EFFECTS OF LIGHT AND DARKNESS ON THE GROWTH OF PEAS

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Abstract

The shoot growth and development of two varieties of peas in light, in darkness, and when transferred from darkness into light and vice versa were studied.

Stem elongation did not continue beyond the sixth internode in dark-grown plants, growth ceasing before the carbohydrate reserves in the cotyledons had run out.

Leaf initiation continued in darkness at the same rate as in light but light was needed for continued initiation beyond leaf 12. Early leaf development occurred in darkness in the same way as in light but development stopped at a very immature stage.

Leaf initiation, further leaf development, and the elongation of internodes higher than the sixth internode resumed when dark-grown plants were transferred into light before they were 24 days old. "Supershortening" of some internodes occurred.

Conversely, leaf initiation, further leaf development, and the elongation of internodes higher than the sixth ceased when light-grown plants were transferred into darkness. Hyperelongation of stems and petioles sometimes occurred.

The response of dark-grown plants to light and the response of light-grown plants to darkness confirmed that the response of a tissue to an external factor very much depended on its physiological age.

I. Introduction

A detailed survey of the morphological and anatomical effects of light on pea seedlings was carried out by Thomson and Miller (1962*a*, 1962*b*, 1963), comparing entirely dark-grown seedlings with those grown under red or white light. They concluded that mature dark-grown leaves, like those grown in weak light, resembled immature light-grown ones and leaves initiated in darkness did not differ from those initiated in light. If they were correct, then mature dark-grown leaves should resume their growth when transferred into light and leaves initiated in darkness, when allowed to develop in light, should mature into leaves similar to entirely light-grown ones. Thomson and Miller did not carry out any transfer experiments.

A few workers (Watson 1942; Morton and Watson 1948; Juhren and Went 1949) have transferred plants from either weak light or darkness into full light and observed that some leaves indeed resumed growth. None of these workers used pea seedlings. Few experiments involving the transfer of light-grown plants into darkness have been recorded in the literature although such experiments are surely necessary to complete our understanding of the role of light in morphogenesis. Juhren and Went (1949) transferred light-grown squash plants into darkness, kept them nourished by feeding them sucrose, and observed that leaf primordia production continued for a while but eventually stopped.

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The transfer experiments described in this paper are an extension of the work of Thomson and Miller. Dark-grown seedlings transferred into light and light-grown seedlings transferred into darkness have been compared with entirely light-grown and entirely dark-grown controls.

II. Materials and Methods

Two varieties of the garden pea, *Pisum sativum* cv. Greenfeast (dwarf) and cv. Telephone (tall) were used. Seeds were supplied by Yates and Co., Sydney.

Seeds were soaked for 3–5 hr in tap water prior to planting in washed vermiculite in small flower pots which were watered every other day. Seedlings were weeded down to five uniform seedlings per pot when they were about 6 days old. Dark-grown seedlings were staked up when they they were about 10 days old and light-grown tall ones a few days later.

Seedlings were grown in constant-temperature rooms maintained at $23 \pm 2°C$, light-grown ones receiving 16 hr of white light daily and dark-grown ones no light except for some dim green light during measurement and staking. A small amount of green light has been shown to have no significant effect on the growth of the dark-grown seedlings (Low 1969).

The first leaf, borne at the first node above the cotyledons, is numbered as 1. The internode between the cotyledons and the first node is numbered as 1. Internodes and leaves were measured to the nearest 0·5 mm with a ruler. Stipule and leaflet measurements were made on the larger of each pair. Ten plants were sampled per treatment. Examination of leaf primordia was carried out by dissecting the shoot apices with dissecting needles under a binocular microscope.

Tissue for examination was fixed in acetic–aclohol (1 acetic acid : 3 ethanol) for 15–45 min, rinsed three times in 70% ethanol, and stored in 70% ethanol until required. Hand-cut sections were made where feasible and such sections were stained in weak, aqueous safranin solution and temporarily mounted in water. Leaf tissue and difficult internode tissue were embedded in paraffin wax, sectioned with a microtome, and subsequently stained in safranin and aniline blue. Cell measurements were made with an ocular micrometer in a microscope. Ten cells were measured from each of three plants for each treatment.

III. Results

Thomson and Miller (1961, 1962*a*, 1962*b*, 1963) worked with cv. Alaska, a tall, early-flowering variety which flowered at the ninth or tenth node. Growth of Alaska peas under all light conditions at 22°C ceased after 16 days from planting. The two pea varieties used here were both late-flowering, flowering occurring at or after the 15th node. At 23°C, growth in darkness of these seedlings did not cease till the 25th day and continued beyond 35 days in the light until terminated by flower formation. The difference between the longevities of the late-flowering varieties used in these experiments and those of the early-flowering variety used by Thomson and Miller probably resulted from the differences in their flowering schedules, since flowering terminated growth.

Seedlings grown in darkness for up to 23 days greened and resumed growth when transferred into light. Light-grown seedlings transferred into darkness before internode 6 had elongated continued to grow in the dark until internode 6 had elongated, those internodes or parts of internodes which elongated in the dark remaining white, not green. Entirely dark-grown plants also had only six elongated internodes. Internode elongation, leaf expansion, and leaf primordia production resumed if seedlings were returned to light within 3 days of growth ceasing in darkness. All leaves became fully green.

Since both varieties responded in a similar manner, only the results for Telephone (tall) peas are presented.

(a) *Internode Growth*

Figure 1 shows the time course of elongation of dark-grown internodes transferred into light. Mature dark-grown internodes were not altered by the transfer to

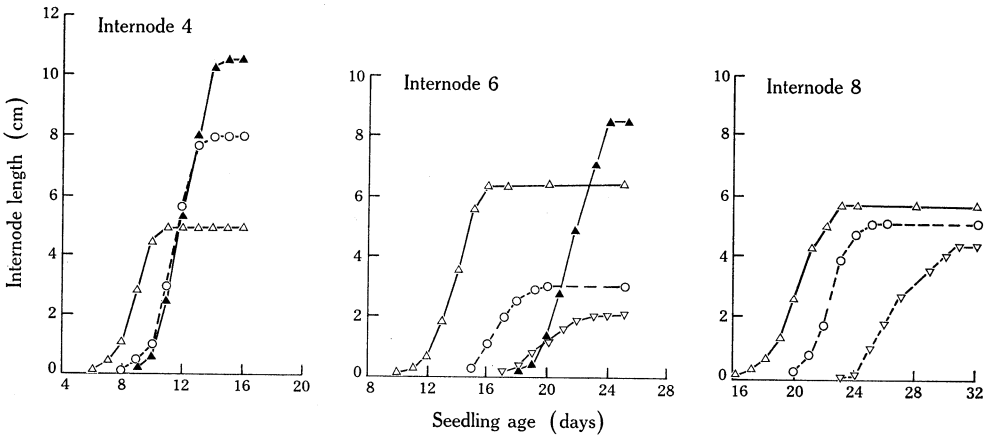

Fig. 1.—Time course of internode elongation: dark → light. ▲ Dark. ○ Dark → light at 13 days. ▽ Dark → light at 16 days. △ Light.

light except for slight greening in the younger ones. The oldest internode still elongating outside the apical bud cut short its elongation; its upper portion became much greener and had much shorter cells than its lower, older portion. The next two

TABLE 1

CELLULAR ANALYSIS OF INTERNODE GROWTH

Analysis of internode 5, which has been "super-shortened" by the transfer from darkness into light, and comparison with internode 3. Each cell dimension is the mean of 30 cells, and each internode dimension is the mean of 10 plants. Coefficient of variation = 10–20%

Treatment	Internode 5				Internode 3		
	Length (cm)	Diam. (cm)	Cortical Cell Length (μm)	Cortical Cell Diam. (μm)	Length (cm)	Cortical Cell Length at Top (μm)	Cortical Cell Length at Base (μm)
Light	6·0	0·25	395	105			
Dark → light at 11 days	2·0	0·20	225	81			
Ratio	0·33	0·80	0·57	0·77			
Light					2·4	248	235
Dark					11·5	482	490
Light → dark at 7 days*					4·1	422	270

* Partly elongated in light, completed elongation in dark.

to four internodes to elongate after the transfer into light became much shorter than their light-grown controls, shortening resulting from fewer and shorter cells (Table 1). Higher internodes did not differ from their fully light-grown counterparts.

Figure 2 shows the time course of elongation of light-grown internodes transferred into darkness. Light-grown internodes which had stopped elongating prior to

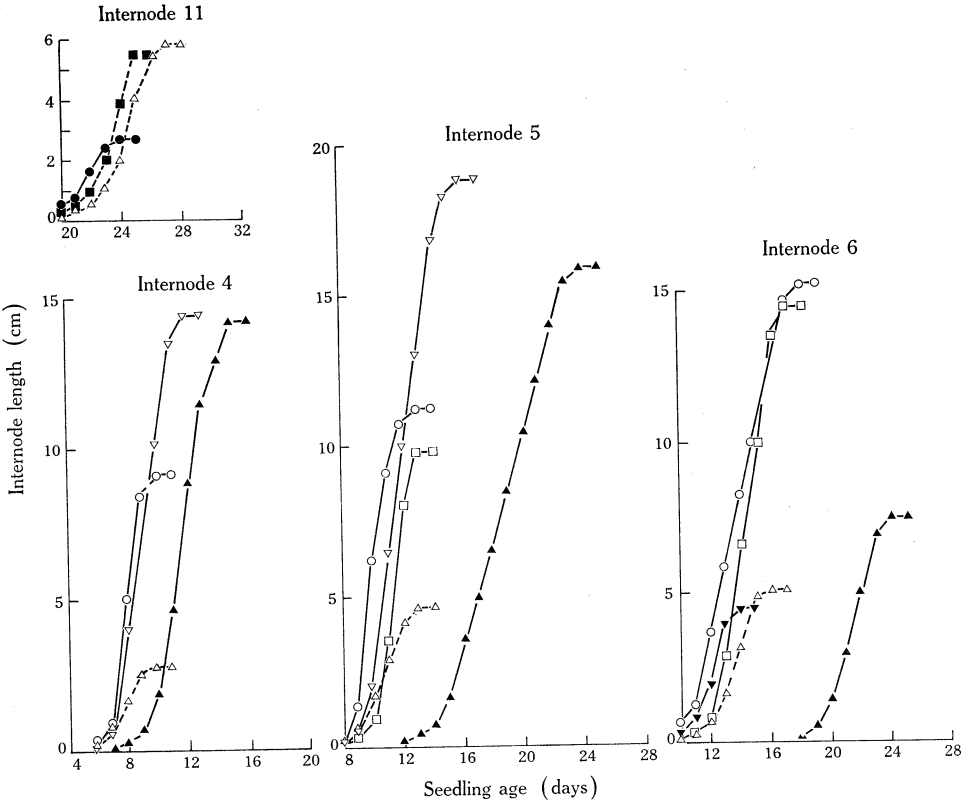

Fig. 2.—Time course of internode elongation: light → dark. △ Light. ▽ Light → dark at 4 days. ○ Light → dark at 7 days. □ Light → dark at 10 days. ▼ Light → dark at 13 days. ● Light → dark at 21 days. ■ Light → dark at 24 days. ▲ Dark.

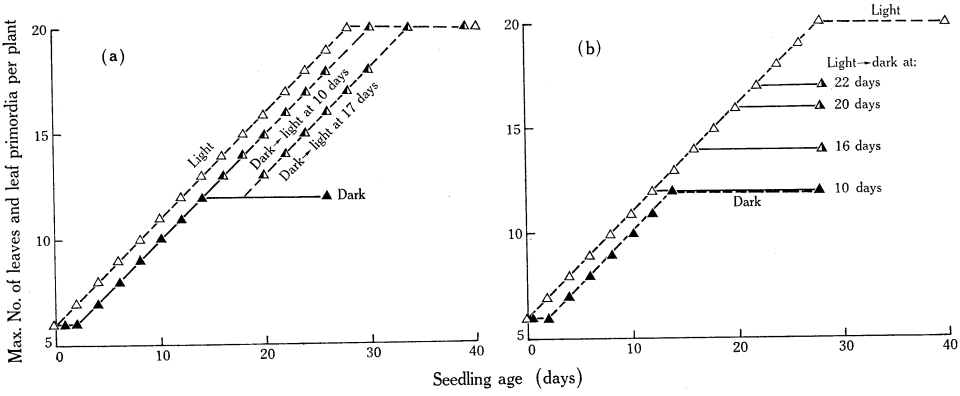

Fig. 3.—Time course of leaf initiation: (*a*) dark → light; (*b*) light → dark.

the transfer into darkness were not affected by the transfer. Partially elongated internodes lower than internode 6 elongated in darkness to become intermediate in

length between fully light-grown and fully dark-grown controls. Internodes lower than internode 6 and less than 0·1 cm long at the time of transfer elongated in darkness to become as long as fully dark-grown controls. Partially elongated internodes higher than internode 6 when transferred into darkness curtailed their elongation and often became shorter than even their fully light-grown counterparts. Internodes higher than internode 6 and less than 0·1 cm long at the time of transfer did not elongate in the dark.

TABLE 2

MATURE LEAF SIZES OF PEAS : DARK → LIGHT

Each length is the mean of 10 plants. Coefficient of variation is 10–20%. m = matured or reached maximum size

Leaf No.	Treatment	Petiole Length (cm)	Leaflet Length (cm)
3 (m in dark at 8 days)	Light	1·1	1·7
	Dark	0·5	0·4
	Dark → light at 7 days	1·1	1·4
	Dark → light at 13 days	0·5	0·4
	Dark → light at 17 days	0·5	0·4
5 (m in dark at 13 days)	Light	2·6	2·3
	Dark	0·5	0·5
	Dark → light at 7 days	2·4	2·1
	Dark → light at 13 days	1·9	2·2
	Dark → light at 17 days	1·4	1·2
6 (m in dark at 20 days)	Light	2·7	2·1
	Dark	0·2	0·3
	Dark → light at 7 days	2·6	2·4
	Dark → light at 13 days	2·5	2·1
	Dark → light at 17 days	1·4	1·3

(*b*) *Leaf Primordia Production*

Peas contain six leaf primordia in their embryos and when germinated they initiate new leaves at the rate of one every other day (Thomson and Miller 1961; Low 1969). The rate of leaf primordia production remains the same in darkness as in light and dark-grown leaf primordia pass through the same sequence of developmental stages as their light-grown counterparts. However, while the late-flowering cultivars Greenfeast and Telephone produced no more than six new leaf primordia in darkness, they produced at least 13 new ones in white light.

Figure 3(*a*) shows the rate of leaf initiation in control plants and in plants transferred from darkness into light. After being transferred into light, dark-grown shoot apices continued to initiate leaves at the rate of approximately one every 2 days. Apices which had stopped leaf initiation in the dark resumed initiation 2–3 days after the transfer to light, regardless of whether they were transferred at 13 days or 23 days. In all treatments the full light-grown complement of 19–22 leaves and leaf primordia was achieved by some plants.

Figure 3(*b*) shows the maximum number of leaves produced by plants transferred into darkness. When light-grown seedlings were transferred into darkness, if leaf 12

had not yet been initiated at the time of transfer, then leaf 11 or leaf 12 would be the last leaf to be initiated in the dark. If leaf 12 had already been initiated prior to the transfer, then leaf initiation either stopped immediately or after the initiation of one

TABLE 3

DIMENSIONS OF MATURE LEAF 3

Each cell dimension is the mean of 30 cells. Each leaflet dimension is the mean of 10 plants. Coefficient of variation = 10–20%

Treatment	Transverse Section of Leaflet				Paradermal Section of Leaflet		
	Lamina Cell No.	Lamina Thickness (μm)	Air Spaces	Palisade Cell Length (μm)	Leaflet Length (cm)	Palisade Cell Diam. (μm)	Epidermal Cell Length (μm)
Light	7·8	180	+	39	1·6	20	89
Dark → light at 7 days	7·8	195	+	34	1·5	21	93
Dark	7·8	107	—	18	0·4	13	30

more leaf. Leaf initiation resumed if plants were returned to light within 3 days of growth ceasing in darkness.

TABLE 4

MATURE LEAF SIZES OF PEAS: LIGHT → DARK

Each value is the mean of 10 plants. Coefficient of variation is 10–20%

Leaf No.	Treatment	Colour	Petiole Length (cm)	Leaflet Length (cm)
3 (matured in light at 10 days)	Light	Green	1·1	1·7
	Dark	Yellow	0·5	0·4
	Light → dark at 4 days	Yellow	1·6	0·8
	Light → dark at 7 days	Light green	1·1	1·4
5 (matured in light at 14 days)	Light	Green	2·7	2·3
	Dark	Yellow	0·5	0·5
	Light → dark at 4 days	Yellow	1·3	0·7
	Light → dark at 7 days	Yellow	4·1	1·1
	Light → dark at 10 days	Light green	2·7	1·6
6 (matured in light at 18 days)	Light	Green	2·7	2·1
	Dark	Yellow	0·2	0·3
	Light → dark at 4 days	Yellow	0·2	0·3
	Light → dark at 7 days	Yellow	0·2	0·7
	Light → dark at 10 days	Yellow-green	4·0	0·8

(c) *Leaf Growth*

Table 2 shows the mean sizes of mature leaves in control plants and in plants transferred from darkness into light. The different component parts of a pea leaf responded to darkness more or less as a unit and hyperelongation, the typical response of stems to darkness, was not exhibited by dark-grown petioles.

Dark-grown leaves that had stopped expanding for more than 6 days prior to the transfer responded to light only by greening, and then only after more than 24 hr in the light. Younger leaves that had stopped expanding in darkness greened more rapidly and resumed cell expansion, but they still remained significantly smaller than their light-grown controls (Tables 2 and 3). Leaves that had not stopped expanding in darkness, when transferred into light usually continued cell division and cell expansion until they became identical with light-grown controls in all respects, although sometimes their petioles were shorter. Even leaves initiated in darkness developed in light into leaves indistinguishable from those initiated in light (Table 3).

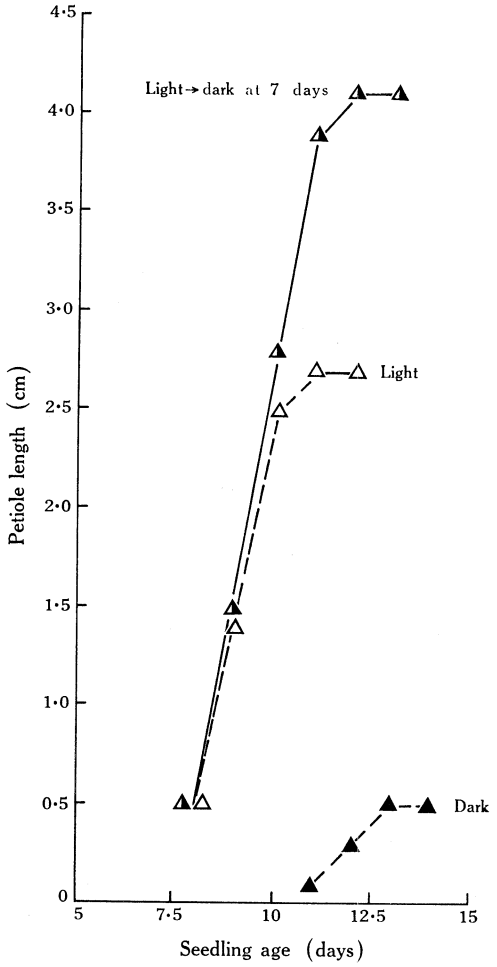

Fig. 4.—Time course of petiole elongation.

When light-grown seedlings were transferred into the dark, leaves already mature prior to the transfer were not affected by the transfer except that senescence was delayed. The less mature a leaf was at the time of transfer, the smaller and more yellow it became after growth in darkness (Table 4). Leaflets immature at the time of

transfer did not open out but remained folded together in the dark. Up to and including leaf 6, the second and sometimes third leaf to expand after the dark transfer showed marked petiole hyperelongation, petioles being much longer than even fully light-grown ones (Table 4; Fig. 4).

Petiole hyperelongation resulted mainly from cell hyperelongation (Table 5). The final leaf to expand approximated the size of the final leaf to expand in dark-grown controls (leaf 5 or leaf 6). If internode 6 had begun elongation prior to the dark transfer, then leaf 7 was the final leaf. If the youngest partially elongated internode at the time of transfer was higher than internode 6, then the leaf immediately above it was the final one to expand in the dark.

TABLE 5

CELLULAR ANALYSIS OF HYPERELONGATED PETIOLE OF LEAF 5

Each cell length is the mean of 30 cells. Each petiole length is the mean of 10 plants. Coefficient of variation is 10–20%

Treatment	Petiole Length (cm)	Cortical Cell Length (μm)
Light	2·7	322
Light → dark at 7 days	4·1	460
Dark	0·5	110
Ratio of light → dark/light	1·52	1·43

IV. DISCUSSION

The effects of light or darkness on a particular tissue, be it stem or leaf, very much depended on its physiological age and its position on the seedling, i.e. whether it was the third or the ninth internode, the fourth or the seventh leaf, etc.

When dark-grown internodes at a certain stage of development were transferred into light, they became even shorter than their entirely light-grown counterparts although younger dark-grown internodes, after growth in the light, did not differ from their light-grown controls. "Supershortening" of the former internodes resulted from decreased cell numbers and decreased cell sizes, indicating that light had accelerated the maturation of these dark-grown cells. Conversely, when some light-grown internodes were transferred into darkness, their cells became longer, indicating that darkness had slowed down their maturation.

In the absence of light, leaf initiation did not proceed beyond leaf 12 even in light-grown plants that had been transferred into the dark: this implied that the limiting substance(s) was not stored. Leaf initiation resumed when the plants received either white light or red light (Low 1969).

Leaf development beyond the very early stages required light and even leaves on light-grown plants did not become fully developed unless they remained in light. However, not all dark-grown leaves developed into normal, light-grown leaves when transferred into light: thus, although darkness slowed down cell maturation, it did not prevent senescence.

Perhaps the most interesting observation was that although petiole hyperelongation was not observed in entirely dark-grown leaves, it occurred in light-grown leaves that had been transferred into darkness at a particular age. This implied that if cells of the petiole had reached a certain stage of development, darkness slowed down their maturation and thus enabled them to continue growing longer in the dark than in the light (see Fig. 4), but entirely dark-grown petiole cells never reached that stage of development.

V. Acknowledgments

Experimental work for this paper was carried out while the author was holding a Sydney University Postgraduate Studentship and is included in her Ph.D. thesis. The author is most grateful to Dr. D. A. Adamson for his helpful guidance and continued interest in this work.

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