

Inhibition of Leaf Processes by *p*-Fluorophenylalanine During Induction of Flowering in the Cocklebur¹

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Received April 20, 1966.

Summary. The amino acid antimetabolite, DL-*p*-fluorophenylalanine (FPA), inhibited induction of flowering in the short-day cocklebur plant, *Xanthium pensylvanicum* Wall., primarily by interfering with processes occurring during the inductive dark period. At the concentrations used the inhibitor had little effect on subsequent vegetative development of the plant.

The inhibition was largely reversed (internally) by L-phenylalanine, but not by D-phenylalanine nor by DL-tyrosine. The FPA strongly inhibited the absorption of labeled phenylalanine, leucine, and glycine, and inhibited the conversion of phenylalanine into protein in experiments where incorporation was separated in time from effects upon absorption. The FPA, too, was incorporated into protein, at nearly half the rate of phenylalanine. Neither D- nor L-phenylalanine significantly interfered with absorption of FPA, showing the FPA did not affect amino acid absorption by simple competition for a common carrier site. It was concluded that FPA may affect flower induction because of its interference with normal enzyme synthesis, although effects on other processes might also be involved.

Several metabolic inhibitors have been found to interfere with flowering in short-day plants (21, 23). Some were applied to the cocklebur, *Xanthium pensylvanicum* Wall., at various times during and after a single inductive dark period to determine their specificity of action. Of these, certain purine or pyrimidine analogs, two amino acid antimetabolites (ethionine and *p*-fluorophenylalanine), and 1 inhibitor of isoprenoid synthesis, were effective only during this dark period (2, 5, 22). Later application, even though still 7 or 8 days prior to determination of the floral response, did not interfere with flowering when these chemicals were used.

Conclusions from some of these studies with the cocklebur are that RNA synthesis is an essential part of induction (3, 5), and, more tentatively, that peptide or protein synthesis is also necessary (5). Induction of *Pharbitis nil* Chois. (Japanese morning glory) requires active DNA multiplication in the bud at the time of arrival of the flowering stimulus (27). Perhaps RNA synthesis in this plant is also essential for induction, since Galun, et al. (10) recently showed that actinomycin D applied to the plumule strongly inhibits its flowering.

In all of the experiments with cockleburs a single leaf was used to provide the flowering stim-

ulus. The need for RNA synthesis during the dark period does not appear to be in the leaf, but is primarily, or perhaps entirely, restricted to the bud (3). This is true even though the plant cannot be induced by darkening only the bud.

If the requirement for RNA synthesis is to form a necessary enzyme or group of enzymes, protein synthesis in the bud would be expected also to be an essential part of induction. We have investigated this possibility using the phenylalanine antagonist DL-*p*-fluorophenylalanine (FPA). FPA inhibited induction 50% at about 0.02 M when the single remaining leaf and bud were treated just before induction (5). Its effect was largely overcome by simultaneous addition of L- or DL-phenylalanine. Inhibition was observed when FPA was applied only to the leaf, and, less consistently, when only the bud (shoot apex) was treated. At the time it was not possible to positively conclude whether this compound interfered with processes occurring in the leaf, the bud, or both.

The present studies were designed to determine whether FPA interferes with induction in the donor leaf or in the bud, or in both, and whether protein synthesis is the sensitive process involved.

Materials and Methods

General Techniques of Handling Plants. Growth, induction, and methods of evaluating the floral response of the *Xanthium pensylvanicum* Wall. (cocklebur) plants were similar to those

¹ Supported by grant R01 GM 06374 from the National Institutes of Health.

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described by Salisbury (20). The plants were grown in soil-containing pots from seed kindly supplied by Dr. James Bonner. They were kept vegetative prior to use by extending the day-length in the greenhouse to about 20 hours with incandescent light of approximately 50-foot candles. When plants reached two months old they could usually be used in the flowering experiments. Those in which the blade of the third or fourth leaf longer than 1.0 cm (numbering from the shoot-tip) was between 6.9 and 8.5 cm long were selected. This leaf is the one capable of providing the maximum flowering response (12). The leaf just above and all older leaves were removed along with their axillary buds. Except where noted, plants were placed on carts and pushed into a dark room for one 16-hour inductive dark period. They were then returned to the greenhouse and maintained for 8 or 9 days under the usual 20 hour photoperiod. At the end of this time the floral stage was determined according to the system of Salisbury (19).

Application and Source of Chemicals. The FPA or other chemicals were applied by dipping the leaves or shoot-apex directly in solutions of the chemicals. Tween 20 (2 drops per 100 ml) was used as a wetting agent to increase penetration. The FPA used was the DL-form supplied by Calbiochem. or by the Nutritional Biochemical Corporation. Phenylalanine and tyrosine were obtained from the same sources. The particular isomer used for various experiments is specified in the Results section.

In the leaf disc experiments a cork borer was used to cut discs 1.2 cm in diameter from the photoperiodically sensitive leaves of several plants selected for uniformity. These discs were pooled in distilled water, removed and blotted, and 10 or 20 placed in each bottle used for the incubation studies. The bottles were either 2-ounce or 4-ounce Skrip ink bottles containing side wells to which 5% KOH was added to collect CO₂. The leaf discs were incubated in 0.02 M sodium-potassium phosphate buffer at a pH of 5.8 to prevent trapping of the CO₂. Radioactive chemicals were dissolved in the buffer. DL-phenylalanine-3-¹⁴C was obtained from the New England Nuclear Corporation. This compound is labeled on the carbon of the alanine side chain adjacent to the ring. L-leucine-1-¹⁴C and DL-glycine-1-¹⁴C were obtained from Calbiochem. DL-*p*-fluorophenylalanine-3-¹⁴C was purchased from Volk Radiochemical Company.

When labeled amino acids were added to the leaves of intact plants, the leaves were first wetted in water containing Tween 20 and allowed to partially dry. Then 0.10 ml of the compounds was spread over the upper surface of the moist leaves using a micro-pipette. The plants were immediately placed in darkness as for flowering induction.

Tissue Analysis. In the leaf disc experiments the ¹⁴CO₂ collected in the wells was analyzed by

a direct plating technique described previously (18). The leaves or leaf discs were thoroughly rinsed in tap water to remove adhering radioactive chemicals. They were then either frozen at -20° for later analysis or were immediately homogenized. Homogenization techniques to extract soluble amino acids and proteins were similar to those of Crook (7). Leaf tissue (frozen discs or an individual leaf) was placed in 25 ml of cold distilled water adjusted to pH 8.5 with NaOH. A Servall omnimixer (Ivan Sorvall, Inc.) was used for grinding, and an ice bath kept the homogenate cold. The cell fragments were filtered off using filter paper and were discarded. Protein was precipitated from the filtrate by adding trichloroacetic acid to a final concentration of 5%. Occasionally the protein was precipitated by heating to 80° for 1 hour. The heating technique gave less protein but its specific activity was the same as that obtained by the acid precipitation when phenylalanine-3-¹⁴C had been incorporated. Acid precipitated protein was washed with 0.5 N trichloroacetic acid at room temperature, then with acetone at room temperature, and finally with boiling acetone. Protein was dissolved in 2.5 ml of 5 N KOH at 50°. The volume was adjusted to 25 ml with water and samples were then taken for determination of radioactivity and for protein analysis by the Lowry procedure (13).

After removal of protein, the remaining extract was analyzed for radioactivity and when chromatography of labeled amino acids and other metabolites was performed, the extract was either air-dried or freeze-dried. Paper chromatography was performed using *n*-butyl alcohol, glacial acetic acid, and water (BAW, 2:1:1 v/v) as the first solvent and *t*-butyl alcohol, methyl-ethyl ketone, water, ammonium hydroxide (5-BKW, 4:3:2:1 v/v) as the second. Chromatograms were placed against Kodak Blue-Brand X-ray film to locate radioactive compounds. The amino acids were detected with a ninhydrin spray.

Radioactive Counting Techniques. All samples for ¹⁴C analysis were plated on stainless steel planchets and counted under a Nuclear-Chicago model D-47 gas flow geiger tube. Self absorption corrections were made for all samples except the soluble extracts where self absorption was negligible and in the case of some of the powders prepared from oven-dried leaf discs or leaves. Where quantitative estimates of the translocation of *p*-fluorophenylalanine were made the tissue was oven dried and aliquots combusted to CO₂ by a wet oxidation technique previously described (25). The CO₂ was collected in KOH, precipitated as BaCO₃, and the Ba¹⁴CO₃ counted and corrected for self absorption.

Results

The Site of Action of FPA. We first attempted to determine whether FPA interfered with proc-

esses in the leaf or in the bud that are essential to flowering. In most of the studies no effect was obtained when only the shoot-tip was dipped in the inhibitor (0.02 M), but occasionally a small inhibition was noted. Leaf application consistently reduced the observed floral stage. When concentrations as high as 0.04 M were used, FPA inhibited flowering when applied to either site. Leaf application was more inhibitory, although in this case approximately 10 times as much chemical was added to the plant due to the difference in wettable surface area.

We found that translocation of FPA- ^{14}C from the shoot-tip to the leaf during the 16 hour inductive period is almost negligible. In 1 experiment, for example, a maximum of 0.02% of the ^{14}C added to the shoot-tip was recovered in the leaf. The possibility cannot be eliminated that some of this radioactivity actually moved as $^{14}\text{CO}_2$ formed from catabolism of the labeled inhibitor, and was incorporated by dark fixation into the leaf. Even if actual translocation of FPA does occur, it seems very unlikely that such traces would be sufficient to account for the inhibition of flowering resulting from shoot-tip application. It is thus probable that FPA, like 5-fluorouracil (3), does inhibit processes occurring in the bud during photoperiodic induction. Additional evidence for this will be cited later.

We also noted in various experiments that the movement of FPA from the leaf to the bud during induction was small. This, along with the greater inhibition of flowering due to leaf treatment, suggested that FPA-sensitive processes also take place in the leaf. Another experiment was designed to determine if this is true. Instead of using plants trimmed to 1 photoperiodically sensitive leaf, as in the above experiments, 2 additional leaves were left on the plants, 1 immediately above and 1 just below.

Table I. *Inhibition of Cocklebur Flowering by DL-p-fluorophenylalanine (FPA).*

FPA was added to the plants by dipping either the covered leaf or the 2 largest leaves above the covered leaf directly in the solution. In no case was the apical bud dipped in the inhibitor. DL-p-fluorophenylalanine-3- ^{14}C (3.5 mc/mmmole) was then added by spreading 0.10 ml over the upper surface of the treated leaves with a micropipette. Black envelopes were used to cover the lowest remaining leaf of all plants and were removed after 16 hours, as described in the text. Continuous lanolin rings were placed around the base of the leaf petioles to prevent creeping of ^{14}C to the apical bud. Buds were harvested immediately after the 16-hour period, oven dried, then wet combusted to CO_2 and radioactivity counted as BaCO_3 .

	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
No. plants used per treatment	25	30	30	30	30
Cpm FPA- ^{14}C added per plant ($\times 10^{-3}$)	93	133	86	113	670
FPA added ($\mu\text{mole/plant}$)					
To uncovered leaves	24	22	21	19	20
To covered leaves	33	35	28	26	28
Cpm found in apical bud					
From uncovered leaves	50	55	3	3	16
From covered leaves	5	0	3	5	29
Avg flowering stage					
Controls	3.2	2.3	2.5	1.0	3.3
FPA on uncovered leaves	2.6	2.5	2.9	1.2	2.8
FPA on covered leaf	1.8	1.2	1.5	0.5	2.1

Three groups of 25 or 30 plants each were induced to flower by covering the lowest leaf (8 to 9 cm long) with black construction paper for 16 hours. With one of these groups, the leaf to be covered with paper was dipped in 0.04 M FPA. With a second group, the 2 leaves just above the 1 to be darkened were dipped in the FPA, and in the third group, no inhibitor was added (controls). By this method the treated plants received about 70% as much FPA when the 2 uncovered leaves were dipped as when only the covered leaf was dipped. Starting at 4 PM, plants were placed under 8 hours of incandescent light of approximately 50 ft-c intensity, then kept in darkness 4 hours, then under normal greenhouse light during the remaining 4 hours.

Results of 5 experiments using this technique are summarized in table I. The floral stages are relatively low in all cases, probably due to the fact that the leaf covered is not optimum for production of the floral stimulus (12), and also because the presence of additional leaves kept in light causes poor flowering (23). Inhibition of flowering due to FPA (35 to 50% in all experiments) resulted only if this chemical was added to the covered leaf in which inductive processes were occurring. Treatment of the 2 leaves immediately above (not including the shoot apex) was never significantly inhibitory.

Translocation of ^{14}C in labeled FPA to the bud was barely detectable in both cases. If inhibition of flowering had resulted only from translocation of leaf-applied FPA to the bud, this inhibition should have been as great when FPA was applied to the uncovered leaves. These results demonstrate that FPA interferes with some reaction essential to flowering which occurs in the leaf providing the floral stimulus. This conclusion is supported by the fact that L-phenylalanine added to the shoot-tip did not

Table II. *Inability of L-Phenylalanine Added to Cocklebur Shoot-Tips to Reverse the Inhibition Caused by Leaf-Applied FPA*

Plants were treated by dipping the leaf or shoot-tip in the indicated solution just before a 16-hour inductive dark period, and the floral stages were measured 9 days later. The no. 3 leaf is that most effective in causing flowering and was the only donor leaf remaining on the plants when induced. Oven-dry weights of this leaf and those which developed above it during the 9 days prior to dissection were measured. All values are means of 20 plants.

Treatment	Avg floral stage	Final dry weights	
		Original no. 3 leaf	Younger leaves
Distilled water (controls)	4.1 ± 1.6*	294 mg	135 mg
0.04 M FPA on no. 3 leaf	2.7 ± 1.1	293 mg	137 mg
0.04 M FPA on no. 3 leaf and 0.04 M L-phenylalanine on shoot-apex	2.7 ± 0.65	295 mg	131 mg

* Standard deviation.

overcome the inhibition caused by FPA added only to a single remaining donor leaf (table II). Table II also illustrates that this compound interferes with flowering without necessarily influencing the increase in dry weight of the leaves.

The Time of Inhibitory Action of FPA. If an inhibitor interferes with flowering only because it affects the induction process, rather than by causing a general inhibition of growth and development, this can be determined by time-of-application curves (20). It was shown previously that 0.02 M FPA, applied simultaneously to the leaf and bud, inhibits flowering only if added during the inductive dark period (5). In more recent experiments we have

used 0.04 M concentrations of FPA, applied to the leaf or bud of separate groups of plants.

These higher concentrations of FPA added to the single donor leaf inhibited primarily, although not entirely, during the dark period (fig 1). Some retardation of floral development occurred upon application even 24 hours after the end of this inductive period. Bud application of FPA caused inhibition regardless of the time of treatment, suggesting that a rather non-specific effect was involved.

It might be suspected that the inhibitions noted from applications after the inductive dark period are due to a general inhibition of growth. This, however, does not appear to be true, since 0.04 M FPA did not inhibit the final dry weights of either the donor leaf or of the combined younger leaves, regardless of the site of application. In 1 experiment, for example, the average final dry weights of the donor leaves at the time of dissection were as follows: A) controls, 285 mg; B) 0.04 M FPA added to donor leaf, 296 mg; C) 0.04 M FPA added to shoot-tip, 299 mg (also see table II). Perhaps FPA interferes with necessary processes occurring after the inductive dark period, such as transport of the flowering stimulus to the bud or differentiation of the bud itself. In any case, FPA interfered most with flowering when added to the donor leaf before or during the inductive dark period.

The Mechanism of Action of FPA. We first determined whether FPA influenced the respiration rate of cocklebur leaves, using a Warburg apparatus. No significant effect upon respiration was observed when donor leaves were dipped in 0.04 M FPA, as in the flowering studies, and the rates measured 9 hours later. Similarly, 4 mM FPA added directly to the Warburg flasks did not significantly influence the oxygen uptake of leaf discs, suggesting that a general interference with respiration is not responsible for the effect upon flowering.

The inhibitory action of FPA upon protein synthesis observed in animal and microbial systems (6, 17) suggested that this compound might interfere with this process in cocklebur leaves. Most of the experiments designed to test this possibility

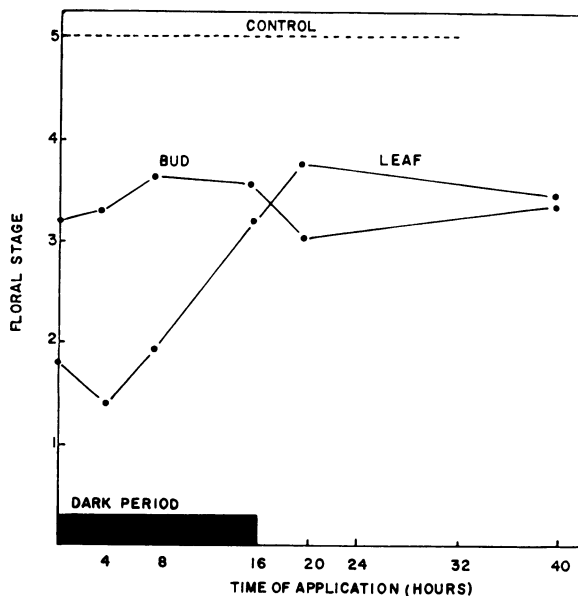


FIG. 1. The inhibitory effect of FPA upon flowering when applied at various times during and after the inductive dark period. Either the leaf or the shoot-tip (bud) was dipped in a solution of 0.04 M FPA at the times indicated. Each point represents a mean of 3 experiments in which each treatment had 15 or 20 plants.

Table III. *Influence of FPA Upon Incorporation of Amino Acids into Protein of Cocklebur Leaves or Leaf Discs*

Leaf results are means from 5 leaves and are representative of 2 experiments. One donor leaf of intact plants was treated with 0.5 μ c of DL-phenylalanine-3-¹⁴C (7.55 mc/mmmole) after dipping in water (controls) or 0.04 M FPA, and was analyzed after 16 hour darkness.

Leaf disc results with DL-Phe-¹⁴C are means of 4 flasks and are representative of 5 experiments. Twenty leaf discs (600 mg fr wt) were incubated 4 hours in darkness at 28°. Flasks contained 10 ml of P₁ buffer at pH 5.8 with 1.0 μ c of DL-Phe-¹⁴C. Leucine and glycine results are means of 6 flasks containing 1.0 μ c each of L-leucine-1-¹⁴C (8.6 mc/mmmole) or DL-glycine-1-¹⁴C (23.6 mc/mmmole), 5.0 ml of buffer, and 10 leaf discs.

Experiment	Labeled amino acid	Inhibitor	Counts/min/flask		
			CO ₂	Soluble extract	Protein
Leaves	DL-Phe-3- ¹⁴ C	None	...	36,100	10,300
		4 mM FPA	...	7580	1500
Leaf discs	DL-Phe-3- ¹⁴ C	None	736	394,000	23,400
		4 mM FPA	408	88,600	3920
	L-Leucine-1- ¹⁴ C	None	68,500	79,800	15,200
		4 mM FPA	9520	15,300	3180
	DL-Glycine-1- ¹⁴ C	None	36,600	78,900	14,100
		4 mM FPA	14,800	16,200	4260

were done with discs cut from leaves of a size and age optimum for causing flowering, because more reproducible results could be obtained than with intact leaves. Nevertheless, some of the results with leaf discs were verified by 1 or more experiments with intact plants.

That FPA strongly inhibited the incorporation of labeled phenylalanine into protein of either leaf discs or intact leaves is evident in table III. However, the total amount of phenylalanine absorbed was also strongly inhibited by FPA, masking any effect on protein synthesis which may have occurred. The effect upon absorption can be seen by comparing the radioactivity in the soluble aqueous extracts, which we have found by paper chromatography to consist primarily of unmetabolized phenylalanine. This fraction contained most of the radioactivity absorbed by the leaf discs, but the insoluble residues obtained when the tissues were homogenized also contained radioactivity, and incorporation into this fraction was similarly inhibited (data not shown). The conversion of phenylalanine-3-¹⁴C into ¹⁴CO₂ was affected less by FPA than was absorption and incorporation into protein.

Because of the marked effect of FPA upon phenylalanine absorption by the tissues, labeled glycine and leucine were used as tracers to measure protein synthesis, since it was suspected that FPA would not appreciably interfere with their uptake. Table III summarizes results of separate experiments showing that the FPA did inhibit uptake of both glycine and leucine in cocklebur leaf discs, the effect being approximately as great as the inhibition of phenylalanine absorption. Thus, a significant difference in protein synthesis cannot be demonstrated by incubating the leaf discs simultaneously with both the inhibitor and any of these amino acids. It was necessary to perform experiments in which uptake of the labeled amino acid

were separated in time from the effect of the inhibitor.

Table IV lists the results of averages from 2 such experiments in which FPA inhibited incorporation of previously absorbed phenylalanine into protein by nearly 40%. If corrections had been made for the amount of incorporation occurring during the first hour when FPA was absent, the inhibition would probably have been even greater, but this was not measured. It may be concluded that FPA does inhibit protein synthesis in cocklebur leaf discs. This experiment was not performed with intact leaves, but it is assumed that FPA would also interfere with protein synthesis in these leaves, especially since the effects on amino acid absorption are the same as in leaf discs (table III).

FPA itself has been shown to be incorporated into protein of certain microorganisms and animal

Table IV. *Inhibition by FPA of the Incorporation of Previously Absorbed Phenylalanine into Protein of Cocklebur Leaf Discs*

Values are means of 2 experiments, each having 4 replications per treatment. Twenty leaf discs were incubated in flasks containing 10 ml buffer and 3.0 μ c of DL-Phe-3-¹⁴C (2.5 mc/mmmole) for 1 hour at 28° in darkness. Tissue was rinsed and placed in fresh buffer without labeled phenylalanine for 3 hours. Half of the fresh buffer solution contained 4 mM FPA, the other half contained no FPA (controls). Analyses were made after the second, 3 hour incubation.

Treatment	Counts/minute		
	Soluble extract	CO ₂	Protein
Control	70,250	228	16,800 (2410)*
4 mM FPA	76,000	256	10,700 (1510)

* Counts/min/mg protein

cells, replacing phenylalanine (1, 11, 17). In *Escherichia coli*, FPA replaced the majority of phenylalanine in the proteins, while synthesis occurred at about half the normal rate (see ref 17). The incorporation of other amino acids into protein was not affected by FPA. Fowden (9), working with radicles of germinating mung bean seedlings, found a phenylalanine-reversible FPA inhibition of growth, but observed no incorporation of FPA into protein. Experiments were therefore conducted to determine if such incorporation occurs in cocklebur leaves, since this phenomenon might lead to the formation of proteins incapable of catalyzing 1 or more of the chemical reactions necessary for flowering.

Table V lists data showing that when equal amounts of labeled FPA and phenylalanine were added to leaf discs, about 94% as much FPA was absorbed, and 44% as much radioactivity from FPA was incorporated into the soluble protein fraction. To confirm that this ^{14}C was indeed in the FPA molecule, the protein was hydrolyzed by boiling 16 hours in 5 N KOH. The hydrolysate was neutralized with perchloric acid, chilled to remove insoluble KCIO_4 , and the amino acids chromatographed 2-dimensionally with unlabeled carrier FPA. FPA moved with phenylalanine in the first solvent (BAW), and was just resolved from phenylalanine in the second solvent (t-BKW). When autoradiographs were made of the chromatograms from the protein hydrolysates of discs incubated with labeled FPA, the single exposed area on the film coincided exactly with the position of FPA on the chromatograms located with ninhydrin. This indicates that FPA can indeed be incorporated into proteins of plant tissues.

Failure of Tyrosine to Reverse the FPA Inhibition of Flowering. The ability of DL-phenylalanine to reverse the inhibition of flowering due to FPA suggests that FPA acts as an inhibitor of functions of endogenous phenylalanine, and likely replaces phenylalanine in proteins to some extent. It was also considered possible that because FPA is similar in structure to tyrosine, it might inhibit flowering because of an interference with tyrosine metabolism. However, concentrations of tyrosine up to 0.05 M had no influence on flowering when applied alone, and did not reduce the FPA inhibition of flowering.

It does not appear that FPA acts as an inhibitor of functions of tyrosine which are involved in induction of flowering of this plant.

The Requirement for L-phenylalanine to Reverse the Flowering Inhibition of FPA. Although our previous results (5) showed effects of FPA to be overcome by approximately equal concentrations of DL-phenylalanine, we had since determined that L-phenylalanine is also capable of this reversing action (unpublished data). When it was observed in the present experiments that FPA strongly inhibited the absorption of phenylalanine by leaf discs or by leaves used in flowering studies, concern arose that phenylalanine might reverse the FPA flowering inhibition only because it similarly interferes with uptake of FPA into the leaves. Consistent with such a possibility was the previously established observation (5) that phenylalanine added simultaneously with FPA completely eliminates appearance of the few necrotic lesions otherwise caused by the latter. If phenylalanine were to overcome the inhibition of flowering due to FPA solely by preventing its absorption by the leaves, this would cast doubt on the conclusion (5) that phenylalanine, and perhaps protein synthesis, are directly involved in the induction process.

Two kinds of experiments show that phenylalanine overcomes the FPA effect by an internal mechanism, and not by competition for absorption. Indirect evidence was first obtained by comparing the ability of D- and of L-phenylalanine to reverse the flowering inhibition of 0.04 M FPA. It was thought that if the L-isomer competes for an uptake site, the D-isomer should, too, and would thus also reverse the effect upon flowering.

We found that L-phenylalanine largely overcame the flowering inhibition caused by FPA, while D-phenylalanine did not. Neither isomer alone was inhibitory. However, both D- and L-phenylalanine were equally effective in preventing vegetative injury to the leaf by FPA. It is not understood how D-phenylalanine can prevent injury symptoms and yet not overcome the effect on flowering. One possibility might be that a slow racemization of the D- to the L-isomer occurs in the cells, which is not completed rapidly enough to affect inductive processes, but which can prevent the injury to the leaves which appear later.

Table V. *Incorporation of ^{14}C from Labeled FPA and Phenylalanine into Protein of Cocklebur Leaf Discs*

Twenty leaf discs were incubated in 10 ml buffer for 4 hours in darkness at 28°. Bottles contained 2.0 μC of DL-Phe-3- ^{14}C or DL-FPA-3- ^{14}C (each at 2.5 mc/mole). CO_2 was collected in 1.0 ml of 5% KOH. Data are representative of 2 experiments.

Amino acid	Counts/min		
	Soluble extract	CO_2	Protein
DL-phenylalanine-3- ^{14}C	374,000	480	18,900 (8880)*
DL- <i>p</i> -fluorophenylalanine-3- ^{14}C	351,000	164	8300 (3800)

* Counts/min/mg protein

Direct evidence that L-phenylalanine prevents the full inhibition of flowering caused by FPA by an internal mechanism was demonstrated by uptake competition experiments using ^{14}C -labeled FPA and phenylalanine. In both intact leaves and leaf discs D- and L-phenylalanine each caused only slight reductions (0 to 15%) in the amount of DL-FPA- ^{14}C absorbed, but FPA significantly decreased the absorption of DL-phenylalanine- ^{14}C . The lack of influence of L-phenylalanine upon absorption of FPA almost surely eliminates the possibility that this is the mechanism of its effective action in reversing the FPA-caused inhibition of flowering. These observations raise an interesting question as to the mechanism of the inhibitory action of FPA upon phenylalanine absorption, although it is clear that a simple competition for an identical carrier site is not occurring. This is to be contrasted with microbial results where it was concluded that phenylalanine and FPA follow the same route into the amino acid pool (17).

Discussion

The results presented here show that FPA is inhibitory to flowering of the cocklebur because of effects occurring in both the donor leaf and in the receptive bud. The time-of-application results indicate that the principal site of action is in the leaf and that the effects on the bud are less specific and less marked. In these respects FPA therefore acts somewhat differently than does 5-fluorouracil (3).

Data in tables IV and V show that FPA blocks the incorporation of phenylalanine into protein and that it is incorporated into cocklebur leaf proteins, probably replacing phenylalanine (17). It is possible that the inhibition of protein synthesis occurs because FPA is incorporated only about half as rapidly as phenylalanine, although other explanations are certainly not eliminated. An inhibition of normal protein formation may well be the mechanism by which FPA interferes with induction of flowering in this plant. If this is true, it suggests that during normal inductive dark periods processes leading to the formation of enzymes specific for flowering occur. These enzymes might be necessary to synthesize the flowering stimulus, or to destroy flowering inhibitors, for example. FPA might act by reducing the rate of their formation or might render them ineffective when it is present as a part of such enzyme molecules.

We have no evidence, however, that protein synthesis is the only process involving phenylalanine with which FPA interferes. As pointed out by Mann (14), conclusions arrived at by the use of metabolic inhibitors in physiological experiments are often only as valid as the specificity of the inhibitor. Perhaps FPA simply blocks transport of

the floral stimulus from the leaf by effects unrelated to protein synthesis.

The interesting, although poorly understood, effects of FPA upon amino acid absorption (table III) suggest that this compound might affect transport of other solutes. The inhibitory influence of FPA upon absorption of phenylalanine, leucine, and glycine is rather similar to that of D-serine upon the uptake of other amino acids, as observed by Ellis (8). D-serine did not inhibit respiration, but reduced the absorption of 6 inorganic ions and several amino acids. It seemed to somehow uncouple salt uptake from respiration. We have not yet determined whether FPA also interferes with the absorption of essential inorganic ions, but did find that L-phenylalanine does not overcome the inhibitory effect of FPA upon absorption of amino acids (unpublished data).

Phenylalanine is presently believed to be a precursor of other aromatic compounds, including *trans*-cinnamic, *p*-coumaric, caffeic, and ferulic acids, and of flavonoids which probably arise, in part, from these acids (15). In pea seedlings (4) and in sorghum mesocotyl (24) red light influences the hydroxylation pattern on ring B of the flavonoids, the ring derived from phenylalanine. The fact that both red light interruptions and FPA applications inhibit induction of flowering in the cocklebur suggests the possibility that both are effective because of influences upon flavonoid formation. Additional possible connections between flowering and phenolic compounds were reported by others (5, 26, 28). That FPA might affect flowering of the cocklebur through an inhibition of normal phenolic metabolism is being investigated.

Recent work suggests that FPA does not inhibit flowering by blocking the conversion of phenylalanine to flavonoid precursors such as *trans*-cinnamic and *p*-coumaric acids. Both of these acids were completely ineffective in overcoming the effect on flowering due to FPA, and had no effect when added alone.

Acknowledgments

The competent technical assistance of Janet Thomas and Annelee Johnson is appreciated.

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