measurement of cotyledon net CO_2 exchange rates

Table I Effect of benzyladenine treatment of either the non-induced or induced cotyledon on $^{14}CO_2$ fixation (10-min exposure) and the subsequent loss after 5h of ^{14}C activity from the fed area of each cotyledon.

All treatments and conditions were as detailed for Figure 4 and the apical ^{14}C counts for this experiment are presented there.

Treatment	$^{14}CO_2$ fixation (cpm per disc)[1]		Loss of ^{14}C activity[2] % of initial activity	
	Non-induced	Induced	Non-induced	Induced
A. BA applied to induced cotyledon	102,904	79,953	36.7	5.8
B. BA applied to non-induced cotyledon	98,232	108,441	18.6	26.6
C. Water control	107,493	108,490	30.6	26.7
D. Non-induced cotyledon removed	-	97,004	-	23.5

[1] No significant differences between treatments from a one-way analysis of variance.

[2] LSD 0.05 = 8.9

Table II Effect of benzyladenine treatment of both cotyledons before a 16-h dark period on the import of ^{14}C-labeled assimilate into the shoot apex.

Exposure of one cotyledon to $^{14}CO_2$ was for 10 min beginning 1h after the dark period. The shoot apex was harvested 5h later. Ten apices were analyzed for each value for the three replicate experiments.

Experiment No	Apex radioactivity (cpm per μg dry wt) Water control	Benzyladenine[1] (4.4×10^{-4} M)	Increase (%)
I	328	415	27
II	407	693	70
III	698	1090	56

[1] All benzyladenine treatments significantly different from control values ($P < 0.01$). There were no interactions between treatments and experiments as tested by a two-way analysis of variance.

Table III Effect of benzyladenine treatment of both cotyledons before a 16-h dark period on $^{14}CO_2$ fixation (10-min exposure 1 h after the dark period) and the subsequent loss after 5 h of ^{14}C activity from the fed area of the cotyledon.

Values are averaged from three replicate experiments (10 plants per treatment) for which shoot apex radioactivity was reported in Table II. Differences between treatments not significant as tested by a two-way analysis of variance.

	$^{14}CO_2$ fixation	loss of ^{14}C activity
	cpm per cotyledon disc	% of initial activity
Water control	180,092	27.1
Benzyladenine (4.4×10^{-4} M)	182,188	34.6

using an IR gas analyzer confirmed the lack of effect of BA on photosynthesis. From three separate measurements comparing groups of six seedlings, net CO_2 exchange rates were 23.1 ± 1.0 and 24.5 ± 0.3 mg CO_2 dm^{-2} h^{-1} for BA-treated and nontreated seedlings, respectively.

The effect of BA treatment of both cotyledons on ^{14}C activity imported by the shoot apex (Table II) may have been associated with a greater loss of ^{14}C activity from the cotyledons (Table III) but variability was high and the differences were not significant. BA application did lead to a significant (20%) reduction in dry matter export over a 5-h period.

Effects of BA on Inductive Processes. Despite our evidence of BA effects on assimilate distribution and, hence, on floral stimulus movement, additional, direct effects on cotyledon inductive processes cannot be excluded. We examined the effect of BA treatment on photoperiodic time measurement and on floral stimulus generation. The timing of sensitivity of flowering to brief (3-min) red irradiations during darkness provides one test of the action of a compound on photoperiodic time measurement (19). BA treatment had no effect on the timing of sensitivity of flowering to these red interruptions of darkness (Fig. 5) and this was confirmed in a subsequent experiment.

Effects of BA on floral stimulus generation were sought by examining the length of the critical dark period for flowering and the time when cotyledon removal treatments show that sufficient stimulus for flowering had been exported. Data illustrating the timing of generation and export of our floral stimulus have been published before for *Pharbitis* (20) and our results (Fig. 6) indicate that BA application had little or no effect on floral stimulus generation and export.

Inhibitory Effects of a Noninduced Cotyledon. Imamura (8) indicated that a noninduced, illuminated cotyledon of *Pharbitis* inhibited flower induction by a darkened (wrapped) cotyledon whether or not the illuminated cotyledon was cut off at the end of the 16-h inductive treatment. This result, he argued, excluded any possible inhibitory effect of the noninduced cotyledon on assimilate and stimulus transport from the induced cotyledon. We found that inhibition is reduced when the inhibitory cotyledon is removed slightly earlier than 16 h (Fig. 7). The timing of appearance of inhibition by the illuminated cotyledon does coincide with, or occurs later than, export of floral stimulus from induced cotyledons: 12 to 20 h for appearance of inhibition (Fig. 7) *versus* 13 to 16 h for stimulus export (Fig. 6). The response is similar for both the water control and following BA treatment of the induced leaf. The results (Fig. 7) could also indicate that the floral stimulus is extremely labile in *Pharbitis*. Apparently, stimulus not exported by 20 h is not retained for later export upon removal of the inhibitory, illuminated cotyledon.

Specificity of Action of Applied Chemicals on Floral Induction. Specific action on inductive processes in the leaf have been suggested not only for BA but for a number of other chemicals

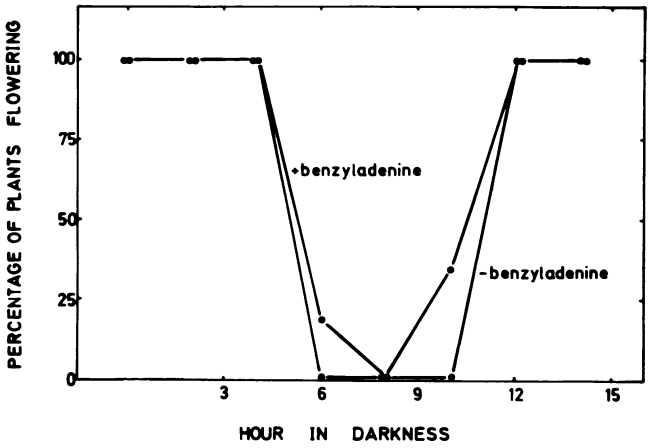

FIG. 5. Percentage of *Pharbitis* plants flowering when a 3-min red irradiation (2.6 w m^{-2} 600–700 nm) interrupted a 14-h dark period at various times. Immediately prior to darkness both cotyledons were painted with a 0.44 mM solution of benzyladenine (●) or with water (○). Treatment effects were no different when flowering was expressed as the number of plants flowering or as a percentage of plants flowering.

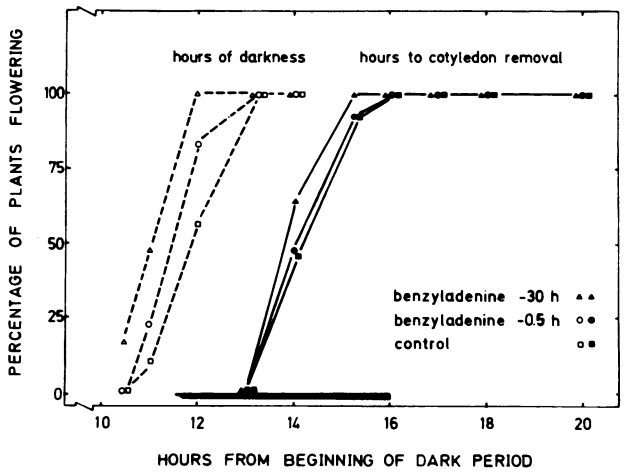

FIG. 6. Flowering response in *Pharbitis* as affected by length of the dark period (△, □, ○) or by removal of the induced cotyledons various times after the start of a dark period of 16 h (▲, ■, ●). Solutions of 0.44 mM BA were applied to both cotyledons 30 h (△, ▲) or 0.5 h before darkness (○, ●) or, water 0.5 h before darkness (□, ■).

(see introductory section). We have tested the specificity of action of some of these compounds by applying them at comparable concentrations to those employed in earlier studies. Applications have been restricted to the illuminated, noninduced cotyledon at

Table IV Effect of applying solutions of cycloheximide (100mg l^{-1}) or SK&F 7997-A_3 (2 g l^{-1}) to non-induced cotyledon on flowering induced by darkening the other cotyledon for 16h and on the rate of net CO_2 uptake of the illuminated cotyledon measured after the inductive treatment. All solutions applied in water at a pH of 7.0.

Compound	Net CO_2 uptake rate % of water control	Flowering % of water control
Cycloheximide	48	189
SK&F 7997-A_3	55	127

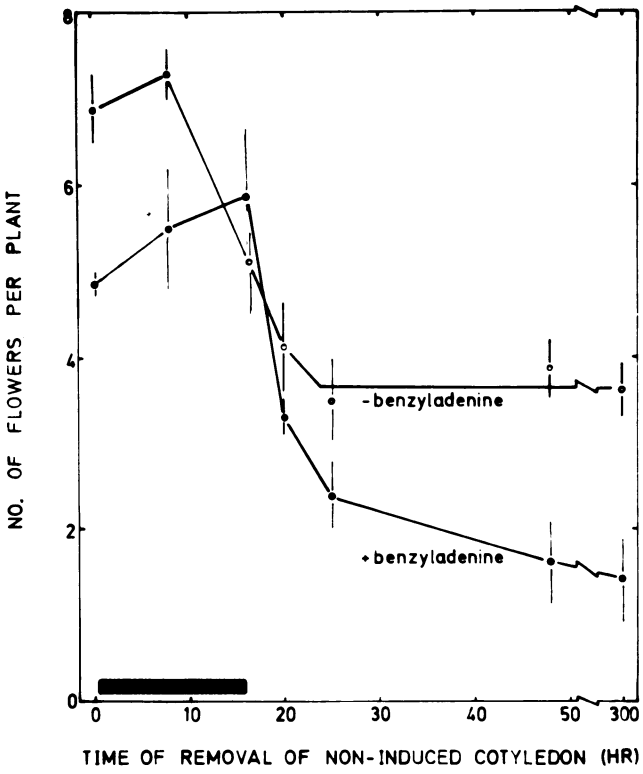

FIG. 7. Effect of time of removal of the illuminated, noninduced cotyledon on the flowering response following exposure of the other, induced cotyledon to a 16-h dark period (darkened bar). A solution of 0.44 mM BA or water was brushed onto the induced cotyledon 2 h before it was darkened.

the time of induction of the other cotyledon. Cycloheximide applied to the illuminated cotyledon at the time the other cotyledon was darkened inhibited net photosynthesis of the treated cotyledon (Table IV) and stimulated flowering almost 2-fold. Applying SK&F 7997-A_3 to the illuminated cotyledon at the time of starting induction had no effect on flowering. The short lived nature of the sensitivity of flowering on applying this compound (2) implies that it may be unstable in the plant. When we applied SK&F 7997-A_3 to the illuminated cotyledon 8 h after the start of the dark period promotion of flowering and inhibition of photosynthesis was found (Table IV).

DISCUSSION

The evidence we have presented here on the site, dosage dependence (Fig. 2), and timing (Fig. 3) of promotion of flowering by applied BA shows clearly that cytokinin action on flowering of *Pharbitis* is localized in the cotyledons and may be on inductive processes, as was suggested earlier by Ogawa (13). We found no effect of BA on photoperiodic time measurement (Fig. 5), or on generation of the floral stimulus (Fig. 6). BA promoted flowering of *Pharbitis* even when applied 5 days before the inductive dark period. Thus, BA action on flowering in *Pharbitis* could be indirect although localized in the cotyledons.

In the past the question of direct rather than indirect action of applied chemicals on photoperiodic induction has not been adequately examined. However, seedlings of *Pharbitis* with their two cotyledons do provide an essentially unambiguous system for assaying for specificity of action. The compound in question if applied separately to an induced or a noninduced cotyledon should give one of four diagnostic responses: (*i*) *no action on flowering*, which implies that the compound is of no interest; (*ii*) *sole action on the induced cotyledon*, which implies that the compound acts on processes required for induction in the cotyledon; (*iii*) *common action*, either inhibition or promotion of flowering, which implies that the compound must be acting outside the cotyledon and most probably at the shoot apex; (*iv*) *opposite action*, inhibition when applied to one cotyledon, promotion when applied to the other, which implies that the action of the compound is indirect on induction via a process which is common to both cotyledons such as assimilate production and transport.

The decisive nature of these criteria is shown by our data for BA effects on flowering and assimilate transport in *Pharbitis* seedlings. The action of BA on flowering of *Pharbitis* was localized in the cotyledon (Fig. 2) but the response was opposite when BA was applied separately to the induced or noninduced cotyledons (Fig. 4; see category iv). Its opposite effects on flowering were associated with a common inhibition of assimilate transport from the treated cotyledon (Fig. 4 and Table I). This response was similar to those previously reported for leaves of bean (7) and grape (16). The normal supply of assimilate from untreated cotyledons to the shoot apex was equivalent for the two cotyledons irrespective of photoperiodic treatment (Fig. 4C and Table IC). However, upsetting this balance of supply by BA treatment of one cotyledon led to a compensatory (2.4- to 3.4-fold) increase in assimilate transported to the apex from the nontreated cotyledon (Fig. 4, A and B). There was no reduction in the total amount of assimilate arriving at the apex (Fig. 4). Inasmuch as floral stimulus is transported with photosynthetic assimilate in *Pharbitis* (9) as in some other species (5, 21), then flowering was promoted or inhibited by application of BA to one or other of the pair of cotyledons. Clearly, BA was acting indirectly on flower induction (category iv).

It follows from the evidence discussed above that BA effects on assimilate-floral stimulus co-transport must be as important in promotion of flowering (both cotyledons induced and treated with BA, Figs. 1–3) as in inhibition of flowering (one of the pair of cotyledons induced and the same one treated with BA, Fig. 4A). When both cotyledons are induced and treated with BA there should be no differential in assimilate-floral stimulus transport.

Nevertheless, total import of ^{14}C-labeled assimilate into the apex was significantly enhanced by BA treatment of both cotyledons (Table II). Also, there was an apparent, although nonsignificant, increase in ^{14}C loss from the cotyledons (Table III). Despite a reduction in total dry matter export following BA treatment (20% less export), promotion of flowering could be expected because of enhanced movement of ^{14}C-assimilate to the apex over the period of export of floral stimulus from the cotyledons. We have no complete explanation for this apparent conflict between dry matter and ^{14}C export data but suggest that: (*a*) the shoot competes more effectively than the root for assimilate so that with a reduction in supply more assimilate and stimulus reaches the apex; (*b*) the specific activity of stimulus was increased per unit of assimilate exported; and (*c*) BA preferentially inhibited remobilization of stored, nonstimulus-containing assimilate so that despite a reduced dry matter export there could be an increase in the relative proportions of current assimilate and of floral stimulus exported to the apex. Irrespective of the final explanation of these data, a 51% increase on average (Table II) in transport of ^{14}C activity to the apex would be of little consequence following BA treatment of one cotyledon only. In the latter situation the resultant 240 to 340% imbalance in transport between the two cotyledons (Fig. 4) would play the dominant role in controlling assimilate flow and flowering.

The implications of our findings of BA action on assimilate-stimulus co-transport are of considerable importance to studies of the biochemistry of floral induction. BA (13), cycloheximide (15), and SK&F 7997-A_3 (2) act indirectly on induction in *Pharbitis* (category iv). All promote flowering when applied to a noninduced cotyledon (Table IV and Fig. 4) and either reduce assimilate export (BA) or inhibit photosynthesis (cycloheximide and SK&F 7997-A_3, Table IV) and thereby reduce assimilate export. Brede and co-workers (3) in a preliminary report have also suggested that SK&F 7997-A_3 influences flowering of *Xanthium* by inhibiting photosynthesis and assimilate export. Ross (15) has also reported that cycloheximide treatment caused necrosis of *Xanthium* leaves so that photosynthesis was probably inhibited as we have found for *Pharbitis*. Whereas sterol or protein synthesis might be a part of induction, all of the data currently available can be explained in terms of assimilate and, hence, stimulus transport. Whether other compounds such as ethylene (17), IAA (14), *p*-fluoro-phenylalanine (4), and even photosynthetic inhibitors such as DCMU (6) act solely on assimilate-stimulus transport awaits testing. Environmental treatments such as temperature shocks, responses to light intensity, anaerobiosis, and CO_2 concentration (see ref. 19) might also be expected to have an effect on assimilate-stimulus transport. Action of applied compounds at the shoot apex itself might also influence assimilate transport. In *Bougainvillea*, but not in *Pharbitis*, cytokinin enhancement of flowering is associated with cytokinin-induced mobilization of assimilate to the shoot apex (18).

Another implication of our findings on assimilate-stimulus co-transport is that the inhibitory effect of noninduced cotyledons in *Pharbitis* may relate to an interference of assimilate from the noninduced cotyledon with transport of assimilate from the induced cotyledon. None of our data (Figs. 4 and 7) compel the conclusion that there is a specific transmissible inhibitor of flowering in *Pharbitis* despite earlier arguments of Imamura (8). There was a direct relationship between inhibition of flowering by a noninduced cotyledon and the transport of assimilate to the apex from the induced cotyledon (Fig. 4, D *versus* C). "Inhibition" in *Pharbitis* can be simply understood in terms of assimilate interference with floral stimulus transport and substantial evidence relating assimilate flow patterns to inhibition of flowering has also been reported for two other short day plants, *Perilla* (10) and *Xanthium* (22). Inasmuch as we cannot finally discount a role for an inhibitor transported along with assimilate, whether examining inhibitory effects of noninduced leaves or responses of flowering to applied chemicals, the transport factor must be examined as one critical link in the chain of events from induction in the leaf to evocation of flowering at the shoot apex.

Acknowledgments—We thank W. Muller (CSIRO) for assistance with statistical analysis and colleagues at CSIRO for comments on the manuscript.

LITERATURE CITED

1. Bodson M, RW King, LT Evans, G Bernier 1977 The role of photosynthesis in flowering of the long-day plant *Sinapis alba*. Aust J Plant Physiol 4: 467–478
2. Bonner J, E Heftmann, JAD Zeevaart 1963 Suppression of floral induction by inhibitors of steroid biosynthesis. Plant Physiol 37: 43–49
3. Brede J, A Kobayashi, JAD Zeevaart 1975 Further studies on the mechanism by which the steroid inhibitor SK&F 7997 inhibits flower formation. *In* Plant Research 74. Annu Rep MSU-AEC Plant Res Lab, Michigan, pp 48–50
4. Collins WT, FB Salisbury, CW Ross 1963 Growth regulators and flowering. III. Antimetabolites. Planta 60: 131–144
5. Evans LT 1971 Flower induction and the florigen concept. Annu Rev Plant Physiol 22: 365–394
6. Evans LT 1976 Inhibition of flowering in *Lolium temulentum* by the photosynthetic inhibitor 3-(,4-dichlorophenyl)-1,1-dimethyl urea DCMU in relation to assimilate supply to the shoot apex. *In* R Jacques, ed, 'Etudes de Biologie Végétale. Hommage au Prof P Chouard. Paris, pp 165–175
7. Fletcher RA, G Hofstra, NO Adepipe 1970 Effects of benzyladenine on bean leaf senescence and the translocation of ^{14}C-assimilates. Physiol Plant 23: 1144–1148
8. Imamura S 1961 The nature of inhibition of flowering by the leaves illuminated continuously during the inductive dark treatment of other leaves in short-day plants. Recent Advances in Botany, Vol 2. University of Toronto Press, pp 1287–1288
9. King RW, LT Evans, IF Wardlaw 1968 Translocation of the floral stimulus in *Pharbitis nil* in relation to that of assimilates. Z Pflanzenphysiol 59: 377–388
10. King RW, JAD Zeevaart 1973 Floral stimulus movement in *Perilla* and flower inhibition caused by non-induced leaves. Plant Physiol 57: 727–738
11. Lindenbaum S, A Kadman-Zahavi 1973 A possible cause of flowering inhibition in partially illuminated seedlings of *Pharbitis nil*. Isr J Bot 22: 199–200
12. Nakayama S, H Tobita, FS Okumura 1962 Antagonism of kinetin and far-red light or β-indoleacetic acid in flowering of *Pharbitis* seedlings. Phyton 19: 43–48
13. Ogawa Y 1961 Über die Wirkung von Kinetin auf die Blütenbildung von *Pharbitis nil* Chois. Plant Cell Physiol 2: 343–359
14. Ogawa Y 1962 Über die photoperiodische Empfindichkeit der Keim Pflanzen von *Pharbitis nil* Chois. mit besonderer Berucksichtigung auf den Wuchsstoftgehalt der Kotyledonen. Bot Mag Tokyo 75: 92–101
15. Ross CW 1970 Antimetabolite studies and the possible importance of leaf protein synthesis during induction of flowering in the cocklebur. *In* G Bernier, ed, Cellular and Molecular Aspects of Floral Induction. Longman's, Green, London, pp 139–151
16. Shindy WW, RJ Weaver 1970 Export of photosynthate effected when leaves are pretreated with growth substances. Nature 227: 301–302
17. Suge H 1972 Inhibition of photoperiodic floral induction in *Pharbitis nil* by ethylene. Plant Cell Physiol 13: 1031–1038
18. Tse ATY, A Ramina, WP Hackett, RM Sachs 1974 Enhanced inflorescence development in *Bougainvillea* "San Diego Red" by removal of young leaves and cytokinin treatment. Plant Physiol 54: 404–407
19. Vince-Prue D 1975 Photoperiodism in Plants. McGraw-Hill, London
20. Zeevaart JAD 1963 Climatic control of reproductive development. *In* LT Evans, ed, Environmental control of plant Growth. Academic Press, New York, pp 289–310
21. Zeevaart JAD 1976 Physiology of flower formation. Annu Rev Plant Physiol 27: 321–348
22. Zeevaart JAD, JM Brede, CB Cetas 1977 Translocation patterns in *Xanthium* in relation to long day inhibition of flowering. Plant Physiol 60: 747–753