

JUVENILE PHOTOPERIODIC SENSITIVITY IN *ANAGALLIS*
ARVENSIS L. SUBSP. *FOEMINA* (MILL.) SCHINZ & THELL.

By L. A. T. BALLARD* and A. E. GRANT LIPP*

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Summary

Exposure to long days of 20 hr light of young plants of *A. arvensis*, when they possess cotyledons and two slightly expanded first leaves, causes flower initiation. One long day evokes no, or only slight, response, two long days a substantial but variable response, and three complete initiation.

By giving varying numbers of long days to plants at different stages of development, and also by removal of plant parts before treatment, it has been shown that cotyledons, as well as leaves, perceive day length and at least contribute to floral induction. While it was shown that small leaves alone are competent in induction, this was not proven for cotyledons, though it is probably true.

An axillary meristem remains labile for only a few days before being determined vegetatively or florally, according to the conditions. At this stage, it probably consists of no more than a few cells.

Anagallis plants become harder to induce as they age, as judged by the amount of flower initiation produced by treatment of (1) leaves of a given rank at different ages (sizes); (2) leaves of comparable size but different rank; (3) laterals inserted at different levels.

I. INTRODUCTION

For some time after Knott (1934), Moshkov (1936), and Chailahjan (1936) showed that the leaves of plants perceive the photoperiodic stimulus it was held that the youngest fully expanded leaf is the most sensitive to it (Borthwick and Parker 1940; Naylor 1941). Later, Khudairi and Hamner (1954) showed that, in *Xanthium*, the half-expanded leaf is most sensitive.

From the incapacity of some very young plants to be induced (Borthwick and Parker 1938) cotyledons were held not to be percipient. While this has been shown to be true in *Xanthium* (Jennings and Zuck 1954), there are now reports of the photoperiodic induction of flowering in juvenile plants, in some of which the cotyledons are clearly effective (Sivori and Went 1944; Zierliacks 1952; Kujirai and Imamura 1958; Cumming 1959), and the situation may not be as uncommon as implied by Evans (1960).

Anagallis arvensis L. subsp. *foemina* (Mill.) Schinz & Thell. is a sensitive long-day plant with fully assimilatory cotyledons (Chouard 1949). It may flower early and at a low node. Objectives of the present work were to assess the contributions made by cotyledons and leaves, and to determine any changes with time in the effectiveness of these organs, or in the sensitivity to induction of potentially floral meristems. Except in *Perilla* (Lona 1959), the necessity for an active meristem to be

* Division of Plant Industry, CSIRO, Canberra.

present at the time of inductive treatment is generally recognized (Carr 1953*a*, 1953*b*; Salisbury 1955; Lincoln, Raven, and Hamner 1958; Kujirai and Imamura 1958). However, in analyses of flowering responses, less attention has been devoted to variation in meristematic activity than to changes in foliar properties.

II. METHODS

The origin of the seeds used and procedures for their germination have been described by Grant Lipp and Ballard (1963). Seeds were imbibed and held in the dark at 25°C until they became maximally sensitive to red radiation, to which they were then exposed in saturating amount. Plant ages and times and durations of treatments are stated in days, starting from the day on which red irradiation initiated germination. Germinated seeds, selected for uniformity, were sown singly, or in groups, in pots containing vermiculite, which were flushed daily with a modified Hoagland's solution.

The plants were grown in rooms held at $20 \pm 1^\circ\text{C}$, under banks of white fluorescent tubes and tungsten filament lamps (wattage ratio 6.1 : 1) providing 1500–1700 f.c. at plant surface during the high intensity light period, and 40–50 f.c. (from tungsten filament lamps only) during supplemental low intensity light periods. The standard conditions used were (a) 8 hr high intensity light followed by 16 hr dark—this is referred to as a short day; (b) 8 hr high intensity light, followed by 12 hr low intensity light, followed by 4 hr dark—this is referred to as a long day. Unless otherwise stated, plants were established and grown under short days until a desired stage was reached, subjected to various long-day treatments, and returned to short days.

Plants were examined under a magnification of 30 diameters 10–18 days after the start of long-day treatment for the presence and distribution of flower initials. Except in one set of experiments, in which lateral axes were specifically investigated, examination was restricted to the primary axis. Flower development was assessed according to a series of stages chosen to give a linear relation with time (Fig. 1), the slope thus representing the rate of development (Salisbury 1955).

Before plants were allotted to treatments, cotyledon and leaf areas were determined. Plants were then selected either for uniformity, or for a desired range, according to requirement. Areas were determined by the use of regression formulae connecting area and maximum breadth, which was measured *in situ* under magnification. Unless otherwise stated the areas cited are those of the two cotyledons, or leaves, at a given node at the beginning of long-day treatment, and they have an accuracy of the order of $\pm 5\%$.

Excision of cotyledons and leaves was done under magnification to ensure that minimal fragments remained.

Analyses of variance were done on values of percentage flower initiation transformed to degrees.

In all experiments, rooms or cabinets designed to be under short-day conditions were monitored by keeping in them control plants which were later examined for flower initiation. No flowers were ever initiated, and the zero entries for all short-day controls have been excluded from the tables.

III. RESULTS

(a) *Growth Habit and Early Development*

This general account of the early development of *Anagallis* in relation to its photoperiodic behaviour provides a background for the interpretation of the detailed data presented later. The descriptions are in terms of what is visible at a magnification of 30 diameters. Valuable histological descriptions of the vegetative and reproductive apices have been given by Vaughan (1955); but these were not closely related to developmental aspects.

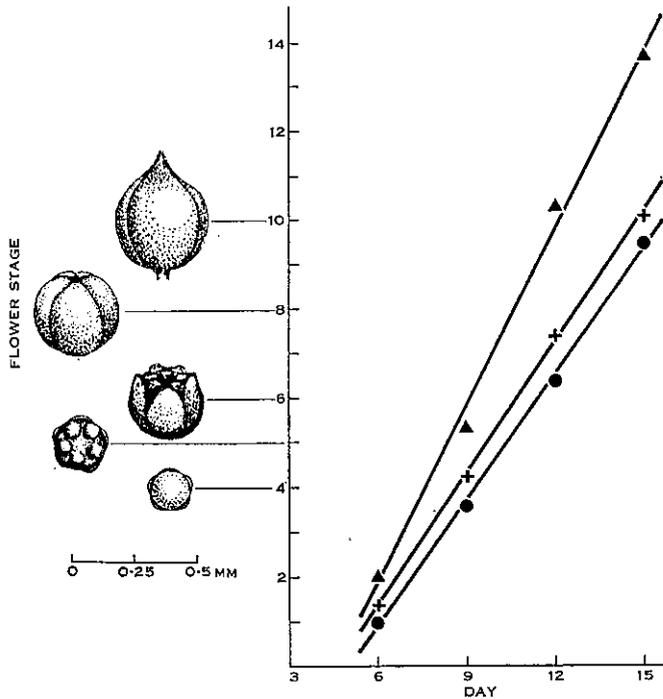


Fig. 1.—Development of the oldest flower initials induced by various long-day treatments. Time measured from start of each long-day treatment. Flower initial development assessed by allotting scores corresponding to selected stages, some of which are depicted. Stages greater than 10 defined by length. ● 2 long days; + 3 long days; ▲ continued long-day treatment. The slope of the continued long-day line differs from that of each of the other two ($P < 0.01$); the slopes of the latter do not differ significantly ($P > 0.05$).

In the seed, the plumular growing point is flat and inconspicuous and no foliar structures other than the cotyledons, which are not storage organs, are differentiated. Cotyledons are carried above ground by the hypocotyl by day 5 or 6 and green rapidly. After this time, a leaf pair is segmented from the apical meristem every $3\frac{1}{2}$ –4 days under the standard short-day conditions. The leaf arrangement is decussate. As the apical meristem assumes the rounded form and the dimensions found in adult plants, two to three pairs of leaf primordia accumulate at the apex. By day 20 there are six

nodes, including the cotyledonary node, and of these four are macroscopically visible; the cotyledons are fully expanded and the first leaves about half-expanded (Fig. 2).

By the time the second leaves have been differentiated, meristems in the axils of the cotyledons are visible. In the vegetative state the relative rates of leaf and axillary meristem production are such that the youngest visible meristem is situated consistently at the second or third leaf behind the apical growing point.

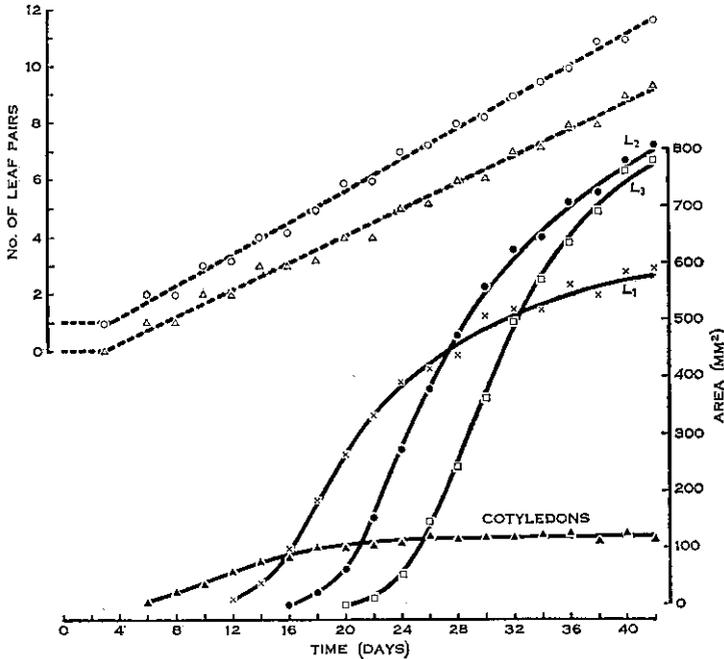


Fig. 2.—Leaf initiation and leaf growth of the young *Anagallis* plant. Time is measured from the irradiation which initiated germination. Areas are for the leaf pair at each node. ▲ cotyledons; × first leaves; ● second leaves; □ third leaves. △ number of leaf pairs (including cotyledons) visible macroscopically; ○ number of leaf pairs (including cotyledons) visible at a magnification of 30 diameters.

Although the conditions on both sides of the growing point, and in leaf axils less than 1 mm distant from it, would be expected to be very uniform, the two leaf primordia of a pair seldom arise precisely at the same time, nor do any two corresponding axillary meristems. From the size disparity apparent shortly after the more retarded one is first visible, the difference which may occur in their ages is estimated to be 1–2 days. As the leaves expand, any obvious disparity in size decreases rapidly and becomes unimportant; but it will later be seen that the initial age difference in axillary meristems situated at the same node affects the initiation of flowers there.

In short days at or above 20°C the plants remain vegetative indefinitely, the axillary meristems producing shoots the organization of which is identical to that of the primary axis. Apical dominance is virtually absent and a plant thus rapidly

develops many orders of branching. Each internode commences to elongate when three leaf pairs above it have been differentiated.

In long days the plants become reproductive (quantitative aspects are considered later). The terminal meristems of any existing axes continue to segment decussately arranged leaves; but the associated axillary meristems become determined as flowers.

It will be shown later that the number of long days required for the initiation of flowers is less than the number of days elapsing between the formation of successive nodes, and that a single partly expanded leaf pair is competent in induction. It is thus possible to determine where meristems which become floral were located during

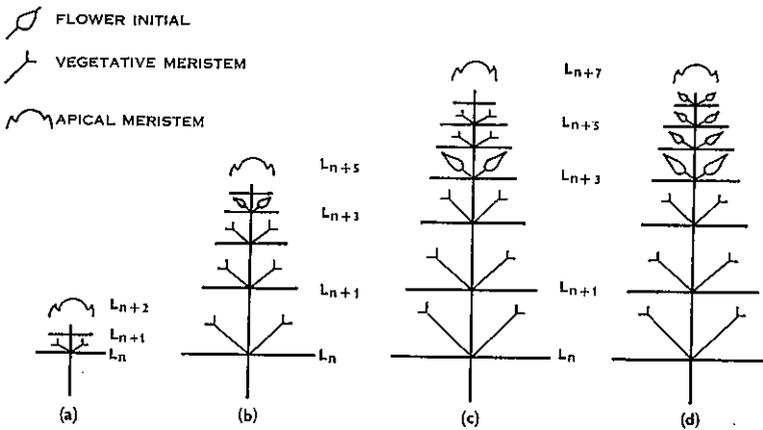


Fig. 3.—Schematic representation of the distribution on the primary axis of vegetative meristems and flower initials following various long-day treatments. For convenience the leaves are depicted in one plane; the phyllotaxis is actually decussate. L_n denotes the third leaf behind an apical meristem at the start of treatment: (a) previously in short days—at the start of treatment; (b) and (c) after 11 and 18 days respectively, the first two of which were long days; (d) after 18 long days.

the long-day exposure. Figure 3 illustrates the result when the third partly expanded leaf pair behind the growing point (shown as L_n in Fig. 3) is exposed to long days. As noted above a vegetative meristem is already visible at that node but not higher. The first flower to arise is situated at the third node above the perceptive leaf pair, i.e. in the axil of a leaf which was not present at the time of induction.

Most theories of photoperiodism incorporate the concept of the transport of some promotive stimulus from the percipient leaves to a meristem, during or shortly after their exposure to inductive conditions, and this initiates further events culminating in the determination of flowers. On this basis, the axillary meristems of *Anagallis* are labile to a certain stage. In the absence of the stimulus from leaves exposed to long days a meristem develops vegetatively, in its presence it becomes determined as a flower.

The fact that meristems not visible at a magnification of 30 diameters (those at L_{n+1} and L_{n+2}) develop vegetatively indicates either that the labile period is of very brief duration, or that the stimulus from the leaf does not reach them, possibly because their subtending leaves act as more effective sinks for the promotive stimulus. At the time of long-day exposure, the labile meristem at L_{n+3} must have existed in a histological sense only, as a designated cell, or, at most, a few cells. Provided that export of the stimulus from the leaves is not more delayed, or its translocation slower, than is usually found in other species—and direct evidence on these points is required—a similar description would be valid for the meristem at the time of its induction.

These findings differ from those of Chouard (1957) who states that the meristems axillary to the youngest leaves are insensitive to a photoperiodic stimulus, whereas those axillary to older leaves are sensitive. In our material the latter are already determined vegetatively, and it is *only* the youngest, in the sense that they will be axillary to leaves not yet differentiated, that remain labile.*

Figure 3 brings out two further points:

- (1) Unlike many other photoperiodic plants, notably *Xanthium*, a floral stimulus is not perpetuated in *Anagallis* at or after induction and sharp reversion to vegetative growth occurs on return to non-inductive conditions.
- (2) Once determined, a floral meristem develops more rapidly than a vegetative meristem, since the former are visible in the axils of the first or second leaf primordia behind the growing point, i.e. one node higher than vegetative meristems. Counts on vegetative apices from plants under short days, and reproductive apices from plants under continued long days, gave the following leaf positions behind the apex at which a developing meristem was visible: vegetative, 2.63; reproductive, 1.69 (L.S.D. = 0.37, $P < 0.001$).

(b) *The Entire Juvenile Plant*

When *Anagallis* plants are exposed continuously to long days from emergence, flowers are formed at the second leaf node. From this it may be inferred that early foliar structures are perceptive of day length, that they need have only a small area, and that only a few long-day cycles are required.

Results from some experiments in which entire plants, consisting of cotyledons and a first leaf pair of area 20–50 mm², were exposed to cycles of long days and then returned to short days are collected in Table 1. Three long days induced flower initiation in nearly all individuals, two produced a substantial but variable response, and one virtually none. Two long days is apparently a threshold stimulus, and small variations in the inducing conditions would, therefore, be expected to have large effects. Part of the variability may also be ascribed to the use of seed from different samples and of variable age. In other experiments involving many hundreds of

* Chouard worked with the red-flowered subspecies (designated in his paper *A. arvensis* L. subsp. *phoenicea* Scop.). While our description refers specifically to the blue-flowered subspecies, we have noted no essential differences between the two subspecies in their photoperiodic behaviour.

plants, we have observed only an occasional plant (less than 1%) to initiate flowers after one standard long day. The response may be greatly increased by using light intensities greater than 40–50 f.c. in the supplemental period, e.g. at 150 f.c. 9% of plants initiated flowers after one long day. *Anagallis* thus differs from many other photoperiodically sensitive plants whose responses are saturated at intensities between 5 and 50 f.c.

Increasing the number of long days not only increases the percentage of individuals which initiate flowers; it also increases the rate of development of the flower initials (Fig. 1).

It should be stressed that all our results refer to flower initiation only. Conditions adequate to induce initials appear to be inadequate for full, normal flower maturation. After 2 and 3 long days we have observed, though rarely, vegetative

TABLE I
FLOWER INITIATION IN YOUNG PLANTS AFTER VARIOUS LONG-DAY TREATMENTS
Plants had cotyledons and a first leaf pair of area 20–50 mm² at start of treatment

Expt. No.	No. of Plants per Treatment	Flower Initiation (%)		
		1 Long Day	2 Long Days	3 Long Days
1	18–26	6	92	100
2	14–15	0	55	—
3	28	0	86	100
4	29	0	79	100
5	36–39	0	36	100
6	33–34	0	24	94

reversions of the types described by Chouard (1950*a*, 1957) and Brulfert and Chouard (1961). In addition, under glasshouse conditions, we have observed the abortion or sterility of flowers and flower parts, unless plants have been exposed to more than 10 long days.

(c) Cotyledons and First Leaves

Three methods, all subject to limitations, can be used to attempt to isolate the action of cotyledons:

- (1) Leaves can be excised prior to long-day exposure. Since *Anagallis* leaves are sessile it cannot be excluded that remaining fragments of basal leaf tissue may contribute to any observed effect. On the other hand, because of their shape, cotyledons can be removed virtually entirely. There is also a minimum size of small leaf or primordium which can be removed without damaging the apex. In practice, we have not removed leaves smaller than approximately 1 mm breadth, such a pair having an area of 2–3 mm². In addition, we have not found it possible to denude the plant of more than three nodes of leaves behind the apex, and maintain normal growth.
- (2) Long-day exposure can be made before leaves develop. Here one is limited to five to six exposures before first leaves of the above size have developed.

- (3) Parts of intact plants can be selectively exposed to long days by shielding. When technically perfect, this method yields the most unequivocal information; but in the very small *Anagallis* plants, completeness of shielding without damage can neither be monitored nor taken for granted any more than the completeness of removal of leaf fragments.

We have therefore used only the first two methods, and data from experiments employing them singly, and in combination, are presented in Tables 2, 3, and 4. All comparisons referred to in the text are significant at $P < 0.05$, or better, unless otherwise stated.

TABLE 2
FLOWER INITIATION IN PLANTS EXPOSED TO 3 LONG DAYS IN RELATION TO POSITION AND SIZE OF THE PERCIPIENT ORGAN

Based on 13-19 plants per treatment. —, organ not yet present; *, organ excised

Time of Long-day Treatment (day)	Mean Area of Organ (mm ²)				Flower Initiation (%)
	Cotyledons	First Leaves	Second Leaves	Third Leaves	
10, 11, 12	34.4 ± 0.4	—	—	—	0
16, 17, 18	92.8 ± 1.9	*	—	—	0
	*	55.3 ± 1.5	—	—	100
	92.6 ± 1.8	61.1 ± 1.8	—	—	100
21, 22, 23	125 ± 3	*	*	—	0
	*	245 ± 4	*	—	77
	*	*	54.9 ± 2.6	—	22
	130 ± 3	252 ± 5	64.8 ± 2.9	—	100
	*	*	251 ± 7	*	6
26, 27, 28	*	*	*	55.5 ± 2.8	23
	*	*	*	51.7 ± 1.5	100
	130	393 ± 8	247 ± 5		

Table 2 shows that when cotyledons only, between one-quarter and fully expanded, were exposed to 3 long days no flowers were initiated. However, entries in Tables 3 and 4 indicate that exposure of cotyledons to 5-8 long days was largely responsible for some of the flower initiation. Thus the plants of treatment 4 (Table 3) carried cotyledons only when exposed to long days and flower initiation at node 6 occurred. Similarly, the plants of treatments 1 and 2 (Table 4), on which cotyledons were the only macroscopically visible foliar structures when long-day exposure started, initiated flowers at node 2, unlike those of treatments 3 and 4, which were not exposed till later. This is consistent with the decrease in flower initiation at node 2 (Table 3, treatment 1 *v.* treatment 2) following reduction of area of cotyledons exposed to long days, though the lowered production of some non-specific substance, as well as of the long-day stimulus may have contributed to this result. The data fall short of demonstrating that exposure of cotyledons to long days in the absence of other leaves can evoke initiation. In Table 3, treatments 3 and 5 were similar

until day 10, at which time very young leaves of area 2-3 mm² were removed from treatment 3 plants but not from treatment 5 plants, since the latter were then returned to short days. Flower initiation at node 2 in treatment 5 plants was similar to that

TABLE 3

DISTRIBUTION OF FLOWER INITIALS IN RELATION TO TIME OF INDUCTIVE LONG-DAY TREATMENT AND ORGANS EXPOSED

Cotyledons emerged on day 6. Based on five replicates (10 for treatment 5), each of 3-8 plants, per treatment

Treatment	Time of Long-day Treatment (day)	Parts Excised and Day of Removal*	Flower Initiation at Indicated Positions (%)				
			Node 2	Node 3	Node 4	Node 5	Node 6
1	6-13 inclusive	Nil	29	98	66	0	0
2	6-13 inclusive	Half each cotyledon, day 6 (10)	0	83	14	0	0
3	6-13 inclusive	First leaves, day 10 (12)	0	51	38	0	0
4	13-20 inclusive	First and second leaves, day 13 (17)	0	0	0	3	17
5	6-10 inclusive	Nil	26	40	0	0	0

* Leaves were removed as completely as possible when they were approximately 1 mm broad. The distal half of each cotyledon was cut off. Where regrowth occurred the operation was repeated on the day indicated in brackets.

in treatment 1 plants (the difference is insignificant, $P > 0.05$), but was zero in treatment 3 plants. This is interpreted to mean that, although such small undeveloped leaves were ineffective on their own (treatment 2 of Table 3), they did produce a

TABLE 4

DISTRIBUTION OF FLOWER INITIALS IN RELATION TO TIME OF INDUCTIVE LONG-DAY TREATMENT Cotyledons emerged on day 6. Based on seven replicates, each of 5-9 plants, per treatment

Treatment	Time of Long-day Treatment (day)	Flower Initiation at Indicated Positions (%)				
		Node 2	Node 3	Node 4	Node 5	Node 6
1	6 onwards	36	100	100	100	100
2	6-12 inclusive	24	99	2	0	0
3	9 onwards	0	73	100	100	100
4	10, 11, 12	0	20	1	0	0
5	14 onwards	0	0	97	100	100
6	14, 15, 16	0	0	94	37	0

stimulus to floral initiation. Subsequent to the last long day, sufficient was exported for it to be effective in the presence of that provided by the cotyledons. At a later stage, however, first leaves on their own were fully competent in floral induction (Table 2).

(d) Meristems

Table 5 illustrates a consistently observed feature, that with 3 long days nearly complete flower initiation occurred at node 4, although substantially less was found for 2 long days. Since leaf competence was not in doubt in these experiments, any differences in flower initiation should be attributed to the meristems, although it is not yet possible to specify closely those properties which could be concerned.

One possibility is that meristems, even at their maximum sensitivity, are intrinsically variable in response to the long-day stimulus, some requiring 3 long days for floral determination and others only 2. However, at least part of the diversity in the patterns of floral initiation should be attributed to differences in the stage of

TABLE 5
DISTRIBUTION OF FLOWER INITIALS IN RELATION TO TIME AND DURATION
OF INDUCTIVE LONG-DAY TREATMENT
Plants had cotyledons and a first leaf pair of area 25-40 mm² at start of
treatment

Expt. No.	Treatment	Time of Long-day Treatment (day)	Flower Initiation* at Indicated Positions (%)	
			Node 4	Node 5
1	1	15	0	0
	2	16	0	0
	3	17	0	0
	4	15, 16	47	0
	5	16, 17	38	16
	6	15, 16, 17	90	27
2	1	16	0	0
	2	16, 17	28	0
	3	16, 17, 18	98	0

* For experiment 1 based on five replicates, each of 4-7 plants, per treatment. For experiment 2 based on 10 replicates, each of 2-5 plants, per treatment.

meristem development in relation to the time of the long day treatment. From Tables 3 and 4 it can be seen that, when exposure to long-days was late, the meristems at lower nodes developed vegetatively, as did those at higher nodes when the exposure was early and then ceased. The data of Table 5 suggest that a similar shift occurred during an interval as brief as one day, i.e. during the day by which treatment 4 and treatment 5 differed, some meristems at node 4 became too old, and correspondingly, at node 5 new ones arose or reached some required minimal development. (The observed trend in this direction at node 4 is not statistically significant, whereas at node 5 it is.)

Nevertheless, provided we are not dealing with meristems absolutely requiring 3 long days for floral determination, the above simple considerations of the timing of the long-day stimulus do not satisfactorily explain the very high initiation at node 4.

Since the two axillary meristems at a node may differ in age by up to 2 days (on a morphological basis) and the labile period under non-inductive conditions is brief, some proportion of the meristems would be expected to be too old for floral determination by the third long day. This may indicate that each element of the long-day stimulus delays a meristem's progress towards vegetative determination and so extends its labile period.

(e) *Age and Position Effects*

Data already presented indicate that when first leaves of area 2-3 mm² were exposed to 2-3 long days no, or slight, flower initiation ensued, but that when they were larger than 20 mm² there was a substantial response. Table 2 shows, for both first and second leaves, that flower initiation fell off sharply as the leaves enlarged (aged). This is brought out more clearly for first leaves by the data of Table 6. Factors other than decrease in leaf efficiency will be discussed later.

TABLE 6
FLOWER INITIATION IN RELATION TO SIZE OF FIRST LEAVES EXPOSED TO LONG DAYS
Based on 13-15 plants per treatment, except treatment 9 in which only four survived surgery

Treatment	Time of Long-day Treatment (days)	Mean Area of First Leaf (mm ²)	No. of First Leaves	Parts Excised	Flower Initiation (%)
1	12, 13	5.1 ± 0.3	2	Nil	7
2	14, 15	19.3 ± 0.6	2	Nil	100
3	14, 15	9.6 ± 0.2	1	One first leaf	20
4	16	44.8 ± 1.9	2	All second leaves	7
5	16, 17	40.0 ± 1.6	2	All second leaves	100
6	16, 17	23.6 ± 1.2	1	One first leaf, all second leaves	40
7	16, 17, 18	45.8 ± 1.1	2	All second leaves	100
8	21, 22	153 ± 5	2	All second and third leaves	0
9	27, 28	395 ± 12	2	All second, third, and fourth leaves	0

Taking together the results of other similar experiments we conclude that flower initiation is already maximal when first leaves, one-twentieth of their fully expanded area or less, are exposed to long days, and that substantial decline, or complete loss in long-day response, has already occurred by the time they are half-expanded.

Table 2 also shows that more plants, on which only first leaves were exposed, initiated flowers than did those carrying only either second or third leaves of comparable area, i.e. greater flower initiation was associated with the leaves of lower ontogenetic rank.

Another type of position effect may be demonstrated with laterals inserted at different levels. In experiments of this type plants were allowed to grow until the

laterals at a particular node carried five macroscopic leaf pairs. They were selected for the uniformity of the terminal three of these, and thus at least one pair had dimensions optimal for flower initiation. These three leaf pairs were retained, the primary axis was decapitated above the node concerned, and cotyledons, all other leaves, and secondary axes were excised. Such plants were then exposed to standard long-day treatment. Later, when in other plants the laterals at a different node had reached a comparable size, this procedure was repeated. The standard photoperiodic treatments were thus administered at different times, at which the axes of different levels were of comparable developmental age. After return to short days both secondary axes of a plant were examined for flower initiation.

One such experiment, based on 12–14 replicates per treatment, each replicate of four plants with two lateral axes, showed that percentage flower initiation on axes arising at the cotyledonary node was 75 and 100, respectively, for photoperiods of 3 and 4 long days, whereas initiation on axes arising from the second leaf node was 48 and 85, respectively. Position comparisons within a photoperiod treatment were significant at $P < 0.01$; photoperiod comparisons within a position treatment were also significant at $P < 0.01$.

IV. DISCUSSION

(a) *Perceiving Organs*

These results demonstrate that, in *Anagallis*, exposure of cotyledons, as well as leaves, to long days leads to subsequent flower initiation. We have not succeeded in demonstrating unequivocally that cotyledons alone will cause flower initiation. This is generally taken to be so when *Pharbitis* seedlings, carrying only expanded cotyledons, are exposed to short days. However, at the stage maximum seedling sensitivity is reached in *Pharbitis*, at least four leaf primordia are present (Kujirai and Imamura 1958; Marushige and Marushige 1963). The oldest of these are even larger than those which contribute to flower initiation in *Anagallis*, and it is possible that they do so also in *Pharbitis*.

Since cotyledons carry out a wide range of syntheses (Lowell Young and Varner 1959; Spragg and Yemm 1959; Lowell Young *et al.* 1960) it is perhaps less surprising that they should act as organs of day-length perception and synthesis of the floral stimulus, than that the very small amounts of tissue involved, both of cotyledons and leaves, should prove sufficient.

The amounts of flower initiation recorded here express the end results of exposing various organs of differing sizes, ages, and position to photoperiodic stimulation. The operation of three factors may contribute to the variations in net effects observed:

- (1) The sensitivity or efficiency of the perceiving organ.
- (2) The transport of a stimulus to the site of floral differentiation.
- (3) Variation in the level of meristematic response. As well as the changes undergone by each individual meristem during its development there may also be changes in the general level of sensitivity corresponding to changes in total plant age.

Complete discrimination between the operation of these three factors is difficult because they may be confounded. In our data the least ambiguous case is the rise in net effect during the period first leaves develop from an early post-primordial stage to when they are one-twentieth expanded. This period is only a matter of days and the distance between perceptor and receptor is small, only few developing organs being inserted between them. For these reasons, we consider significant variation in (3) and (2), respectively, to be less likely than that, over this range, the first leaves increase in sensitivity. From comparisons of two leaves *v.* one leaf of the same age (Table 6), it further seems that area (amount of tissue) is responsible. Leaf area has been found to limit induction in *Oenothera biennis* (Chouard 1950*b*) and in *Lolium temulentum* (Evans 1960). Our conclusion that size and not age (within this range) is important is at variance with that of Khudairi and Hamner (1954), though, as indicated by Evans (1960), age and position were confounded in these authors' experiments.

On the other hand, the variations in net effect between young and older leaves of similar rank, and between leaves and axes of similar ages but different rank, could be contributed to by any or all of the three factors.

Taken together, these variations in net effect, suggest that an *Anagallis* plant should become harder to induce as it ages. This situation is unlike that usually found in other plants, where the photoperiodic requirements become less stringent with age, sometimes to the point of indifference, e.g. *Hyoscyamus* (Lang and Melchers 1943), *Scabiosa ukratica* (Chouard 1950*b*), *L. temulentum* (Evans 1960), and *Echium plantagineum* (Ballard, unpublished data).

(b) Meristems

Some indication of the nature of the required activity in meristems before they can respond to a photoperiodic stimulus is given by the findings that multiplying DNA must be present in the terminal bud in *Pharbitis* (Zeevaart 1962*a*), and that RNA synthesis is necessary in *Xanthium* (Bonner and Zeevaart 1962). In *Anagallis* it is noteworthy that the activity which is critical should occur so early, and involve so few cells. Apparently some minimal concentration of a stimulatory substance is required to effect floral determination, and it is immaterial from what source or sources it is derived. By means of treatments which reduce growth rate, such as low temperature and restricted water supply, we can induce flowers at the first leaf axils, i.e. lower than is otherwise the case. The critical factor thus appears to be the rate of meristem development in relation to rate of receipt of the stimulus (and any inactivation it may possibly suffer). This result also provides evidence that, in the seed, the "axillary" meristems are either not present or not determined, at least beyond the cotyledonary node.

From studies of vegetative reversion in this species, Chouard (1950*a*, 1957) and Brulfert and Chouard (1961) have postulated a dynamic equilibrium between reproductive and vegetative states, rather than a fully determined, absolute condition of either. Under some conditions vegetative reversion is accompanied by morphological evidence of previous activity towards flower initiation. The extension

of the labile period, suggested by the present data, may be a weaker manifestation of the same phenomenon.

Our data provide no information on the question of possible variation in meristematic competence with total plant age. Evidence would have to be secured by reciprocally grafting variously aged meristems and lower portions of plants, as effectively done by Zeevaart (1958, 1962*b*). In *Bryophyllum daigremontianum*, Zeevaart showed that meristems of juvenile plants were competent before leaves of the same age; but the question of any later variation remains open.

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