

GROWTH PERIODICITY IN PINUS SYLVESTRIS L., WITH SPECIAL
REFERENCE TO THE EFFECT OF DAY-LENGTH.

Thesis submitted for the degree of Doctor of Philosophy
in the University of London

by

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PART I. INTRODUCTION.

In recent years there has been a great revival of interest among plant physiologists throughout the world in problems associated with growth and development in plants. Most of this recent work has been carried out with annual species since it is generally considered more convenient to work with such plants. For this reason our knowledge of the physiology of growth in woody species falls short of that for herbaceous plants. There can be no doubt, of course, that many of the results obtained from the study of herbaceous plants hold also for woody species. For example, evidence will be brought forward below to support the view that the mechanism of photoperiodism is the same for both woody and herbaceous species. Similarly, it has been shown (Czaja, 1934) that auxin is present in the growing shoots of certain woody species, just as in herbaceous plants.

Nevertheless, there are many features of growth peculiar to woody species and not found in typical herbaceous plants. For example, growth of the shoot in herbs is normally terminated by flower formation, whereas in most woody species the annual cycle of growth in a high proportion of the growing points is ultimately terminated not by a flower bud, but by a vegetative resting bud. Investigation of features of growth

Great Britain are (1) temperature; (2) light intensity; (3) length of day.

The probable importance of temperature and light intensity in affecting the annual cycle of growth in woody species has long been recognized, and in the past these, together with rainfall, have generally been considered to be the over-riding factors. The discovery, however, that such processes as the duration of extension growth and time of leaf-fall in woody species may be affected by length of day under experimental conditions (Ganer and Allard, 1923) raises the question as to how far this factor is important in affecting the annual rhythm of growth in nature. Very little definite information on this subject is available at present, and it was therefore decided to carry out an intensive study of the annual cycle of several growth processes in a single species, with special reference to the role of photoperiodic effects in the annual cycle of growth. For this purpose the species, Pinus sylvestris L, appeared to have several advantages, viz.:

- (1) Various other species of the genus Pinus were already known to show marked photoperiodic effects (Bogdanov, 1931, Kramer, 1936, Jester & Kramer, 1939), and there were grounds for expecting that P. sylvestris would also prove to be 'photoperiodic' (Sylven, 1942).
- (2) It is a species which is indigenous to Great Britain (at least in the North, and is naturalised in southern England).

Hence observations with respect to the annual cycle of growth under natural climatic conditions could be regarded as 'normal' for this species.

An intensive study of the annual cycle of growth, in the light of more recent advances in the physiology of development, does not appear to have been made previously for a woody species, although it has been done for various herbaceous plants, including the onion (e.g. Heath, 1945) and certain cereals (e.g. Purvis and Gregory, 1937).

Before the experimental work is described, a brief account of the normal cycle of growth in Pinus sylvestris will be given, since a knowledge of this is essential before an attempt is made to discover the factors which determine it.

PART II. THE NORMAL CYCLE OF GROWTH IN PINUS SYLVESTRIS.

Although Pinus sylvestris is commonly used as a 'type' species for the teaching of elementary botany, there is very little accurate and detailed information as to its various phases of growth, either in textbooks or elsewhere. The following account is based upon the personal observations of the writer over a period of three years on experimental plants grown in pots and on trees of all ages growing under naturalised conditions at Oxshott Heath, Surrey, which was visited on numerous occasions throughout this period. Where the observations of other authorities are drawn upon, this is duly indicated.

1. Germination of the seed.

The ripe seed is normally shed from mature cones during dry, fine weather in early spring, usually in March (Groom, 1907). In forestry practice the cones are normally collected in winter, and stored in a warm, dry place, which brings about opening of the cone scales and releases the seed. Such seed is normally sown the same spring, without any prior 'stratification' and normally about 90% of the seed germinates.

Emergence of the seedling occurs in late April, or early May, and is 'epigeal', the testa being carried up on the tips of the cotyledons.

2. First-year seedlings.

The emergence of the cotyledons, of which there are

4 - 9, is followed by the production of linear 'juvenile' leaves, of the type well known in conifers (Chamberlain, 1935). The initial period of leaf-formation is not at first accompanied by any elongation of the internodes, so that a "rosette" stage is formed. This lasts for about six weeks, after which extension of the internodes commences between the leaves at the base of the rosette. From then onwards, there is a continuous extension of successive internodes between the basal leaves of the rosette, and this process keeps pace with the continued production of new leaves at the centre, so that a rosette of leaves continues to exist in the apical region throughout growth. This pattern of behaviour, in which a rosette stage precedes the extension of the shoot has also been observed by the writer in seedlings of other conifers, e.g. Picea sitchensis, Larix spp., Cedrus atlantica, etc.

Growth of the shoot, with continuous leaf-formation and internode extension, continues until late August or early September under favourable conditions, and then ceases with the formation of a terminal resting-bud at the centre of the apical rosette of leaves. The apical rosette remains present throughout the period of dormancy. The appearance of normal first-year seedlings is shown in Figure 1.



Fig.1. Mature first-year seedlings.

In the development of the root-system of first-year seedlings, a long radicle is formed which soon gives rise to secondary roots. These secondary roots bear fine tertiary roots which potentially are capable of giving rise to mycorrhizal 'short-roots' if infection occurs. Under favourable conditions, mycorrhizal roots first appear in July or early August and continue to increase in number throughout the autumn. Growth of primary and secondary roots also continues until October or November.

3. Growth of the shoot in the second and later years.

After the formation of a terminal resting bud at the end of the first season's growth the plants remain dormant until the following spring. Towards the end of March or early April elongation of the resting buds may be detected in young trees (or some weeks later in older trees). In second-year seedlings, however, the initial stages of extension of the new shoots consist of elongation of the internodes between the juvenile leaves forming the terminal rosette of the first year's growth. Thus we have the unusual occurrence of a lapse of six months or more between the formation of the leaves and the extension of the associated internodes. Further growth of the shoot in second year seedlings consists of the extension of the internodes between the scale leaves of the resting-bud, the whole process occupying a period of 6 - 8 weeks. When this period of extension has been completed there is no further growth by the continued activity of the apical meristem, as in other species e.g. Robinia pseudacacia (Büsgen, 1929). Extension growth of the annual shoot in Pinus sylvestris therefore consists solely of expansion of initials already laid down in the bud, (Wight, 1933) and is completed by the end of May or early June. Thus, after the first year of growth the number of nodes formed by the annual shoot is predetermined by the number of scale-leaves formed in the terminal resting bud in the previous year.

By the time that extension of the shoot has been completed, the green leaves (borne in pairs on dwarf shoots in the axils of the scale-leaves) have attained a length of approximately only 1 cm. These leaves grow by means of a basal meristem, which remains active long after extension of the shoot has been completed. Tolsky (1913) showed that in S. Russia the leaves of P. sylvestris continue growth in length until August, and the writer has found that in Southern England growth continues until about the last week in August (see page 91 below).

4. Growth of buds.

Information on the growth of the resting buds is very slight and the following observations of the writer are only of a preliminary nature. According to Wight (1933), by November there is visible within the obvious resting bud of P. sylvestris a minute rudimentary resting bud of the next season. While certain evidence described below tends to confirm this observation, if purely vegetative buds of young trees are dissected in winter, a continuous series of scale-leaves may be found, right to the minute rudimentary scales of the apex itself. Because of this continuity in the series it becomes impossible to draw any visible distinction between the scales which will form the terminal nodes of the next season's shoot and those which will form the lowermost scales of next season's resting buds. During the elongation of the young shoot in the spring, however, the limits of the new resting bud soon become

recognizable, for the paired leaves of the dwarf-shoots grow out of the axils of the scale-leaves at an early stage except at the extreme apex; it is later seen that the internodes of this latter region do not extend and that the apical scales are the lowermost of those of the new buds.

Regular inspection of this new terminal bud from June onwards shows a slow increase in size for several months. The gradual increase in the size of the buds is shown in Figure 2, which gives the mean length of the terminal buds of second year seedlings grown in the open ground, the data being obtained by taking samples from about 30 plants on the successive dates.

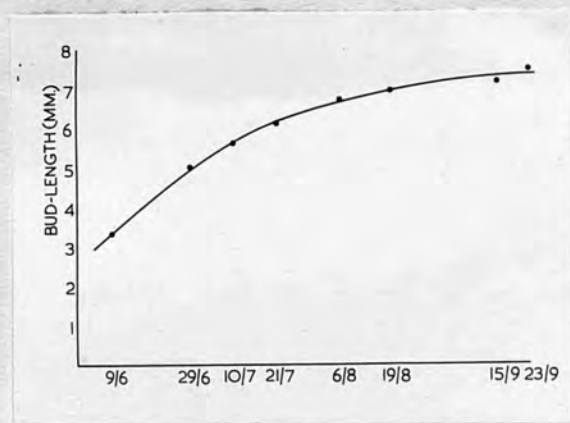


Fig.2. Growth in length of terminal buds.

Dissection of the buds at successive intervals shows that the growth consists not only of an increase in size of the dwarf-shoot rudiments in the axils of the scales, but also of the continued formation and growth of new scales and dwarf-shoot initials. Elongation of the buds continues even after leaf-growth has ceased, but by the end of September appears

practically to have ceased. If the terminal buds of female-cone-bearing branches are dissected, it is found that the rudimentary female cones of the following spring are just discernible early in September. Now it is well known that the female cones are borne at the extreme apex of the annual shoot, so that in buds of this type, there can be no further formation of new initials once the female cone initials have been formed at the beginning of September.

5. Root-growth.

No systematic study of extension growth in the roots of Pinus sylvestris is known to the author, but according to Wight (1933) "the roots of P. sylvestris show two periods of growth in length. The first is early in the season (May-June) and the latter at the end of August and the beginning of September. In the former period the production of fibrous roots seems to predominate, though there is also some growth in length of the stout root leaders. These grow in length most vigorously during the later period of growth." These conclusions were apparently based upon observations of a "qualitative" nature made upon the roots of trees growing under natural conditions. Under such conditions, however, the general picture is complicated by variations in soil-water content. Observations of a preliminary nature on root-growth were carried out by the writer on plants growing under both culture and field conditions. As Brown (1915)

has pointed out, in conifers, within a space of 1 cm. of the root tip a brown colouration appears, "so that new growth can be detected without any difficulty. Furthermore, after cessation of growth in the autumn, this brown mantle approaches nearer the root-tip, so near, in fact, that one can be reasonably sure as to the presence of new growth".

Observations on root-growth were carried out in 1948 by transplanting third-year seedlings from pots to 'root-observation boxes', provided with sloping glass-sides. These boxes were 12 inches in length and depth and 10 inches in width at the top. The glass was normally kept covered by means of metal sheets which could be fitted on the outside and removed as necessary for observation. The plants were transferred to the boxes in April, and at this date it could be seen that a small amount of new growth had been made by a few roots. The "ball" of roots, as removed from the pot, was kept intact and placed in the soil in the boxes so that it rested against the glass on one side. Since the old ball of roots was still clearly distinguishable, any new growth into the surrounding soil could also be clearly seen. (Figure 3). Four 'pots' of plants were transferred to each box, of which there were six in all. Observations were made at frequent intervals throughout the growing season until the end of October. Under these conditions it was found that very little root-growth occurred during April and the first two weeks of May, when shoot growth was taking place. A few new

roots were formed by some of the groups of plants, but in many cases new root-growth was entirely absent. During the second half of May, however, as shoot growth was terminating, root activity became very general in all groups of plants. This activity involved the formation of long secondary roots from which finer branches bearing mycorrhizal shoot-roots arose, and was maintained, without any observable slackening, until well into October.

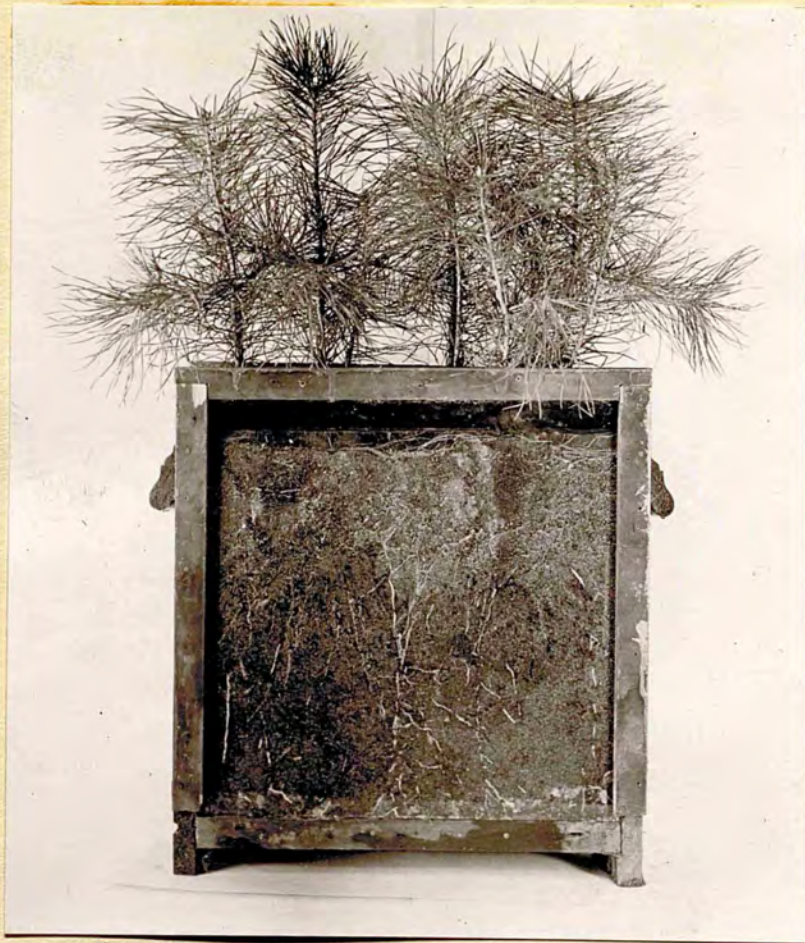


Fig.3. Root-observation box.

An entirely comparable picture was obtained by inspecting the root activity of pot-grown plants throughout the same period. An attempt was made in 1948 to obtain quantitative data

on the normal growth of the roots by digging up at 2-weekly intervals second-year seedlings growing in the open ground in the nurseries of the Forestry Commission at Wareham, Dorset. Unfortunately the penetration of the roots into a hard 'pan' from which they could not be removed intact made it impossible to carry out the original intention of obtaining quantitative data on the amount of root-growth, but sufficient roots were extracted to confirm fully the 'qualitative' results obtained from the observation boxes, viz. that there is limited root activity until extension of the shoot has ceased, after which root-growth occurs actively until the autumn.

Finally, observations were carried out at regular intervals on the roots of mature trees growing at Oxshott, Surrey, by excavating to a depth of several inches, and tracing the roots to their apices, which were then examined for evidence of recent new growth. In selecting sites for excavation of the roots, as far as possible positions where the soil was moist were chosen. On 25th April the long roots showed 3-4 cms. of new growth and new short (mycorrhizal) roots were present. From early May until mid-June, however, there was very little trace of root activity. From 29th June until the autumn root activity remained continuous in situations where the soil was moist.

Thus although Wight's observations that root-growth

becomes markedly active in late May and June is confirmed, under conditions of artificial culture no evidence could be obtained of any reduction in activity until the end of the autumn. It seems very probable that the apparent rhythmic activity observed by Wight was due to variation in soil-water content.

No evidence was found in second year seedlings of the differentiation observed by Wight between the relative activity of "fibrous roots" (by which is presumably meant lateral roots of higher orders) and "root leaders", at different times of the growing season.

Occasional signs of root activity may sometimes be detected in pot plants of P. sylvestris during the winter, but such activity is generally only slight. Moreover, in plants kept under favourable temperature conditions in a greenhouse throughout the winter the roots as well as the shoots remain completely dormant. Under natural temperature conditions, frequent signs of renewed root activity are generally evident in March, before any external signs of shoot growth are apparent. As already described, however, root-growth does not become fully active until the end of May or early June, when the new shoots have appeared.

6. Cambial activity.

The annual cycle of cambial activity in Pinus sylvestris

was followed in detail by Wight for both shoot and root. Commencement of cambial activity in the spring, both in the trunk and in the buds, precedes the breaking of the buds. The most vigorous growth in the trunk occurs from May to early July. After this, slow but continuous cambial activity continues until the beginning of October.

Cambial activity in the roots starts some weeks later than in the shoot, and then commences uniformly along the length of the root. Cambial activity in the thinner distal parts of the roots is over comparatively early and growth ceases first at the root apices. In the proximal parts of the roots cambial activity continues almost as long as that in the shoot.

The observations of the writer (page 124) confirm the observations of Wight with respect to the duration of cambial activity in the shoot.

7. Summary of the annual growth cycle in *P. sylvestris*.

The annual cycle of growth and development (after the first year) outlined above, is summarised in Table I.

It is seen that growth of the shoot commences with the onset of more favourable conditions in the spring. Extension growth of the shoot ceases in 6-8 weeks, but leaf growth continues for much longer by the activity of a meristem at the base of each leaf. Cambial activity is maintained from spring until well into October. Growth of the buds continues from early June until September. Extension growth of the roots may

commence early in the spring, but the period of greatest activity commences at the end of May or early June, and continues well into the autumn. Cambial activity in the distal portions of the root continues for a limited period only in June and July.

Table 1. Annual Growth Cycle in *P. sylvestris*.

Phase of growth.	Date of commencement.	Date of cessation.	Remarks.
<u>Shoot.</u>			
(1) Extension growth.	April/early May.	end May/early June	Amount of growth predetermined by number of initials in the bud.
(2) Leaf growth.	April/early May.	end August	
(3) Cambial activity.	April/early May.	October.	
(4) Bud growth.	June.	September.	
<u>Root.</u>			
(1) Extension growth.	March.	Oct/Nov.	Greatest activity commences in May/June.
(2) Cambial activity.	June.	July.	Applicable to distal roots. Proximal parts of roots as for shoot.

These observations agree closely with those made by Brown (1915) and Kienholz (1934) on *Pinus strobus* and *P. resinosa*, in N. America, except that in these latter species extension growth of the shoot continues somewhat longer (until early August) than in *P. sylvestris*.

III. PHOTOPERIODISM IN PINUS SYLVESTRIS.

A. INTRODUCTION.

1. General aspects of photoperiodism in Woody Species.

The existence of a capacity for photoperiodic response in woody species was first shown by Garner and Allard for Liriodendron tulipifera and Acer negundo in 1923. Since that date a number of other instances of photoperiodism in woody species have been reported. This work has recently been reviewed by the writer (Wareing, 1949) and only the main results need be summarised here. Altogether some 50 species in 30 different genera have so far been investigated (Appendix I) and these have all, without exception, been found to exhibit some form of photoperiodic response, although not all the various types of response are shown by every species. In general, however, it may be said that short photoperiods (of the order of 10 hours duration) hasten the onset of dormancy in seedlings of all species so far investigated, with one exception (Quercus alba (Kramer, 1935)). The "onset of dormancy" is here taken to include the cessation of extension growth, with or without the formation of a terminal resting bud (according to the normal behaviour of the species in question) and followed by leaf fall in deciduous species.

With longer photoperiods the onset of dormancy is delayed and in some species e.g. Robinia pseudacacia,

Liriodendron tulipifera, Pinus taeda (Kramer, 1936), there is a certain critical length of photoperiod above which growth may be maintained continuously for at least 18 months. In other species, however, e.g. Fraxinus americana, Quercus borealis (Kramer, 1936) and Acer pseudoplatanus (writer, unpublished), there appears to be no 'critical photoperiod' above which growth may be maintained indefinitely, and in such species dormancy is not appreciably delayed even under continuous light, as compared with 'long days', although short days bring hastened dormancy.

In a third type of response it is found that when seedlings are grown under a series of different photoperiods, with increasing day-length there is a certain optimum photoperiod for extension of the shoot, and at day-lengths both below and above this optimum, growth is reduced e.g. Pyrus ussuriensis, Salix babylonica (Moshkov, 1932).

One of the characteristic features of photoperiodism in herbaceous plants is that the effects are usually fully evoked by quite low light intensities, so that 'long-day' effects result from supplementing a period of natural daylight of, say, 10 hours by a further period of illumination at 10 foot-candles. Indeed, there is good evidence that the 'saturation' intensity in some plants is only 1 foot-candle or less (Withrow and Benedict, 1936).

There is equally good evidence that light of very low intensity exerts profound photoperiodic effects in woody species also. Thus Kramer (1937) found that light of only 1 foot-candle intensity from street-lights caused shoots of Abelia grandiflora to continue growing in the autumn after similar shoots under natural day-lengths had become dormant.

The question arises as to whether seasonal changes in length of day have any influence on the duration of growth of woody species under natural conditions. The writer has pointed out (Wareing, 1949) that three major categories may be recognized among woody species, according to the duration of the growing period in relation to natural day-length conditions, viz.:

- (1) Species in which growth continues throughout the summer until September or October. It has been shown that the duration of growth in certain species of this type is ultimately controlled by the onset of shorter days in the autumn e.g. Robinia pseudacacia, Liriodendron tulipifera etc. and this is no doubt true for many other species which continue growing late in the season.
- (2) Species in which growth ceases in June, July or early August, before there has been any appreciable fall in the natural length of day e.g. Acer pseudoplatanus, Tilia europaea. In such species extension growth occurs under effectively constant day-length conditions, and ceases when

a certain stage of development has been attained, of which the number of leaves formed on the annual shoot may be used as an index. It is evident that in such species cessation of growth cannot be controlled by any change in natural day-length conditions.

(3) Species in which growth of the annual shoot is restricted to expansion of initials already laid down in the resting bud. It is clear that in these species, after the first year of growth, the day length conditions cannot directly affect the duration of the extension growth, or the number of leaves developed. Many species of Pinus belong to this category, including Pinus sylvestris (p.8), after the first year of growth. Nevertheless, the duration of cambial activity in P. sylvestris has been shown to be affected by natural day-length changes in the autumn (Wareing 1949 b).

The time of leaf-fall in certain species is also affected by day length conditions in the autumn and may be inhibited if the natural day length is artificially lengthened (Garner and Allard, 1923; Matzke, 1936).

Thus the extent to which day-length changes may control the growth-cycle in woody species depends upon the growth-pattern of the species in question, but short-day effects of some type ^{may} occur in the autumn in species belonging to any of the three categories described above.

2. Photoperiodism in the genus Pinus.

Turning to a consideration of what is known regarding photoperiodism in the genus Pinus, we must draw a distinction between any effects observed in first-year seedlings when extension growth is not pre-determined, and possible effects after the first year when growth is pre-determined in many of the species.

Bogdanov (1931) included seedlings of certain species^X of Pinus in his experiments, in which it was found that short days hastened dormancy in all the species studied. Bogdanov also found that races of Pinus spp. from southern Russia when grown in Leningrad, tended to show two periods of extension growth in a single season, instead of the normal single period.

Kramer (1936) found that short days hastened dormancy in seedlings of P. taeda, whereas under long days (18 hour photoperiods) or continuous light it could be maintained in growth throughout the winter. (He does not state whether or not growth was continuous throughout this period, but his illustrations suggest that growth occurred as a succession of "flushes").

Jester and Kramer (1939) investigated the effects of (a) short days ($8\frac{1}{2}$ hours), (b) normal days, (c) long days (15 hours),

X The writer has been unable to refer to the original paper of Bogdanov, whose work is known to him only through the review of Gevorkiantz and Roe (1935), and who do not give the species actually studied.

and (d) continuous light, on a number of woody species, including P. resinosa, P. banksiana, P. echinata and P. caribaea. It was found that 'short-days' brought about reduced growth in height, whereas 'long-days' caused increased height, as compared with 'normal days'. Under continuous light, P. resinosa and P. banksiana remained growing throughout the winter. No indication was given as to whether differences in height were due to differences in number of nodes or length of internode, and it was not stated whether the prolonged growth of P. resinosa and P. banksiana was continuous or occurred as a series of "flushes".

Thus, although several experiments have previously been carried out with various species of Pinus, detailed accounts of the precise mode of growth under different day-lengths are not reported, and no attempt has been made to determine the effects of natural changes in day-length in the normal cycle of growth.

A further photoperiodic effect in P. taeda was reported by Phillips (1941) who found that dormant seedlings brought into the greenhouse in the autumn could be induced to break dormancy immediately if given supplementary low-intensity illumination (with red light) to extend the natural day length to 18 hours, whereas similar seedlings kept in the greenhouse under normal days remained dormant until February.

Gustafson (1938) found that plants of P. resinosa which had been allowed to remain out-of-doors during the winter broke

dormancy in the normal way under natural day-length conditions in the spring, but plants which had been protected from low temperatures during the winter showed no new shoot growth under natural spring day lengths, but did so if the photo-period was increased to 16 hours. From these observations it is evident that although a period of low-temperature exposure is necessary for the normal breaking of dormancy in species of Pinus, in the absence of such exposure breaking of dormancy may be brought about when the photoperiod exceeds certain values.

Although photoperiodism has not previously been demonstrated in P. sylvestris, Sylven (1942) has drawn attention to the earlier work of Schotte (1905) and Engler (1913), who found that seedlings of strains of this species from North Sweden showed reduced growth and 'stunting' when grown at more southerly latitudes. On the basis of similar results obtained with different geographical races of Populus tremula, Sylven concluded that the effects described for P. sylvestris were probably photoperiodic effects resulting from differences in natural length of day when seedlings were transplanted from northern to southern latitudes.

Langlet (1942-3) has shown that day-length is probably a factor in determining the age at which female reproductive organs are first initiated in P. sylvestris, and that strains from the north of Sweden show accelerated reproductive

development when grown in more southerly latitudes.

The suggestion that P. sylvestris exhibits photoperiodism had thus already been put forward before the present work was undertaken, but a clear demonstration and a detailed analysis were still lacking. In these circumstances a thorough study of photoperiodic phenomena in P. sylvestris promised to be of considerable interest.

3. Scope of the present work.

The investigations were carried out during the years 1947 to 1949 inclusive, and included observations on more than 2,500 individual experimental plants. Experimental work with this type of plant is necessarily somewhat slow, since the growing of a single batch of first-year seedlings occupies a whole growing season, while experiments with older plants involves growing them over a period of 12 months or more before such experiments can be commenced, and here again only a single growth-cycle can be obtained in any one season. The work was carried out mainly at Bedford College, London, but certain experiments, which involved the control of day-length conditions by hand-operation of light-proof covers in the early morning and late evening over a period of several months, were more conveniently carried out in the private garden of the writer, in 1947 and 1948 at Teddington, Middlesex, and in 1949 at Raynes Park, London.

Although the work was directed primarily towards the elucidation of the role of photoperiodism in the normal cycle of growth, since so little is known regarding the general features of photoperiodism in woody species, the investigations included also experiments on first-year seedlings designed primarily as a contribution towards an understanding of the mechanism of photoperiodism in this type of plant.

B. EXPERIMENTAL.

1. Photoperiodism in first-year seedlings of *Pinus sylvestris*.

Experiments on first year seedlings of *Pinus sylvestris* were carried out in the years 1947-1949. As has already been pointed out, first-year seedlings of *P. sylvestris* differ in two important respects from older plants of this species.

(1) The green leaves are 'juvenile' leaves borne on the main stem and are determinate in growth, as contrasted with the paired needles, of indeterminate growth and borne on 'dwarf shoots', of older plants.

(2) The duration of growth of the shoot in the first year is not predetermined by the number of initials laid down in a resting bud, as in the older stages.

In these two respects, therefore, the growth of first-year seedlings is unique in the life-history of an individual of *P. sylvestris* and hence any photoperiodic effects observed in the seedlings will not necessarily be equally applicable to the later stages. Nevertheless, because the duration of

growth is not predetermined in first-year seedlings, they provide a convenient material for the study of the effect of day-length on the duration of growth in a woody species, since relatively large numbers can be grown in pots in a small area, and, as will be shown, they prove to be highly sensitive material.

1.1. Experiment 1.

The first experiment, conducted during the summer of 1947 at Teddington, Middlesex, was designed to explore as fully as possible the effects of two different lengths of photoperiod on various aspects of growth of the root and shoot in first-year seedlings of P. sylvestris.

Methods. The experimental plants were grown in 3½" pots containing soil obtained from Wareham Forest, Dorset, to which had been added 25% by volume of a compost (known as "C 5/10") used in nurseries of the Forestry Commission and containing bracken refuse, spent hops and dried blood.

The seed was supplied by H.M. Forestry Commission and was stated to be derived from parent trees grown in East Anglia. Seven or eight seeds were sown in each pot on 26th May 1947. Germination followed rapidly and on 15th June, by which date 50% of the seedlings had cast the testa, the 'photoperiodic' treatment was commenced. Fifty pots of seedlings were placed in each of two light-proof boxes constructed of a wooden framework and covered with a patent roofing-material. Each

box was provided with a detachable lid which was removed for a specified period each day. One series of plants was exposed from 6 a.m. to 4 p.m. (G.M.T.)^x thus giving a 10-hour photoperiod ("S.D." series) and the other was exposed from 5 a.m. to 8 p.m. (G.M.T.), giving a 15-hour photoperiod ("L.D." series). From 8th August the natural day-length fell below 15 hours and supplementary illumination was provided from a 100-watt electric lamp suspended 2 feet above the plants, giving an intensity of illumination at the level of the plants of about 20 foot-candles. The validity of this procedure is discussed in detail below.

Samples consisting of the plants from five pots were taken from each series for dry-weight determination at intervals of 2 weeks throughout the growing period. The plants were removed from the pots, washed free of soil and killed by immersing in boiling 90% alcohol^{xx} for 5-10 minutes. The plants were then dried in an oven at 100°C for 24 hours. The length of the stem between the cotyledons and the apex, and the number of leaves 1 cm. or more in length were determined for each plant. (The arbitrary standard of 1 cm for leaf-length was

^x All the times of day quoted hereafter refer to G.M.T.

^{xx} The use of boiling alcohol for killing purposes probably led to some loss of weight due to the solution of resin in the alcohol, but as all plants were similarly treated, for comparative purposes this is not important.

chosen because it included all mature leaves and excluded only very young developing leaves).

Results.

Development of shoots. The emergence of the seedlings was followed in both series by a period of growth in which there was a steady production of leaves, not accompanied at first by any detectable elongation of the stem, so that the leaves formed a close "rosette". This phase continued for approximately 6 weeks from the time of emergence of the seedlings. There was no significant difference in leaf number between the two series during this period, but there was a noticeable difference in the length of the leaves, those of the S.D. plants being markedly shorter than those of the L.D. series (see below).

By 27th July extension of the lowest internodes was discernible in a number of plants of the L.D. series, and from this date there was a period of continued stem extension and leaf formation. In the S.D. series extension of the internodes was not observable until about one week later than in the L.D., and the length of the extended internodes was much less in the S.D. plants (see below).

The growth of the shoot ultimately ceased with the formation of a terminal 'winter resting bud' in both series of plants. In the S.D. series, however, dormancy commenced much

earlier than in the L.D. series, 50% of the S.D. plants showing terminal buds on 7th September, the corresponding date for the L.D. plants being 5th October, so that dormancy was delayed by 4 weeks under 15 hour photoperiods.

The internodes of the terminal region remained unextended in both series, so that a terminal rosette of leaves was formed, in the centre of which was the resting bud.

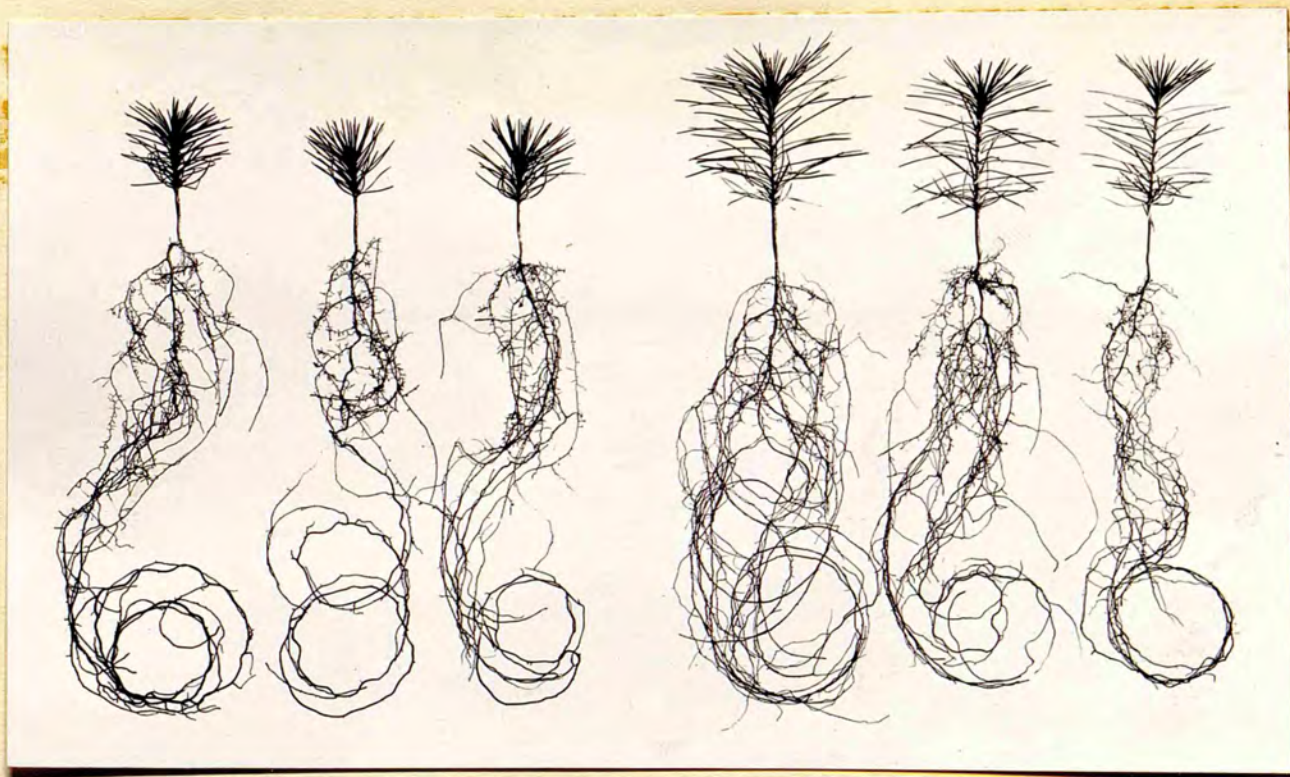


Figure 4. First year seedlings grown under 10-hour (left) and 15-hour (right) photoperiods respectively.

The observations on stem extension and leaf-formation throughout the growing period are summarised in Figures 5 and 6, and the data for the mature plants is given in Table 2. It will be seen that the final difference with respect to total leaf-number between the two series was due mainly to the difference

in duration of growth, there being little difference between the rates of leaf production in the two series.

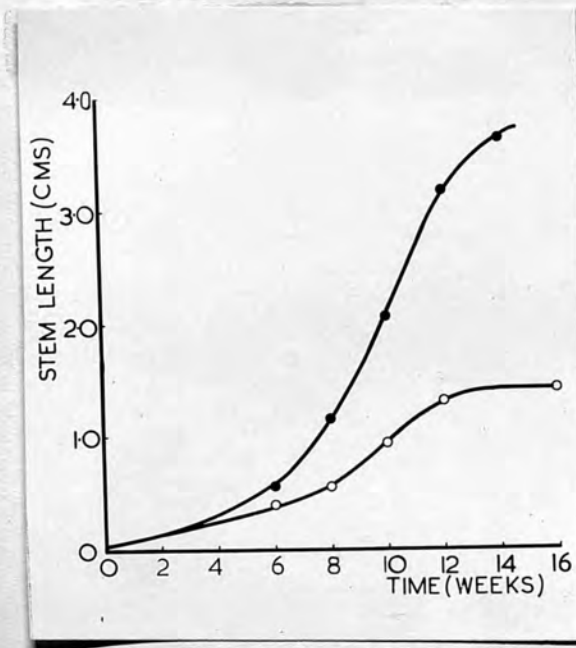


Fig. 5. Stem-lengths.

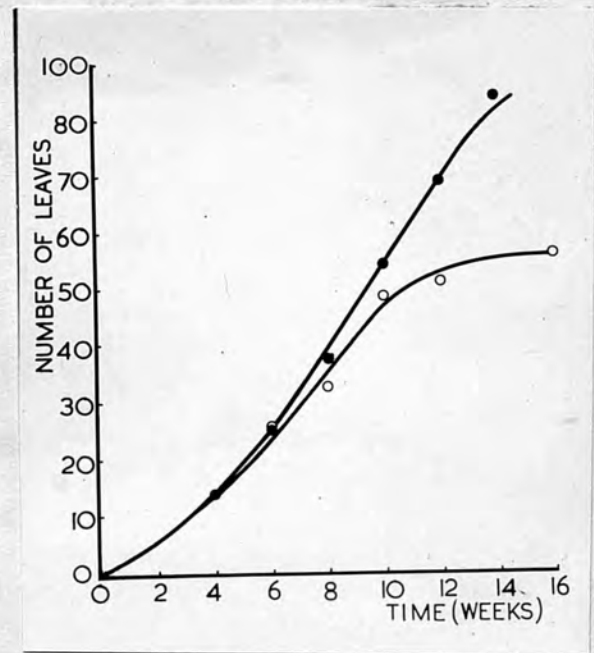


Fig. 6. Leaf numbers.

Increases in Stem-length and Leaf-number in plants grown under 10-hour (open circles) and 15-hour (closed circles) photoperiods respectively.

Table 2. Data for mature seedlings.

Series	No. of plants	Duration of growth (days)	Mean number of leaves.	Mean length of stem. (cms.)	Length of first 25 internodes. (mm.)
S.D.	25	84	$56.2 \pm 2.6^*$	1.41 ± 0.08	0.32 ± 0.02
L.D.	30	112	86.4 ± 2.5	4.20 ± 0.20	0.60 ± 0.02

* In this and all later tables the Mean \pm Standard Error are quoted.

The difference in mean length of stem, however, was due partly to the difference in the number of internodes (= number of leaves), and partly to the fact that the internodes were much shorter in the S.D. plants. This is shown by the data for the mean lengths of the first 25 internodes for each series.

It is seen that the mean internode length in the L.D. plants is nearly double that in the S.D. plants. This difference is clearly seen in Figure 4. The difference in leaf-length referred to above was maintained throughout the period of growth. The length of successive leaves on any one plant increases until a maximum is reached at about the 25th leaf, and thereafter remains constant except in the apical region. Data for the mean maximum leaf-length for each series are given in Table 3. It is seen that the leaves of L.D. plants are significantly longer than those of S.D. (with 't' test, P 0.001).

Table 3. Maximum leaf-length.

Series.	Number of plants.	Number of leaves examined.	Mean leaf-length.
S.D.	24	65	2.4 ± 0.04 cms.
L.D.	23	70	3.0 ± 0.04 cms.

In a few S.D. plants it was observed that premature growth of 'dwarf-shoots' bearing pairs of leaves occurred in the axils of the juvenile leaves. This did not occur in any of the L.D. plants.

Thus under 'short-days' as against 'long days', the following differences in the growth of the shoot were observed:-

- (1) Cessation of growth occurred sooner;
- (2) fewer total leaves were formed;
- (3) the internode length was reduced;
- (4) the leaf-length was reduced.

The question as to whether these are true 'photoperiodic' effects, as opposed to effects arising from differences in amount of assimilation, is considered below.

Development of Roots. Observations on the development of the roots were made throughout the period of the experiment. On 15th June, at the commencement of the experiment, the roots consisted of simple unbranched radicles. By 13th July secondary roots were generally present, with a mean number of 15.3 per plant on the S.D. plants and 15.5 per plant on the L.D. 'Short roots' were observable in both series on 27th July, and by 10th August a few branched mycorrhizal short roots were present. The development of mycorrhizal roots on the samples taken on 7th September is shown by the data of Table 4.

Table 4. Development of Mycorrhizal roots.

Series.	No. of plants examined.	No. of plants showing mycorrhizas.	Mean No. of mycorrhizal roots per plant.
S.D.	29	21	16
L.D.	30	17	22

Thus, up to the time when the S.D. plants became dormant there were no clear morphological differences in the root development of the two series.

Until the onset of dormancy of the shoot there was active growth of both primary and secondary roots in both series, but after the S.D. plants had become dormant a very strong growth of the primary roots became apparent. This effect was not observable in the L.D. plants, but the latter did not become dormant until 5th October, after which date temperature and light conditions were not so favourable for growth as they had been following the onset of dormancy in the S.D. plants.

Unfortunately no quantitative observations were made on the relative development of primary and secondary roots during the active growth period, but the data of Table 5 give such data for the mature plants. These data indicate the greater development of the primary roots in the S.D. plants, whereas the mean length of the secondary roots was greater in the L.D. plants. These differences are significant (with 't' test, $P < 0.05$).

Table 5. Root lengths in mature plants.

Series	No. of plants.	Length of primary root. (cms)	Mean length of secondary roots (cms).
S.D.	14	46.4 ± 2.65	9.03 ± 0.72
L.D.	15	40.2 ± 1.69	12.01 ± 1.11

The root systems of typical L.D. and S.D. plants are shown in Figure 4.

Relative growth of root and shoot. In order to investigate whether differences in length of day have any effect on the relative development of root and shoot in seedlings of P. sylvestris, the increases in dry weight of these organs were followed throughout the period of the experiment. The data are summarised in Table 6 and Figures 7-9. The percentage difference in total dry weight between the two series is not proportional to the difference in photoperiod, and on 24th August, before dormancy of the S.D. plants began to accentuate the differences between the two series, the dry weights of the S.D. plants (expressed as a percentage of those of the L.D. plants) were as follows:-

Total plant.	Shoots.	Roots.
77.6%	76.1%	80.3%

The differences in length of stem between the two series on this date were, however, greater than the differences in dry weights of shoot, the stem length for the S.D. plants being less than 50% that of the L.D. plants.

Consideration of the dry weight curves for the roots shows a steady increase in both series throughout the period of growth, the 'exponential' phase lasting somewhat longer than for the shoots. It will be seen that the general rate of dry weight increase in the S.D. plants was not noticeably affected

by the onset of dormancy of the shoot during the 12th week of the experiment.

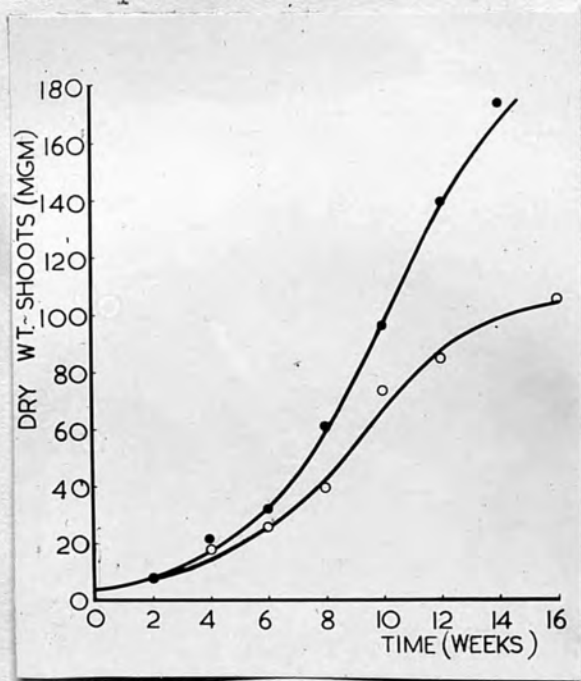


Fig. 7. Dry Weight of Shoots.

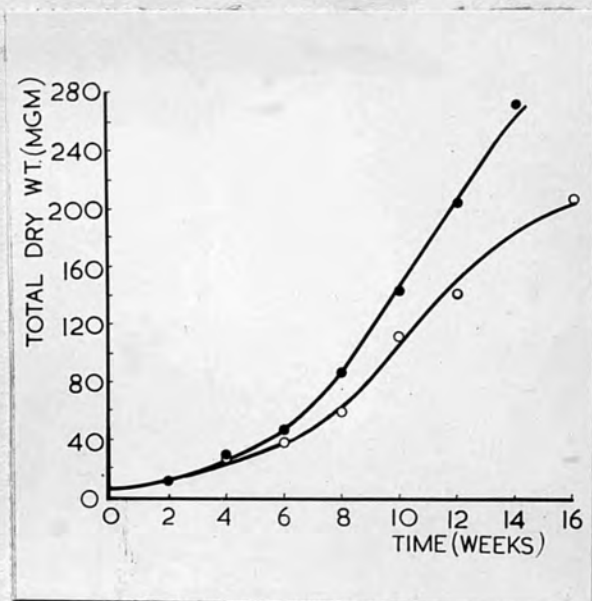


Fig. 8. Total dry weight.

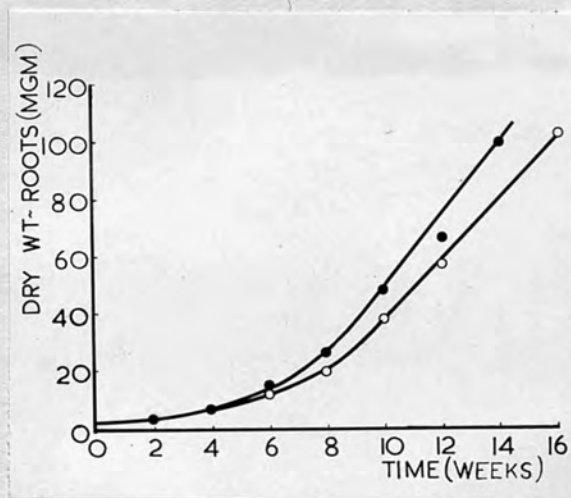


Fig. 9. Dry Weight of Roots.

Dry Weight Data for seedlings grown under 10-hour (open circles) and 15-hour (closed circles) photoperiods respectively.

Table 6. Dry-weight data.

Time from commence- ment of experiment	Long day.			Short day.		
	Total plant (mgms)	Shoots (mgms)	Roots (mgms)	Total plant (mgms)	Shoots (mgms)	Roots (mgms)
0	4.65	3.79	0.87	4.65	3.79	0.87
2 weeks	10.15	7.58	2.57	9.94	7.53	2.41
4 weeks	28.1	21.5±1.0	6.6	23.8	17.5±0.8	6.3
6 weeks	46.8	31.9±1.6	14.9±0.6	37.0	25.3±1.1	11.7±0.6
8 weeks	86.5	60.6±2.1	25.9±1.2	58.4	39.0±1.9	19.4±0.9
10 weeks	142.9	95.6±2.9	47.3±2.1	110.8	72.8±3.0	38.0±2.0
12 weeks	203.7	138.0±5.7	65.7±3.4	140.7	84.2±4.4	56.5±2.7
14 weeks	270.6	171.6±7.2	99.0±6.3	-	-	-
16 weeks	-	-	-	205.7	103.9±6.4	101.8±4.3

The relative growth of shoot and root is frequently measured by the ratio: weight of shoot / weight of root, this being the so-called "shoot/root ratio". This ratio was calculated for the two series of plants from the dry-weight data on successive sampling dates, and is seen to vary throughout the period of growth (Table 7.)

Table 7. Shoot/Root Ratio.

<u>Date.</u>	<u>L.D.</u>	<u>S.D.</u>
15/6/47	4.4	4.4
29/6	3.0	3.1
13/7	3.3	2.8
27/7	2.2	2.2
10/8	2.3	2.0
24/8	2.0	1.8
7/9	2.1	1.5
21/9	1.7	-
5/10	1.7	1.1

The value of the shoot/root ratio falls progressively in both series and in the early stages there is no marked difference between them. The greater fall in the value of the ratio for the S.D. series after 24th August is to be explained by the onset of dormancy in the shoot while the roots continued to grow actively.

Pearsall (1927) has shown that for many plants there is

a linear relation between the logarithms of the shoot and root weight, viz.

$$S = CR^k$$

$$\text{or } \log S = k \log R + C \quad \dots\dots\dots (1)$$

where S = shoot weight

R = root weight

and k, C are constants.

Figure 10 shows the results of plotting log shoot-weight against log root-weight for the two series of plants of the present experiment.

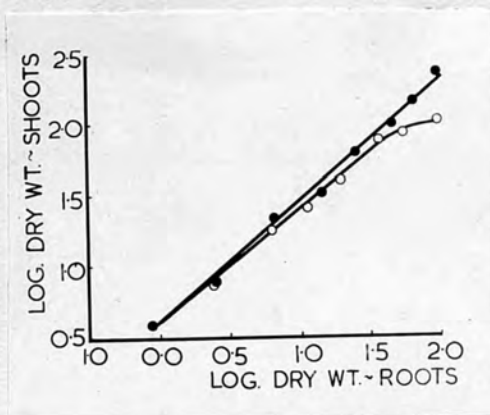


Fig.10. Relative Growth of Shoot and Root, in 10-hour - (open circles) and 15-hour - (closed circles) day seedlings respectively.

It is seen that the rule holds very closely for both L.D. and S.D. plants during the period of active growth of the shoot, but there is deviation from the linear relation in the later stages of the S.D. series, due of course, to the cessation of growth of the shoot. The regression coefficients for log shoot weight on log root weight are found to be 0.8193

and 0.7494 for the 'long day' and 'short day' series respectively. Applying a 't' test for the difference in the values of these regression coefficients, it is found that they do not differ significantly ($t = 0.88$ corresponding to a probability level of approximately 0.4 for 10 degrees of freedom). This indicates that during the period of active growth the relative balance of shoot and root development is unaffected by differences in length of day, that is to say, the value of k (given by the slope of the curve) is unaffected by the length of the photoperiod.

Huxley (1932) has pointed out that on differentiation of the equation (1) we obtain

$$\frac{dS}{dtS} = k \frac{dR}{dt.R} \dots \dots \dots (2)$$

Now $\frac{dS}{dtS}$ & $\frac{dR}{dtR}$ are the relative rates of growth of shoot and root respectively, and hence equation (2) indicates that there is a constant ratio between the relative rates of growth of shoot and root. The results indicate that this ratio is unaffected by length of day under the conditions of the experiment.

1.2. Experiment 2.

Since the conditions of Experiment 1 were such that the differences between the long day and short day plants might possibly have been due to differences in photosynthesis, a

simple experiment was carried out in 1948 to test the effect of reducing the intensity of natural daylight by 50% but keeping the length of the natural photoperiod unchanged. The plants grown under such conditions were compared with (a) plants grown in full daylight and natural day length and (b) plants grown in full daylight but with a reduced photoperiod of 10 hours.

At the same time a further series of plants was grown under natural day-light supplemented by low intensity illumination during the hours of darkness, so that plants were exposed to continuous light. Since the supplementary illumination was of such a low value (25 f.c.) as to have a negligible effect on assimilation, any effects observed could be regarded as 'photoperiodic' and not due to any appreciable changes in assimilation.

Methods. The culture methods were the same as those described in Experiment 1. The observations on dry-weight were discontinued, however, since it had been found in Experiment 1 that a difference in day-lengths had no detectable effect on the relative development of shoot and root, and that dry-weight data relating to shoot growth added little to the information obtained from observations on leaf-number and stem length.

The seed was sown on 20th April 1948, and on emergence of the seedlings they were divided into four series of 12 pots each and exposed to different treatments as follows:-

- Series A. Full natural daylight and normal daylengths.
- Series B. Full natural daylight but reduced photoperiod of 10 hours, obtained by covering the plants from 4 p.m. to 6 a.m. (G.M.T.)
- Series C. Natural daylight of reduced intensity, obtained by placing over the plants a wooden framework covered with several layers of cheese-cloth to give a light intensity equal to 50% of that of full daylight. The day-length conditions were 'normal'.
- Series D. Natural daylight of normal daylength supplemented during the hours of darkness by artificial illumination of approximately 25 foot-candles at the level of the plants.

Observations were made to determine the dates on which 50% of the plants of each series showed terminal resting buds. When all plants had become dormant the mean length of stem and the mean number of leaves were determined and from these data the mean internode lengths for the extended portion of the stem (i.e. the portion below the terminal rosette) were calculated. The data are summarised in Table 8. The mean duration of growth was obtained by determining the date on which 50% of the plants in any given series showed terminal resting buds.

Representative plants from each series are shown in Figure 11.

Table 8. Data for mature plants of Experiment 2.

Series	No. of plants	Mean length of stem (cm)	Length of 'internode' (mm)	Mean number of leaves	Mean duration of growth (days)
Normal day A	47	3.47 ± 0.13	0.86	64.7 ± 2.0	116
15 hr day full day B	49	0.69 ± 0.04			89
N.D. low light 50% C	41	3.28 ± 0.17	0.94	57.3 ± 2.2	116
Constantly kept arb. light etc. D	49	5.90 ± 0.23	1.37	53.4 ± 1.6	103

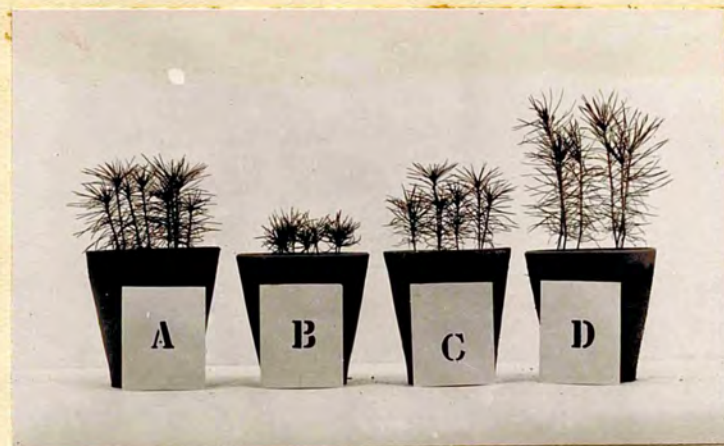


Fig. 11. Representative samples of the four series of plants of Experiment 2. (For description of treatments see p.42).

Results. The natural daylength during the period of the experiment (early May to mid-September) ranged from 15 hours to $16\frac{1}{2}$ hours at mid-summer and then diminished to approximately 13 hours by 12th September. (These times are from sunrise to sunset and do not include a photoperiodically active twilight

period of variable duration). The day-length conditions for series A and C may thus be described as 'long-day'. From the data it is seen that the plants of series A and series B resembled the 'long-day' and 'short-day' plants of Experiment 1. The appearance of dwarf shoots bearing pairs of leaves in the S.D. plants was more prevalent than in the previous experiment.

The plants of series C, which were grown under normal day-length but with reduced light-intensity, closely resembled those of Series A with respect to duration of growth, leaf number and internode length, and were sharply contrasted with the plants of Series B, although the latter must have received a greater quantity of incident light. Although the plants of Series C had significantly fewer leaves than those grown under full day-light (for 't' test, P between 0.02 and 0.01), the length of the internodes was greater in the shaded plants, which thus showed slight 'etiolation'. This latter effect is in marked contrast with the reduced internodes of short-day plants, thus indicating that the differences observed between the 'long-day' and 'short-day' plants of Experiment 1 were not due to differences in assimilation, but were probably true photoperiodic effects.

The plants of series D, grown under continuous illumination, showed the following differences from the 'normal-day' plants of series A:-

- (1) the mean duration of growth was 13 days shorter under continuous illumination;
- (2) the number of leaves was significantly fewer; (with 't' test, $P < 0.001$);
- (3) the total length of stem, however, was greater due to the very much longer internodes under continuous illumination;
- (4) there was no terminal 'rosette' of leaves, nearly all the internodes, practically to the apex of the stem, being fully extended;
- (5) the lateral 'dwarf-shoots' grew out in the axils of the uppermost juvenile leaves, and bore pairs of needles, just as had been noted in the S.D. plants;
- (6) the terminal buds were very much smaller in size than those of the other three series.

It should be noted that all these effects were produced by artificial illumination of only 25 foot-candles, and hence must be true 'photoperiodic' effects. The significance of these effects will be further discussed below.

1.3 Experiment 3.

Evidence will be adduced below in support of the view that photoperiodism in woody species has essentially the same physiological basis as in herbaceous plants. One of the characteristic features of photoperiodism in herbaceous species is that it is the duration of unbroken darkness which determines

d. p. exp Table 10 p. 55!

whether or not flowering will occur under any given day-length conditions, and that a short 'light' break during the dark period completely modifies the nature of the response (see, for example, Gregory, 1948). When the dark period is interrupted by a 'light-break' so as to give two dark periods of unequal length, then the response appears to be determined by the longer of these two dark periods (Harder and Bode, 1943). On the other hand, interruption of the photoperiod by a period of darkness does not appreciably affect the time of flowering in herbaceous species. As these effects have not previously been reported for woody species, the following experiment was carried out to determine whether they are also observable with this type of plant.

Methods. The culture conditions were the same as those described for the preceding experiments. The seed was sown on 20th April 1948 and on emergence the seedlings were divided into four series of eleven pots each, which were treated as follows:-

Series A - Exposed to a 15-hour daily photoperiod from 6 a.m. to 9 p.m. using natural daylight supplemented as necessary by artificial illumination from a 100-watt filament lamp, giving an intensity of approximately 20 f.c. at the plants.

Series B - Exposed to an 11-hour photoperiod from 6 a.m. to 5 p.m. using natural daylight.

Series C - Exposed to two periods of light in each 24 hour period - (a) from 6 a.m. to 1 p.m. (b) from 5 p.m. to 9 p.m. There were thus two daily periods of darkness, of 4 hours and 9 hours duration respectively, separated by light periods of 7 hours and 4 hours duration. For the second light period from 5 p.m. - 9 p.m.) natural daylight was supplemented by artificial illumination as for series A. The plants of series C were thus exposed to the same conditions as those of series A, except for the dark period from 1 p.m. to 5 p.m.

Series D - Exposed to a photoperiod of $10\frac{1}{2}$ hours from 6 a.m. to 4.30 p.m. The dark period was interrupted by 30 minutes of illumination at 20 foot-candles from 8.30 - 9.0 p.m., thus giving two dark periods of 4 and 9 hours' duration respectively. The conditions were thus the same as for series B, except for the 'light-break' during the dark period.

The mean dates of dormancy were observed in each series and on maturity the mean stem length, total leaf-number, and internode length were determined. The data are summarised in Table 9, and representative plants from each series are illustrated in Figure 12.

Results. The data of Table 9 show that the plants of series A and B under 15 and 11 hour photoperiods respectively,

Table 9. Data for mature plants of Experiment 3.

Series	No. of plants	Mean length of stem (cm)	Length of 'internode' (mm)	Mean number of leaves	Mean duration of growth (days)
A	52	4.62 ± 0.21	1.07	66.1 ± 2.1	119
B	47	1.51 ± 0.08	0.62	38.2 ± 0.9	86
C	54	5.69 ± 0.20	1.00	79.1 ± 1.8	135
D	50	3.54 ± 0.11	0.99	49.3 ± 1.7	95

cf. p. 53.
Length of stem

showed the type of response which can now be regarded as typical for 'long-day' and 'short-day' seedlings of Pinus sylvestris. The interruption of the photoperiod by 4 hours' dark in the case of series C did not reduce the total leaf number as compared with series A, but actually resulted in a longer duration of growth and a greater total leaf-number. This result was unexpected and was the subject of further investigation which is described below.

The plants of series D, which received a 'light-break' of only 30 minutes' duration at low intensity, showed a marked response to this treatment, the total leaf-number being significantly greater than that of series B. (for 't' test, $P < 0.01$) while the internode length was practically equal to that of series A.

Thus, the interruption of the dark period by a short 'light break' produced a response approaching that of plants

grown under 'long-day' conditions, as in short day herbaceous species. Similarly the results obtained when the photoperiod was interrupted with a period of darkness agree with the observation that in herbaceous species such interruption does not reduce the effect of long photoperiods, but the enhanced 'long-day' effect (as shown by the increased leaf-number) in *P. sylvestris* appears to be a new phenomenon. stat

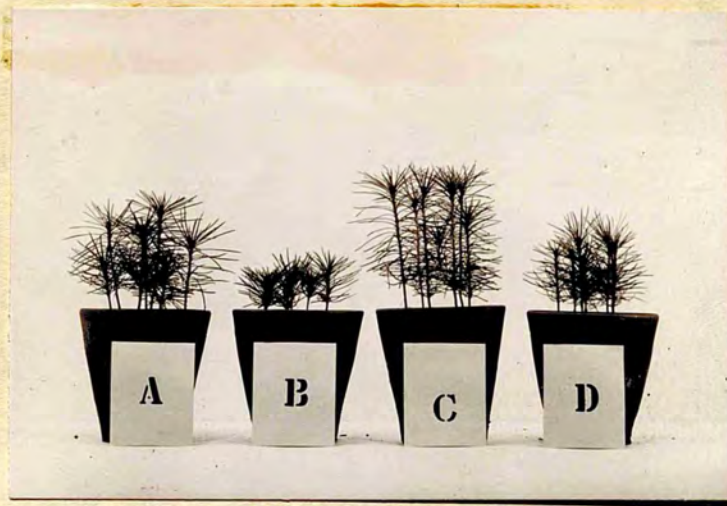


Fig.12. Representative samples from the four series of plants of Experiment 3. (For description of treatments see pages 46-47).

It is of interest to compare the growth of series C and D which received the same total period of light (11 hours) and the same dark periods of 4 hours and 9 hours respectively, for whereas the number of leaves formed in series C was greater than in series A the leaf-number of series D did not

attain that of series A, (although greater than that of series B.)

The difference between the responses of series C and series D would appear to be due to the difference in amount of light received during the second period of illumination - 4 hours of which part was daylight in the case of series C, and only 30 minutes at a low intensity in the case of series D. This matter is further discussed below.

1.4 Experiment 4.

Among the results of Experiment 2 and 3 in 1948 were the observations that (1) the greatest leaf-number is attained under a photoperiod of duration less than 24 hours (i.e. continuous light); (2) seedlings which received a photoperiod interrupted by 4 hours darkness in addition to a 9-hour dark period each day attained a greater leaf-number than plants which received only one period of darkness of 9 hours' duration per day. These two observations suggested that a high leaf-number may actually be favoured by a short period of darkness and that if two such periods are given in a single 24-hour cycle, then their effects may be cumulative. In order to test this hypothesis two further experiments were carried out in 1949. In the first experiment seedlings were grown under various photoperiods, ranging from 12 hours to continuous light, in order to determine under what duration of dark period the maximum leaf-number is attained.

Series Methods. The seedlings were grown under the same conditions as in the previous experiments. Owing to the very poor germination of seed sown in April, a complete re-sowing had to be made on 15th May 1949. Emergence of the seedlings had been completed by 12th June, on which date the plants were divided into nine series of 10 pots each, of which seven series were used in the present experiment, and were exposed to the following photoperiods:-

Series 1. - 12-hour photoperiod; plants covered from 7 p.m. to 7 a.m.

Series 2. - 14-hour photoperiod; plants covered from 9 p.m. to 7 a.m.

Series 3. - 16-hour photoperiod; plants covered from 15½ hours after sunrise until dark. From middle of August it was necessary to supply artificial illumination of 20 f.c. intensity to supplement the natural daylight. The period of 15½ hours was chosen, since there is a twilight of approximately 30 minutes' duration before sunrise, during which the light intensity is sufficient to evoke a photoperiodic response.

Series 4. - 18-hour photoperiod; plants not covered, but exposed to natural daylength plus additional illumination at approximately 20 f.c. in the evening to give a total photoperiod of 18 hours from half-an-hour before sunrise.

Series 5. - 20-hour photoperiod; plants exposed to natural day-length, supplemented by artificial illumination from 3 a.m. until sunrise, and from sunset until 11 p.m.

Series 6. - 22-hour photoperiod; plants exposed to natural day-length supplemented by artificial illumination from sunset to sunrise, but covered from 5 p.m. to 7 p.m.

Series 7. - 24-hour photoperiod; plants exposed to natural day-length, supplemented by artificial illumination from sunset to sunrise.

The duration of artificial illumination for series 4 and 5 was controlled by time-switches, otherwise the photoperiods were controlled by hand. It will be observed that in the case of series 1 - 3 the full photoperiod consisted of natural daylight, except for a short period of additional illumination from mid-August, to give a 16 hour photoperiod in the case of series 3. The plants of series 4 - 7, on the other hand, received the full natural day, (except that the plants of series 6 were covered from 4 p.m. to 6 p.m.), supplemented by artificial illumination for varying periods.

A number of plants of series 4 and 5 had still not completed their growth by the end of October and hence these series were transferred to a greenhouse maintained at 15°C, on 31st October. All growth was completed by the end of November.

When shoot growth had ceased, determinations were made

Table 10 Data for Mature Plants of Experiment 4

Photoperiod	10	12	14	16	18	20	22	24
Total stem length (cms.) \bar{x}	0.76 +0.05	1.06 +0.06	1.50 +0.09	1.78 +0.10	4.10 +0.20	6.32 +0.23	3.91 +0.20	3.68 +0.17
Total no. of leaves \bar{x}	36.7 +1.53	44.9 +1.95	53.2 +2.3	61.6 +1.3	71.9 +2.2	74.7 +2.7	51.4 +2.1	50.6 +1.6
Leaves in terminal rosette \bar{x}	-	-	29.2	28.8	19.5	7.7	11.5	11.6
No. of extended internodes \bar{x}	-	-	24.0	32.8	52.4	67.0	39.9	39.0
Stem-length to "rosette" \bar{x}	-	-	1.12	1.36	3.75	6.07	3.74	3.47
Internode length (mm.) \bar{x}	-	-	0.466	0.413	0.716	0.906	0.935	0.889
Mean date of cessation of growth (50% of plants).	(13/8)	25/8	2/9	10/9	7/10	29/10	4/9	26/8
Mean duration of growth (days)	(90)	87	94	102	129	151	96	87

\bar{x} Mean of 30 plants.

for each series of the mean leaf-number, stem-length, length of internode etc. The data are summarised in Table 10 and Figure 13. (These data also include a series of plants grown under a 10-hour photoperiod as part of Experiment 6. The plants were derived from the first sowing made on 17th April 1949, but otherwise the culture conditions were identical with those of the present experiment).

Each series contained approximately 50 plants, but the data for leaf-number and stem length were determined for 30 plants only in each case. The method adopted in choosing these plants was to select 6-7 pots at random from each series and then to count all the plants in each pot until the required number had been obtained.

Results. The total height attained by the plants of all series in 1949 was considerably less than that attained under the corresponding daylengths in 1948 (compare, for example, the heights of plants under 10 hr, 15 hr, and 24 hr photoperiods shown in Tables 2, 8 and 9 with those given in Tables 10). This reduced height was partly due to lower leaf-numbers (i.e. fewer nodes), but was mainly due to greatly reduced internode lengths in 1949. The summer of 1949 was remarkable for its high sunshine record and it is possible that high light intensity was responsible for the reduced internode length, since this effect is well-known in other plants (Shirley, 1936). Alternatively, the abnormally high temperatures may have been

supra-optimal for the growth of the seedlings.

In Figure 13 the mean leaf numbers are plotted against the length of the daily period of darkness. It is seen that the maximum leaf number is attained with a daily dark period of 4 hours (20-hour photoperiod) and that with only 2 hours darkness or with continuous light the maximum leaf-number falls off sharply. Similarly, with dark periods greater than 4 hours, the leaf number falls linearly with increasing length of dark period. The duration of the growing period also shows a maximum at 4 hours darkness, but the greatest internode length was at 2 hours darkness, with only a slightly diminished value at 4 hours dark and continuous light. (Table 10).

One of the most notable features of these results is the striking difference between the plants under a 22-hour and 20-hour photoperiods respectively. The former resembled very closely the plants grown under continuous light, which showed the features already noted for the corresponding treatment in 1948 viz. relatively short growing period, reduced leaf-number, high internode length, small terminal buds and premature growth of 'dwarf-shoots' in the axils of the juvenile leaves. The 20-hour photoperiod plants, however, showed a very long growing period, a markedly higher leaf-number, large terminal buds and very little tendency for the growth of dwarf shoots. A few plants under the 20-hour day resembled the 22-hour day plants

(e.g. in growth of dwarf shoots and small terminal buds) and vice versa. Evidently there is a relatively sharp transition from one type of response to the other during the 3rd and 4th hours of darkness.

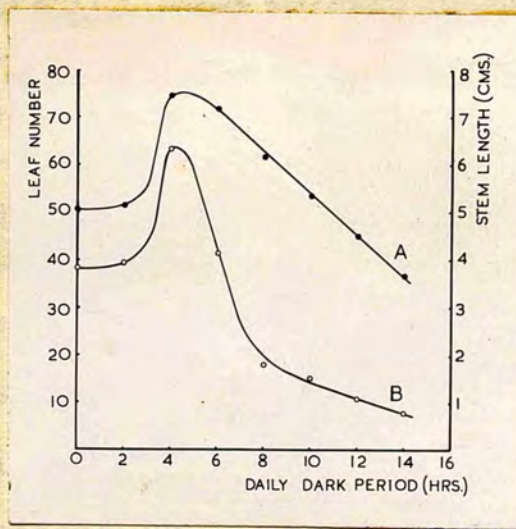


Fig.13. Variation in leaf-number (A) and stem-length (B), with length of dark-period.



Fig.14. Representative samples from each of series of plants of Experiment 4.

Although the greatest final height and leaf-number were attained with a 20-hour photoperiod, the greatest rate of

growth in height was attained under continuous light. This is shown by the data of Table 11, giving the heights of the plants for each series on 5th August, on which date all plants were still growing.

Table 11. Mean Heights of plants on 5th August.

Photoperiod	14 hr	16 hr	18 hr	20 hr	22 hr	24 hr
Mean Height (cms)	0.94	0.89	1.03	1.57	1.68	1.79

A further point of interest is that the number of unextended internodes forming the terminal 'rosette' of leaves was a minimum in the 20-hour day plants, and was greatest with the shorter photoperiods. In the 10-hour and 12-hour day series the extended internodes were so short that it was impossible to determine the precise limits of the terminal rosette.

The results of this experiment will be discussed below, and the chief point of interest to note here is that a daily dark period of 4 hours results in the greatest final leaf-number

1.5 Experiment 5.

A further experiment was carried out using the two remaining series of pots from the same batch of seedlings as those used for the preceding experiment. This experiment was designed to test the second part of the hypothesis put forward above, viz. that a short dark period promotes a high leaf-

number and that if two or more such periods are given in a single 24-hour cycle, then the effects are cumulative. Following up the results of Experiment 3 in 1948, the short dark period used was of 4 hours' duration, but the present experiment differed from Experiment 3 in that all dark periods were of the same duration. One series of plants received two dark periods of 4 hours' duration, separated by 2 light periods of 8 hours; the other series received 3 dark periods of 4 hours each day, separated by 3 light periods of 4 hours. The growth of these two series of plants was compared with the 20-hour day plants of Experiment 4, which received only one such 4-hour dark period per day (These will be referred to as "Series C"). It was not known, of course, at the outset of this experiment that the 20-hour photoperiod would give the greatest leaf-number in Experiment 4, and the choice of 4-hour dark periods in the present experiment proved to be more fortunate than could have been foreseen!

Methods. The two series of plants were treated as follows:-

Series A. - Plants exposed to two light periods each of 8 hours duration (1) from 3 a.m. to 11 a.m. (2) from 3 p.m. to 11 p.m. Light periods separated by two dark periods, each of 4 hours' duration. Supplementary artificial illumination was given from 3 a.m. to sunrise and from sunset to 11 p.m.

Series B.- Plants exposed to three light periods each of 4 hours duration: (1) from 3 a.m. to 7 a.m. (2) from 11 a.m. to 3 p.m. (3) from 7 p.m. to 11 p.m. Light periods alternated with 4-hour dark periods. Artificial illumination was given as for series A.

Series C.- Plants receiving 20-hour photoperiod in Experiment 4.

The plants of series A and B were grown alongside the plants of series C, and the artificial illumination arrangements were common to all three series of plants.

At the end of October a few of the Series A plants were still growing and they were transferred to the greenhouse at the same time as the plants of Series C. At this date over half the series B plants were still growing, and as it was not



Fig.15. Representative samples of plants of series C and A of Experiment 5. (For description of treatments see text).

practicable to continue the experimental treatment for this series in the greenhouse, this part of the experiment was

abandoned and the plants were allowed to remain out-of-doors under natural day-length conditions from the end of October.

A?

Results. Growth in the plants of series B proceeded vigorously throughout the period of the experiment, and the plants soon became noticeably taller than those of series C. The data for the mature plants are given in Table 12, and corresponding data for the series C plants are repeated from Table 10 for comparison. These data are for 30 plants taken from each series.

Table 12. Data for mature plants.

Series.	Duration of growth	Total leaf-number	Height (cms)	Mean internode length (mm)
8L → 4D → 2L → 4DA	138 days	98.7 ± 4.4	10.12 ± 0.42	1.125
20L → 4D C	147 days	74.7 ± 2.7	6.32 ± 0.23	0.906

Table 13. Dry weight data for mature plants.

Series	Mean dry weights			S/R Ratio
	Shoot (gms)	Root (gms)	Total plant (gms)	
A	0.217 ± 0.010	0.0517 ± 0.0028	0.269 ± 0.013	4.2
C	0.193 ± 0.010	0.126 ± 0.010	0.319 ± 0.018	1.5

It is seen that although the mean duration of growth was 13 days shorter for the series A plants, these attained a greater total leaf number, and showed a greater internode length than those of series C. The difference in mean total

height between the two series is very striking, and is clearly indicated in Figure 15. Since the duration of growth of series A was actually less than that of series C, it is clear that the rates of leaf-production and stem elongation were greatly accelerated by the introduction of two dark periods into the 24 hour cycle.

The dry weights of the shoots and roots were determined for the plants of both series when all shoot growth had ceased. The data (Table 13) show that the mean dry weight of the shoots of series A was greater than that of series C, but the difference is not significant (with 't' test, $P = 0.25$). The mean dry weight of the roots of series A was significantly less than that of series C ($P < 0.05$), however, those of the former being very poorly developed. The total dry weight for plants of series A was significantly less than that of series C ($P < 0.05$), a fact which must probably be ascribed to reduced period of daylight to which series A was exposed. Because of the poor root development, the shoot/root ratio of series A was much higher than in series C.

The plants of series A also differed from the majority of plants of series C in that they showed poor development of the terminal bud, accompanied by premature growth of 'dwarf-shoots' in the axils of leaves in the terminal region. This may be seen in Figure 15. In this respect they resembled the plants under 22- and 24-hour photoperiods.

These results seem clearly to indicate that whereas, with a single daily alternation of light and dark periods, the greatest growth is obtained with 20 hours' light and 4 hours' dark, if two dark periods of 4 hours duration are given in the 24 hour cycle, then growth (as indicated by leaf-number and height) is even further enhanced. The additional dark period results in a greatly increased value of the shoot/root ratio, indicating that during the first 4 hours of darkness growth of the stem is greatly promoted as compared with that of the root system.

The plants of series B showed obviously reduced growth throughout the period of the experiment, as compared with those of series A, and, to a lesser extent, as compared with the plants of series C. The growth of these plants was particularly slow during September and October (as indicated by the rate at which the plants became dormant), when the duration of natural daylight was diminishing rapidly. When it is considered that the plants were covered each day for a total of eight hours during the period of natural daylight throughout the experiment, it seems very probable that insufficient photosynthesis was limiting the growth of these plants. It is also possible, however, that the limitation of the photoperiod to 4 hours' duration, part of which was at low light intensity (before sunrise and after sunset), was important. This matter is discussed below.

1.6 Experiment 6.

Moshkov (1932) reported the occurrence of a 'photo-periodic after-effect' in certain woody species. Thus, if one-year cuttings of Salix viminalis were subjected to short photoperiods for 15, 25 or 36 days respectively and then transferred to long photoperiods the total height attained by such plants was less than that of plants grown under long-days throughout. In the case of a similar experiment with S. alba exposure to short-days for periods of 15 and 25 days duration increased the total height-increment when the plants were subsequently transferred to long-days. 'Photoperiodic after-effects' shown by differences in leaf-shape have been reported in Robinia pseudacacia, and Morus alba (Scepotjev 1948a, 1948b).

In order to ascertain whether a 'photoperiodic after-effect' occurs also in Pinus sylvestris, and to obtain more information regarding this phenomenon, the following experiment was carried out.

Methods. Thirty five pots of seedlings were grown under the same conditions as described in the previous experiments. On emergence the seedlings were divided into two lots (containing (a) eighteen and (b) seventeen pots) which were subjected to 10-hour and 15-hour photoperiods respectively from 28th May. After 15 days (12th June) 9 pots were transferred from 15-hour to 10-hour photoperiods. After a further 15 days

these latter plants were transferred back to 15-hour days, and at the same time 8 pots which had until then remained continuously in short-days were transferred to long-days. The day-length conditions were then maintained unchanged until all plants had become dormant. The mean heights and leaf numbers of all plants were then determined. There were thus four series of plants exposed to day-length conditions as follows:-

Series A - 10-hour photoperiod throughout.

Series B - 15-hour photoperiod throughout.

Series C - 15-hour photoperiod throughout except for 15 short photoperiods from 12th June to 27th June.

Series D.- Exposed to 10-hour photoperiods for first 30 days from emergence, and then transferred to 15-hour photoperiod.

Results. The data for the four series of plants are summarised in Table 14.

Table 14. Data for mature plants of Experiment 6.

Series	No. of plants	Length of stem (cms)	Leaf-numbers		Total leaves	Mean duration of growth
			Leaves on extended portion of stem.	Leaves in terminal rosette.		
A	30	0.76 ±0.05	-	-	36.70 ±1.52	77 days
B	50	1.45 ±0.06	27.8 ±0.7	28.3 ±0.9	56.22 ±1.36	87 "
C	49	1.47 ±0.07	26.2 ±1.1	21.2 ±1.1	47.45 ±1.65	77 "
D	49	1.39 ±0.09	24.5 ±1.1	19.4 ±1.1	43.86 ±1.72	77 "

Considering first the leaf-numbers of the various series, it is seen that both series C and D, which received periods of exposure to short days, formed fewer leaves, than did those which received a 15-hour photoperiod throughout. Statistical analysis shows that these differences are highly significant (for 't' test $P < .001$).

Now both series C and D continued growth under a 15-hour day for some six weeks after the short-day exposures for both series ceased on 27th June. The question now arises as to whether this effect of exposure to short days, which hastened the onset of dormancy and reduced the total number of leaves, was a true photoperiodic after-effect, or whether in fact the morphological differentiation of the terminal resting bud had already commenced at the time they were transferred from short-days. The occurrence of a prolonged period of further growth with continuous formation of new leaves after removal from short days alone suggests that we have a case of 'photoperiodic after-effect', but the possibility is not excluded that the new leaves which appeared were actually laid down at the end of the short-day exposure. The following considerations indicate that the latter alternative is not the case, however. Comparison of the plants of series D with those grown under a 10-hour photoperiod throughout shows that there is a difference in total leaf-number (43.9 as against 36.7) between the two series which is highly significant ($P < .001$). Now both these series received

continuous short-days from emergence until 27th June, when the plants of series D were transferred to long-days. If differentiation of the terminal resting-buds had already commenced at that date there could have been no difference in final leaf-number between the two series. The fact that the plants of series D formed a greater number of leaves than those of series A indicates that a further period of leaf formation occurred in the plants of series D after transfer to long days. Nevertheless, the effect of the previous exposure to short-days was made evident by the hastened dormancy and fewer total leaves of series D as compared with plants grown under long days throughout (series B). It is thus evident that we have here a true photoperiodic after-effect. The same argument holds equally for the plants of series C, unless these had already formed a greater number of leaves than the plants of series A, at the time of short-day exposure, an assumption for which there is no evidence.

If we compare the number of leaves (1) on the extended portion of the stem, and (2) in the terminal rosette, in the plants of series C and D with those grown under long days throughout, it is seen that the differences in respect of the former are only slight, and that the differences in total leaf-number are mainly due to differences in the number of leaves in the terminal rosette. Moreover, there are only slight differences with respect to the length of the extended portion

of the stem in the three series in question. It appears, therefore, that under a given length of photoperiod the extension of a certain maximum number of internodes is possible, and that if there are many additional leaves already present above the point of maximum extension, the internodes in the apical region must remain unextended to give a terminal 'rosette'.

Additional evidence in support of this interpretation is afforded by the results of the following further experiment.

1.7 Experiment 7.

Methods. Twelve pots of seedlings were kept under a 10-hour photoperiod from emergence until the first signs of dormancy became apparent (as indicated by the appearance of terminal buds) on 4th August. Six of the pots (series A) were then transferred to natural day-length conditions (on this date corresponding to about a 15-hour photoperiod), while the remainder were continued under a 10-hour photoperiod. In 2 - 3 weeks all plants showed terminal resting-buds and all growth had ceased. The leaf-numbers and stem lengths of both series were determined and the data are given in Table 15.

Results. There was clearly no significant difference in total leaf-number between the two series, indicating that the formation of a terminal resting-bud was already pre-determined, (although buds were not visible in the great majority of plants) at the time of transfer of the series B

Table 15. Data for Mature Plants of Experiment 7.

Series	Number of plants	Length of stem	On extended portion of stem.	Leaf numbers In terminal rosette.	Total
A	30	1.13 ±0.09	21.8 ±1.1	17.3 ±1.6	39.1 ±1.9
B	30	3.35 ±0.26	35.1 ±1.7	4.4 ±0.5	39.5 ±1.7



Fig.16. Representative samples of plants of Experiment 7. "A" grown under 10-hour day throughout, "B" transferred from 10-hour day to natural day-length on 4th August.

plants from short-days to long-days. Nevertheless, the total length of stem was very much greater in the plants of series B and this was due partly to the greater number of extended internodes (35.1 as against 21.8), and partly to the greater internode length, in the plants of series B. Whereas the number of unextended internodes in the plants of series A was 17.3,

there were only 4.4 such internodes in those of series B. Evidently the transfer of series B plants to long-day conditions permitted the extension of a greater number of internodes than under short days, although the total number of leaves formed was already pre-determined at the time of transfer, and hence the increased number of extended internodes was at the expense of the number of leaves in the terminal rosette.

1.8 Discussion.

The occurrence of photoperiodic effects in seedlings of Pinus sylvestris seems clearly established by the results of the experiments described above. In general, with 'short-days' (e.g. 10-hour photoperiod) the duration of the growing period is reduced, fewer total leaves are formed, and the mean internode length is very much shorter as compared with corresponding plants grown under 'long-days' (e.g. 15-hour photoperiod). As the length of the photoperiod is still further increased a maximum for duration of growth and total leaf-number is reached with a photoperiod of 20 hours, and with photoperiods longer than this the duration of growth and total leaf-number fall sharply. There is, however, no such clearly marked optimum photoperiod for internode length, there being an ill-defined 'maximum' with a photoperiod of about 22 hours. That these are true 'photoperiodic' effects and not due to variations in amount of photosynthesis resulting from exposure

to different periods of illumination is clearly shown by the following evidence:

- (1) Reduction of the intensity of natural daylight by shading does not produce the typical 'short-day' effects, but the plants closely resemble those grown under full light of 'normal' day-length. (Experiment 2).
- (2) A period of supplementary illumination of quite low light intensity (20-25 foot-candles), on the other hand, has marked effects. For example, there is a very great difference between plants grown under the full natural day-length supplemented by low intensity illumination to give 18,- 20,- and 24-hour photoperiods respectively. The difference in the amount of light received by the 18,- and 20-hour day plants amounted to only 2-hours at 20-25 foot-candles, and yet significant differences in respect of duration of growth, total leaf-number, etc. were observed. Even more marked differences occurred between the 20- and 24 hour day plants.
- (3) If the daily light period is divided into 2 portions of 7-hours and 4-hours' duration respectively (Experiment 3), then the resulting plants do not resemble 11-hour day plants, but show enhanced 'long-day' characteristics. Similar results were obtained in Experiment 5.

Reference has already been made to the fact that certain photoperiodic effects in woody species are evoked by quite low

light-intensities. The evidence adduced under (2) above shows that this is true equally for photoperiodic effects in Pinus sylvestris. This conclusion justifies the experimental procedure of supplementing natural daylight by low-intensity artificial illumination to obtain the required length of photoperiod. In order to avoid the possibility of effects due to differences in amount of photo-synthesis the procedure is often adopted of growing both 'short-day' and 'long-day' plants under the same period of illumination by natural daylight, and supplementing this with low-intensity illumination to obtain the long photoperiods. This practice is adequate in experiments where both series of plants are grown for approximately the same period, but in the present series of experiments the 'long-day' plants continued growing much longer than the 'short-day' plants and the assimilation conditions (including temperature and light-intensity) inevitably became less favourable during the latter part of the growing period of the 'long-day' series. In these circumstances no clear advantage would have ensued by subjecting all series to the same period of natural daylight, and the simpler procedure of supplementing a varying natural photoperiod with artificial illumination to give a constant total photoperiod was therefore adopted.

It is of interest to compare the type of photoperiodic response found in seedlings of P. sylvestris with that found in herbaceous species. Such a comparison appears most appropriate

for herbaceous species in which the growth habit is 'determinate' (i.e. in which vegetative activity is finally terminated by the conversion of the apical meristem into a flower primordium) for seedlings of P. sylvestris are also 'determinate' in habit, but form a terminal resting-bud instead of a flower-bud. If we then apply the usual classification into 'long day' and 'short day' types, it is seen that seedlings of Pinus sylvestris, in common with other woody species, must be regarded as 'short day' plants in that the period of active growth is reduced by short days and prolonged by long days (at least for photoperiods up to 20 hours). Seedlings of P. sylvestris differ from many typical 'short day' herbs, however, in that even under long days vegetative growth cannot be maintained indefinitely, but ultimately a terminal resting bud is formed under all photoperiods. In this respect P. sylvestris resembles those varieties of soybean (normally regarded as a typical 'short day' species) which ultimately form flower primordia even under long days or continuous light (Borthwick and Parker, 1939).

The type of response found in seedlings of P. sylvestris, in which maximum height and leaf number is reached at a photoperiod below that of continuous light is not well-known in herbaceous species. This may be due partly to the comparative infrequency of experiments in which continuous light has been used, and partly to the fact that even where such experiments

have been carried out the responses have been measured by reference to the initiation of flowering, rather than in terms of height and leaf number. Reduced growth of several species under continuous light has however been reported by various observers (for example Redington (1929), Arthur (1936)) and in the case of tomato, which is normally regarded as a 'day-neutral' species with respect to flowering response, the effects of photoperiods above 18 hours are definitely deleterious (Withrow and Withrow, 1949). It appears, therefore, that the occurrence of maximum vegetative development at some photoperiod below continuous illumination is not uncommon in herbaceous species.

Now from the fact that a greatly increased leaf-number is obtained in P. sylvestris when the dark period is interrupted by a short 'light-break', it seems clear that the reduced leaf-number under 'short-days' is primarily due to the long dark periods occurring under such conditions. Thus, we may regard long dark periods (greater than 4 hours' duration) as tending to inhibit continued growth of the shoot. The precise stage of development (as indicated by leaf-number) at which this inhibition becomes effective depends on the duration of the dark period. From Figure 13 it is seen that with dark periods longer than 4 hours, the mean leaf-number decreases linearly with increasing length of dark period. With

photoperiods of less than 4 hours' duration there is a sharp fall in the mean leaf-number, reaching a minimum with continuous illumination.

Now it is clear that with continuous illumination the formation of a terminal resting bud cannot be controlled by any inhibitory effect of a dark period and it seems indicated, therefore, that with dark periods of less than 4 hours a different mechanism controls the initiation of a terminal resting bud. This is suggested by the shape of the curve for leaf-number shown in Figure 13, which appears to represent the integrated responses of two distinct reactions, represented by curves "X" and "Y" in Figure 17.

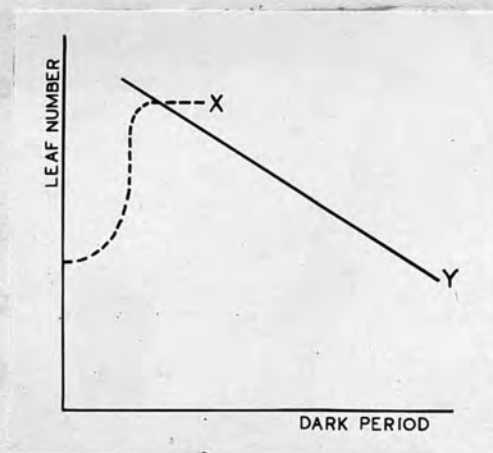


Figure 17.

Moreover, this view is supported by the observation that the terminal resting buds of plants grown under continuous illumination and 22-hour photoperiods are abnormally small, and by the premature growth of the axillary 'dwarf shoots'

(indicating a loss of apical dominance) under such photoperiods.

A single daily dark period of 4 hours' duration results in an increase in total leaf-number by 24.1 (74.7 minus 50.6, Table 10) as compared with exposure to continuous illumination. The results of Experiment 5 indicate that when two such short dark periods, separated by 8-hour photoperiods are introduced into the 24-hour cycle, the effects are cumulative and a further increase in leaf-number by 24.0 (98.7 minus 74.7, Table 12) is obtained.

These results indicate that it is the first 4 hours of dark immediately following a period of illumination which result in increased leaf-number. This is clearly seen when the leaf-number (98.7, Table 12) of plants receiving two daily dark periods of 4 hours, separated by 8-hour photoperiods, is compared with that (61.6, Table 10) of plants receiving 8 hours of unbroken darkness and a single photoperiod of 16 hours per day.

We thus have to explain how it is that

- (1) the first 4 hours of darkness have a growth-promoting effect, while any further period of dark (which is continuous with the first) has an inhibitory effect;
- (2) a daily period of 4 hours' dark has a greater growth promoting effect than continuous illumination;
- (3) two daily 4-hour dark periods result in greater growth than a single dark period.

① It is suggested that the explanation of the first phenomenon is that some substance, which we may call "A", is formed during the light period, and this has a growth-promoting effect. During the first 4 hours of dark there is still a supply of A available as a carry-over from the light period, but after 4 hours this is exhausted or is removed from some field of action, thus permitting an inhibitor-system to come into effect.

② To meet the second phenomenon we must postulate that provided a supply of A is still available from the light-period, then dark actually favours its growth-promoting action.

③ The third phenomenon suggests that the production of A reaches saturation after a few hours of daylight, and that when this has occurred dark is actually more favourable to growth than a further period of illumination.

The present suggestion is thus that the type of photo-periodic response obtained under any given conditions depends upon the relative influence of two mutually antagonistic systems - (1) a system promoting continued growth of the shoot, which depends upon a photocatalysed reaction, and which has a 'carry-over' effect for only a limited period in the dark; (2) a second, antagonistic, inhibitory system which becomes operative when the dark period exceeds the duration of the

'carry-over' effect of the first system. The nature of these systems is discussed in more detail below.

We may ask, what is the minimum intensity and duration of illumination required prior to a dark period to bring about the full increase in leaf-number? In Experiment 3 the plants of series D received a short 'light break' of 30 minutes' duration at approximately 20 foot-candles, so that there were two dark periods of 4 hours' and 9 hours' duration respectively, but the total growth of such plants was less than that of plants grown under a single daily cycle of 15 hours light and 9 hours dark. On the other hand, the growth of plants of series C, which were similarly exposed to dark periods of 4 hours and 9 hours, but separated by a light period of 4 hours (mainly daylight) actually exceeded that of plants grown under a 15-hour photoperiod and 9 hours dark. Evidently the intensity and/or duration of illumination of the 'light break' in the case of series D were too small to permit a sufficient carry-over effect during the first 3-4 hours of the following dark period, to obtain the full increased leaf-number.

It was noted in Experiment 4 that the number of unextended internodes forming the terminal "rosette" varied with the length of the photoperiod. From Table 10 it is seen that the proportion of leaves contained in the terminal "rosette" decreases progressively as the length of the photo-

period increases from 14 to 20 hours. These differences do not become apparent until the end of the growing period, for all plants, irrespective of the day length conditions, show a well marked "rosette" during the stages of active growth; but whereas under 'short day' conditions leaf formation and internode extension appear to cease at approximately the same time, under a 20-hour photoperiod the upper internodes continue extending even after the presence of a terminal bud has become apparent and leaf-formation has obviously ceased.

It thus appears that for any given daily photoperiod a certain mean number of internodes can undergo extension, and this number does not necessarily coincide with the total number of leaves formed. The independence of the two processes is further shown by the results of Experiments 6 and 7. In Experiment 6 it was found that plants which were exposed to short days during the early stages of growth, and were then transferred to long days, showed a significantly smaller total leaf number than plants grown under 'long days' throughout, but there was very little difference in the number of extended internodes between the various series of plants. Again, in Experiment 7 plants which were grown under short days throughout showed a well-marked terminal 'rosette' of leaves, but plants transferred to 'long days' at a time when the terminal buds were developing continued to show extension of the internodes,

although the formation of new leaves had ceased. Thus the apical meristem and the region of internode extension appear to show distinct and independent responses to the length of the photoperiod.

The question arises as to whether the number of extended internodes also shows a maximum response under a 20-hour photoperiod, as does leaf-number. The response of internode extension to different photoperiods can only be studied provided the maximum possible number of extended internodes is not limited by the total number of internodes laid down i.e. by the total number of leaves formed. Now this condition is fulfilled for photoperiods of less than 20 hours' duration, for under such conditions there is still a terminal 'rosette' of leaves with unextended internodes. As the day-length is increased the number of leaves in the terminal rosette decreases, and it appears that with a 20-hour day the total number of leaves formed corresponds closely with the maximum possible number of extendable internodes. With photoperiods greater than 20 hours, the total leaf-number falls, but although there are a number of short internodes at the extreme tip of the stem, the leaves in this region do not form a close rosette as they do under shorter photoperiods. It seems possible, therefore, that with photoperiods greater than 20 hours, the number of leaves formed is actually limiting the number of extended internodes.

Now it is interesting to compare these observations with the effects observed in a species, such as Robinia pseudacacia, which does not form a true terminal resting bud, but has a 'sympodial' type of growth habit (Büsgen, 1929) i.e. when growth of the shoot ceases the apical, unextended, portion of the shoot withers and is abscised, the uppermost axillary bud functioning as a terminal bud. It has been pointed out above (p.19) that the duration of growth in R. pseudacacia increases with the length of the photoperiod, as in P. sylvestris and when the day-length exceeds a certain 'critical' value, growth may be maintained for an apparently indefinite period. At day-lengths below the critical, extension of the internodes proceeds up to a certain stage and then ceases abruptly (and is followed by abscission of the apical portion). It thus appears that under short days the response in R. pseudacacia corresponds closely to the 'internode response' in P. sylvestris, and that this is the only type of response present in the former species. This conclusion affords added interest to the suggestion that the 'internode response' curve in P. sylvestris does not show a 'maximum' at a 20-hour photo-period, but continues to rise at photoperiods greater than this, as in R. pseudacacia.

It may be asked whether the existence of a 'critical' photoperiod, above which growth may be maintained indefinitely,

is a feature peculiar to woody species with the 'sympodial' habits. The answer would appear to be in the negative, for Liriodendron tulipifera, which is stated (Garner and Allard, 1923) to remain in continuous growth under long photoperiods or continuous illumination is a species with a true terminal resting bud.

If the duration of growth in seedlings of P. sylvestris (and other woody species) is controlled by some inhibitor system which is operative under long dark periods, it may be asked why the action of this system does not become effective until a certain stage of development has been reached, and what is the mechanism of this inhibition. Since defoliation of certain woody species in the early summer, when extension growth has ceased, is known (see Klebs, 1914) to result in premature breaking of dormancy of the buds, leading to a further period of leaf-formation, some inhibitory action of the leaves on the further development of the buds seems to be indicated under normal conditions. It appears possible therefore that a progressive increase in this inhibitory action as the total number of leaves increased, might constitute the factor determining the ultimate cessation of growth. In order to test this hypothesis experiments were carried out with a number of woody species (described in Appendix II), which involved continuous defoliation of the older leaves from below,

so that the total leaf-area at any one time was maintained at a low level. It was found in all species, including seedlings of P. sylvestris, that the duration of growth and the total number of leaves formed on the annual shoot was not affected by such treatment. Thus, it was clear, that it is not the attainment of a certain leaf-area which determines the cessation of growth.

A further experiment was carried out with Pyrus malus and Platanus acerifolia to determine whether, if all further young leaves were removed when a certain number of mature leaves had been developed, the duration of growth is affected. Although the experiments were of a preliminary nature, significant increases in the duration of growth and total number of leaves formed were obtained by such treatment. These results seem to indicate that when a certain stage of development has been reached the expanding leaves have an inhibitory action on the further growth of the apical meristem. Inasmuch as it is the high auxin content of expanding leaves which appears to be one of the major factors in the phenomenon of 'correlative inhibition' it appears likely that an auxin mechanism may also be involved in these effects obtained by defoliation of young leaves.

2. Effect of day-length on shoot-growth after the first year.

A further series of experiments was conducted to determine the effect of day-length upon growth of the shoot in young plants of P. sylvestris, after the first year. As has already been pointed out, after the first year of growth, extension of the annual shoot is restricted to expansion of initials already laid down in the resting bud, and it is clear, therefore, that the day-length conditions prevailing during this process cannot affect the number of nodes developed. In order however to find whether the extension of the internodes is still affected, as in first year seedlings, the following experiment was carried out.

2.1 Experiment 8.

(a) Twelve pots, each containing 3-4 two-year old plants of P. sylvestris, were brought into the laboratory early in January, and were divided into two series of 6 pots each. Both series were allowed to stand at a window of southern aspect during a period of 8 hours each day. At night, one series was subjected to 16 hours of darkness by enclosure in a light-proof cover constructed of a wooden framework covered with black cloth. The second series was exposed to a further 6 hours additional illumination from a 100-watt lamp giving a light-intensity of about 20 foot-candles at the level of the plants. The room temperature remained at about 15°C both night and day. One

series was thus exposed to an 8-hour daily photoperiod, and the other to a 14-hour photoperiod.

The terminal buds of both series showed signs of breaking dormancy one month after the commencement of the experiment, and there were no observable differences between the two series in this respect. The length of the photoperiod thus had no detectable effect on the rate of breaking of dormancy under the conditions of the experiment.

(b) Methods. Both series of plants were then transferred to a greenhouse, and the day-length conditions remained as before. Both series were exposed to natural daylight from 9 a.m. to 5 p.m., and each series was then transferred to one of two identical light-proof covers consisting of a wooden framework covered with black cloth. Each cover comprised a lower section (about 2 feet cube) and an upper section which was separated from the lower by an asbestos sheet. A 75-watt lamp was housed in the upper section of each cover, above an aperture, 3 inches square, in the asbestos sheet. This aperture was covered with a sheet of glass, which in the case of the cover housing the 'short-day' series, was coated with an opaque layer of black unvarnished paint. A water-screen consisting of a large crystallizing dish containing a depth of 5 cms. of water was interposed between the lamp and the aperture in both covers. Thus, the only difference between the two

covers was that in one case the glass over the aperture was opaque.

A water screen of 5 cms. thickness in conjunction with a 100-watt lamp removes effectively all infra-red radiation below 10,000 Å (Brackett, 1936). Hence, except for the 'near infra-red' from 8,000 - 10,000 Å, the only additional radiation penetrating to a 'long-day' plants was in the visible spectrum, and gave an intensity of illumination of 10-12 foot-candles at the level of the plants. Any other heating effects from the lamps were common to both covers, and actual tests showed that there were, in fact, no detectable differences in temperature.

The temperature of the greenhouse was maintained at 15°C as far as possible, but in bright weather temperature tended to rise above this.

The experimental plants showed a fair amount of variation within each series, but the difference between the mean initial heights of the plants of each series (Table 16) is found not to be significant (for 't' test, $P < 0.20$).

Results. Growth of the young shoots, following the 'breaking' of the buds, was rapid and was completed in 6-8 weeks. At first there appeared to be no detectable difference in the rate of extension between the 'long-day' and 'short-day' series, but after several weeks it became clear that extension

of the new shoots of the 'short-day' plants was appreciably reduced as compared with those under 'long-days'. When extension of the shoots had been completed (which was determined by measuring the lengths of the new shoots at intervals) the mean length of the new growth of the 'leaders' was determined for each series. Differences were also observable between the two series with respect to the length of the leaf, which was obviously much reduced under 'short-day' conditions. The mean leaf-length was determined for each series, and for this purpose the mean length of three leaves borne at the mid-point of the new shoot was determined for each plant separately. (This method was chosen since the length of any given leaf appears to depend partly upon its morphological position on the shoot). The data are summarised in Table 16. Representative plants of the two series are shown in Figure 18.

Table 16. Data for 3rd year plants grown under (a) 8-hour (b) 14-hour, photoperiods respectively.

Series	Photoperiod	No. of plants	Initial height (cms)	Mean length of new shoot (cms)	Mean length of leaves (cms)
A	8 hours	20	10.61±0.43	5.86±0.43	2.20±0.09
B	14 hours	23	9.87±0.42	7.65±0.53	4.29±0.22



Fig.18. Third-year seedlings: A - 8-hour day,
B - 14-hour day.

It is seen that there is a difference of 1.8 cms. between the mean length of the new shoots of the two series, and this difference is fully significant (for 't' test, $P < 0.01$). Now when it is considered that the additional period of illumination received by the 'long-day' plants was at 10-12 foot-candles only, it is clear that this is a photoperiodic effect, and that extension of the internodes is reduced under 'short-days', just as in the first year of growth. It is noteworthy, however, that the relative difference in mean internode length between 'long day' and 'short day' plants is much less in older plants than in first-year seedlings.

The difference in leaf length between the two series is also clearly significant, and is dealt with below.

2.2 Experiment 9.

In order to obtain confirmation of the above results, and to obtain further information on certain matters arising from them, the following experiment was carried out.

Methods. In early April, two-year old plants, contained in $4\frac{1}{2}$ inch pots, were transferred from the pots to the root-observation boxes described on page 12. Approximately 15-20 plants (transferred from 4 pots) were contained in each box, and there were 4 such boxes used in the present experiment. The plants were divided into two series, each of two boxes. One series ('long-day') was exposed to natural day-length conditions (ranging from approximately 15 to 17 hours) during the period of the experiment (April-May) and the other series ('short-day') was exposed to a photoperiod of 10 hours, by covering with a light-proof cover from 5 p.m. to 7 a.m. Both series were grown out-of-doors. In order to determine whether day-length conditions affected the activity of the roots, regular observations on the growth of the roots were carried out.

Results. (a) Shoot extension. The mean length of the extending shoots was determined for both series at weekly intervals from the time of bud-break; the data are summarised in Figure 19.

As in the previous experiment, under short days the extension of the new shoots was significantly reduced. Very

little difference between the two series was evident during the first 2 weeks but thereafter the growth increment of the short day plants was approximately only half that of the 'long day' series.

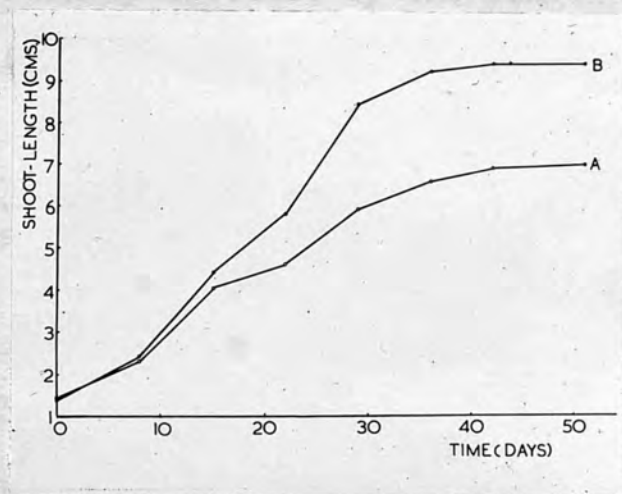


Fig.19. Shoot extension under long days (B) and short days (A) respectively.

(b) Apical Dominance. In order to determine whether the length of the photoperiod affected the relative growth of the 'leaders' and lateral shoots, the mean length of the lateral shoots arising at the base of the new 'leader' growth was determined when extension growth had been completed. The mean length of the laterals on each plant was determined and from this the mean lateral-length for each series was calculated. From the data so obtained the ratio: mean length of leaders/mean length of laterals, was determined for each

series. The values for this ratio were 1.65 and 1.60 for the 'long-day' and 'short-day' series respectively. The close agreement between these two values indicates that the laterals of the 'short-day' series showed proportionately the same reduced extension as the leaders, and that apical dominance was not appreciably affected by the length of the photoperiod.

Table 17. Effect of photoperiod on shoot-extension and leaf-growth.

Length of photo-period.	No. of plants	Mean length of new 'leader' growth. (cms)	Mean length of new lateral growth. (cms)	Mean leader growth/mean lateral growth. (cms)	Mean leaf length (cms)
10 hrs.	30	7.23 ± 0.35	4.52	1.60	3.0 ± 0.15
Natural daylength	40	9.90 ± 0.33	5.97	1.65	7.0 ± 0.25

(c) Leaf-growth. By 3rd June, extension of the shoots had effectively ceased, but it was observed that on that date the leaves of both series were still very short (1-2 cms. in length), and indeed the presence of a soft, succulent region at the base indicated that growth by activity of the basal meristem was still continuing. Continuous observations were therefore carried out on the further growth of the leaves. By mid July the basal portions of the leaves of the 'short-day' series had become fully differentiated, so that the soft meristematic region became tough and resistant, like the

remainder of the leaf, and it was evident that leaf-growth in this series had then ceased.

Active leaf-growth continued in the 'normal day' series until early September, when the same changes occurring at the leaf-base indicated that leaf-growth had ceased. Confirmatory evidence that the presence of a succulent light-coloured region in the basal region of the leaf indicates that leaf-growth is still active was obtained by making a mark on the leaf with Indian ink at the point where the leaf-base emerges from the 'sheath'. In this way any growth of the leaf at the base was easily observed, and it was found that in the 'normal day' series there was still active extension of the leaves in early August, but by the end of August growth was markedly diminished and simultaneously with the visible changes occurring in the basal region, further growth ceased. Thus, the observation of Tolsky (1913) that leaf-growth of P. sylvestris continues long after extension of the shoot has ceased, was confirmed. This conclusion was also confirmed by observations carried out on trees of various ages growing under naturalised conditions at Oxshott Heath, Surrey, in 1948 and 1949. Differentiation of the basal meristem of the leaf again did not occur until early September, and hence, it would appear this behaviour is 'normal' for P. sylvestris.

The marked difference in final leaf-length between the 'long day' and 'short day' plants (Table 17) in the present

experiment is thus to be ascribed partly to the reduced growing period of the leaves under 'short day' conditions. This fact had escaped notice in the previous experiment.

(d) Root growth. Simultaneously with the observations on shoot-growth, regular inspection was carried out on the growth of new roots in both series of plants. As has already been described (p.13), under natural day-length conditions root-growth remains very limited during the period of extension of the shoot, but towards the end of this period root growth increases markedly and remains active until the autumn. The same pattern of growth was observed in the short-day series, in which, however, the onset of increased activity was delayed slightly. This root-activity in the short-day plants continued at least until the end of July, when the short-day treatment was discontinued.

Thus no marked differences in the behaviour of the roots could be detected between the 'long-day' and 'short-day' plants over the period covered by the experiment.

2.3. Experiment 10.

The results of the foregoing experiment clearly indicate that day-length conditions affect the duration of growth of the leaves of P. sylvestris. It is not clear from the facts so far described, however, whether the shorter duration of growth of the leaves is a direct effect of the day-length conditions on

leaf-growth, or whether the effect is a secondary one resulting from the differences in extension of the stem. The solution of this problem is of importance in relation to the possible control of the duration of leaf-growth with the incidence of short days in the autumn, for if the effects of short days observed above resulted indirectly from the reduced extension of the stem they could not occur under natural day-length conditions, since shoot extension normally occurs only under long days.

In order to obtain direct information on this point, a further experiment was carried out.

Methods. Second-year plants in pots were allowed to grow under normal day conditions during the spring, until elongation of the shoot had ceased. Early in June they were divided into three series of six pots each, and the plants of each series were removed from the pots and planted together in a box of soil. All series of plants thus started with stems of equal length. From early June, however, each series received a different day-length treatment as follows:-

Series A - 12 hours.

Series B - 14 hours.

Series C - Normal day.

Observations were made as to the duration of growth, by removing single leaves and examining the basal meristem (as

described in Experiment 9). Using this criterion, the date on which leaf growth ceased in each series was determined. After leaf growth had ceased in all plants, the mean length of the leaves of each series was determined by measuring the lengths of 3 leaves from each plant, taken at random. The data are summarised in Table 18.

Table 18. Effect of day-length on leaf-growth.

Series	Photoperiod	No. of plants.	Mean leaf-length (cms)
A	12	53	5.18 ± 0.17
B	14	54	6.20 ± 0.15
C	'Normal'	56	8.68 ± 0.18

Results. Leaf growth ceased earliest (end of July) under a 12 hour day, under which the final leaf-length (5.2 cms) was also the shortest. Under a 14 hour photoperiod growth continued only slightly longer (early August), to give a mean leaf-length of 6.2 cms. Under normal day, however, growth continued until the end of August (when the natural photoperiod is rather less than 15 hours) and the mean total growth attained was 8.7 cms., which is thus considerably greater than under either of the reduced photoperiods. These differences in leaf-length are all statistically significant (for 't' test, $P < 0.001$). Direct photoperiodic control of the amount and duration of leaf-growth may thus be regarded as established.

2.4 Experiment 11.

Since under normal day-length conditions, leaf growth was observed to cease at about the end of August in trees of various ages and under a variety of cultural conditions (both in pots and in nature) the question naturally arises as to whether cessation of leaf-growth under natural conditions is controlled by the falling natural day-length in August. In order to obtain information on this point two experiments were carried out in successive years. On 8th August 1948 6 pots of second year seedlings were divided into 2 series of 3 pots each (containing a total of about 12 plants in each series). All plants had previously been grown under normal day-length conditions, but from 8th August one series was allowed to remain under normal days, whereas the other received additional artificial illumination from dusk until 9 p.m., giving a total photoperiod of between 17 and 16 hours' duration. Both series of plants ceased growth simultaneously at the end of August however. A similar experiment was carried out in 1949. Twelve pots, each containing 4-5 second year plants were selected on 15th August, when leaf growth was still active. They were divided into 2 equal series and one was exposed to normal photoperiods, while the other was given supplementary illumination at about 15 foot-candles to extend the natural photoperiod to 16 hours. The natural photoperiod at the commencement of

the experiment was about $15\frac{1}{2}$ hours. Leaf-growth continued in both series until approximately the first week in September, when the natural photoperiod is about $14\frac{1}{2}$ hours, but by that date it had ceased simultaneously in both series.

In both these latter experiments, therefore, additional illumination to extend the natural day-length to about 16 hours during the latter part of August failed to prolong the duration of leaf-growth as compared with 'normal' days, and there is, therefore, no evidence that in the experimental plants cessation of leaf-growth was controlled by a reduction in the natural length of day.

The failure to prolong the duration of leaf-growth by additional illumination in the present experiments might be due to either of the following causes:-

(1) The additional period of illumination (approximately $1\frac{1}{2}$ hours) was insufficient to effect any appreciable change in the duration of growth. In this connection it may be noted that in Experiment 10 there was only a slight difference in the time of cessation of leaf-growth between the 12-hour and 14-hour series. If this is the case, the natural changes in day-length occurring during the month of August are unlikely to be sufficient to affect the duration of growth appreciably, and presumably cessation of leaf-growth would occur at approximately the same time even if day-length conditions were maintained constant from the beginning of August.

(2) Possibly the dates of commencement of the additional illumination (8th and 15th August respectively) were too late to affect the duration of leaf growth, the cessation of which was already 'pre-determined' on those dates.

2.5 Experiment 12.

From the previous experiment it is seen that under constant day-length conditions growth of the leaves continues until a certain mean leaf length has been attained. Cessation of leaf-growth must in this case be brought about by some 'internal' method of control, as must also be the case with extension growth in first-year seedlings grown under a constant photoperiod. It seems possible that the attainment of a certain leaf-length or stem length might itself be the controlling factor. In this case it should be possible to prolong growth by experimentally removing some of the tissue already differentiated, so that the limiting size is never attained. This cannot ordinarily be done for stems, since removal of part of the stem at the base involves separation of the apical meristem from the roots. In the case of a leaf with a basal meristem, however, removal of part of the leaf at the distal end does not interfere with the meristem and provides suitable material for testing the hypothesis put forward above. The following simple experiment was performed accordingly.

Methods. Twelve pots of second-year seedlings were

selected on 24th July and divided into two equal series each containing 25-30 plants. All plants had been grown under natural day-length conditions up to that date and leaf-growth was still proceeding actively. The mean leaf-length for all plants at that date was 6.54 cms. The leaf-length in one series was reduced to 2-3 cms. by cutting off the distal portions of the leaves, while the leaves of the second series were allowed to remain intact. From 24th July both series of plants were subjected to a 12-hour photoperiod. Now, it was known from the results of Experiment 10 that the maximum leaf-length possible under a 12-hour day in comparable plants is approximately 5 cms. Thus the leaf-length in the plants with reduced leaves was below the maximum possible under a 12 hour day, while that of the untreated plants exceeded the maximum. If the attainment of a certain leaf-length is itself the determining factor, growth should continue in the experimental plants and cease in the 'control' plants under a 12 hour photoperiod.

Results. Close observations were kept upon the basal meristematic tissue in both series of plants, and the leaves were marked with Indian ink as described in Experiment 9. Very little further leaf-growth was made by either series of plants, however, and within approximately 3 weeks the basal meristematic region had become fully differentiated tissue in

both series of plants. These results therefore afforded no support for the hypothesis that the limiting leaf-length under a given photoperiod is determined by the amount of tissue already differentiated.

2.6 Experiment 13.

It is well-known that the location of photoperiodic perception in herbaceous species is in the leaves (Cailachjan, 1936), the most sensitive being the youngest mature leaves. It is of some interest, therefore, to know whether photoperiodic perception in older plants of P. sylvestris occurs in the young extending shoot, or in the older leaves of the previous year's shoot. Prima facie considerations suggest the latter alternative, for in the growth of the new annual shoot extension occurs very rapidly and has been completed by the time the new leaves are little more than 1 cm. long. In order to obtain information on this question, the following experiment was carried out, in which defoliation of the old leaves was effected before the plants were exposed to two different day-length treatments. It was to be expected that such plants would make reduced growth, if only because of the absence of current assimilation, but since in many woody species extension of the shoot is at the expense of stored food reserves, rather than of current assimilation, (Büsgen, 1929) it was hoped that sufficient growth would be made to permit detection

of any effect due to the influence of the different day-length conditions.

Methods. The plants used were two-year old seedlings which previously had been grown in pots under natural day-lengths and had been allowed to remain in the open during the winter. Twenty-four pots, each containing 4-6 plants, were brought into the greenhouse in January, and equal numbers of plants were transferred to each of two boxes of soil. The temperature of the greenhouse was maintained at 13-15°C. In approximately one month the plants began to show signs of breaking dormancy, and on 25th February, half the plants in each box were completely defoliated. Normal 2-year seedlings possess a number of lateral buds at the apex of the shoot, in addition to the main terminal bud. In order to reduce the drain upon food reserves in the defoliated plants, the lateral buds of all plants were removed, leaving only the single terminal bud.

From 25th February, both boxes of plants were exposed to 8 hours' daylight from 9 a.m. to 5 p.m. At 5 p.m. both boxes were transferred to the dark covers described in Experiment 8, and one series was kept in complete darkness until 9 a.m. while the other series was exposed to supplementary illumination, at 10-15 foot-candles' intensity, for 6 hours and then remained in darkness until 9 a.m. Thus, one box of plants was exposed to a daily photoperiod of 8 hours and the other box to one of 14

hours. Measurements of the lengths of the new shoots were taken at weekly intervals from the commencement of the experiment, and by 19th April it was evident that extension growth had effectively ceased. The final lengths of the new shoots for the respective series of plants are shown in Table 19.

Results.

Table 19. Final lengths of new shoots.

Treatment.	Number of plants	Mean length of new shoots.
Long day (1) Intact	27	12.18 ± 0.62
" " (2) Defoliated	23	5.01 ± 0.27
Short day (1) Intact	28	10.18 ± 0.44
" " (2) Defoliated	19	4.82 ± 0.45

The growth of the defoliated plants was very slow from the commencement of the experiment and continued for approximately the same period as in the 'intact' plants. Growth of the leaves on the new shoots occurred in both defoliated and 'intact' plants during the period of extension.

Reduced extension was apparent in all the internodes of the new shoots of the defoliated plants. The final length of the new shoots of the defoliated plants, under both 'short' and 'long' photoperiods, were very reduced as compared with those of the 'intact' plants and there was no significant

difference between the two series of defoliated plants, although there was a significant difference between the two 'intact' series, as in previous experiments. The very poor growth made by the defoliated plants rendered it impossible to answer the original question, as to what is the site of photoperiodic perception, but the reason for the failure of

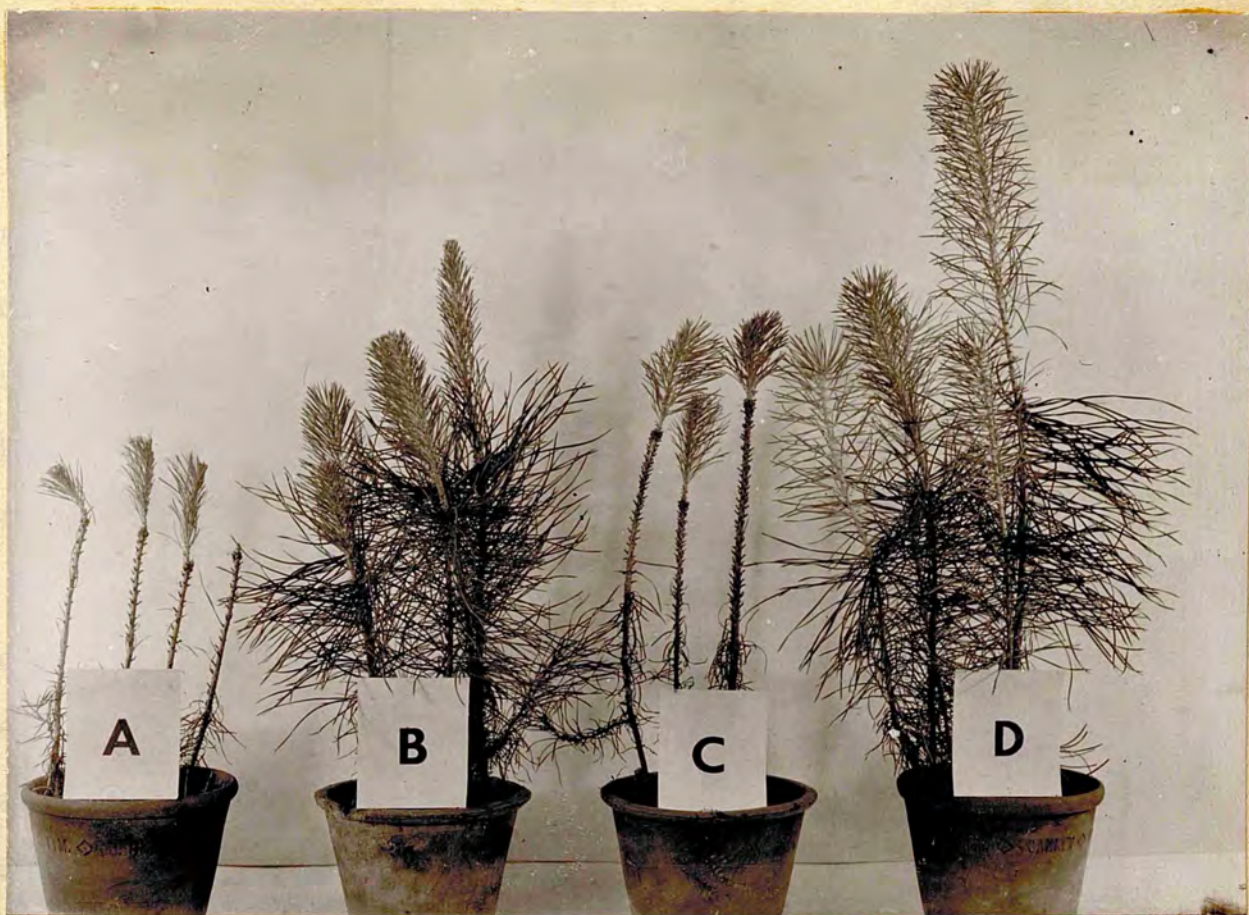


Fig.19A. Third-year seedlings. A - defoliated, short day.
B - Intact, short day. C - Defoliated, long day,
D - Intact, long day.

normal growth is itself a point of some interest. Moreover, in approximately 1/5th of the plants of both series the buds had failed to break dormancy even by the end of the experiment,

whereas this phenomenon was absent from any of the 'intact' plants. Evidently the presence of older leaves is necessary for the normal breaking of the buds and normal extension of the shoots in P. sylvestris, which thus differs in this respect from deciduous species.

2.7 Experiment 14.

Since the previous experiment seemed to indicate that the presence of the old leaves is necessary for normal extension of the shoot, and since the extension is greater under 'long days' than under 'short days', it would appear that normal extension is dependent upon some reaction occurring in the light in the old leaves. It is of interest to know, therefore, what is the effect of complete darkness on extension of the new shoots in plants which still retain the old leaves.

Now in many species, when the daily quantity of light received by the plants is reduced below a certain minimum, 'etiolation' effects become apparent, so that in continuous darkness there is actually elongation of the internodes as compared with those of plants grown in the light. These etiolation effects include certain disturbances in the morphogenesis of the leaves when the plants are kept in complete darkness, but the development of the leaves and internodes can be rendered more nearly 'normal' by exposure to quite small quantities of light (Priestley, 1925). In the following

experiment therefore, plants were kept (1) in complete darkness, and (2) under a daily alternation of 12 hours' low intensity illumination at 15-20 foot candles and 12 hours darkness. Now it has been shown that in herbaceous species a daily light period entirely at low intensity is ineffective for photoperiodic induction of flowering, and it was assumed therefore that in the present experiment the effect of such illumination should be without 'photoperiodic' effects on extension of the shoot, but should reduce the etiolation effects resulting from complete darkness.

Methods. Twelve pots of 2-year old plants from the same stock as those used in Experiment 13 were brought into the laboratory dark-room on 6th April, when signs of breaking of dormancy were becoming apparent. Six of these pots of plants were then kept in continuous darkness, and the remaining six were exposed to a daily alternation of 12 hours' illumination at 15-20 foot-candles from a 100 watt lamp and 12 hours darkness. Watering of the plants kept in complete darkness was carried out every 2-3 days, and during this operation the plants were exposed for a few seconds to a photographic safe-lamp, fitted with a "Wratten" No.2 filter for observation.

Results. Growth of the shoots was very rapid in both series of plants and had effectively ceased within 3 weeks. At this time, however, not all the internodes in the apical

region had extended, and it therefore seems likely that further extension was limited owing to the exhaustion of some essential factor. The final lengths of the new shoots in both series are given in Table 20.

Table 20. Final lengths of new shoots.

Treatment.	Number of plants.	Mean length of new shoots.
Continuous darkness	29	6.64 ± 0.50
12 hours' illumination + 12 hours dark.	26	6.53 ± 0.51

It is seen that there was no significant difference in the lengths of the new growth of the two series. Leaf-growth occurred on the new shoots of both series, the leaves attaining a length of approximately 2 cms. The new shoots of the plants maintained in darkness were colourless, whereas in those grown with a daily exposure to 12 hours light the stems and leaves were green. There were no other observable morphological differences between the two series, both of which were remarkably 'normal' in appearance. Thus P. sylvestris appears not to show any gross etiolation effects when kept in complete darkness or low-intensity illumination.

When the growth of the new shoots is compared with that of the defoliated plants of Experiment 13, certain well-marked differences appear. Firstly, the total length of the new shoots (approximately 6.5 cms.) in the present experiment was

considerably greater than that attained by the defoliated plants of Experiment 13 (approximately 5 cms.). Moreover, the new shoots in the present experiment grew very rapidly from the commencement of the experiment, and ceased extension in 3 weeks, whereas those of the defoliated plants grew slowly over a period of 8 weeks, although a strict comparison is not



Fig.20. Third-year seedlings. A, B - Grown under low-intensity illumination for 12 hours per day; C, D, grown in complete darkness.

possible as temperature conditions were not identical in both experiments. Further, whereas the defoliated plants showed reduced internodes throughout the length of the new shoot, the plants of the present experiment showed long internodes in the

basal region and unextended internodes in the apical region. Thus the effects of defoliation are in marked contrast to the effects of keeping the leaves in darkness, and since more rapid growth occurred under the latter conditions, it appears that the presence of the leaves promotes extension of the shoot even in complete darkness. It would appear that during the initial stages of shoot extension the leaves contain a reserve of some essential growth factor, which, however, ultimately becomes exhausted so that full extension of the apical internodes can only occur in presence of a sufficient quantity of light.

2.8 Experiment 15.

The results of Experiments 8 and 9 indicate that 'long days' produce greater extension of the shoot in P. sylvestris than 'short days', and yet on the other hand it is clear from Experiment 14 that even in complete darkness considerable extension of the shoot may take place.

Now, evidence has already been produced from the responses of first-year seedlings, that long dark periods have an active inhibitory effect on growth of the shoot, including internode extension. It is well-known (Mann, 1940) however, that the dark period must nevertheless be immediately preceded by exposure of the plant to a certain minimum quantity of light in order to be effective. It seems possible, therefore, that the rapid growth of plants kept in continuous darkness is due

to the absence of the inhibitory effect which results when there is a daily alternation of light and dark. The following experiment was therefore carried out to test this hypothesis, on the assumption that photoperiodic 'perception' occurs in the leaves of the previous year.

Methods. Eighteen pots of 2-year old plants were brought into the laboratory on 3rd April, when 'breaking' of the buds had commenced, and were divided into three equal series. In one series (A) the stems of the plants were individually wrapped in black cloth, so that the old leaves were kept in continuous darkness, while the terminal buds were left exposed at the tips of the stems. The lateral buds at the apex were removed from the plants of all series, leaving the single terminal bud. All series of plants were exposed to illumination at 400-500 foot-candles from a battery of eight 80-watt fluorescent lamps suspended 9-12" above them, for 6 hours daily. The plants of series A, and also of a second series (B), were kept under a dark cover in the laboratory between the periods of illumination, while the third series (C) received a further period of illumination at 25 foot-candles from a 100-watt filament lamp for a further 10 hours, giving a 16-hour photoperiod, and 8 hours darkness. In order to eliminate any heating effect during the period of low intensity illumination of series C, a water screen consisting of a large

crystallizing dish containing 4-5 cms. of water was placed between the lamps and the plants. There were thus no appreciable temperature difference between the plants under the dark cover and those receiving additional illumination. The room temperature remained nearly constant at 15°C, except during the period of illumination by the fluorescent lamps, when it rose to approximately 20°C.

The three series of plants were thus exposed to light conditions as follows:-

Series A - Old leaves in continuous darkness, new shoot under 6-hour photoperiod at high intensity.

Series B - Old leaves and new shoots under 6-hour photoperiod at high intensity.

Series C - Old leaves and new shoots under 16-hour photoperiod, consisting of 6 hours at high intensity and 10 hours at low intensity.

The length of the new shoot was measured individually for each plant in all series at the commencement of the experiment, and at intervals of 3 days thereafter.

Results. The arrangements of the experiment were thus such that the effects of continuous darkness and 'short days' on the old leaves could be compared, while the light conditions for the new shoots remained the same. In this way any differences due to possible etiolation effects when the new

shoots are kept in continuous darkness were eliminated. The third series of plants under 'long day' was introduced in order to verify that the quantity of light received under the fluorescent lamps was sufficient to evoke photoperiodic effects. The growth of the plants is shown by the graphs of Figure 21, and the final data are given in Table 21.

Table 21. Final lengths of new shoots.

Series	Number of plants.	Length of new shoot (cms)	Length of leaves at end of experiment.
A	33	5.37 ± 0.27	1.27 ± 0.11
B	31	6.33 ± 0.34	2.15 ± 0.07
C	31	11.03 ± 0.44	2.66 ± 0.07

*leaves in bud
new shoots in bud
leaves & new
shoots in bud
leaves & new
shoots in
bud
high + slow*

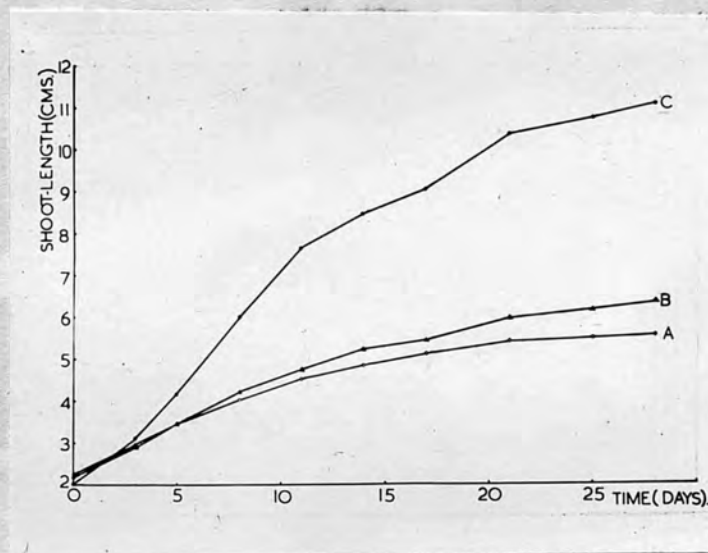


Fig.21. Extension growth of shoots.

Representative plants of each series are shown in Figure 22. It is seen that there was very little difference between the growth of series A and B during the first 17 days of the experiment, the slightly reduced growth in series A over this period possibly being due to the absence of current assimilation in these plants. Thus, during the first 17 days of the experiment, covering of the leaves of the previous year's shoot had no appreciable effect on the growth of the current year, as compared with that in plants of which the old leaves received 6 hours' light per day. The plants of both these series showed the marked reduction of internode extension already found to be associated with short photoperiods.



Fig.22. Representative plants of Experiment 15. (For respective treatments, see text).

On the other hand, the shoots of plants exposed to a 16-hour day showed a very much greater extension of the internodes, and an increased rate of extension was perceptible from the beginning of the experiment. Thus the previous results obtained when a short period of illumination at high light intensity was supplemented by a period of low intensity illumination were confirmed, and it was evident that the quantity of light received from the fluorescent lights was sufficient to evoke a photoperiodic response.

It is seen from Figure 21, that whereas the rate of extension decreased slightly in all series of plants between the 11th and 17th days, this was followed by a second period of increased growth-rate in series B and C, but not in series A. (A second increase in growth-rate was also observed in Experiment 9 - see Figure 19.) Now during the period of reduced rate of extension between the 11th and 17th days there was a marked growth of the leaves in all series, but this was least in the case of series A, so that marked differences in the length of the leaves became apparent between the plants of series A and B. It would appear, therefore, that the second period of increased growth-rate in series B and C was associated with the development of the new leaves in these two series, and that the failure of series A to show a second rise in growth-rate was connected with the poor growth of the

new leaves. Thus, covering the old leaves of series A ultimately resulted in differences between series A and B, probably due to the absence of current assimilation in the former.

A matter of considerable interest was the observation that whereas during the initial stages of extension the young shoots were strongly geotropic, during the period of reduced extension there was a marked tendency to curvature in the young shoots, so that the apical portions pointed in all directions. During the second rise in growth rate, however, these shoots again assumed a vertically erect position. These observations suggest that the period of reduced rate of extension was connected with an auxin deficiency, due to the exhaustion of a reserve of auxin (or auxin precursor) on which the initial period of extension depends, and that the second period of increased rate of extension is due to the current production of auxin by the young growing leaves.

The close similarity between the responses of series A and B during the first 17 days could be due to one of two causes viz. either (1) inhibition of extension is a maximum under a 6-hour photoperiod, and further inhibition cannot be obtained even when the old leaves are kept in continuous darkness; or (2) the day-length conditions to which the older leaves are exposed do not affect the extension of the new shoot

and photoperiodic perception is located in that shoot itself. This latter possibility appeared unlikely in view of the fact that differences were detectable between series A and B on the one hand, and series C on the other from the commencement of the experiment, at which time leaves were still absent from the new shoots, being only 1-2 mm in length and retained within the basal sheath.

Unfortunately lack of a sufficient number of plants of the same origin had prevented the inclusion of a fourth series of plants (with old leaves covered and exposed to a 16-hour day) so that the design of the experiment was 'orthogonal'. The following further experiment was therefore carried out to test the possibility of a direct effect of day-length upon the extension of the current shoot.

2.9 Experiment 16.

Methods. Twelve pots of 2-year old seedlings were divided into two equal series, in both of which the leaves of the previous year were wrapped in black cloth, as in series A of the previous experiment. The lateral buds were removed, leaving a single terminal bud on each plant.

One series of plants (A) was exposed to 6 hours' daily illumination under the fluorescent lamps (already described) and to 18 hours dark; the other series (B) was exposed to 6 hours high intensity and 10-hours low-intensity illumination,

under the same conditions as the 16-hour day series of the previous experiment. Thus the only difference in treatment between the two series was the exposure of the young shoots to 10 hours' illumination at 25 foot-candles in the case of series B. This additional illumination was given from above, in order to preserve the same conditions as in Experiment 15, although this method of illumination was clearly not the most suitable for the erect expanding buds.

The growth of the new shoots was measured for each plant at intervals of 2-3 days. The data are given in Table 22 and Figure 23.

Results. It is seen from Figure 23 that the rate of extension of the shoots of series A. was greater than that of

Table 22. Data for Experiment 16.

Series	Number of plants	Length of new shoots.	
		On 9th day	At end of experiment
A	19	3.05 ± 0.20	4.31 ± 0.32
B	19 ^x	4.02 ± 0.31	6.67 ± 0.49

^x Three of these became accidentally broken during the later part of the experiment and are not included in the final shoot-lengths.

series B from the commencement of the experiment, and on the 9th day there was already a highly significant difference in

shoot length between the two series (for 't' test, $P < 0.01$). At this time the young leaves of the new shoots had still not emerged from the basal sheath in the majority of the plants, and in the remainder the tips of the leaves projected for only

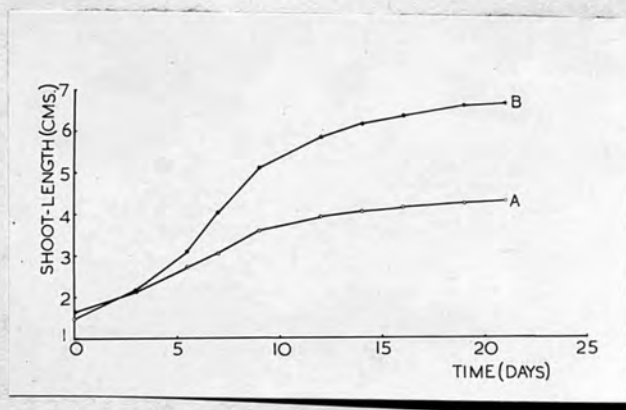


Figure 23. Extension of new shoots.

1 mm. beyond the sheaths. Thus a greater extension of the young shoots occurred under long-day conditions even during the very early stages, at a time when the 'needles' were still in a very rudimentary state. There seems no doubt, therefore, that the additional low-intensity illumination to which the plants of series B were exposed had a direct effect upon the extension of the internodes. This appears the more remarkable when it is considered that the illumination was directed from

above, so that the intensity of the incident light on the sides of the young shoots must have been very much less than 25 foot-candles. The difference in rate of shoot-extension between the two series was maintained for a further 14 days, when growth in both series had effectively ceased.

As in the plants of the previous experiment in which the leaves were covered (series A), there was no second increase in growth-rate in either series of the present experiment, and this resulted in a lower final shoot-length in both long- and short-day plants than is the case when the old leaves are not covered. Nevertheless, the final difference between the two series was comparable to that obtained under long- and short-days in previous experiments, and this result, together with those of Experiment 15, (in which it was shown that covering of the old leaves made little difference during the early stages of extension), indicates that the effect of day-length upon shoot-extension is a direct one, and is not brought about through the agency of the previous year's leaves.

2.10 Discussion.

It has been demonstrated in the experiments described above that, although after the first year the duration of growth is pre-determined by the number of node-initials laid down in the bud, nevertheless the extension of the internodes is affected by day-length conditions, short days producing

reduced internode extension. In this respect the effects observed in P. sylvestris agree with the general observation that short days produce shortened internodes and a tendency to a 'rosette' habit in both long-day and short-day herbaceous species (Garner, 1936). This effect on internode extension appears to be a direct one, and is not a secondary one arising from a photoperiodic 'perception' located in the leaves, as is generally held to be the case in herbaceous species. A direct response of the stem to photoperiodic stimulus has been reported by Dostal (1944) for the rhizomes of Circaea intermedia. Similarly Harder and Westphal (1944) observed that some inhibition of flowering occurs when the defoliated stems of the 'short day' species Kalanchoë Blossfeldiana are illuminated. In these last two cases, however, it was fully differentiated stem tissue which was the site of perception, whereas in P. sylvestris the effect appears to be directly upon meristematic tissue which is in the process of extension.

If the reduced internodes under 'short days' are due to the inhibitory effect of the long dark periods as was suggested in connection with the effects observed in first year seedlings, it might be expected that in the limiting case of exposure to continuous darkness, growth would be completely inhibited. As we have seen, however, extension can occur rapidly in complete darkness and in fact full extension of the

lower internodes occurs, although growth ceases before the upper internodes have extended. The observation that exposure to low-intensity illumination does not have any marked effect on the development of the new shoots in P. sylvestris (apart from the development of chlorophyll) is in marked contrast to the effects resulting from exposure to small quantities of light in other species. P. sylvestris thus does not show any obvious 'etiolation' effects, beyond a possible slight lengthening of the internodes and absence of chlorophyll.

From these considerations it would appear that as the length of the daily photoperiod is reduced, at a certain day-length extension of the internodes will be at a minimum and at still shorter day-lengths this inhibitory effect on internode extension will be less effective. The observation that exposure to 12 hours' low-intensity illumination per day had no observable effect on internode extension, whereas if such low-intensity illumination is used to supplement a period of exposure at a higher intensity, it has a marked effect ('long-day' plants of Experiments 8, 9 and 15), agrees with the observation that in herbaceous species low-intensity illumination alone is not effective for photoperiodic induction of flowering (Mann, 1940) although it has marked effects when used to supplement a period of exposure to daylight. Thus, when the daily quantity of light received by the plant falls below a certain minimum, the photoperiodic effects resulting

from long dark periods are also absent, including, apparently, the inhibitory effect on internode extension.

It has been shown that although the day-length conditions to which the leaves of the previous years' shoot are exposed have little or no effect on the extension of the current year's shoot, nevertheless the older leaves apparently contain some material in the absence of which the breaking of the buds and extension of the shoots occurs only with great difficulty. In view of the importance of auxin in shoot extension (Thimann and Skoog, 1934) it seems possible that a reserve of auxin or auxin precursor is normally present in the old leaves, and on which extension of the new shoots is dependent, but the possibility that other factors are also involved is by no means excluded. It seems clear that the function of the leaves in this connection is not simply that of current photosynthesis, since active extension of the shoot can occur even in the dark, provided that the old leaves are left intact.

The effect of day-length upon the duration of growth of the leaves appears to present the same problem as photo-periodic control of the duration of growth in first year seedlings viz. how it is that under a constant day-length growth may proceed for a certain period and is then inhibited. The observation that artificial shortening of the leaves did not

affect the duration of growth indicates that the attainment of a certain area of leaf is not itself the determining factor, thus agreeing with the results of the defoliation experiments described in Appendix II.

Since the preliminary observations of Experiment 9 yielded no evidence of any effect of day-length upon the growth of the roots, and since quantitative data upon root-growth are not easily obtained, this aspect of the work was not pursued further.

3. Effect of day-length on the duration of cambial activity.

3.1 Introduction.

Reference has been made above (p.16) to the observation by Wight (1923) that cambial activity^{*} of the shoot in P. sylvestris continues until well into October, after extension growth and leaf-growth have ceased. The duration of cambial activity in woody plants varies greatly according to the species. Thus, in oak (Büsgen, 1929) the formation of secondary wood continues until September, whereas in sycamore (Elliott, 1935) it ceases in July.

The factors determining the duration of cambial activity are not fully understood, and although several authors (e.g. Jost 1893, Priestley, 1936) have noted that in many trees the

* In this section the references to cambial activity relate to the formation of new xylem, and do not include phloem-formation

period of cambial activity appears to be correlated with that of extension growth of the branches, nevertheless in certain trees, cambial activity continues long after extension growth of the shoots has ceased, as, for example, in oak and P. sylvestris. In these latter species, in which cambial activity continues until the occurrence of reduced natural photoperiods in the autumn, the possibility of direct photoperiodic control is suggested.

Length of day is well-known to affect the activity of the cambium in herbaceous species, but the effects so far observed in such cases appear to be associated primarily with the change from the vegetative to the reproductive state (Roberts and Struckmeyer, 1948, Melikjan, 1946) which tends to obscure any direct effect of day-length upon the activity of the cambium. The following series of experiments were therefore carried out to determine whether day-length conditions affect the duration of cambial activity in P. sylvestris.

Concurrently with the experiments, observations were carried out upon the duration of cambial activity in the annual shoot in young trees growing at Oxshott Heath, Surrey, and in 2-year seedlings from the nurseries of the Forestry Commission. The method of determining the duration of cambial activity used in these observations, and also in the following experiments, was that described by Wight (loc.cit), who pointed out that in

transverse sections of the stem a dormant cambium can be distinguished from an active one by the fact that in the former the cambium cells abut directly upon fully lignified tracheids of the wood, whereas in the latter there is a transitional zone occupied by partially lignified elements. (Figures 24, 25).

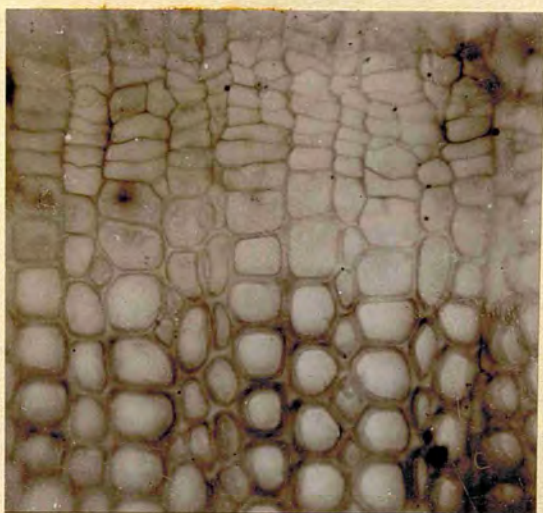


Fig.24. Active Cambium.

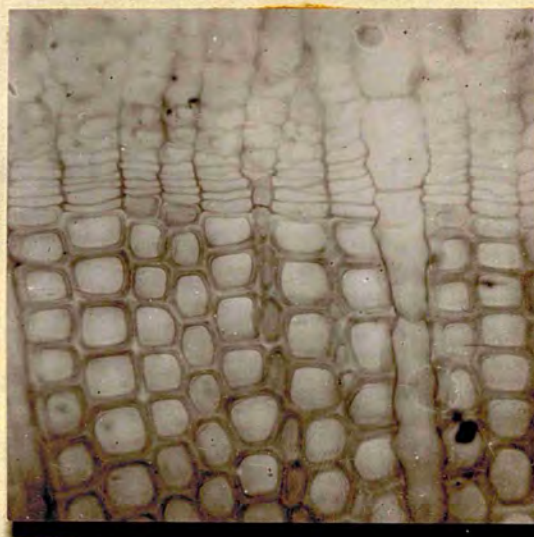


Fig.25. Dormant Cambium.

During the summer, when the cambium is fully active, the transitional zone at the base of the current year's shoot is some 3-4 cells in width, but as the cambium approaches dormancy the number of partially lignified cells is gradually reduced to zero.

The transverse sections used in these observations were cut by hand, stained in safranin and light green, and mounted in Canada balsam. In such preparations the incompletely

differentiated tracheids in the transitional zone stain green, and can be clearly distinguished from the fully lignified elements stained with safranin.

Using this technique, it was found that cambial activity in the annual shoot of young trees under natural day-lengths (whether in pots or under naturalised conditions) in 1948 and 1949 continued until about the third week in October, thus confirming Wight's observations.

3.2 Experiment 17.

Methods. Three different series of second and third-year plants were used. Two of these series (A and B) were grown in pots, while the third had been grown in 'root-observation' boxes (as described above) from the spring. All the plants had been grown under natural day-length conditions until 31st August, 1948. Commencement of the experiment was delayed until this latter date, in order that leaf-growth should first be completed. This precaution was taken since it was already known that length of day may affect the duration of leaf-growth, and it was desired to eliminate any secondary effects upon the cambium which might have resulted if different day-length conditions had been imposed prior to the cessation of leaf-growth.

On 31st August each series was divided into two equal groups and exposed to day-length conditions as follows:-

Series A, Group (1) Natural day-lengths.

Group (2) Natural day-lengths + artificial illumination to give total photoperiod of 15 hours.

Series B & C, Group (1) 10 hour photoperiod.

Group (2) Natural day-lengths + artificial illumination to give a total photoperiod of 15 hours.

All plants of series A were allowed to remain in the open. The plants of series B and C exposed to a 10-hour photoperiod were enclosed in a light-proof cover, constructed of patent roofing-felt, from 5 p.m. to 7 a.m. As these light-proof covers afforded a measure of protection against low night temperatures during September and October, a similar degree of protection was given to the plants exposed to the 15-hour photoperiod by enclosing them in a cover constructed of a patent transparent material, known as "Windolite", for the same daily period as the 'short-day' plants were enclosed in the light-proof covers. Owing to over-shadowing by buildings the 'long day' plants received no direct sunlight during the period they were enclosed in the cover, and temperature conditions remained effectively the same in both 'long day' and 'short day' covers.

The additional period of artificial illumination to extend the natural photoperiod was obtained by suspending a

100-watt lamp outside the cover over the 'long day' plants, and was found to give an intensity of approximately 20 foot candles at the upper leaves of the plants. The heating effect from the lamp in the 'long day' cover (the dimensions of which were approximately 6' x 3' x 2') was very slight and it was found that the temperature difference between both covers did not exceed 1°C during the period of additional illumination.

Results. Samples were taken from all series of plants at approximately 2-weekly intervals for sectioning and observation of the cambium. These sections were taken from base of current year's shoot. Little difference could be detected between the 'long day' and 'short day' plants of each series for several weeks after the commencement of the experiment, the cambium remaining still active in all plants, as shown by the presence of a zone of partially lignified tracheids. By early October, however, noticeable differences began to arise between the 'long day' and 'short day' plants of each series, and it was evident that in the 'short day' plants cambial activity was reduced, since the transitional zone of partially lignified elements was only one or two cells in width or entirely absent, while in the 'long day' plants it remained 3-4 cells in width. By the third week in October the observations on sample plants showed that cambial activity had ceased in most of the 'short day' plants, whereas in most

of the 'long day' plants the cambium still remained active. During the 3rd and 4th weeks of October, samples of 12 plants from each of the six groups were sectioned for examination of the cambium. It was found that while the cambium in the majority of plants of the 'long day' groups was still active, in a few plants it was dormant, and conversely, while the cambium was dormant in the majority of 'short day' plants, in a few there was still slight cambial activity. Moreover, in some plants the cambium was quite active over one sector of the stem, but dormant over the remainder.

In order therefore to obtain a quantitative measure of comparison between the 'short day' and 'long day' groups of each series, the following method of 'scoring' was adopted. One 'unit' was awarded for each complete ring of undifferentiated tracheids between the cambium and fully lignified elements, and one-half of a 'unit' was awarded where such a ring was incomplete.

The total 'score' was then calculated for each group of 12 plants, the data being summarised in Table 23.

From these data it is clear that the length of the photoperiod affects the duration of cambial activity in P. sylvestris. That this is a true photoperiodic effect, and is not the result of increased assimilation under 'long day' conditions is clearly shown by the fact that the treatment of

Table 23. Activity of Cambium at end of October.

Series.	Photoperiod.	Number of plants		Total score
		Cambium active.	Cambium dormant.	
A	(i) 'normal'	4	8	3.5
	(ii) 15 hours	10	2	31.0
B	(i) 10 hour	4	8	2.5
	(ii) 15 hour	11	1	18.5
C	(i) 10 hour	0	12	0
	(ii) 15 hour	6	6	18.5

the long day plants of series A differed from that of the 'normal' day plants solely in the fact that the former received an additional period of illumination of only 20 foot-candles intensity, for a daily period which ranged from about 1 hour at the beginning of the experiment to about $4\frac{1}{2}$ hours in the later stages. In the case of the long day plants of series B and C, the additional illumination (5 hours) received by the 'long day' plants included approximately 4 hours of daylight at the commencement and $\frac{1}{2}$ hour at the termination of the experiment.

It was observed that those plants of the various 'long day' series which showed a dormant cambium were mainly smaller specimens suggesting that nutritional factors may also influence the duration of cambial activity.

An important feature of these results is the long period of time (6-7 weeks) which elapsed between the commencement of the short day treatment and the incidence of dormancy. This suggests that the effect of day-length on the activity of the cambium may be indirect.

A further point of interest is that the 'normal day' plants of series A became dormant at approximately the same time as the 10-hour day plants of series B and C, although the day-length for the former ranged from approximately 14 to 10½ hours during the experiment, whereas the latter received a constant photoperiod of 10 hours throughout.

3.3 Experiment 18.

In order to obtain confirmation of the results obtained in Experiment 17, a further experiment was carried out in 1949. This experiment also incorporated observations on the effect of 'de-budding' on the activity of the cambium, for the fact that growth of the buds continues into September (p.10) suggests that possibly this might have some influence on the duration of cambial activity.

Methods. The material used consisted of second-year plants, growing in 7-inch pots, each pot containing 4-5 plants. All plants had been grown under natural day-length conditions prior to the commencement of the experiment. On 6th September, when leaf-growth had ceased, the plants were divided into

4 groups each containing 12 pots, and subjected to day-length conditions as follows:-

Series A - 15-hour day.

Series B - 10-hour day.

Series C - Natural day-length.

Series D - 15-hour day, plants de-budded.

The plants of series B were covered with a light-proof cover from 5 p.m. to 7 a.m., and the plants of series A and D were covered in a transparent cover for the same period, as in the previous experiment. Supplementary artificial illumination for series A and D was provided from two 150-watt lamps suspended outside the cover, and arranged so as to give as uniform illumination as possible over the plants. The plants of series C were allowed to remain out-of-doors.

Samples from each series were taken at regular intervals for observation of the cambium. Experience gained in the previous experiment made it possible to recognize an active cambium without the necessity for staining, and the sections were simply mounted in glycerine between a slide and coverslip, for inspection.

Results. As in the previous experiment, no difference could be detected between the various experimental series of plants for several weeks after the commencement of the experiment. By the second week in October, however, all series,

except those under a 15-hour photoperiod, showed signs of reduced cambial activity and by the 4th week samples from these plants showed that the cambium was dormant. On 25/26 October, therefore, sections were cut from 20 plants of each series (except in the case of the 'normal day' series, of which only 10 plants were examined). It is seen from the data of Table 24 that the results of Experiment 17 are fully confirmed, and indeed the results obtained previously are even more clearly marked in the present experiment. As the differences between series A and the others were clearly significant, the method of 'scoring' was not adopted. The effect of day-length on cambial activity in *P. sylvestris* may

Table 24. Activity of cambium at end of October.

Series	Photoperiod	Number of plants with	
		Active cambium.	Dormant cambium.
A	15 hour	17	3
B	10 hour	2	18
C	'Normal'	2	8
D	15-hour (de-budded)	4	16

then be regarded as fully established. Moreover, the fact that cambial activity may be prolonged beyond the normal date of cessation by artificially prolonging the natural photoperiod

indicates that natural changes in day-length in the autumn control the duration of cambial activity in P. sylvestris (at least under the conditions of the experiment).

The comparatively long interval between the commencement of 'short day' treatment and the final cessation of cambial activity, observed in Experiment 17, was again observed in the present experiment. Also, cambial activity again ceased at the same time in both 'normal day' and 810-hour day' series.

The cessation of cambial activity in de-budded plants, even though kept under long photoperiod, is a matter of some interest which is discussed later.

Although the majority of plants under a 15-hour day still showed an active cambium on 25th October, the width of the 'transitional zone' in many plants was noticeably narrower than was observed earlier in October, suggesting that even the 'long day' plants were beginning to show reduced cambial activity. In order to test how long cambial activity could be prolonged in these 'long day' plants under favourable temperature conditions, on 26th October the remaining plants were transferred to the greenhouse, which was maintained at 12-15°C, the photoperiod being continued at 15 hours. A further sample of 10 plants from this series was examined early in November, and it was found that the cambium was still active in the majority of these plants. A further sample taken on

25th November, however, showed that all the plants were then dormant.

Thus it was not possible to maintain cambial activity indefinitely under the conditions of the experiment, but this may have been due to the fact that conditions for photosynthesis during November were very unfavourable. Apart from a high proportion of cloudy days the plants were able to receive direct sunshine for only a relatively short period each day owing to overshadowing by tall buildings as the altitude of the sun decreased. It thus remains an open question whether cambial activity could be maintained indefinitely with long photoperiods and under favourable conditions for assimilation.

3.4 Experiment 19.

The observation that short days in the autumn bring about hastened dormancy of the cambium raises the question as to the effect of short days in the spring, when there is normally a renewal of cambial activity. Examination of the cambium in the 'short day' and 'normal day' plants of Experiment 9, in which two different day-length treatments were applied during the period of extension of the shoot, showed that a new annual-ring had been formed in both series of plants, although the amount of secondary growth appeared to be less in the 'short day' than in the 'normal day' plants.

The cambium of the 'short day' plants still showed some activity at the end of July, when leaf-growth had ceased. Thus, the application of 'short days' in the spring did not prevent the normal renewal of cambial activity at that time. It is well-known, however, that the renewal of cambial activity in the spring appears in many cases to be correlated with extension of the young shoots (e.g. Priestley, 1930), and since shoot extension occurred in both 'short day' and 'long day' plants in the experiment in question, the following further experiment was carried out to test the effect of day-length on the renewal of cambial activity in plants which had been de-budded, so that shoot extension was suppressed. Experiments of this type have been carried out by various authors in the past, for example, Jost (1893) found that if P. Laricio was de-budded in March (leaving the leaves on) the shoots so treated underwent some secondary growth, but the wood was of an abnormal type, mainly parenchyma, with occasional deformed tracheids.

Methods. Eleven pots of 2-year old plants which had remained out-of-doors throughout the winter were divided into two series of 6 and 5 pots respectively. They were de-budded on March 6th, on which date sections of sample plants showed that the cambium was still dormant. One series of plants was allowed to remain under natural day-length conditions, while

the other was exposed to an 8-hour day. Both groups were kept out-of-doors. During April, when normal plants showed breaking of the buds, growth of the apical region of the dwarf-shoots was observed to be occurring in both groups of plants, and such new growth was cut out with the point of a knife, so that all extension-growth was suppressed.

Results. On 1st May, sections of sample plants from both series were cut, and these showed that no new secondary wood had then been formed, although normal plants (with buds) were found to have formed quite a wide band of new wood by that date. Thus under the conditions of the experiment, no cambial activity was obtained in de-budded plants, whether under natural or short-day conditions.

Observations were also carried out on plants which had been allowed to remain in the greenhouse throughout the winter. By 1st May the cambium of these plants was still dormant, although starch was exceptionally abundant in the cells of the cortex.

It was found that the majority of the defoliated plants of Experiment 13 had formed no new secondary wood below the current year's growth, while in a very few slight cambial activity had occurred over a small sector of the stem. Thus, here we find a correlation between feeble extension growth and the presence of cambial activity. On the other hand, the plants

of Experiment 14 which were kept in the dark but allowed to retain their leaves showed rapid extension growth and active formation of new wood. All these observations agree with the conclusion that there is a close connection between the renewal of cambial activity and extension growth in P. sylvestris.

3.5 Discussion.

From the results of Experiments 17 and 18 it is clear that the length of the photoperiod affects the duration of cambial activity in P. sylvestris. This appears to be the first instance so far reported, of photoperiodic control of the duration of cambial activity in a woody species. Moshkov (1935) has described a difference in the amount of secondary wood in Robinia pseudacacia under 'long' and 'short' days respectively. In this instance, however, there were definite differences in the amount and duration of extension growth of the shoot, under 'long' and 'short' days, and these differences in shoot growth must in turn have had an effect on the amount of secondary growth^x, so that it becomes impossible to determine any direct effect of the length of the photoperiod on cambial activity. Similarly, as has been pointed above (p.109), differences in cambial activity under 'long' and 'short' days in herbaceous species are complicated by the change from

^x The influence of extension growth on cambial activity is discussed below.

vegetative to reproductive growth, and here again a direct effect of day-length upon cambial activity cannot be studied.

In P. sylvestris, however, both extension growth and leaf growth, which might be expected to affect the activity of the cambium, cease considerably sooner than cambial activity, and hence the effects of different photoperiods upon cambial activity cannot be secondary effects arising from differences in duration of these other growth processes. The problem of what determines the duration of cambial activity in woody species has been the subject of much study in the past (see Büsgen, 1929), but many aspects still remain unsolved. In his masterly analysis, Jost (1891, 1893) came to the conclusion that cambial activity is in some way connected with the presence of developing leaves in many species. In line with this, a correlation between extension growth and cambial activity has been observed in many woody species (Priestley, 1930). Thus, in species with 'diffuse porous' wood, cambial activity does not commence until the buds break, and then a 'wave' of renewed cambial activity travels basipetally from the buds down the branches and trunk (Coster, 1927, Priestley, 1930). If the buds are removed before the breaking of dormancy in such species there is no cambial activity (Coster 1927) as was found to be the case also for P. sylvestris. Similarly, a general correlation between the duration of extension growth and that

of cambial activity has been observed for certain 'diffuse porous' trees (see Priestley, 1930). Thus, for 'diffuse porous' trees a close connection between cambial activity and extension growth is indicated. This conclusion becomes intelligible in the light of later work on the role of auxin as a 'cambial stimulus'. Thus, Snow (1935) showed that auxin stimulates cambial activity in Helianthus, and Reinders-Gouwentak (1949) has shown that the application of auxin in the spring to debudded twigs of several species including the diffuse porous types Salix and Populus, produces typical cambial activity (see below). In view of the well-known fact that young leaves contain a high auxin-content (Thimann and Skoog, 1934) and the demonstration that a growth-hormone is produced in the apical region of shoots of Pyrus malus and Aesculus spp. (Avery et al., 1937), it seems highly probable that the observed correlation between cambial activity and extension growth in 'diffuse porous' trees is due to the fact that in such species the maintenance of cambial activity is dependent upon a continued supply of auxin from the apical region, and that when extension growth ceases, the resulting drop in auxin production (Avery et al., 1937) results in the cessation of cambial activity.

A similar close connection between radial growth and extension growth in P. sylvestris is indicated, at least for the renewal of cambial activity in the spring, by the

observation that cambium of de-budded plants remains dormant (Experiment 19). On the other hand, cambial activity in P. sylvestris is maintained long after extension growth has ceased, and this has been observed also in certain 'ring porous' Dicotyledons e.g. Quercus, Fraxinus, (Bütsen, 1929). Coster (1927) and Lodewick (1929) observed also that in such 'ring porous' trees renewal of cambial activity is not closely correlated with the breaking of the buds, but may actually precede it. In such species, although the production of 'spring wood' appears to be correlated with expansion of the new shoots, (Priestley, 1936) the production of summer wood is not dependent upon the continuance of extension growth. Following the argument that "all radial growth owes its inception to the activity associated with leaf-production on the extending shoot" Priestley was able to trace a direct connection between the vessels of the summer wood and those of the differentiating members of the buds (which undergo a limited growth during the summer, but do not expand, of course, until the following spring). Thus, he postulated that even in such 'ring porous' species, maintenance of cambial activity, if not dependent upon extension growth, is still associated with meristematic activity at the apices. Now the work of Reinders-Gouwentak (1949) has shown that in the 'ring porous' species Fraxinus ornus the production of spring wood can be

induced in de-budded twigs by applying auxin at the tip, and there is thus a presumption that the production of spring wood is normally dependent upon a supply of auxin from the expanding shoots. If the production of summer wood is similarly dependent upon an exogenous supply of auxin, then this may possibly come from the developing (but not expanding) buds, since the production of ^{'free'}auxin appears usually to be associated with meristematic activity (Went and Thimann, 1937). In this case, the evidence of Priestley of a connection between the development of the buds and summer wood production is rendered intelligible. It is thus of interest to consider whether the maintenance of cambial activity in P. sylvestris is possibly dependent upon continued growth of the buds.

The results of Experiment 18, in which it was found that cambial activity ceased in de-budded plants even under 'long day' conditions, certainly indicate that the presence of terminal buds is necessary for continued cambial activity even when day length conditions remain favourable. The question then arises as to whether or not the buds must be actively growing in order to stimulate cambial activity, or whether they retain this property even in the dormant state. The observations on the growth of the buds under natural conditions (p.10) seem to indicate that elongation has effectively ceased by mid-September whereas cambial activity

can be observed for a further 4-5 weeks, but the procedure of taking relatively small samples for measurement at intervals is not sufficiently accurate to detect slight growth-changes in the buds. The effect of de-budding on cambial activity however is not immediate, for a zone of partially lignified tracheids was observable for a further 5-6 weeks after the operation of de-budding, so that the cambium of de-budded 'long day' plants became dormant at approximately the same date as intact plants maintained under 'short day' conditions. This long time-lag between the effect of removal of the buds and dormancy of the cambium would account for the observation that under natural conditions cambial activity can be observed for several weeks after bud-growth has apparently ceased.

If, in fact, maintenance of cambial activity is dependent upon continued bud-growth, is the effect of long photoperiods on cambial activity primarily an effect upon bud-growth? In order to test this possibility, an attempt was made to detect any differences as between the growth of the buds of plants under 'long days' and 'short days' respectively, by painting the outside of the buds with 'nail varnish' during September, and observing any subsequent cracking of the coat due to swelling of the buds. Although growth of the buds could be detected by this method in both series of plants during early September, growth in both series after 22nd September was so

slight as to render the observations inconclusive. Further observation is thus needed on this point. Assuming however that bud-growth had ceased in both series by late September, two alternatives would seem to exist with respect to the effect of 'long days' in prolonging cambial activity viz. (1) under both 'long' and 'short' day conditions cambial activity cannot be maintained indefinitely once bud-growth has ceased, and the effect of long days is merely to prolong the interval between the cessation of bud-growth and the incidence of dormancy in the cambium. This hypothesis is in accordance with the observation that cambial activity could not be prolonged indefinitely even under long day conditions, but had ceased by end of November. (2) Maintenance of cambial activity is actually independent of growth of the buds under long day conditions, and can be maintained indefinitely under favourable conditions. In this case the failure to prolong cambial activity indefinitely during the winter was due probably to poor light conditions. Further experiments are required to determine which of these alternatives is correct.

4. Effect of day-length and temperature on the breaking of dormancy.

It is well-known that many woody species, on becoming dormant in the autumn, require a period of exposure to low temperatures (5-8°C) during the winter before normal breaking

of dormancy can occur in the following spring, and that if they are protected from exposure to chilling by being kept in a greenhouse throughout the winter, normal breaking of the buds is delayed or is irregular (Howard, 1910; Colville, 1920). The requirement for a period of low-temperature exposure does not appear to have been previously demonstrated for P. sylvestris, but the following simple experiment shows that such treatment is, indeed, necessary for normal development of this species.

4.1 Experiment 20.

At the end of October approximately 20 two-year plants growing in 4 pots were brought into the greenhouse (which was maintained at approximately 13°C), while 12 other pots containing 50-60 similar plants were allowed to remain out-of-doors. Both series of plants remained dormant throughout the winter. In mid-February, the plants left in the open were brought into the greenhouse and all these plants broke dormancy regularly early in March. At this time all the plants which had remained in the greenhouse throughout the winter were quite dormant. During early April, however, the majority of these latter plants began to show signs of breaking dormancy also. At this time there was a spell of fine sunny weather and the temperature of the greenhouse rose to 20-25°C on some days. It seems probable that the increased temperature and/or light intensity stimulated the breaking of dormancy in these plants.

Nevertheless, it is evident that a period of exposure to low temperatures hastens and facilitates the breaking of dormancy in P. sylvestris.

4.2 Experiment 21.

Reference was made above (p.23) to the observations of Gustafson (1938) who found that plants of P. resinosa which were protected from exposure to low temperature during the winter failed to break dormancy unless exposed to a day-length of 16 hours. Similarly, Philips (1941) found that premature breaking of dormancy could be induced in P. taeda in December, by extending the natural photoperiod to 18 hours by low-intensity supplementary illumination. The following experiments were carried out to test the effect of continuous light on the breaking of dormancy in P. sylvestris:-

- (a) Twelve pots of 2-year seedlings were divided into two equal series on 27 June and from this date one series was kept under 'normal' photoperiods, while the other series received additional illumination at approximately 15-20 foot-candles from sunset to sunrise. Within 2-3 weeks the plants receiving continuous illumination showed signs of 'breaking' of the buds and this was followed by a period of extension of the shoot. The plants were maintained under continuous illumination until early October, when the terminal buds on the newly formed shoots of some plants had again broken dormancy and showed the initial stages of

a third 'annual shoot'. The plants under natural photoperiods remained dormant throughout the experiment.

- (b) Six pots containing 2-year old plants were transferred from a large batch growing under 'normal days' to continuous illumination at 15-20 foot-candles on September 4th. Within approximately 3 weeks all plants (approximately 25) under continuous illumination broke dormancy and formed a new annual shoot, while comparable plants under natural day-lengths remained dormant.

From the results of these experiments it is evident that dormancy can be broken prematurely in P. sylvestris, without prior exposure to low temperature, simply by exposure to low-intensity continuous illumination.

5. The Mechanism of Photoperiodism in P. sylvestris.

It has been shown that the photoperiodic responses of first-year seedlings of P. sylvestris suggest that the duration of growth is controlled by an interaction between a growth-promoting system whose action is dependent upon light and an inhibitor-system which becomes effective when the dark period exceeds 4 hours. It has been shown, also, that in both first-year and older seedlings the length of the photoperiod affects the extension of the internodes. Also, in older plants the duration of cambial activity is affected by length of day.

Now extension of internodes and cambial activity are processes

known to be affected by auxin supply (Thimann and Skoog, 1934; Snow, 1935), auxin deficiency having the same effect as short photoperiods. Moreover, in certain deciduous woody species, abscission of the leaves occurs under short photoperiods (p.21), and this a process also known to be affected by auxin supply. On these grounds the suggestion was put forward by the writer (Wareing 1949b) that these photoperiodic effects in woody species "result from the action of light on the production or availability of auxin within the plant, and that quite low light intensities are effective". Now attention has been drawn by various authors to evidence that light appears to be necessary for the production of auxin (Van Overbeek, 1933; Thimann and Skoog, 1934, Avery, 1935, Zimmerman and Hitchcock, 1936, Oortwijn, 1938). On the other hand, Skoog (1944) found that tobacco callus tissues may be grown in darkness, and that such cultures produce auxin extractable with ether (though not free auxin), auxin synthesis being independent of the light. As Skoog points out, however, these results do not exclude the possibility that light may cause an increase in free diffusible auxin.

The suggestion that the effect of light on auxin-production or availability may be important in photoperiodic phenomena does not appear to have been put forward previously. Cailachjan and Zdanova (1938) showed that the amount of

diffusible auxin in the stems of several species was greater in 'long days' than in short, but concluded that auxin is not important in the initiation of flowering. Leopold (1949) showed that the leaves of Coleus produced 78% more auxin when grown under a 16-hour day than under a 10-hour day. He found that auxin applied to the leaves of barley reduced tillering, thus having the same effect as long days. On the other hand, triodo-benzoic acid and coumarin, which are known to be auxin antagonists, increased the amount of tillering and thus had the same effect as short days. He suggested that the differences in tillering under long and short days result from differences in the amount of auxin produced.

It is clear, however, that even if auxin is the substance produced in the light phase, differences in photoperiodic response under 'long' and 'short' days cannot result simply from differences in amount of auxin produced as a result of long and short periods of illumination respectively, since the length of the unbroken dark period also plays an active role in determining the type of response. This latter phenomenon suggests that the effects of dark are not due simply to the absence of auxin production but that there is an active inhibition of the effects of auxin during long dark periods. Evidence that an active growth-inhibitor, which is antagonistic to the effects of auxin, is involved in the phenomena of

correlative inhibition, was produced by Snow (1939, 1940). Stewart (1940) was able to isolate such a substance from the cotyledons and leaves of radish, and further instances of the occurrence of growth inhibitors are mentioned below. Now many of the problems of correlative inhibition, such as the early cessation of growth of lateral shoots, are clearly related to the problem of what determines the cessation of growth in seedlings of P. sylvestris.

The following extension of the hypothesis put forward to account for the results in seedlings of P. sylvestris (p.76) is therefore suggested:

- (1) During the light period there is a production of auxin or an auxin-precursor, which is the basis of the growth-promoting effect of the light-phase, postulated above. During the first hours of darkness the auxin level within the plant is still high as a result of production during preceding photoperiod, but after some hours of dark the auxin level becomes reduced.
- (2) There is an active auxin-inhibitor system within the plant which becomes effective after several hours of dark, when the auxin level has fallen.

The reduction in the auxin level during darkness might be due to its transport from some field of action, such as the leaves or the apical meristem. Thus, a diurnal rhythm in auxin

content has been found in the leaves of Carica papaya (Yin, 1941). The possibility is not excluded, however, that during darkness the auxin itself becomes converted into an inhibitor, for evidence of such a conversion has been produced (Funke and Söding, 1948). Also, Stewart (loc.cit) found that the inhibitor present in the leaves of radish seedlings was easily converted by hydrolysis into an active growth promoting substance. Again, Veldstra (1944) on theoretical grounds, has suggested that plant growth-inhibitors may be structurally related to growth-substances.

The type of mechanism postulated is also indicated by a consideration of the conditions for the breaking of dormancy in P. sylvestris. It has been shown by Hemberg (1949a, 1949b) that in dormant potatoes and resting buds of Fraxinus excelsior an auxin-inhibitor is present and that during the winter there is a gradual disappearance of the inhibitor. The ability to break dormancy is correlated with the disappearance of the inhibitor, and it would appear that the effect of low-temperature exposure required by both these plants is in some way to remove the inhibitor. If the same mechanism forms the basis of the requirement for a period of low-temperature exposure in P. sylvestris, then it is of great interest to note that in this species dormancy may equally well be broken by exposure to continuous illumination, suggesting that the inhibitor is also

removed in the presence of light. Moreover, it has been shown by Klebs (1914) for beech and Gulisashvili (1948) for certain other species, that dormancy can be broken prematurely even in the leafless winter conditions by exposure to continuous illumination and without exposure to low temperatures.

If light is necessary for auxin production, as has been suggested above, it is at first sight difficult to understand how it is that growth of the new shoots in P. sylvestris can proceed for some time even in complete darkness. It is possible, however, that after low-temperature exposure during the winter with consequent disappearance of an inhibitor, a reserve of auxin or an auxin-precursor is made available. This must certainly be the case with deciduous woody species, in the expanding buds of which abundant auxin is present although no leaves have yet been expanded (Czaja, 1934).

It is of interest to consider the implications of this postulated mechanism of photoperiodism in P. sylvestris for the problem of photoperiodic induction of flowering in herbaceous species, for there are good grounds for the view that the underlying mechanism is the same in both woody and herbaceous species. The evidence for this view may be summarised as follows:-

- x
- (1) In both woody and herbaceous species, photoperiodic effects are evoked at quite low light-intensities.
 - (2) In herbaceous species it appears that normally it is the length of unbroken darkness which determines the type of response, and this appears to be true also of P. sylvestris. A short 'light-break' during the dark period completely modifies the response in both herbaceous species and P. sylvestris.
 - (3) In both types of plant there is usually a reduction of the internodes, tending towards a 'rosette' growth habit, under short days.
 - (4) There is a photoperiodic 'after-effect' in both herbaceous and woody species when they are transferred from 'short day' to 'long day' conditions.
 - (5) Just as in certain herbaceous species there is an interaction between vernalisation and day-length effects (for example, in unvernalsed winter rye reproduction may be hastened by short days), so in P. sylvestris dormancy may be broken both by low temperature and very long days. Similarly, certain varieties of peach which have been germinated without prior stratification, become dormant prematurely and have abnormally short internodes, but these effects may be removed by exposure to continuous light. (Lammerts, 1943).

The parallelism between photoperiodic phenomena in herbaceous and woody species is thus sufficiently close to warrant the assumption that the underlying mechanism is the same in both cases. This being the case, it would appear unlikely that the initial stages in photoperiodic induction of flowering in herbaceous species involve the production of a specific 'flower-forming' hormone, since in seedlings of woody species photoperiodic responses may be observed which affect purely vegetative processes and which appear not to be connected with any effects on the reproductive state. Thus any substances formed directly by the light- and dark-reactions are likely to be much more generalised in their effects, which include not only the initiation of flower buds, but extension of internodes, apical dominance, leaf-fall, cambial activity etc.

A number of theories have been put forward to account for the phenomena associated with photoperiodic induction of flowering in herbaceous species, the best-known probably being those of Hamner (1942) and Gregory (1948). According to the theory of Gregory a substance "A" is produced in the leaves in the light phase, and in short day plants is converted to "B" in the dark. The substance "B" is transported from the leaves to the apical meristem and is there converted to "C", the 'flower hormone'. "B" is light-sensitive whilst in the leaf, and in the presence of a 'light-break' during the dark period,

is reconverted to "A". Thus a dark period is necessary for the formation of "B", and the effect of a 'light break' in preventing the initiation of flowering is due to the reconversion of B to A.

Gregory suggests that in 'long day' plants the substance "A" is formed in the leaves in the light, and is transported to the meristem where it is converted to "C". Thus "B" is held not to be found in long-day plants. It is suggested that during the dark, substance "A" is converted to "X", which plays no part in flower initiation.

We may, however, equally well interpret the effects in herbaceous species according to the hypothesis put forward for P. sylvestris, if we postulate that in 'short day' plants day-length conditions favouring high auxin levels are inimical to flower initiation and that conditions tending to reduce the auxin level promote flower initiation. Flower initiation in 'short day' plants is then due primarily to the inhibition of auxin which occurs in long dark periods. Evidence in support of this hypothesis is provided by the experiments of Galston (1947), Bonner (1949) and Bonner and Thurlow (1949). Galston showed that treatment of soybeans with tri-iodobenzoic acid, which is a known auxin antagonist, induced the formation of flower buds in plants maintained under a day-length unfavourable for flowering.

Bonner found that application of the auxin antagonists 2,4 - Dichloranisole and tri-iodobenzoic acid to vegetative Xanthium plants resulted in initiation of 'flower-like' buds. Bonner and Thurlow showed that the photoperiodic induction of flowering by short days in Xanthium is inhibited by spraying the leaves with auxin, or by placing cuttings in solution of auxin. On the other hand when 2,4 - Dichloranisole, is supplied to cuttings floral development is hastened. In the pineapple also it has been shown that flower initiation may be induced by treatment with auxin (Van Overbeek, 1946).

There is thus good evidence in support of the view that in herbaceous species the auxin level within the plant is an important factor in the initiation of reproduction and that in short day plants a low auxin level is favourable to flower initiation.

It is suggested that 'long day' plants are those in which flower initiation is favoured by conditions leading to a high auxin level, and that failure to flower in short days is due to the predominance of the inhibitory system under such conditions.

A highly speculative scheme, which has certain points in common with the present hypothesis has been put forward by Rezende (1949), who postulates that flowering is determined by the attainment of a certain difference in level between auxin

and anti-auxin (= bound auxin) within the plant. No suggestion is made, however, as to how this scheme is to be related to the phenomena of photoperiodism.

According to the hypothesis put forward here, in both long day and short day plants the substance "A" postulated by Gregory and Hamner as found in the light is auxin or an auxin precursor. In long dark periods an auxin-inhibitor "B" is formed, possibly from the auxin formed in the light phase. The question then arises as to the effect of a 'light break' during the dark period. According to Gregory the substance B formed in the dark is reconverted to A, and this suggestion would agree with the apparent interconvertibility of auxin and an inhibitor, to which reference has already been made. The destruction of an inhibitor by light is suggested by the effect of continuous illumination in breaking dormancy of the buds in P. sylvestris. Thus Gregory's scheme is quite compatible with the scheme put forward here if we postulate that although A is a necessary precursor for B, nevertheless A and B have mutually antagonistic effects, A being inimical and B favourable to flowering. Alternatively we may suggest that the effect of a light break is to produce more "A" from a substrate " ∞ " which is produced by a non-photochemical reaction, and which accumulates during the dark period preceding a 'light break' (Withrow and Withrow, 1944). On this view, the effect of a

'light break' is to produce more auxin, which is inimical to flowering in short-day plants at certain levels of concentration.

While such a scheme as this leaves many difficulties unsolved, it has the support of much evidence and has the merit of embracing a much wider range of photoperiodic phenomena than earlier schemes, including those in both woody and herbaceous species.

PART IV. FACTORS CONTROLLING THE ANNUAL CYCLE OF GROWTH IN
P. SYLVESTRIS.

We are now in a position to summarise and discuss what has been demonstrated as to the factors, both 'internal' and 'external', controlling the normal pattern of the annual cycle of growth in P. sylvestris. (This discussion will be confined to plants in the second or later years of growth).

Commencing with a consideration of plants which have become completely dormant in the autumn, it has been shown that further development is dependent upon a period of low-temperature exposure, for plants which are transferred to a greenhouse in October remain dormant at least until the following April, even though temperature conditions apparently remain favourable for growth throughout the winter, whereas plants allowed to remain out-of-doors during November and December, break dormancy readily when transferred to the greenhouse in January. Evidently the requirement for a period of low temperature exposure has normally been satisfied by January, and further development is then dependent upon an increase in temperature, so that conditions become favourable for growth. Under natural conditions breaking of dormancy does not occur until April and there seems little doubt that the natural rise in temperature in the spring is the major factor determining the time of bud-break in P. sylvestris, since the

process can be induced to take place sooner simply by transferring the plants to a warm greenhouse. There is no evidence that the breaking of dormancy in P. sylvestris under normal conditions is affected by natural changes in day-length conditions in the spring.

The results of Experiment 13 indicate that the presence of leaves is necessary not only for normal extension of the shoot, but also for the breaking of dormancy, for when plants were defoliated at the first signs of bud-break, a high percentage of plants failed to break at all, whereas nearly 100% of plants with leaves ultimately broke dormancy. In this respect P. sylvestris stands in marked contrast with deciduous species. The role of the leaves in this connection needs further investigation.

The breaking of the buds is immediately followed by a period of extension of the annual shoots, which proceeds rapidly and is completed in about 2 months. This process involves only the expansion of initials laid down in the bud, so that the period of extension growth is 'internally' determined. This extension growth occurs during a period when natural day-lengths are relatively 'long' (13-16 hours) so that the photoperiodic effects observed under experimentally shortened photoperiods will not normally be important under natural conditions.

Growth of the leaves continues much longer than that of the stem, by the activity of basal meristem, and is not normally completed until the end of August or early September. In so far as the duration of leaf-growth has been shown to be reduced by short days, and since during August the natural day-length is falling rapidly, the possibility of photoperiodic control of leaf-growth is indicated. Experimental lengthening of the natural day-length during August, however, failed to prolong the duration of leaf-growth, but the duration of additional illumination may have been insufficient (p.96). It is possible, therefore, that the maximum leaf-length corresponding to the prevailing natural photoperiods is being approached by early August, and that leaf-growth would cease shortly afterwards even if day-length conditions remained constant, just as in Experiment 10 leaf-growth ceased in early August under a constant photoperiod of 14 hours.

Cambial activity, as indicated by swelling of the cambial cells, can be detected both in the buds and in the main trunk before breaking of the buds has occurred. The formation of new wood, however, commences at the apex of the trunk and at the tips of the branches and travels basipetally (Wight, 1933). The evidence summarised above (Experiment 19) supports the view that in P. sylvestris, renewal of cambial activity is dependent upon expansion of the buds.

Continuous cambial activity is maintained throughout the summer until October, at which time shoot extension, leaf-growth and bud-growth have ceased. Since the duration of cambial activity can be prolonged by extending the natural photoperiod with low intensity artificial illumination, it is evident that the falling natural day-length in the autumn plays an important part in bringing about the cessation of cambial activity. For some reason, which is not at present clear, the maintenance of cambial activity under artificially lengthened days is dependent upon the presence of terminal buds, which thus probably play an important role in the maintenance of cambial activity under natural conditions.

The buds themselves show slow but continuous growth from early June (when extension of the shoot has ceased), until approximately mid-September, and the question arises as to what determines the cessation of bud-growth at this latter date. Possibly the development of the buds is 'internally' determined and not controlled either by external environmental factors or by interaction with other processes in the tree. Certain considerations, however, suggest that possibly the cessation of bud-growth is connected with the cessation of leaf-growth. Firstly, reference has already been made (p.11) to the observation that the rudiments of female cones may first be detected in the buds in early September, shortly after the

cessation of leaf-growth. It is well-known that these female cones are formed at the tip of the annual shoot, so that in such buds the formation of further nodes must have ceased at about the time of cessation of leaf-growth. Secondly, an unusual feature was observed in the buds of certain plants of Experiment 10, in which it was shown that the duration of leaf-growth may be affected by the length of photoperiod to which they are exposed. Now, certain portions of the rudiments at the tip of the bud give rise to the terminal bud of the following season (p.10), which will also be surrounded by a variable number of subsidiary buds, forming the lateral shoots. Normally the portions of the bud-tip which will next season form respectively terminal and lateral buds cannot be distinguished, but in 10% of the plants of Experiment 10 exposed to 12 and 14-hour photoperiods, and in which leaf-growth ceased at the end of July and in early August respectively, the tip region of the buds was divided into a number of separate apices, obviously corresponding to the terminal and lateral buds of the next season, which had developed further than normal. Out of some 50 plants grown under ^{normal} day, however, not one showed this feature. It is suggested that the explanation of this phenomenon is that in such plants the development of the buds and determination of the apical region into portions which would form next season's

terminal and lateral buds occurred unusually early, corresponding to the early cessation of leaf growth, but temperature and light conditions still remaining favourable for growth, these rudiments underwent further development than is normal, during the later part of the growing season.

If the formation of new nodes within the bud ceases at the time of cessation of leaf-growth at the end of August, the further elongation of the buds observed during the first 2-3 weeks of September must be due merely to enlargement of the existing initials at the tip of the bud.

Thus the possibility exists of direct or indirect photoperiodic control of the duration of growth of the buds. This conclusion is of considerable importance in relation to the possible experimental photoperiodic control of reproduction in P. sylvestris, for the male cones are formed at the lower nodes of the bud, which are developing during the early part of the summer (when day-length is at a maximum), while, as already pointed out, the female cones are formed at the tip of the bud, during late August or early September, at a time when the natural day-length has shortened considerably. These facts clearly merit a closer examination of the effects of day-length conditions on the development of the bud, but since reproductive organs do not normally appear in seedlings of P. sylvestris until they are 5-7 years old, a direct experimental

approach is a matter of some difficulty. It is of interest to note, however, that strains of P. sylvestris from Northern Sweden when grown in more southern latitudes, first show female cones at the age of 3 years instead of the normal 5-7 years, and Langlet (1942-3) has suggested that this is a photoperiodic effect resulting from the shorter natural day-lengths at more southern latitudes.

The later stages in the reproductive cycle also show a well-marked rhythm which appears to be related to seasonal climatic changes. For example, after pollination the further growth of the pollen tubes is soon inhibited, and the female cones increase in size, but fertilisation remains inhibited until the following spring. Again, the female cones remain green throughout the second summer, and do not ripen until the following spring. The elucidation of the physiological basis of this rhythm presents problems of great interest, but which remain unsolved at present.

The observations on the growth of the roots were not sufficiently detailed to permit any conclusions to be drawn. Evidence for some periodicity in the growth of the roots has been described, and suggests that root-growth is greatly stimulated when the extension of the new shoots has occurred in the spring. It would be of interest to know whether root-growth is affected by the onset of dormancy in the shoot; the

observation that root growth remains very limited in plants kept in the greenhouse throughout the winter suggests that this may be the case. The annual periodicity in growth of the roots is a subject which requires further detailed investigation.

It is of some interest to consider the possible biological significance of photoperiodic phenomena in a woody species, such as Pinus sylvestris. Reference has already been made (p.24) to the work of Schotte (1905) and Engler (1913), who found that distinct geographical races exist within the species P. sylvestris and that first-year seedlings from parent trees growing at northern latitudes in Sweden show reduced growth and have short internodes when grown in Germany. Sylven (1940) suggested that this response is a photoperiodic effect, resulting from differences in natural length of day under different latitudes. This view is supported by the observations of the writer that experimentally reduced photoperiods do indeed result in the same sort of effects as were observed in first year seedlings when grown in more southern latitudes. Moreover, in older seedlings (after the first-year) Langlet (1942-3) observed a reduction in the length of the annual shoot and of the needles when races from northern Sweden were grown in the south. The suggestion that these also are photoperiodic effects is again confirmed by the results obtained in the present work. There is thus strong evidence that

geographical races, which differ with respect to photoperiodic response, exist within the species P. sylvestris, just as has been shown for Populus tremula (Sylvén loc.cit.), and this implies that under natural conditions the individuals growing in any one locality show a delicate adjustment to the day length conditions prevailing there. This conclusion implies in turn that the photoperiodic response in P. sylvestris has an important biological significance.

Now the capacity for photoperiodic response can only have significance in relation to the natural seasonal changes in day-length which occur in any one locality - if the natural day-length remained constant throughout the year the capacity for photoperiodic response could have no biological advantage. The effects produced experimentally on transferring members of a given geographical race from one latitude to another are the result of a change in the mean day-length throughout the growing season, and will not have any direct significance for the normal annual cycle of growth of an individual in response to seasonal changes in day-length conditions at a given locality. Inasmuch as no evidence has so far appeared to indicate that day-length changes in the spring exert any important effect in P. sylvestris it would appear that we must look primarily to the significance of the effects of decreasing natural day-lengths in the autumn. So far, the chief processes found to be controlled by reduced photoperiods at the end of

the growing season are the cessation of cambial activity and possibly cessation of leaf-growth and bud growth. Although attention has been drawn to the fact that the initials of the male and female cones respectively occur at different times of the year and therefore under different day-length conditions, the male cone initials, which are formed during the early growth of the bud in midsummer, clearly do not arise in response to any change in natural day-length conditions. The female cones are formed under reduced day-length conditions in late August, but here again there is no evidence of direct photoperiodic control and the formation of female cone initials appears rather to be a response to the cessation of further growth of the bud (which may, however, be an indirect effect of the photoperiodic control of leaf-growth, as suggested above - p.162). Moreover, photoperiodic control of the initiation of female cones at the end of August would appear to confer no direct biological advantage, since further development is arrested until the following spring. It is suggested, therefore, that photoperiodism in P. sylvestris is connected primarily with the onset of dormancy, rather than control of the reproductive phase.

This conclusion is suggested also by a consideration of photoperiodic effects in other woody species, in which the times of cessation of extension growth and of leaf-fall

may be controlled by the onset of short-days. It has been shown that in certain shrubs e.g. Hibiscus, Buginvillea, (Allard, 1935), Sesamum (Rhind, 1935) the onset of flowering is controlled by natural changes in day-length, but in many woody species of temperate regions the rudiments of the flower-buds are laid down in the summer of the preceding year and the time of formation of these initials does not appear to be related to any natural day-length changes, but frequently occurs after the cessation of extension growth in the summer. (Busgen, 1929).

The suggestion is therefore put forward that in many woody species, as in P. sylvestris, the significance of photoperiodism lies in the control of the onset of dormancy in the autumn. The biological advantage of this control is that it provides a mechanism whereby dormancy is brought about prior to the onset of unfavourable conditions. In those species in which extension growth continues until the autumn, severe frost damage may result if the day-length is artificially lengthened, so that growth is prolonged beyond the normal date (Moshkov, 1932, Kramer, 1937). In P. sylvestris this danger does not exist with respect to extension growth, but it is well known (Büsgen, 1929) that an active cambium may suffer severe damage when exposed to frost, so that the effect of short days in hastening the dormancy of the cambium reduces the probability of such damage.

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APPENDIX I.

Woody Species shown to exhibit Photoperiodic Responses.

In all woody species which have been tested so far, it is found that the duration of extension growth is affected by the day-length conditions. In many cases the effect of 'short days' as against natural daylengths has been compared, while in the other cases the effect of extending the natural photoperiod by artificial illumination has been tested. In all cases (except Quercus alba) in which shortening of the natural day-length has been carried out, it has been found to reduce the duration of extension growth, and in all cases (except Fraxinus americana, F. pennsylvannica and Quercus borealis) in which natural day-length has been extended, the onset of dormancy has been delayed.

Table 1. Species in which the duration of extension-growth is reduced by 'short days':-

<u>Species.</u>	<u>Authority.</u>
Acer negundo	Garner and Allard.
Acer sp.	Bogdanov.
Aesculus sp.	"
Ailanthus sp.	"
Alnus sp.	"
Caragana sp.	"
Fagus grandifolia	Kramer.

<u>Species</u>	<u>Authority.</u>
Fraxinus americana	Kramer.
Fraxinus sp.	Bogdanov.
Juglans sp.	"
Larix sibirica	Moshkov.
Larix sp.	Bogdanov.
Liquidambar styraciflua	Kramer.
Liriodendron tulipifera	"
Phellodendron amurense	Moshkov.
Pinus caribaea	Jester and Kramer.
P. sylvestris	Wareing
P. taeda	Kramer
Pinus sp.	Bogdanov.
Populus tremula	Syiven.
Prunus sp.	Bogdanov.
Pyrus sp.	"
Pyrus ussuriensis	Moshkov.
Quercus borealis	kramer.
Q. stettata	"
Rhus sp.	Bogdanov.
Robinia pseudacacia	Moshkov) Kramer)
Salix babylonica	Moshkov
Syringa vulgaris	Chouard.
Ulmus sp.	Bogdanov.

Table 2. Species in which the duration of extension growth is prolonged by extending the natural day-length.

<u>Species.</u>	<u>Authority.</u>
Abelia grandiflora	Kramer.
Acer campestre	Gulisashvili.
Acacia melanoxylon	"
Betula verrucosa	"
Carpinus betulus	"
Catalpa speciosa	"
Fagus sylvatica	Klebs.
Liquidambar styraciflua	Kramer.
Liriodendron tulipifera	Garner and Allard) Kramer)
Phellodendron amurense	Gulisashvili.
Pinus banksiana	Jester and Kramer.
P. caribaea	" "
P. resinosa.	" "
Quercus alba	Kramer.
Q. macranthera	Gulisashvili.
Q. stettata	Kramer.
Q. suber	Gulisashvili.
Robinia pseudacacia.	Kramer.
Taxus baccata.	Gulisashvili.

It has also been shown that the time of leaf-abscission may be delayed in certain species by extending the natural

day-length in the autumn.

Table 3. Species in which leaf-abscission is delayed by 'long days'

<u>Species.</u>	<u>Authority.</u>
Platanus acerifolia	Matzke.
P. occidentalis	"
Populus canadensis	"
Rhus copallina	Garner and Allard
R. glabra	" "
Salix fragilis	Matzke.

It appears that the earlier cessation of extension growth under short days is usually accompanied by earlier leaf-abscission, but the authors quoted in Table I are not explicit on this point.

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APPENDIX II.

It was pointed out in Section 3A above that in certain woody species (e.g. Acer pseudoplatanus, Betula spp., Carpinus betulus, etc.) growth of the annual shoot ceases during the height of summer, and that in such species the duration of growth is determined neither by a natural change in day-length nor by limitation to expansion of initials already laid down in the bud (as in Pinus sylvestris). The question thus arises as to why growth should proceed actively for a certain period and then cease abruptly, at a time when external conditions would appear to be still favourable for growth. Since the cessation of growth does not appear to be controlled by any regular seasonal change in external conditions, it would appear that such control must be 'internal'.

In species which have a 'sympodial' growth habit, where the apical region withers and is abscised when a certain stage of development has been reached, an active inhibition of further growth is strongly suggested. Now it is known that with certain species which cease growth early in the summer, if artificial defoliation of the shoots is carried out shortly after expansion has been completed, the newly-formed resting-buds may be induced to break dormancy prematurely, leading to a fresh expansion of leaves. This observation suggests that the expanded leaves exert an inhibitory effect on the further

development of the young buds. Thus it seems possible that the cessation of growth of the annual shoot might also be brought about by an inhibitory action of the leaves, and that as the number of expanded leaves increases cessation of growth is ultimately determined when the total inhibitory action becomes over-riding. This hypothesis implies that it is the attainment of a certain total leaf-area by the annual shoot which ultimately determines its duration of growth. In order to test this hypothesis the following experiment was carried out, to determine the effect of continuous defoliation of the annual shoot on the duration of growth.

Experiment 1.

In a preliminary experiment, first year seedlings of P. sylvestris were used. Six pots of seedlings growing under 'normal' day-length conditions were used for defoliation. On 25th July 1948, the lowermost 10 leaves of the plants were cut off with scissors, leaving approximately 5 fully formed younger leaves remaining, together with the terminal 'rosette' of immature leaves. On 9th and 17th August respectively, further defoliations of five leaves were carried out on all plants, so that a total of 20 leaves was removed from the lower region of each plant. The subsequent growth was compared with 'intact' plants growing under similar conditions. The date on which each plant first showed a terminal resting bud was determined

for both treated and control plants. It was found that the date on which 50% of the plants of each series showed terminal resting buds, was 18th September for the treated, and 13th September for the control plants, respectively. Thus defoliation made little difference to the total duration of growth.

Since the removal of a large proportion of the mature leaves of a seedling must seriously reduce the capacity of the plant for assimilation, further experiments were carried out with other species, using the annual shoots of older trees, on the assumption that the reduction in photosynthesis in the defoliated shoots would be partly compensated by translocation from other parts of the tree. Evidence in support of this assumption is provided by the observation that even colourless shoots of chimaeral plants are able to make considerable growth.

Methods. Annual shoots of three species (Forsythia spp. Laburnum vulgare, Philadelphus sp.) with the 'determinate' growth habit, and two species (Tilia europaea, Ulmus campestris) with a 'sympodial' growth habit, were used. Since the total number of leaves formed on the annual shoots varies considerably and is affected both by its morphological position on the branch and by the age of the branch on which it is borne, the following procedure was adopted in selecting the shoots for defoliation and the 'control' shoots for comparison. Shoots of corresponding size and morphological position were selected

in pairs usually from the same parent branch and both were labelled. One of these shoots was defoliated (as described below) and the other allowed to remain intact. Thus the 'control' shoots were selected at the same time as the treated shoots, so that any subsequent differences in the two shoots which were not due to the treatment were random for both the defoliated and control series. With each species, the leaves were removed from the lower portions of the 'experimental' shoots, leaving only 2-3 mature leaves in the upper part. As additional leaves matured, further defoliations were carried out at intervals. Observations were made as to the date of cessation of growth in both defoliated and intact shoots.

Experimental details for the individual species are as follows:-

(1) Forsythia sp. Shoots of a single large shrub were used. The shoots used were the laterals on long shoots, and the two members of the pairs selected were in all cases on the same parent shoot. Defoliation commenced 10th May, mean date of cessation of growth in both series 16th June.

(2) Laburnum vulgare. The shoots used were on a single bush, which had been rather severely pruned back during the previous winter. Defoliation commenced 5th May, growth ceased in both experimental and control shoots on 20th May.

(3) Philadelphus sp. Experiments carried out on a single shrub. the shoots used were laterals and the members of each pair were

on the same parent shoot. Defoliation commenced 7th May, growth ceased in both experimental and control series approximately 16th June.

(4) Ulmus campestris. Experiments carried out with two young trees (estimated age 12-15 years) growing in close proximity. The shoots used were the uppermost laterals. The shoots used were in all cases from the second axillary bud of the previous year's shoot and were chosen in pairs from the same parent branches. Defoliation commenced 6th May, growth ceased in both defoliated and control shoots on approximately 7th July.

(5) Tilia europaea. Experiments carried out with shoots which had arisen from the main trunks of two trees from which the branches had been cut off one year previously. All shoots were thus of closely corresponding morphological position and they were selected in pairs of similar size. Defoliation commenced 2nd May, growth ceased approximately 22nd June in both defoliated and intact shoots.

When growth had ceased, the total number of leaves removed, and those remaining, were determined for each experimental shoot, and similarly the total number of leaves on each 'control' shoot was counted. The data for each species are given in Table 1.

Results. It has already been indicated that with all species the date of cessation of growth was the same in both defoliated and control shoots. From the data of Table 1 it is

Table 1. Data for Experiment 1.

Species	Defoliated shoots.			'Control' shoots		
	Number of shoots	Number of leaves removed.	Number of leaves remaining.	Total No. of leaves.	Number of shoots	Total No. of leaves.
Forsythia sp.	20	10.6	9.95	20.55 ±0.69	20	20.15 ±0.76
Laburnum vulgare	11	6.0	7.0	13.0 ±0.19	11	12.7 ±0.27
Philadelphus sp.	9	8.9	11.6	20.5 ±1.2	9	22.3 ±1.1
Ulmus campestris	20	8.1	5.85	13.95 ±0.38	20	13.50 ±0.30
Tilia europaea.	20	7.45	4.95	12.4 ±0.33	20	12.8 ±0.34

evident that in no case did defoliation bring about any significant difference in the total number of leaves formed, as compared with that of the control shoots. Indeed, it is remarkable that the removal of a high proportion of the leaves had so little effect on the total duration of growth and total number of leaves formed. Thus, no evidence was obtained in support of the hypothesis that it is the attainment of a certain total leaf-area on the shoot which determines the duration of growth.

In these circumstances it was of interest to test whether possibly it is only the uppermost leaves, immediately

below the apical region, which are effective in inhibiting further growth when a certain stage of development has been reached. In the foregoing experiment a small number of mature leaves immediately below the apical region had been allowed to remain, and hence the possibility that they had exerted an inhibitory effect was not excluded. The following further experiment was therefore carried out, using a cultivated apple-tree, and trees of Platanus acerifolia, which were by that date the only suitable species available in which growth was still active.

Experiment 2. (a) Pyrus malus.

Methods. The tree used for this experiment had been severely pruned during the previous winter, and this had resulted in an abundant growth of strong vegetative shoots at the top of the branches. These were selected in pairs of similar size as in the previous experiment. A label was attached to the uppermost mature leaf of each shoot. From one member of each pair were removed the young leaves which had attained the stage at which the lamina had begun to unfold. All subsequently developed leaves were removed at the same stage. Defoliation commenced on 7th July, and active growth continued in the control series until approximately 15th August by which date 50% of these shoots formed a terminal bud. The defoliated shoots, on the other hand continued growing until the

end of August and early September. When all shoots had ceased growth the initial number of leaves, and the number of leaves formed since the commencement of the experiment were determined for each shoot of both the 'experimental' and the 'control' series. The initial length of the shoots and the increment during the period of the experiment were also determined for each shoot. The data are summarised in Table 2. It is seen

Table 2. Effect of defoliation in *Pyrus malus*

	Initial No. of mature leaves (1)	New leaves formed (2)	Total leaves (3)	Initial length of shoot (inches) (4)	Further extension of shoot (inches) (5)	Mean \bar{x} internode length (inches)
Treated	23.1 ± 0.55	19.1 ± 1.3	42.2 ± 1.3	27.3	12.2	0.64
Control	22.7 ± 0.44	12.2 ± 1.4	34.9 ± 1.7	27.7	13.3	1.09

\bar{x} This is the mean internode length for the portion of the stem formed during the experimental period.

that there was no significant difference between the mean initial leaf-numbers and stem lengths of the two series. On the other hand, the mean number of leaves formed by the defoliated shoots during the period of the experiment is noticeably greater than the corresponding number for the 'control' series. Since the shoots were originally selected in pairs of comparable size and morphological position, an analysis of variance was carried out, and from this a value for 't' was obtained for the

difference in total leaf-numbers between the two series:-

Total leaf-numbers : Analysis of Variance.

Item.	Sum of squares	Degrees of freedom	Mean square	't'	Probability
Experimental treatment	455	1	455.1	4.89	< 0.001
Pairs	970	17	57.07		
Error	324	17	19.05		

It is seen that the difference due to the experimental treatment (defoliation) is highly significant. Thus removal of the young leaves resulted in a significantly greater total leaf-number than in the control shoots.

The mean length of the defoliated portion of the stem was actually less than the corresponding portion of stem in the control series, for although there was a greater number of internodes in the defoliated portion the length of the internodes was much reduced by defoliation (Table 2).

(b) Platanus acerifolia.

This species was chosen as an example of a species with the 'sympodial' growth habit. The shoots used were those on the lower branches of large trees. Since it was not possible to select pairs of comparable shoots located in close proximity, as in the previous experiments, no attempt was made to obtain quantitative data, but approximately similar 'control' shoots were labelled at the commencement of the experiment for further

observation. Defoliation of the young leaves in one series of shoots was commenced on 15th June, the operation being carried out at the stage when the lamina emerged from the nodal sheath. (at which stage the lamina was approximately 1.5 - 2.0 cms. in diameter). The successive emergence of new leaves continued in both series of shoots until the end of July, when the apical region of the 'control' shoots withered and was abscised in the normal manner, but the further emergence of new leaves continued slowly in the 'experimental' shoots for a further 2-3 weeks. Further extension of the experimental shoots then ceased, but there was no immediate abscission of apical portion as occurs normally, but instead the apical region remained fresh for a period of some 14 days, after which the whole of the defoliated portion slowly died back, but there was still no abscission. Thus removal of the young leaves prolonged the normal period of growth and prevented normal abscission of the apex of the shoot. Although these observations must be regarded as merely of a preliminary nature, nevertheless they agree with the results already observed in Pyrus malus.

DISCUSSION.

The results of the foregoing experiments clearly indicate that whereas removal of mature leaves in the basal region of a growing shoot has no appreciable effect upon the duration of growth and the total number of leaves formed, nevertheless,

removal of the young leaves (before expansion of the lamina has occurred) does have a noticeable effect. These results apparently imply that young expanding leaves have an inhibitory effect upon the growth of the apical meristem, but that this inhibitory effect does not become over-riding until a certain stage of development has been reached. It is of interest to note that in those species in which the terminal bud of the shoot normally dies e.g. Syringa vulgaris, if defoliation of the uppermost leaves of the shoot is carried out, the life of the terminal bud is prolonged (Berthold, 1904). Several other instances are known in which a growing member is found to have inhibitory effect upon a neighbouring young one, for example, in Solidago sempervirens (Goodwin, 1937), in which a rapidly-expanding leaf retards the development of the younger leaves. This retarding effect was demonstrated by removing the rapidly-growing leaf, thereby hastening the elongation of the next succeeding leaves, and was reproduced by applying auxin to the petiolar stub of such an amputated leaf. It thus appears that the inhibitory effect of an expanding leaf is due to its high auxin content. This conclusion agrees with the observation of Snow (1938) that a region of high auxin-concentration applied below the apical region has an inhibitory effect on the latter. Such a mechanism does not, however, explain why it is that such inhibition only becomes effective when a certain stage of

development has been reached.

The fact that removal of the young leaves brings about prolongation of the period of extension-growth in the treated shoot, when other 'intact' shoots upon the same tree have already become dormant, seems to indicate that each annual shoot of the tree is, to some extent at least, autonomous and is not controlled by some condition which is general throughout the whole tree.

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Photoperiodic Control of Leaf Growth and Cambial Activity in *Pinus sylvestris*

It has long been known¹ that geographical races of *Pinus sylvestris* from northern latitudes show reduced growth of the shoot when planted in more southern localities. Sylven² and Langlet³ suggested that this is probably a photoperiodic effect, arising from differences in the natural length of day between different localities. My experiments have shown that *Pinus sylvestris* is indeed photoperiodic. As in many other species⁴, short photoperiods (10 hr.) induce earlier cessation of growth of the shoot in first-year seedlings of *P. sylvestris* as compared with long photoperiods (15 hr.), so that fewer leaves are formed before the formation of a terminal resting-bud. There is also a reduction in the length of the 'internodes', leading to a rosette type of growth, under short days.

After the first year, however, growth of the annual shoot consists solely of expansion of the initials already laid down in the terminal bud⁵, and hence in older plants day-length can have no direct effect on the duration of growth of the shoot or on the number of leaves formed. There is, however, as in first-year seedlings, a reduction in the extension of the stem under short days, leading to a shorter distance between successive dwarf shoots.

The leaves of *Pinus sylvestris* are organs of 'indeterminate' growth, and activity of the meristem at the base of the needles results in their continuous growth in length until late August under natural day-length conditions; under a 10-hr. photoperiod, however, they cease growth six to eight weeks sooner, so that their final length is much reduced. It seems probable that cessation of needle-growth in the autumn is controlled by the reduced day-length conditions then prevailing.

Day-length is also found to affect the duration of cambial activity. Two series of two-year-old seedlings of *P. sylvestris* were kept under (a) natural and (b) 15-hr. photoperiods respectively from the end of August (when leaf-growth had ceased), the longer photoperiod being obtained by giving supplementary low-intensity (20-25 foot-candles) illumination to extend the natural photoperiod. Wight⁶ has pointed out that in *P. sylvestris* a dormant cambium can be distinguished from an active one by the fact that in the former there is a sharp boundary between the

cambial cells and fully lignified xylem elements, whereas in the presence of an active cambium there is a zone of partially lignified tracheides between the cambium and the fully differentiated elements. Using this criterion, an examination of the two series of plants at the end of October showed that under the natural day-length conditions the cambium was dormant or showed only slight activity, whereas in the majority of the plants under the 15-hr. day the cambium was still very active. Confirmation of these results was obtained in other experiments in which constant photoperiods of 10 hr. and 15 hr. respectively were used.

In poor specimens the cambium was dormant irrespective of the day-length treatment, and hence it would appear that nutritional conditions also affect the duration of cambial activity. Provided these other conditions are not limiting, however, cessation of cambial activity in *P. sylvestris* is evidently controlled by the reduced natural day-length in the autumn, and is probably only one indication of the incidence of a general state of dormancy, which in deciduous species includes photoperiodic induction of leaf abscission⁶. The inhibition of cambial activity by short days is effective only at the end of the growing season, for two-year plants grown under a 10-hr. photoperiod in the spring showed definite (though reduced) secondary growth, at least until the cessation of leaf growth.

Cambial activity, leaf abscission and extension of the stem are processes well known to be controlled^{7,8} also by auxin supply, auxin deficiency having the same effect as short photoperiods. It seems possible, therefore, that these photoperiodic effects in woody species result from the action of light on the production or availability of auxin within the plant, and that quite low light intensities are effective.

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¹ For example, Schotte, G., *Medd. Statens Skögsförsöksanstalt*, 2 (1905).

² Sylven, N., *Svensk Papperstidning*, 43 (1940).

³ Langlet, O., *Medd. Statens Skögsförsöksanstalt*, 33 (1942-43).

⁴ Moshkov, B. S., *Bull. App. Bot. Gen. and Plant Breed.*, 23 (1930).

⁵ Wight, W., *New Phytol.*, 32 (1933).

⁶ Garner, W. W., and Allard, H. A., *J. Agric. Res.*, 23 (1923).

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PHOTOPERIODISM IN WOODY SPECIES

By P. F. WAREING

INTRODUCTORY.

IT is well known that length of day exerts a decisive effect on the onset of the reproductive phase in many herbaceous plants, and the physiological basis of this 'photoperiodic' response has been the subject of much investigation in recent years (for a review of the subject see R. O. Whyte (24)). It is not so generally realized, however, that the capacity for photoperiodic response appears to be at least as widespread among woody species as among herbs, such processes as the onset of dormancy and leaf-fall being controlled in many trees by natural changes in day-length. Although our knowledge of photoperiodism in trees is not yet so complete as it is for herbaceous plants, it is already clear that variation in day-length is an important factor in the annual cycle of growth in woody species, and that many species show a delicate adjustment to the day-length conditions of their natural habitat.

It is thus clear that photoperiodism has an important bearing on various problems associated with forestry, and it is hoped therefore that the following review will serve to make the subject better known.

EFFECT OF DAY-LENGTH ON THE DURATION OF GROWTH.

As long ago as 1914, Klebs (10) reported that under conditions of continuous artificial illumination, beech (*Fagus sylvatica* L.) could be induced to break dormancy in September and under such conditions could be kept growing continuously throughout the winter. Similarly, Garner and Allard (5) found that plants of *Liriodendron tulipifera* L. transferred to a greenhouse in September and exposed to normal daylight supplemented by electric light until midnight promptly renewed active growth and remained growing continuously for 18 months, whereas plants under similar conditions, but without additional illumination to prolong the natural day-length, remained dormant throughout the winter. These workers also found that under a 10-hour photoperiod¹ growth of seedlings of *Acer negundo* L. in the summer was very small as compared with that under normal summer day-lengths. On the other hand, grafts of apple (*Malus pumilla* Mill.) exposed to short days (12-hour) made more rapid growth than similar grafts exposed to normal day-length.

Experiments on the effect of day-length on seedlings of a wide variety of woody species were carried out independently by Bogdanov (2, 6) and Moshkov (17, 18) in the region of Leningrad. Several species of the following genera were used: *Acer*, *Aesculus*, *Ailanthus*, *Alnus*, *Caragana*, *Corylus*, *Fraxinus*, *Juglans*, *Larix*, *Phellodendron*, *Pinus*, *Prunus*, *Pyrus*, *Rhus*, *Robinia*, *Salix*, and *Ulmus*. The plants were grown either under the full summer day-length or under various shorter

¹ The term 'photoperiod' is applied to the daily period of exposure to light.

photoperiods down to 9 hours, obtained by covering the plants with light-proof covers for appropriate periods. It was found that with the majority of species short days hastened the onset of dormancy as indicated by earlier cessation of extension growth and earlier leaf-fall. For example, Moshkov found that under the natural Leningrad day, *Robinia pseudacacia* L. had not completed its growth after 5 months, so that the young growing-tips were destroyed by the autumn frosts; but with photoperiods of 14 and 10 hours the growing period was reduced to 2½ months and 1 month, respectively. With the earlier onset of dormancy the annual growth made by the plants was correspondingly less, and the frost-resistance of such plants was considerably greater than that of plants of the same species grown under the natural Leningrad day-length. In the case of *Salix babylonica* L. and *Pyrus ussuriensis* Maxim. it was found that the duration of the growing period and the height attained by the plants was greater under a 14-hour day than under the longer photoperiods of the natural day. With these species, therefore, it appears that there is an optimum photoperiod for maximum growth of the shoot, whereas with the other species the greatest growth was obtained with the longest days.

Results in agreement with those described above have been obtained by Kramer (11) with seedlings of various North American species, including *Fraxinus* spp., *Fagus grandifolia* Ehrh., *Robinia pseudacacia* L., *Liriodendron tulipifera* L., *Liquidambar styraciflua* L., *Quercus* spp., and *Pinus taeda* L. It was found that dormancy was hastened by short days in all species except *Quercus alba* L., and that long days delayed dormancy in the majority of species, as compared with normal day-lengths. *Robinia pseudacacia* L., *Liriodendron tulipifera* L., and *Pinus taeda* L. could be maintained in growth throughout the winter under long days or continuous illumination. The growing periods of the two species of *Fraxinus* and *Quercus borealis* var. *maxima* Sarg., however, were not affected by long days. Similar results were obtained by Jester and Kramer (9).

From the above experiments, which cover a considerable number of species belonging to a wide variety of genera, it may be taken as well established that dormancy is hastened by short days in seedlings of the majority of woody species and delayed by long days. Indeed, with some species, when the day-length exceeds a certain 'critical' value growth may be maintained continuously throughout the winter. At day-lengths below this 'critical' daily photoperiod the duration of the growing period increases as the length of the daily photoperiod increases (17, 18). That is to say, for any given constant daily photoperiod the annual shoot is able to attain a certain stage of development and then growth ceases with the formation of a terminal resting-bud. The (unpublished) results of experiments carried out by the writer with first-year seedlings of Scots pine (*Pinus sylvestris* L.) indicate that under any given cultural conditions there is for each value of photoperiod a certain mean number of leaves developed before the terminal bud is formed. It is not clear whether the attainment of a certain specific number of leaves is itself the factor controlling the cessation of growth, or whether leaf number is merely correlated with certain 'internal' factors controlling development. The observation that artificial defoliation may prolong the duration of growth (1, 16, 22) is,

however, apparently evidence in support of the former alternative. From the data already considered it is evident that there is a 'specific leaf number' corresponding to each day-length below the 'critical' photoperiod. This idea of 'specific leaf number' corresponds with the concept of 'minimum leaf number' used by Gregory and Purvis (7) as an index of 'ripeness-to-flower' in annual plants.

Woody species do not readily fit into the classification of 'short-day' and 'long-day' species generally accepted for the photoperiodic induction of flowering in herbs, for most woody species (whether adapted to regions of relatively short natural day-length or of long day-length) resemble most closely the short-day type of annual plant in that the duration of the vegetative growing period in both groups is reduced by short days and prolonged by long days, and may even be continuous under very long photoperiods.

It is characteristic of photoperiodism in herbaceous plants that the responses are fully evoked by quite low light-intensities, which can have no appreciable effect on assimilation. This is also true of photoperiodic effects in woody species, intensities as low as 1 foot-candle apparently being effective for some species (12, 15).

PHOTOPERIODISM AND THE ANNUAL GROWTH CYCLE.

The facts described above suggest that length of day may play a part in determining the duration of the growth period of woody species in nature. Because, in general, short days promote early incidence of dormancy, it is clear that the falling day-length in the autumn will ultimately bring about cessation of growth in any shoots still active at that time.

Not all woody species normally remain in active growth until there has been any appreciable fall in the natural length of day, however, many species from northern latitudes completing their annual extension growth by May, June, or July (4, 13). Klebs (10) made observations on the length of the growing season in 165 woody species growing at Heidelberg, and found that they formed a continuous range from those which ceased growth in May to those which continued active growth until 'well into the autumn' (October). He found, however, that the duration of growth depends upon the age of the tree, the position of the shoot, and the cultural conditions. Young trees and young shoots arising directly from the base or main trunk frequently cease growth much later than the tips of the main branches of an older tree.¹ Hence the natural day-length conditions during the period of extension growth will be different according as to whether we are considering younger or older trees.

If we classify woody species according to the duration of the growing period in relation to the natural day-length, we may recognize three main groups:

1. Species in which growth remains continuously active until September or October, when the natural day-length is very much shorter than during the summer months. This group includes many shrubs and woody climbers, together

¹ Burger (3), on the other hand, found that the period of extension in some trees increases with age.

with the young stages of many larger trees, e.g. *Viburnum opulus* L., *Cornus sanguinea* L., *Forsythia viridissima* Lindl., *Ligustrum vulgare* L., *Rhus* spp., *Prunus laurocerasus* L., *Vitis vinifera* L., *Clematis vitalba* L., *Lonicera periclymenum* L., *Hedera helix* L., *Salix* spp.; also the young stages of *Sambucus nigra* L., *Robinia pseudacacia* L., *Alnus glutinosa* Gaertn., *Chaemacyparis lawsoniana* Parl., and other species of which the older stages are appropriate to the second group.

With these species there is every indication that reduced day-length in the autumn ultimately determines the cessation of growth, for if, for example, young plants of *Robinia pseudacacia* L. growing out of doors are given supplemented illumination to extend the natural day in the autumn, the growing period is prolonged (12) and ultimately growth ceases owing to the unfavourable temperature conditions. It seems probable that the natural day-lengths during the summer exceed the 'critical' value for these species, which continue growth indefinitely under photoperiods equal to those of midsummer.

2. Species in which growth ceases in July or August, when day-length conditions have not changed appreciably from those prevailing during the early stages of growth in April and May. This group includes *Alnus glutinosa* Gaertn., *Carpinus betulus* L., *Betula* spp., *Picea abies* Karst., *Larix decidua* Mill., *Pseudotsuga taxifolia* Brit., *Platanus orientalis* L., *Robinia pseudacacia* L., *Liriodendron tulipifera* L., *Ailanthus altissima* Swingle, and the young stages of *Acer pseudo-platanus* L.

As we have already seen, studies of the duration of growth at constant day-length show that for every species there is a certain maximum amount of growth (as indicated by the leaf number) possible at any given photoperiod. It would appear, therefore, that species of this second group cease growth in July and August because by then they have attained the specific leaf number corresponding to the day-length then prevailing. That is to say, in contrast to the species of the first group, with these species even the maximum summer day-lengths do not exceed their 'critical photoperiods'. If this interpretation is correct, the problems involved in the determination of dormancy are not fundamentally different in the two groups.

3. Species in which growth ceases in May or June. In many, and probably all, these species the annual growth of the shoot is limited to expansion of the initials already laid down in the bud, and no new leaves are formed which were not already present in the bud (4). This group includes many of the trees endemic to northern Europe, e.g. mature trees of *Fagus sylvatica* L., *Quercus petraea* Liebl., *Aesculus hippocastanum* L., *Juglans regia* L., *Fraxinus excelsior* L., *Rhamnus cathartica* L., *Pyrus communis* L., *Sorbus aucuparia* L., *Acer pseudo-platanus* L., *Pinus sylvestris* L., *Pinus nigra* Arnold, and *Abies* spp.

¹ The majority of these examples are taken from the observations of Klebs (10) at Heidelberg (Germany) and of Burger (3) at certain localities in Switzerland. Corresponding data for species growing in Great Britain do not appear to be readily available, and although the writer has confirmed that many of these continental observations are applicable also for southern England, the assignment of the various species to particular groups must be regarded as subject to confirmation.

In some of these species, e.g. *Pinus sylvestris* L. (4, 25), the leaf initials are laid down *two* years before the unfolding, so that within the obvious resting-bud the minute resting-bud of the following year is already formed. In all those species in which the total number of nodes or leaves of the annual shoots is predetermined in the bud it is clear that the duration of growth is affected, not by the external conditions during the expansion of the bud, but by those prevailing in the previous year, when the shoot primordia are being laid down (4).

In these species in which the amount of the annual growth is predetermined in the bud, it must be supposed that the actual number of leaf initials laid down corresponds approximately with the 'specific leaf number' for the prevailing summer day-length conditions. There is, however, as Klebs pointed out, a tendency in these species to the production of a second crop of 'Lammas'-shoots under certain conditions. This is particularly true of young trees, where instead of the prolonged continuous growing-period found in young trees of the first two groups, additional growth is effected by the production of 'Lammas'-shoots; in older trees of this type the majority of shoots have only one period of extension each year. If the day-length conditions are abnormally long, the tendency to the production of 'Lammas'-shoots is still further enhanced. Thus Bogdanov (2) found that species of *Pinus* from south Russia produced 'Lammas'-shoots when grown under the longer days of Leningrad, whereas normally there is only one annual period of extension growth. Gustafson (8) obtained similar results in *Pinus resinosa* Ait., with a 16-hour day as compared with only one period of growth under the shorter natural day-length, while Phillips (20) was able to induce premature breaking of dormancy in December in plants of *Pinus taeda* L. exposed to an 18-hour day. Similarly, Klebs (10) obtained three successive periods of shoot elongation in beech exposed to continuous illumination in winter. These observations afford a partial explanation as to why 'Lammas'-shoots are most liable to occur during the height of summer, when the natural day-length is greatest.

Although the above classification of woody species is a convenient one in considering the duration of the growth period, it will be realized that each category probably intergrades into the next, and indeed in certain species the young stages may pass from the 'Group 1' to the 'Group 2', or from the 'Group 2' to the 'Group 3' type of growth, as they attain maturity. Moreover, other environmental conditions, particularly soil moisture and nutrient supply, have an important effect, the growing period being longer under favourable conditions than under unfavourable (10).

The biological advantage of the photoperiodic reaction in those species whose growth continues well into the autumn is easily understood, for it appears to provide a means whereby the dormant condition is attained before the severe conditions of winter set in. Young growing shoots are well known to be much more susceptible to frost damage than dormant shoots. Hence a species of the 'Group 1' type grown in a latitude where the natural day is longer than that of the normal region of growth will be liable to frost damage in the autumn. Thus Moshkov (17) found that under the natural day-lengths of Leningrad, *Robinia pseudacacia* L. continued growing until it was damaged by autumn frosts, whereas

under short days in the same region it became dormant sooner and was then frost-resistant. Kramer (12) kept two series of plants of *Abelia grandiflora* Rehd. out of doors in the autumn, and exposed one series to normal day, while the other received additional illumination to give a photoperiod of 14½ hours. Very little further growth was made by the plants under normal day, and they proved frost-resistant, whereas the plants under long days produced succulent new growth which was destroyed by frost. Thus the effect of day-length on the duration of the growth period has an important bearing on the problem of frost-damage in those species which continue growth late into the autumn (19).

The same significance, however, does not appear to be applicable to the species of the second and third groups, in which extension growth of the shoot ceases long before the advent of the autumn frosts. It must be remembered, however, that dormancy in the full sense involves not only cessation of extension growth but also leaf-fall (in deciduous species), dormancy of the cambium, and certain physiological changes in the tissues as yet little understood.

Now it is known that the time of leaf-fall is indeed controlled by day-length in many species. Thus Garner and Allard (5) brought plants of *Rhus glabra* L., *R. copallina* L., and *Liriodendron tulipifera* L. into the greenhouse in September and provided half the plants with additional artificial illumination until midnight, while the remainder received no additional illumination. The plants of *Rhus glabra* L. and *Liriodendron tulipifera* L. receiving additional illumination retained their leaves all winter, whereas the control plants lost their leaves just as soon as those growing out of doors. In the case of *Rhus copallina* L. the long-day conditions delayed leaf-fall for several weeks, but not indefinitely. Experiments by other workers have given similar results, and it has been shown (15) that illumination of intensity as low as 1 foot-candle from street lights may delay leaf-fall in the autumn. Inasmuch as leaf-fall is associated with dormancy of the cambium and generally increased resistance to unfavourable conditions, it is clear that the time of its incidence in relation to the onset of winter conditions must play an important part in the adaptation of a species to any given region.

This possibility, however, is not applicable to those species of the second and third groups which are *evergreen*. The best examples of this category are seen in the evergreen conifers, e.g. *Pinus* spp. Geographical races have long been known in *Pinus sylvestris* L., and in a number of experiments in which races from northern latitudes have been grown in more southern regions it was found that such races showed reduced growth in height and reduction of needle-length as compared with the growth attained in more northern regions (see Sylven (23) for references). Sylven suggested that this might be a photoperiodic effect, resulting from the reduced day-length at lower latitudes. The experiments of the writer (unpublished) have established that *P. sylvestris* L. is indeed photoperiodic, and that first-year seedlings respond to short days by hastened dormancy and also by a reduction in internode length and leaf-length. After the first year of growth, however, the duration of extension growth is unaffected by day-length since in *P. sylvestris* L. the number of 'dwarf shoots' is predetermined in the bud, and elongation of the shoot ceases in June, when full expansion of the stem initials

laid down in the buds has occurred. The leaf, however, continues growing for a much longer period by means of a meristem at its base, and it is found that the duration of growth of the needles is affected by day-length so that under reduced photoperiods they cease growth much sooner than normally. It is difficult to see what biological advantage accrues to the tree from photoperiodic control of the duration of needle-growth, and it seems possible that this is only a secondary effect arising from photoperiodic control of a more fundamental process which has so far escaped observation.

We have thus evidence of at least three processes affected by day-length in different woody species, viz. (1) duration of extension growth, (2) time of leaf-abscission in deciduous species, and (3) duration of leaf-growth in certain ever-green conifers. It seems probable that as our knowledge of photoperiodism in woody species is extended, yet other processes will prove to be affected by day-length. Indeed Langlet (14) has reported that northern strains of *P. sylvestris* L. grown under the shorter days of more southern latitudes first show female reproductive organs at an earlier age than is the case in their normal region of growth. Hence there is indirect evidence that day-length may affect the initiation of the reproductive phase in woody species, as in herbaceous plants. In this connexion the suggestion of Garner and Allard (5) that the reduced vegetative growth of apple during long summer days may favour the formation of fruit buds is of interest.

EFFECT OF DAY-LENGTH ON BREAKING OF DORMANCY.

The experiments of Klebs (10) on the effect of continuous illumination of beech (*Fagus sylvatica* L.) seedlings in the winter showed that whereas all efforts to break dormancy by methods which are successful with many other species (e.g. hot-water treatment, ethylene, &c.) are ineffective with beech, a period of exposure to continuous artificial illumination at 1,000 foot-candles rapidly brings about unfolding of the buds, even when the plants are in the leafless winter condition. Provided the shoots receive the necessary preliminary period of illumination, their subsequent unfolding can take place equally well in the light or dark. Klebs examined the effect of the length of the daily period of illumination on the breaking of dormancy and obtained no difference whether he used a 12-hour or an 18-hour day, and he came to the conclusion that the *quantity* of light received (intensity \times time) was the determining factor. As the phenomenon of photoperiodism had not been discovered at the time of Klebs's experiments, however, it is possible that he too easily dismissed any possible effect of length of day. However, Garner and Allard (5) found that a number of small trees and shrubs, including *Acer negundo* L., *Acer rubrum* L., *Cornus florida* L., and *Rhus glabra* L., which were exposed to an 8-hour day beginning early in January, successfully unfolded their leaves at about the same time as did the 'controls' exposed to normal day. Kramer (11), on the other hand, found that breaking of dormancy of leafless seedlings of various species brought indoors in January was hastened under long days, but nevertheless ultimately occurred also under short days. Kramer found, however, that seedlings of woody species kept *out of doors* in the

spring ultimately broke dormancy at the same time in April, whether under long or normal days, and concluded that, normally, by the date when temperature has risen sufficiently to permit growth, the length of the natural day is no longer a limiting factor.

It would appear, therefore, that while some species, such as beech, require a minimum period of illumination before breaking of dormancy can occur, there is no evidence that in any species the length of day normally controls the time of unfolding of the buds in the spring, and probably in the majority of species the seasonal rise in temperature is the determining factor. The premature breaking of buds in the production of 'Lammas'-shoots in the summer by abnormally long days seems to be a distinct phenomenon and to have no relevance to the question at present under discussion. It is well known, on the other hand, that the breaking of dormancy in the spring is in many species dependent upon a previous exposure to low temperatures during the winter. An interesting case is reported by Gustafson (8), who found that plants of *Pinus resinosa* Ait. which had been allowed to remain out of doors during the winter broke dormancy in the normal way under natural day-length conditions in the spring, but plants which had been protected from low temperatures during the winter showed no new shoot growth under natural spring day-lengths, but did so if the photoperiod was increased to 16 hours. It was thus possible to achieve the breaking of dormancy by exposure either to low temperatures or to long days.

PRACTICAL IMPLICATIONS OF PHOTOPERIODISM.

While it does not seem feasible as yet to apply artificially shortened or lengthened photoperiods in manipulation of the growth of woody species for economic purposes (as is the practice with certain horticultural crops), the possibility that this may one day be practicable is by no means excluded, particularly in nursery work. Nevertheless, knowledge of the phenomena of photoperiodism is not without present value to the forester, as, of course, is the case with all advances which increase his understanding of the growth-processes in woody species. It is now evident that the adaptation of a species to the climatic conditions of any given geographical region will usually include adjustment to the natural day-lengths of that region, as well as to other factors such as temperature, rainfall, &c., which have long been recognized as important. This fact must be borne in mind when considering the introduction of new species into any region. It was pointed out above that some species from southern latitudes grown in more northerly regions tend, because of the longer natural photoperiods, to continue growing longer than in their region of origin, and thereby become liable to damage by frost. On the other hand, species indigenous to northern latitudes must be expected to show reduced growth in more southern regions.

Moreover, not only do differences exist between species with respect to their photoperiodic responses, but within certain species having a wide geographical range we find the existence of distinct races differing from each other with respect to their day-length requirements. Reference has already been made to evidence for the view that geographical races within *Pinus sylvestris* L. differ in respect

of their photoperiodic responses. Similarly Sylven (23) has shown that within the species *Populus tremula* L. the races from northern Sweden differ in their photoperiodic responses from those of southern Sweden, and that when northern strains are growing in the south they show reduced growth and stunting, while southern strains grown in the north show delayed dormancy and are damaged by autumn frosts. Local races differing with respect to the length of the growing period are known for a considerable number of other species (3, 4), and there seems little doubt that further study will reveal genetic differences between such races with respect to photoperiodic response. It is thus evident that photoperiodism is of great importance in relation to problems of provenance.

The question naturally arises as to the order of latitude-difference at which differences in natural day-length become important. For example, it may be asked whether the difference in natural day-length between southern England and Scotland is sufficiently great to cause a differentiation of geographical races with respect to photoperiodic response. Obviously no satisfactory answer can be given to this question in the present state of our knowledge, and it may be anticipated that each species will behave differently in this respect. It is of interest to note, however, that strains of *Populus tremula* L. from various localities in Sweden differing in latitude by 2-3° showed genetic differences with respect to photoperiodic response.

Differences in the effective day-length may arise not only directly from differences in latitude, however, but also indirectly from differences in spring temperature, which affect the time of unfolding of the buds. Where the buds unfold early in the season the natural day-length will be appreciably shorter than for those buds which unfold later. Thus a delay of 4 weeks in the breaking of dormancy from 1st April to 1st May in the latitudes of southern England will mean a difference in day-length of nearly 2 hours. This may have an important effect on those species in which the annual extension growth is completed in 6-8 weeks, which is the case with a number of trees. The chief causes of differences in spring temperature will be altitude and latitude. Thus Romell (21) observed differences in the time of commencement of growth in *Pinus sylvestris* L. due to differences in altitude and latitude. Hence local races from different altitudes may be expected to show differences in respect of photoperiodic response, even though the latitude may be constant. Similarly the fall in mean temperature generally associated with increased latitude will result in the growth of the annual shoots commencing later, and hence under longer day-length conditions, and this effect will augment the direct effect of increased day-length at higher latitudes.

Finally, reference may be made to the implications of photoperiodism for any breeding programme. In breeding for improved types, a knowledge of the effect of day-length on the duration of the growth period is obviously of great importance, for new types must show the same delicate adjustment to day-length as is found in nature.

SUMMARY.

The capacity for photoperiodic response has been shown to be present in a wide variety of woody species. In seedlings grown under experimental conditions the length of day affects the period of growth of the shoot, the time of leaf-fall in many deciduous species, and the period of growth of the leaves in certain evergreen conifers.

The effect of natural seasonal changes in day-length on the annual cycle of growth is discussed. Many species show a delicate adjustment to the natural day-lengths of their normal region of growth.

Photoperiodism has important implications for certain matters associated with forestry, such as frost resistance, problems of provenance, and the breeding of new types.

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