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SHOOT GROWTH AND HETEROPHYLLY IN GINKGO BILOBA[1]

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ABSTRACT

Ginkgo biloba resembles other woody plants with long and short shoots in having variable leaves, and this variability in shape and other characteristics is closely related to the specialization of the shoots. The unlobed or bilobed *early* leaves of short shoots are preformed in the winter bud, and their nearly synchronous expansion in the spring is not accompanied by stem elongation. The leaves clustered at the base of long shoots are like short-shoot leaves in origin, time of appearance, and form, but they are succeeded by a second set of leaves whose internodes elongate. These multilobed *late* leaves develop at intervals of several days, and their production sometimes continues throughout the summer. The early and late leaves differ in the circumstances and continuity of ontogeny, and their differences in form originate at an early stage. The similarity of the late leaves to the deeply cut leaves of seedlings appears to be due to a common mode of ontogeny, rather than to any tendency to revert to a juvenile or ancestral state, as suggested in the past. The developmental events described here are strongly correlated with the pattern of auxin production found by earlier workers, and it is suggested that auxin is the principal hormonal intermediary between the production of a second set of leaves on long shoots and the elongation of those shoots.

Introduction

Among the remarkable features of *Ginkgo biloba* L. are its foliage leaves, which are unique among the leaves of seed plants in their fan shape and dichotomous venation. They are also highly variable in form, and for many years botanists have been aware that much of this variability is somehow associated with the specialization of the shoot system into long and short shoots. The leaves of short shoots are undivided or slightly bilobed, but vigorous long shoots bear many leaves which are deeply cut into two or more lobes. Most seedling leaves also have deeply incised blades. The resemblance of seedling and long-shoot leaves to each other, and to the deeply divided leaves of some extinct Mesozoic relatives of *Ginkgo* (FLORIN 1936), has made this tree a favorite illustration of HAECKEL's biogenetic "law": that ontogeny tends to recapitulate phylogeny. BAILEY (1897), for example, described the incised leaves of vigorous long shoots as "fitful recollections of an ancient state," and TAKHTAJAN (1959, p. 83) cited *Ginkgo* in a modern restatement of recapitulation.

Although the form of *Ginkgo* leaves is not duplicated in other living trees, an association between heterophylly and shoot specialization much like that in *Ginkgo* is fairly widespread among other deciduous woody plants of the temperate flora. *Populus trichocarpa* typifies this group (CRITCHFIELD 1960), which includes representatives of *Betula* (CLAUSEN and KOZLOWSKI 1965); *Cercidiphyllum* (TITMAN and WETMORE 1955); and *Liquidambar* (SMITH 1967). The nature of the link between shoot specialization and heterophylly in *Ginkgo* has not been worked out, although many other aspects of its long- and short-shoot development were investigated by GUNCKEL

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and his co-workers (GUNCKEL and WETMORE 1946*a*, 1946*b*; GUNCKEL and THIMANN 1949; GUNCKEL, THIMANN, and WETMORE 1949). This relationship is described here, and *Ginkgo* is compared with other woody plants exhibiting this type of heterophylly.

Material and terminology

Measurements of leaves and internodes were made at 4-day to 4-week intervals during two growing seasons on four 6- and 7-year-old trees (A–D) in the Arnold Arboretum nursery at Weston, Massachusetts. Individual shoots are numbered as in table 2 throughout this paper. In 1958 leaf length was measured to the base of the notch which partly bisects the blade. In 1959 separate measurements were made of petiole length and lower and upper blade length (the base of the blade to the notch, and the notch to the tip of the longer lobe flanking it). The 1959 observations were terminated in early September, before the last leaves had completed their development. The young trees in the Weston nursery supplied only small numbers of buds, and additional buds and mature shoots were collected from several much older trees in the Arnold Aboretum, Jamaica Plain, Massachusetts, and on the Berkeley campus of the University of California.

Terminology largely follows that of an earlier paper (CRITCHFIELD 1960). The *early leaves* expand when the buds open in the spring; the *late leaves* are produced subsequently. In *Ginkgo* there is no sharp discontinuity between these two kinds of leaves in either time of appearance or morphology, and leaves were classed as early if they appeared at the time of bud opening and matured no more than 2 days after the preceding leaf. The *transitional leaves* are intermediate between early and late leaves in time of appearance and form. Leaves are numbered from the base of the annual shoot, and an internode has the same number as the leaf at its upper end. The leaves in the winter bud are arbitrarily designated *embryonic leaves* if the petiole and blade are distinguishable (fig. 1, *1–9*); earlier stages are *primordia* (fig. 1, *10–12*). The blade and petiole are set off from each other by a constriction around the top of the petiole when the leaves are 200–300 μ long.

The *phyllochron* (BOND 1945) is the time interval between corresponding developmental stages of successive leaves, excluding initiation (to which *plastochron* refers). The stage on which the phyllochrons are based here is leaf maturation, defined as 90% of final leaf length. The 1958 measurements to the base of the notch were converted to estimates of total leaf

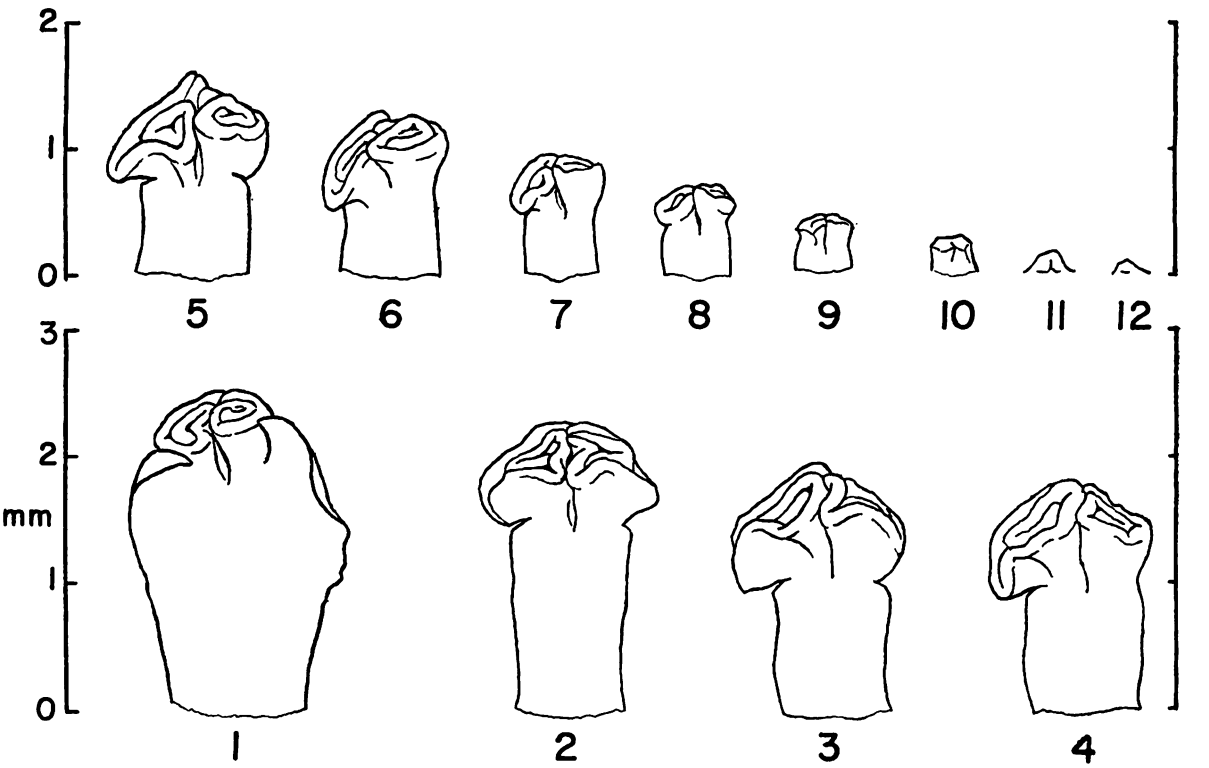


FIG. 1.—Contents of the terminal bud of a long shoot collected from tree C in mid-April 1959. "Leaf" *1* is transitional between a bud scale and embryonic leaf. Leaf *13*, a low mound flanking the apical meristem, is not shown.

length, using correction factors derived from 1959 measurements to both notch base and lobe tip.

Characteristics of mature leaves were measured on the same shoots used for growth observations. Portions of the adaxial surface of the blade with at least four stomata per square millimeter were considered stomatiferous. Resin cavities and veins were observed in partly cleared blades. The length of the longest resin cavity was measured in a strip of upper blade extending from the midpoint of the upper margin of half the blade to a point halfway to the base of the blade. This strip was five interveinal areas wide at the upper margin, decreasing (because of vein dichotomies) to three to four at the lower end. The mean distance between veins, based on the separation of 10 veins, was measured at the lower end of this strip. Xylem development was observed in embryonic leaves cleared in dilute NaOH, usually followed by chloral hydrate.

TABLE 1

CONTENTS OF WINTER BUDS

	Terminal buds		Axillary buds
	Long shoots	Short shoots	
Buds dormant:[a]			
No. of buds	6	5	14
No. of leaves:			
Embryonic	7–11	6–8	4–6
Primordia	3–4	3–4	3–4
Total	11–14	9–12	7–10
Buds swelling:[b]			
No. of buds	22	17	18
No. of leaves:			
Embryonic	7–12	6–11	4–8
Primordia	3–5	2–5	2–4
Total	10–16	9–15	7–12

[a] Collected early October–mid-March from trees B–D and one older tree.
[b] Collected mid-April from tree C and three older trees.

Observations

THE WINTER BUD.—The short and long shoots produced by a *Ginkgo* tree during a single season are sharply distinct in stem length. The short shoots are only 1–2 mm; the long shoots range from 2–3 cm to at least 75 cm. Except in young trees, short shoots greatly outnumber long shoots and bear most of the foliage leaves on the tree, but the long shoots are almost entirely responsible for building up the woody framework of the shoot system.

The sharp distinction between the two shoot types is not reflected in their terminal buds, which are externally similar and of about the same size (3–4.5 mm high). The embryonic shoot of the bud is enveloped by 8–14 scales which are modified petioles. The innermost scales often have vestigial leaf blades at their apices, and transitions between scales and leaves are fairly common (fig. 1, *1*).

Axillary buds are confined to the widely spaced nodes of long shoots. The closely spaced basal and terminal nodes lack them, and so do short shoots. Most axillary buds are smaller (2.5–3.5 mm) than terminal buds, occasionally ranging in size down to rudiments no more than 1 mm high, but in other respects they are like terminal buds.

Dormant buds sampled from October to March contained a total of 7–14 leaves (table 1). All buds had three to four primordia surrounding the apical meristem, but they differed in number of embryonic leaves. Terminal buds had two to five more embryonic leaves than axillary buds, and terminal buds of long shoots had one to three more than buds of short shoots.

The embryonic leaves range in length from 2 mm to 0.2 mm, successive leaves decreasing in size. Length is a crude measure of embryonic leaf development, however. The blades of the first few leaves are about equal in size and development, and the differences in total length are due to differences in length of the petioles (fig. 1, *2–5*). The embryonic leaves are glabrous, and their blades are involute.

Most embryonic leaves of dormant buds lack xylem cells. If xylem is present, it is usually restricted to the lower part of the two parallel vascular bundles that enter the petiole. In buds collected from two trees in October, the smallest leaf with xylem was 1.45 mm long, and the largest leaf without xylem was 1.90 mm. GUNCKEL and WETMORE (1946*b*) reported similar data for a long-shoot terminal bud collected at the end of the growing season. They found no xylem in leaves up to 1.42 mm, but in a leaf 1.90 mm long the xylem extended into the blade.

By mid-April, about 3 weeks before the bud scales separated, many buds began to enlarge. In the most advanced buds sampled at this stage (from an Arnold Arboretum tree), the maximum number of leaves had increased to 16 (table 1). This increase was in embryonic leaves, although the number of primordia was somewhat more variable in enlarging buds (two to five). The largest leaves had begun to produce hairs along the margins and on the adaxial face of the petiole.

Xylem formation proceeded rapidly in swelling buds, even before the leaves began to increase appreciably in length. In buds of two trees sampled at this stage, the longest leaf without xylem was 0.90 mm and the shortest leaf with xylem was 0.75 mm. In leaves 1.9–2.25 mm long, the most advanced veins with xylem extended well into the blade and had dichotomized three to five times. (At maturity these leaves have an average of 5.5–6.3 dichotomies

per leaf trace.) The blades of leaves *1–3* in figure 1 had 18, 11, and 2 vein endings with xylem, respectively, leaves *4–6* had xylem only in the petiole, and leaf *7* and succeeding leaves lacked xylem.

Any winter bud of *Ginkgo*, whether terminal or axillary, can produce either a long or short shoot during the growing season. Short shoots may develop from the terminal buds of long shoots, and vice versa. However, the kind of shoot a bud will produce can sometimes be predicted, and GUNCKEL and THIMANN (1949) made use of such buds in their investigation of auxin production of young shoots. These regularities in bud behavior depend on tree age, the nature and age of the shoot axis, and the position of axillary buds on the previous season's long shoots (GUNCKEL et al. 1949). The leaders of young trees, for example, are almost always perennial long shoots, and the terminal buds of axes that have been short shoots for many years rarely switch to long-shoot production.

GROWTH OF THE SHOOT.—By the end of April the buds were green and approaching their maximum length of 10–14 mm. During the first half of May the scales separated and reflexed, exposing the young shoot. Most of the scales were deciduous within 2 weeks, but some transitional appendages developed very small blades (less than 1 cm^2), and in a few instances produced petioles several centimeters long.

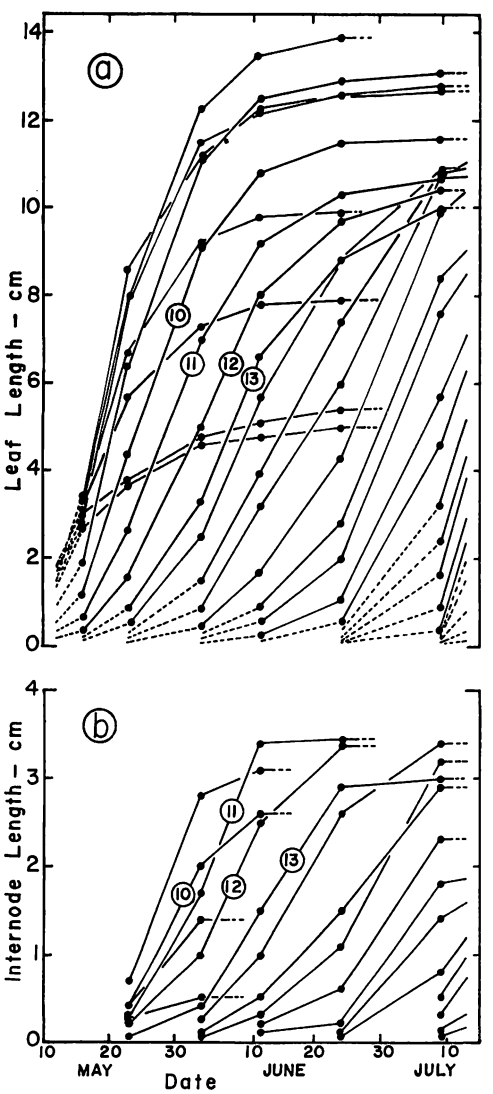


FIG. 2.—*a*, Growth of leaves of a long shoot from bud opening to midsummer (shoot 4). *b*, Growth of internodes of a long shoot from bud opening to midsummer (shoot 4). Transitional leaves and internodes numbered.

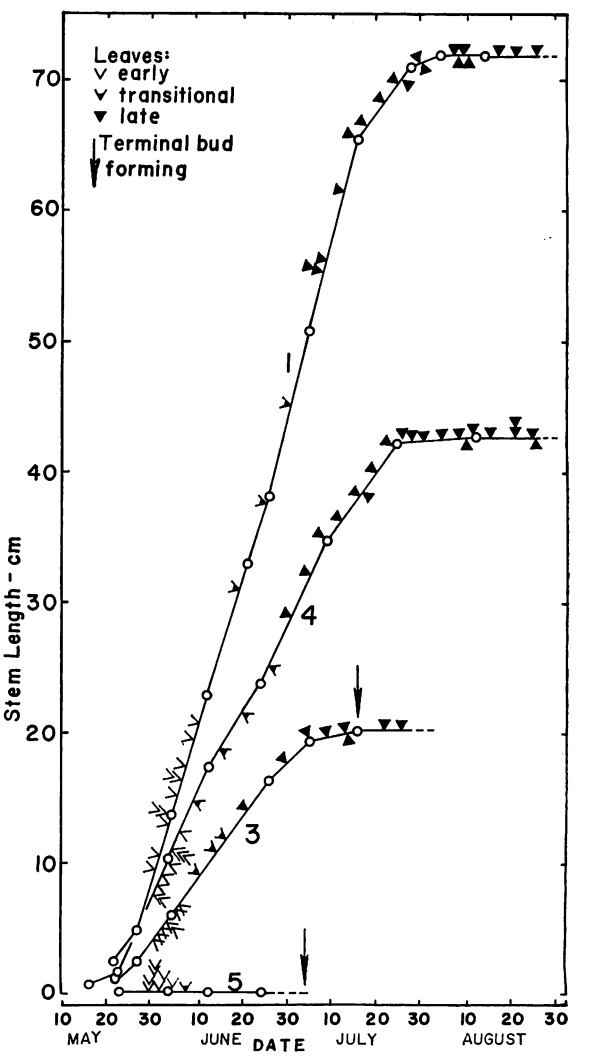


FIG. 3.—The development of *Ginkgo* shoots. Timing of stem elongation and leaf maturation of one short shoot (*5*) and three long shoots (*1*, *3*, *4*) in 1958–1959. Triangles and *V*'s indicate approximate dates on which leaves reached 90% of their final length. Terminal buds of shoots *1* and *4* developed in early September.

These appendages, which occasionally persisted on the shoot, were not counted as leaves.

The early leaves of all shoots expanded rapidly and almost simultaneously (fig. 2, leaves *1–9*; fig. 3), often maturing out of sequence. Their number coincided closely with the number of embryonic leaves in comparable winter buds (table 2). The 6–11 early leaves on individual shoots matured within a 5–10-day span, with phyllochrons averaging less than a day (table 3). All early leaves reached maturity between May 30 and June 10, a month or less after the shoots had emerged from the buds.

During the first stages of shoot development the petioles of the early leaves grew much faster than the blades. The blades unrolled when they were 0.6–1.0 cm long, within a few days of bud opening. At this stage the petioles were 1.3–2.5 times as long as the blades. Between May 19 and 27 the petioles completed 90% of their growth. The blades were about half their final length at this time (42%–59%), and did not reach the 90% point until early June, 11–18 days after the petioles.

The expansion of all or part of the embryonic leaves in the winter bud, and the development of a new bud, completes the growth of most short shoots. The two short shoots measured (table 2, shoots 2 and 5) each produced a single transitional leaf which matured 3 and 11 days after the last early leaf. Terminal buds were observed about 4 weeks later. The delayed production of transitional leaves is not typical of the great majority of short shoots on older trees, however. Most short shoots produce only four to six leaves, less than the total number of embryonic leaves in most buds, but about equal to the number with well-developed blades. In 14 terminal buds of short shoots collected from three older trees, the number of embryonic leaves averaged 2.3 more (range 0–4) than the number of leaf scars on the previous season's shoot. On such shoots the most distal embryonic leaves eventually lose their small blades by abscission and develop into the outer scales of the new terminal bud.

The distinction between short and long shoots was evident soon after the separation of the bud scales. The stems of future long shoots began to elongate and additional leaves began to appear at the

TABLE 2

NUMBERS OF LEAVES ON SHOOTS AND IN WINTER BUDS[a]

YEAR AND TREE	SHOOT NO.	PREVIOUS SEASON'S SHOOT	NO. OF LEAVES ON SHOOT				SHOOT LENGTH	NO. OF BUDS	NO. OF LEAVES IN BUD[b]		
			Early	Tr.	Late	Total			EL	P	Total
1958:											
A	1	LS	11	3	18	32	71.7	3	8–11	3–4	12–14[c]
B	2	LS	9	1	0	10	0.2	1[d]	7	3	10
B	3	SS	7	3	8	18	20.3	1[d]	7	3	10
1959:											
C	4	LS	9	4	26	39	42.5	6	7–9	3–4	10–13
C	5	LS	7	1	0	8	0.2				
B	6	−(AB)	6	5	8	19	23.5	2	5–6	3–4	9

[a] Abbreviations: AB = axillary bud; LS = long shoot; SS = short shoot; EL = embryonic leaves; P = primordia; Tr. = transitional.
[b] Collected early October 1958 and mid-April 1959 from same tree and same shoot type except as noted.
[c] No other long shoots on tree A; long-shoot buds of tree D substituted.
[d] Terminal bud of 1958 shoot.

TABLE 3

PHYLLOCHRONS OF LEAF MATURATION ON GINKGO SHOOTS[a]

SHOOT NO.	LEAF NUMBER																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20+
1	$\bar{X}$=0.9											*9*	*6*	*6*	7	0	1	4	3	$\bar{X}$=3.0
2	$\bar{X}$=0.9									*11*										
3	$\bar{X}$=1.0						*4*	*4*	*2*	5	9	5	4	4	1	8	4			
4	$\bar{X}$=0.8								*3*	*6*	*5*	*6*	5	4	4	3	4	1	$\bar{X}$=2.9	
5	$\bar{X}$=0.8						*3*													
6	$\bar{X}$=1.0						*2*	*4*	*2*	*4*	*7*	9	8	1	3	3	4	2	3	

[a] Expressed in days and based on 90% of final leaf length. Transitional leaves italicized. $\bar{X}$ = mean.

shoot tip, initating the second phase in the growth of the long shoot.

Each long shoot produced three to five transitional leaves following the expansion of the early leaves. Most of the transitional leaves developed from primordia present in the winter bud: the total number of early plus transitional leaves was about equal to the total number of leaves in comparable buds (table 2). A few transitional leaves may have originated from the smallest embryonic leaves or the first primordia initiated in the spring. The transitional leaves expanded at a slower rate than the early leaves (fig. 2*a*). They matured in succession, at intervals averaging 4.8 days (range 2–9).

Following the expansion of the transitional leaves, the first late leaves developed. These leaves were not present in the winter bud, but were initiated after growth resumed in the spring. The initiation of late leaves probably began before the buds opened in early May (table 1).

A slow rate of leaf production persisted in the first part of the late-leaf series. The phyllochron of the first late leaf ranged from 5 to 9 days (table 3). The rate then accelerated, leveling off at an average phyllochron of about 3 days (table 3, fig. 3). The expansion of late leaves was completed by the end of July on the less vigorous long shoots (fig. 3, *3*), but others continued to produce leaves through the summer (fig. 3, *1*, *4*).

The long shoots of young trees produced 8–26 late leaves by the termination of shoot growth (table 2). Many long shoots of older trees produce only three to four, however, and a few may not produce any. A possible example of the latter was a leafless long shoot, 2.8 cm in length, which had developed from the terminal bud of a short shoot. It bore only 10 leaf scars and may have produced only early and transitional leaves from embryonic leaves and primordia present in the bud.

By comparison with the early leaves, the late leaves were highly precocious in the initiation of xylem and pubescence. Leaves 500μ long had four-lobed blades and abundant hairs (*see* FANKHAUSER 1882, figs. 15, 22). The hairs, often multicellular and branched, were mostly restricted to the adaxial face of the petiole and the adjacent (abaxial) surface of the inrolled blade. At about this stage xylem began to appear in the base of the petiole. In shoot tips collected in mid-May to mid-June from two young trees, the smallest leaf with xylem was 0.55 mm long and the largest without xylem was 0.40 mm. The veins developed rapidly, and leaves 1–2 mm long had 4–14 vein endings with xylem in the blade. Blades less than 1 cm long, just beginning to unroll, appeared to have produced their full complement of veins.

The petioles of transitional and late leaves, like those of early leaves, matured much earlier than the blades. On one long shoot (table 2, shoot 4) the petioles of leaves 10–29 reached 90% of their final length 6–19 days before the blades (mean: 11.8 days). On the same shoot, the lower blade (below the middle notch) matured an average of 6.9 days before the upper blade. This difference was extremely variable, however (range 2–14 days), because the relative depth of the notch was itself highly variable (see next section of this paper).

The stems of long shoots were 1 cm long within 1–2.5 weeks of bud opening. Throughout their early development they conformed closely to the developmental schedule described by GUNCKEL and THIMANN (1949), who also made their observations in eastern Massachusetts. Stem elongation accelerated rapidly, and by the end of May it had reached a fairly constant rate which was sustained for several weeks (fig. 3). The rapid growth of the stem in late May and early June contrasted with the slow rate of transitional and late-leaf production during this period.

Both the rate and duration of stem elongation varied among long shoots. Shoot 1, ultimately the longest (table 2), maintained a higher maximum rate (1.2 cm per day) for a longer time (7 weeks) than the others. Shoot 3, more representative of most long shoots on older trees in its final length and leaf production, sustained its maximum rate of 0.4 cm per day for only 4 weeks.

Stem elongation was maintained at a high level during the first part of the late-leaf sequence (fig. 3), then declined abruptly. At maturity the uppermost late leaves, usually four to seven in number, were clustered around the terminal bud. The terminal bud of shoot 1 was visible about 4 weeks after the maturation of the last leaf in late August. Shoot 4 aberrantly continued to produce leaves long after stem elongation had ceased. By September 9, when observations of this shoot were discontinued, a remarkable total of 16 leaves had accumulated around the terminal bud, and the uppermost were not yet mature.

Individual internodes of the long shoot matured before the corresponding leaves. On shoots 4 and 6 the elongate internodes completed 90% of their growth in length an average of 4.4 days before the leaves at their upper ends (range 1–11 days). This difference remained fairly constant throughout the elongate portion of the shoot, from the upper part of the early leaf sequence to several nodes below the terminal bud.

The basal internodes of the long shoot did not elongate appreciably. At maturity most of the early leaves were clustered at the base, below the first long internode (1 cm or more). In a sample of 30 long shoots with 14–31 leaves collected from eight trees

of varying age in Massachusetts and California, there were four–seven leaves (mean 5.3) in the basal whorl. This part of the stem was 0.3–1.9 cm long (mean 1.0). On long shoots that developed from axillary buds, transitional leaves were sometimes included in the basal cluster.

On mature shoots the final length of internodes increases rapidly above the basal whorl. It then falls off for one–three internodes before increasing sharply for a second time. This two-peaked pattern of internode length is highly characteristic of *Ginkgo* long shoots (fig. 4). In the same 30-shoot sample referred

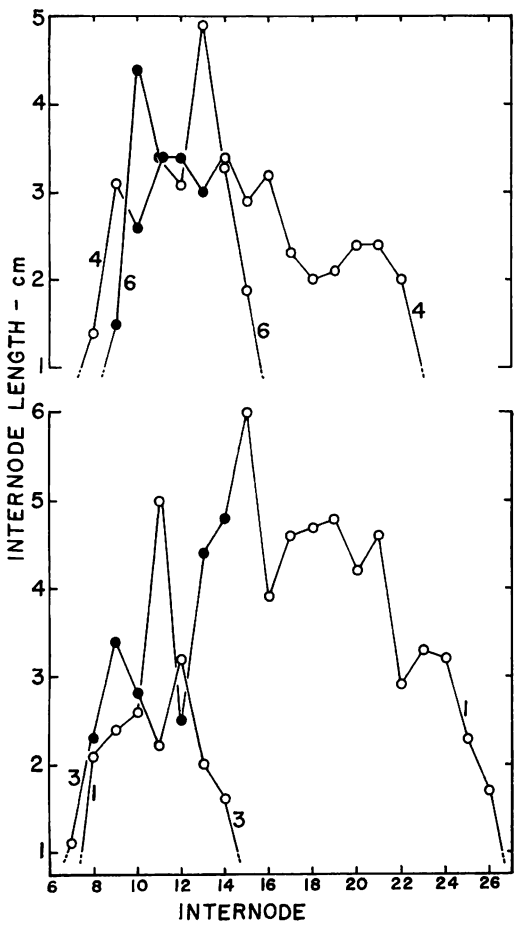


FIG. 4.—Final length of internodes on mature long shoots. Black dots are transitional-leaf internodes.

to above, internodal curves of all but three shoots had two peaks a mean of 2.5 internodes apart. On all long shoots with two peaks, the first fell in internodes 6–11 and the second in internodes 8–15. The second peak was the longest internode on the majority of shoots (fig. 4, shoots *1*, *6*). The first long internode was terminated by the uppermost early leaf or a transitional leaf, the second by a transitional leaf or one of the first late leaves (fig. 4).

In a small-scale defoliation experiment, the removal of part of the transitional and late leaves at early developmental stages drastically reduced stem elongation without adversely affecting the subsequent production of leaves. Two long shoots which had originated from axillary buds of tree B were partly defoliated; the control was shoot 6 on the same tree (tables 2, 3). In early June, leaves 8–12 and 8–13, 7.5–1.0 cm long, were cut off at the base of the petiole and the cut surface was covered with lanolin. The basal leaves were left intact. Six and five additional leaves 0.6–2.0 cm long were later removed at intervals. Defoliation was terminated in early July, when the elongation of the control shoot tapered off. The partly defoliated shoots produced two and 10 small leaves above the defoliated region, their total leaf production exceeding the control by one and 11.

Stem elongation decreased abruptly soon after the initial defoliation (fig. 5). Between early June and the

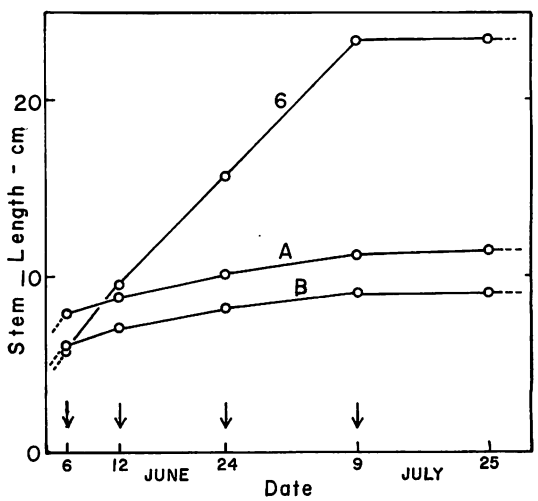
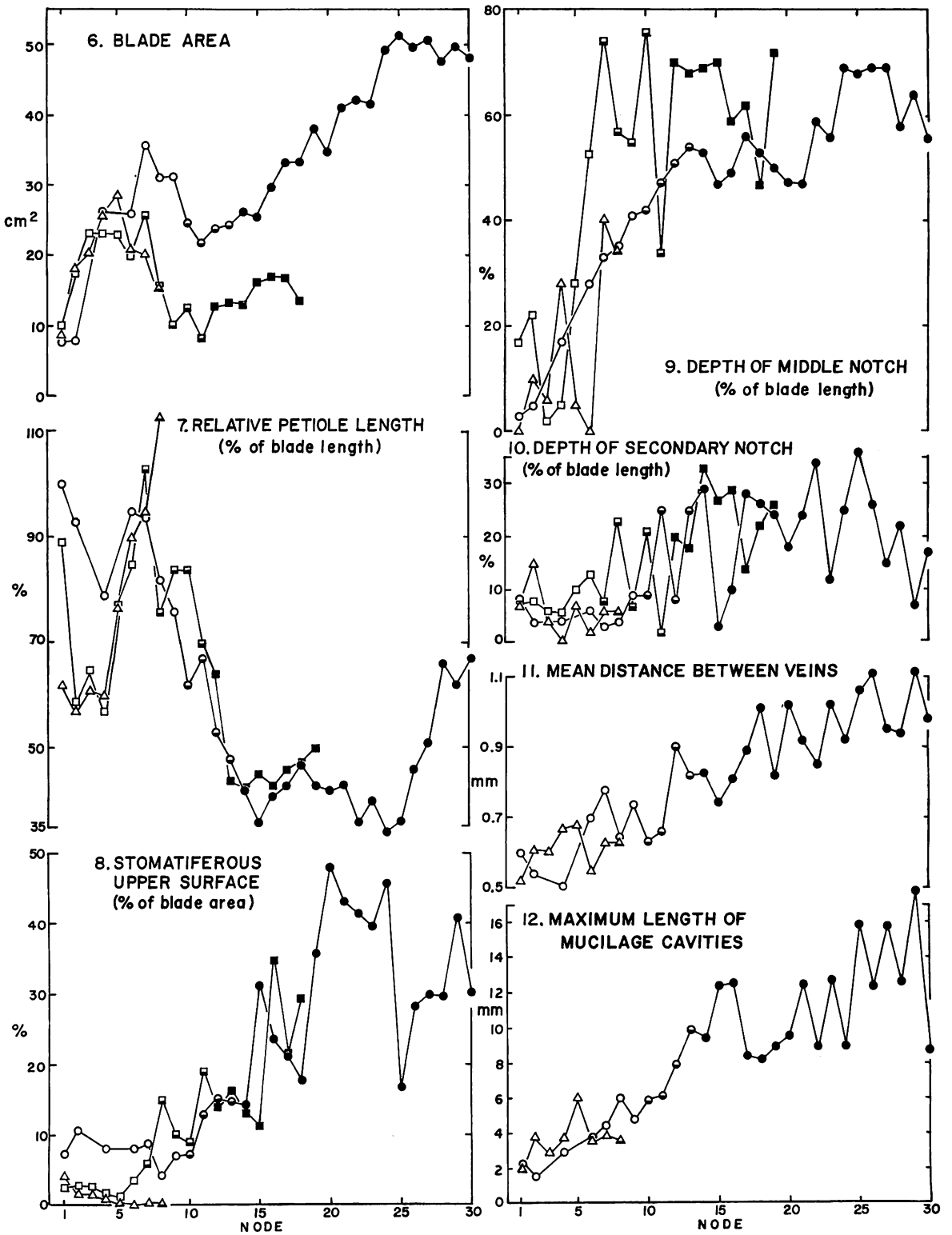


FIG. 5.—The effect of partial defoliation on stem extension. Shoot *6* not defoliated. Arrows indicate dates on which developing leaves were removed from shoots *A* and *B*.

end of extension growth, the treated shoots increased only 47% and 52% in length, compared to a 317% increase in the length of the control shoot.

VARIATION IN LEAF MORPHOLOGY.—Differences in leaf size from node to node closely reflected the two-phase development of the long shoot. The blade area of early leaves increased sharply above the basal one to two nodes, peaking at nodes 5–7 and then decreasing (fig. 6). On long shoots, blade area reached a low point at a transitional-leaf node and then increased, with a second maximum in the upper part of the late-leaf sequence. Vigorous long shoots produced much larger late leaves (fig. 6, shoot *4;* fig. 13) than long shoots with fewer leaves (fig. 6, shoot *6*).

The petioles of most early leaves were relatively



FIGS. 6–12.—Variation in leaf morphology on long and short shoots. Shoot 4 (*circles*) is a vigorous long shoot; 6 (*squares*) an average long shoot; and 5 (*triangles*) a short shoot. Transitional and late leaves are shown by half-black and all-black symbols. Figs. 9, 10. Depth of central and secondary notches expressed as percentage of blade length measured along the axis of the notch.

long—generally more than half the length of the blade (figs. 7, 13, 14). The transitional leaves were highly variable in petiole length, and sometimes had the longest petioles on the shoot. Most late-leaf petioles were less than half the length of the blade, but the closely spaced uppermost leaves had longer petioles than the widely spaced leaves preceding them (fig. 7).

The blades of *Ginkgo* leaves are strikingly variable in shape, and single vigorous long shoots encompass much of this range. On the sample shoots, the first few early leaves had broadly fan-shaped blades,

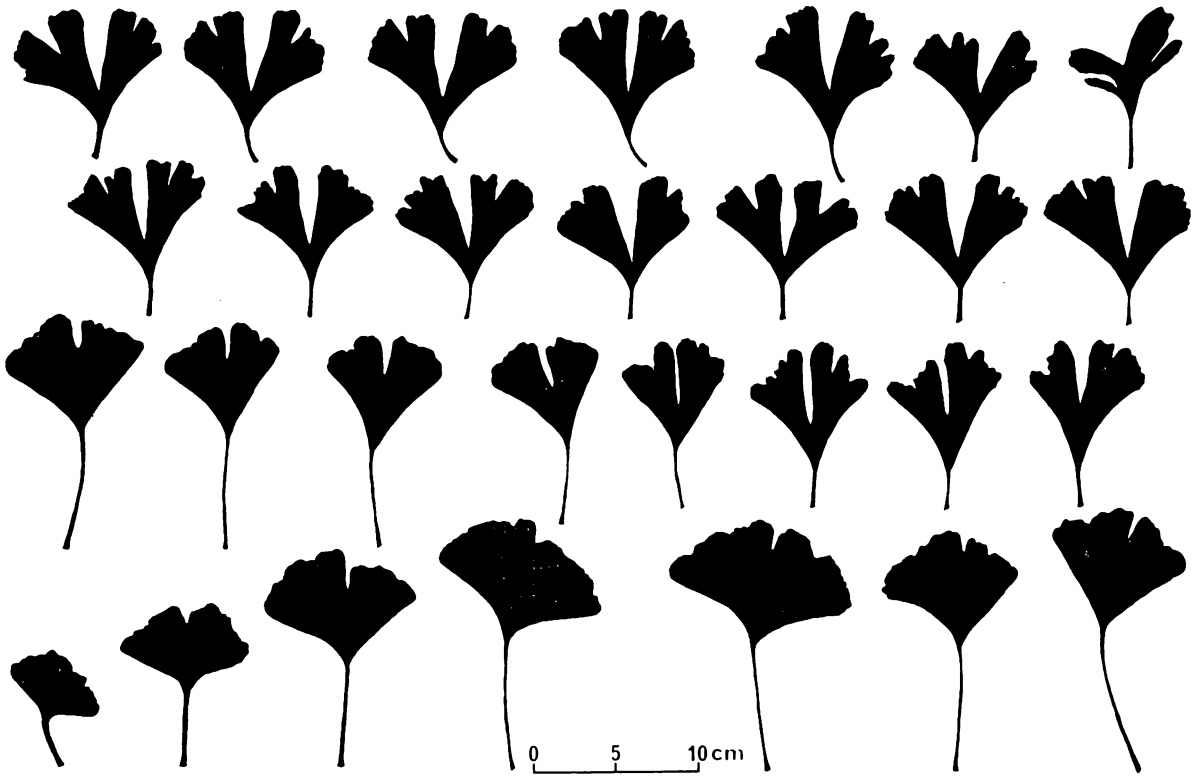


FIG. 13.—Leaves of a long shoot. Photographs of leaf tracings. Leaves of shoot 1, with leaf 1 (lowermost) in lower ~~right~~ left and 31 in upper ~~left~~ right. Leaves 2, 4 and 32 are missing.

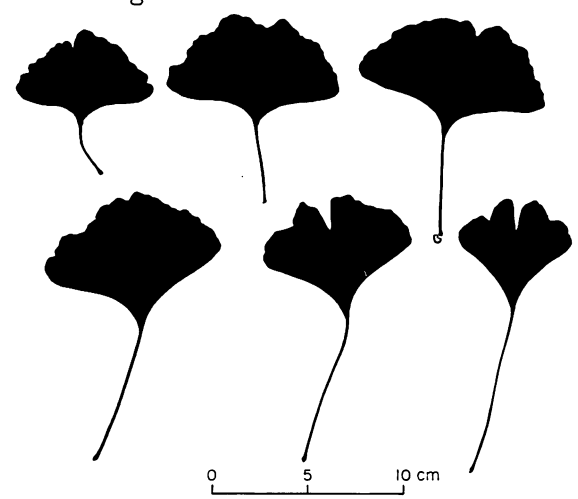


FIG. 14.—Leaves of a short shoot (tree B). Leaf 1 in upper left. Photographs of leaves.

much wider than long (figs. 13, 14). The blades were entire or moderately bilobed (fig. 9), and secondary notches were inconspicuous or nonexistent (fig. 10). Relative blade width decreased and the depth of the central notch increased in the uppermost early leaves.

The highly variable blades of the transitional leaves were sometimes the narrowest (fig. 13) or mostly deeply cut (fig. 9) of any of the long-shoot leaves. Some transitional leaves had more than two lobes, the depth of the secondary notches differing markedly between leaves at adjacent nodes (fig. 10).

The late leaves often had wider blades than the transitional leaves, but they never attained the broad fan shape of mostly early leaves (fig. 13). Their blades were always dissected, and most were distinctly four-lobed. The central notch extended about $\frac{1}{2}$–$\frac{2}{3}$ the length of the blade (fig. 9), and the depth of

the lateral notches ranged up to one third of the blade length (fig. 10).

Compared with the leaf venation of many dicotyledons, the dichotomous venation of *Ginkgo* leaves is extremely sparse. The early leaves of shoots 4 and 5 had 48–75 vein endings in each half of the blade, and the veins were an average of 0.50–0.78 mm apart (fig. 11). The generally larger late leaves of shoot 4 had only 40–59 vein endings per half blade, spaced 0.74–1.11 mm apart. This wide spacing of the veins is in marked contrast to average intervascular intervals of 55–337μ reported by WYLIE (1939) in a wide variety of woody and herbaceous dicotyledons.

The presence of stomata in the upper epidermis of leaves on long shoots of *Ginkgo* was reported by SPRECHER (1907). FLORIN (1936) described them as "in part aborted" but did not give details. In his illustration (p. 19, text-fig 6g) they are somewhat smaller but otherwise similar to the more numerous stomata of the lower epidermis.

The blades of most early leaves of the sample shoots had some stomata in the upper epidermis. They were concentrated at the base of the blade, in a small triangular region which covered up to 11% of the blade (fig. 8).

Stomata were much more widely distributed in the upper epidermis of most late leaves. The stomatiferous area extended upward from the base of the blade, sometimes to the upper margin, and covered 11%–48% of the blade (fig. 8). Also sporadically present were much smaller triangular or wedge-shaped strips of stomatiferous epidermis which extended downward from the upper margin and had the appearance of displaced segments of lower epidermis.

A single file of resin or mucilage ducts is present in each interveinal area of *Ginkgo* leaves. Their maximum length in the upper blades of early leaves sampled was 1.6–6.0 mm, compared with 8.0–17.6 mm in late leaves (fig. 12).

The first two to five leaves of first-year *Ginkgo* seedlings are scalelike or have very small blades, but succeeding leaves resemble the leaves of vigorous long shoots in several features. Considerable phylogenetic significance was attached to this resemblance by advocates of the theory of recapitulation around the beginning of this century (SEWARD and GOWAN 1900; SPRECHER 1907). They reported that the petioles of seedling leaves are short and the blades multilobed, with a deep central notch. SPRECHER also noted that seedling leaves are like long-shoot leaves in having stomata on the upper surface.

The few seedling leaves available in this study were much thinner than the leaves of older plants. They had relatively short petioles (37%–43% of the blade length), four to six lobes defined by a deep central notch (62%–82% of blade length), and secondary notches up to 20%–23% of the blade length. The venation was extremely sparse, with only 25–30 vein endings per half blade. The veins were 1–2 mm apart except at points of branching. The longest glands in the seedling leaves were 9–11 mm. These leaves had very few stomata in the upper epidermis, unlike those described by SPRECHER.

Discussion

The long shoots of *Ginkgo biloba* originate in much the same way as those of *Populus trichocarpa* (CRITCHFIELD 1960). In both species, the stem fails to elongate if leaf expansion is limited to the relatively well-developed leaves of the winter bud. All future long axes produce a second set of leaves. This set includes leaves that develop from primordia present in the winter bud, and may occasionally be limited to such leaves. *Ginkgo* long shoots with 10–12 leaves probably fall into the latter category. More commonly, however, most of the leaves produced during the second phase of long-shoot development are initiated and expand during the same season.

The possibility that shoot elongation in *Ginkgo* may be a consequence of the continued production of leaves after the expansion of the bud leaves was suggested by GUNCKEL and THIMANN (1949) in this statement: "It is only after a week or more that certain of the shoots add more leaves and undergo internodal elongation, giving rise to long shoots." They did not deal further with leaf production, and this aspect of their work has often been misinterpreted. HATCHER (1959), for example, erroneously cited the long shoot of *Ginkgo* as an example of elongation from a telescoped condition.

The sequence of developmental events outlined in this paper closely parallels the changes in auxin production described by GUNCKEL and THIMANN (1949) in young shoots of *Ginkgo*. They found that the yield of diffusible auxin increased rapidly in enlarging buds, reaching a peak in late April or early May. The auxin production of short shoots then declined permanently, but putative long shoots showed only a transient dip, followed by a rapid increase to much higher yields.

The first auxin peak is earlier than the peak production of diffusible auxin by preformed shoots of most woody angiosperms (*see* ROMBERGER 1963 for review). *Ginkgo* is also more precocious than angiosperms in the production of its sparse leaf venation, and the period of rapid xylem production in the blade approximately coincides with the first peak in auxin production. Angiosperm leaves, in contrast, continue to produce new veins and vein endings throughout most of their expansion (PRAY 1963). A causal relation in *Ginkgo* between these two events—peak auxin and peak xylem production—is suggested by

the work of JACOBS and MORROW (1957). They concluded that auxin is the limiting factor for xylem differentiation in *Coleus* leaves, and possibly in the leaves of other plants. GUNCKEL and THIMANN did not obtain much auxin from *Ginkgo* leaves, but their defoliation experiments indicated that the leaves probably supply an auxin precursor to the stem.

The first peak in the production of auxin by putative long shoots of *Ginkgo* is slightly later and higher than the single peak of short shoots. This shift may reflect differences between long and short shoots in the number and average size of embryonic leaves that expand into mature leaves. Buds that produce long shoots tend to have more embryonic leaves than short-shoot buds, and they all expand. On many short shoots, by contrast, only the largest of the embryonic leaves develop into mature leaves.

The auxin production of putative long shoots temporarily decreases about the time the shoots are first visible, but after the stem begins to elongate its auxin production soon surpasses the first peak. The longest shoots assayed by GUNCKEL and THIMANN (14.1 and 16.3 cm) yielded 20–30 times as much auxin as the first peak. These shoots of greenhouse-grown plants were about the length that vigorous long shoots of field-grown trees reach in early June (fig. 3). By this stage the continuous production of late leaves in and above the region of greatest elongation is well under way (fig. 2).

The yield of auxin along the longest shoots studied by GUNCKEL and THIMANN showed a bimodal tendency similar to the two peaks in final internode length of mature long shoots. Shoots more than 9 cm long had two auxin peaks in internodes 5–9 (GUNCKEL and THIMANN 1949, tables 5 and 6), below the double peak in internode length described here. The most advanced shoots assayed by GUNCKEL and THIMANN were still actively elongating in all but their basal internodes, and the data are insufficient to establish the nature of the relationship between these two bimodal tendencies.

These correlations between leaf production, auxin production, and stem elongation in *Ginkgo* lead to the conclusion that the cause-and-effect relationship between the production of a second set of leaves on some shoots and the extension of those shoots is mediated primarily by auxin. In the two decades since the work of GUNCKEL and THIMANN, other hormones such as the gibberellins have been shown to influence the elongation of woody-plant shoots (FULFORD et al. 1968). It is now generally accepted that growth processes as complex as stem extension are under the control of balanced systems of interacting hormones. Nevertheless, the coincidence between the developmental events described here and the data of GUNCKEL and THIMANN provides additional circumstantial evidence that auxin plays a dominant part in controlling the extension of *Ginkgo* long shoots. The evidence for this conclusion can be summarized in these points:

1. Only long shoots produce a second set of leaves, and their internodes comprise most of the elongate part of the shoot.
2. Only long shoots have a second—and much higher—peak of auxin production.
3. If the leaves of young long shoots are removed, auxin yield drops to low levels (18%–33% of the controls) within 2 days (GUNCKEL and THIMANN 1949).
4. If the second set of leaves is removed at early developmental stages, further elongation of the stem is only about one fifth that of undefoliated shoots.

The similar origin of long and short shoots in *G. biloba* and *P. trichocarpa* has produced some striking similarities in shoot topography and leaf morphology between these unrelated woody plants. In terms of shoot development, the principal difference between them is the presence of a discontinuity between embryonic leaves and primordia in the winter buds of the poplar, and its absence in *Ginkgo* buds. The poplar buds generally contain only leaves more than 5 or less than 1 mm long, and the three–eight embryonic leaves and two to three primordia are self-defining categories. The developmental discontinuity between them is reflected in the much sharper definition of the two phases of long-shoot development. The first late leaf on the poplar shoot does not mature until 2.5–4.5 weeks after the last early leaf, although subsequent phyllochrons average less than 1 week. The change from early to late leaves is correspondingly abrupt on the poplar shoots, which lack transitional leaves. In *Ginkgo*, by contrast, there is a continuous gradation between the most and least developed leaves of the winter bud, and the long shoot bears a series of leaves which are transitional between early and late leaves in form and time of appearance.

The two-phase development of the long shoots of both species is reflected in bimodal changes in leaf size along the shoot. In the poplar, the low point separating two peaks of leaf length is at the first one to two late-leaf nodes. The low point in blade area on *Ginkgo* long shoots is at a transitional-leaf node.

Compared with early leaves, the late leaves of both species have more abundant and more widely distributed stomata on the upper surface of the blades. They also tend to have better developed systems for the production of resin or mucilage. The resin-secreting marginal glands of the late leaves of poplar are much larger and more numerous than those of the early leaves, and the mucilage cavities in the

blades of *Ginkgo* late leaves average three to four times the length of those in early leaves.

On the long shoots of both species, the physical separation and exposure to light of the leaf blades are maximized by an inverse relationship between the length of petioles and internodes. Most of the early leaves are clustered at the base of the shoot. They have longer petioles than the much more widely spaced leaves above them. The two sets of leaves are separated by one or more of the longest internodes on the shoot in both species. Near the terminal bud the spacing between late leaves decreases, and their petioles tend to increase in length. This trend is more pronounced in *Ginkgo* than in poplar.

One of the most remarkable points of similarity between *Ginkgo* and poplar is the high incidence of two peaks in internodal length along the shoot. The peaks are two to four internodes apart on the long shoots of both. On *Ginkgo* shoots, the peaks are at or near the end of the early-leaf series and the beginning of the late-leaf series. The first peak on poplar shoots separates the two sets of leaves and the second is in the late-leaf sequence. These regularities in the location and spacing of the peaks suggest that the first may be associated with the expansion of the largest leaf primordia in the winter bud, and the second with the development of the first leaf or leaves initiated during the current season. This suggestion offers no explanation of the special potency of these leaves in influencing stem elongation, however, nor does it explain the transient falling-off of internode length between the two peaks.

The early and late leaves of *Ginkgo*, like those of *P. trichocarpa* and other woody plants of this type, undergo their early ontogeny in fundamentally different circumstances. The late leaves develop uninterruptedly at a shoot tip, like the leaves of seedlings and all leaves of annual plants. During the critical early stages of development, such leaves are highly vulnerable to external influences. The abundant production of mucilage, the early production of pubescence, and perhaps the precocious development of xylem by the late leaves of *Ginkgo* may be adaptive consequences of their greater exposure to the environment throughout early ontogeny.

The early leaves of *Ginkgo*, on the contrary, develop in the very different microenvironment of an enclosed bud. Their ontogeny is sharply discontinuous, unlike that of the late leaves. The first phase is a distinct and prolonged period of what SACHS (1893) called *morphologische Ausgestaltung* (putting-into-shape), during which the blade and petiole are blocked out and the form of the blade is largely determined. As SACHS pointed out, nature has imposed a sharp boundary between the embryonic and expansion phases of leaves in winter buds. The early leaves of *Ginkgo*, like the preformed leaves of other woody plants, complete the embryonic phase and the first critical stages of expansion before they are directly exposed to the external environment.

After the renewal of growth in the spring, the first leaves to reach the form-determining stages of ontogeny are the primordia in the winter bud, which develop into the transitional leaves of the shoot. The variability of these leaves may illustrate a widespread tendency of serial plant organs to be most variable at the start of a series (PEARL 1907). Among the unusual features of the transitional leaves is a relatively high frequency of vein anastomoses. ARNOTT (1959) found that the leaves at nodes 10–13 of *Ginkgo* long shoots averaged 0.80–0.82 anastomoses per leaf, compared with only 0.19–0.59 at nodes 1–9 and 14–20. ARNOTT thought that the high incidence of anastomoses at nodes 10–13 might be related to high auxin production in this part of the shoot, but GUNCKEL and THIMANN (1949) obtained the highest yields of auxin below this region, at internodes 6–9. It is more likely that the high frequency of anastomoses is a further expression of the developmental irregularities of the transitional leaves on this part of the shoot.

In many plants, including most woody plants, the juvenile and adult stages are markedly different, and the adult plant sometimes produces reversions to the juvenile state. This pattern of plant ontogeny was termed "heteroblastic development" by GOEBEL (1900). He interpreted heteroblastic development in terms of physiology rather than phylogeny, at a time when recapitulationist interpretations of plant development were prevalent. GOEBEL stressed the importance of limited nutrition in the juvenile state and in reversions to it. His views were later extended to include special emphasis on the size and nutritional status of the apical and subapical meristems (*see* ALLSOPP 1965 for review).

A nutritional interpretation hardly seems applicable to the type of heteroblastic development shown by *G. biloba*, *P. trichocarpa*, and other woody plants like them. In these plants, the sequence of leaf forms is repeated each season. The second set of leaves tends to resemble, in varying degrees, the leaves of seedlings, but this second set is produced only on those shoots in which the extension of the shoot system is concentrated. In *Ginkgo*, particularly, the resemblance to seedling leaves is often the most pronounced on shoots of the greatest vigor, in terms of stem length and leaf production. A nutritional explanation is even less tenable in *P. trichocarpa*, which produces extremely vigorous sprouts bearing only leaves of the late-leaf type. Nor is heterophylly in *Ginkgo* a consequence of changes in

the size of the apical meristem. There is unanimous agreement that the size of *Ginkgo* shoot apices varies little, either seasonally or by shoot type (FOSTER 1938; GUNCKEL and WETMORE 1946*a*; CLOWES 1961, table 1). Neither do seasonal influences seem to play any significant role in the origin of this type of heterophylly. Future early leaves in developing buds enter the form-determining stages of ontogeny throughout the summer, and late leaves at the tips of elongating shoots enter the same critical phase of development from the time of bud opening until at least midsummer.

The morphological similarities of the late leaves of *Ginkgo* and the leaves of first-year seedlings appear to be a consequence of their common pattern of ontogeny. Both kinds of leaves develop under similar circumstances at the growing tip of a shoot, and the development of both is continuous from initiation to maturation, lacking the well-defined embryonic phase of the early leaves. The circumstances and the continuity of leaf development are inseparable in the intact plant, but factors associated with the latter are probably of more fundamental importance in their influence on final leaf form. Shortening the embryonic phase of leaves, or altogether eliminating it as a distinct phase, produces "reversions" in several kinds of plants (*see* review in CRITCHFIELD 1960). This ontogenetic interpretation of the type of annual heterophylly encountered in *G. biloba* offers no clues to the nature of the external and internal factors that are ultimately responsible for these differences in leaf form, but it provides a context in which the experimental manipulation of leaf form might provide such clues.

LITERATURE CITED

ALLSOPP, A. 1965. Heteroblastic development in cormophytes. Encycl. Plant Phys. **15**:1172–1221.

ARNOTT, HOWARD J. 1959. Vein anastomoses in the leaves of long shoots of *Ginkgo biloba*. Nature **184**:1336.

BAILEY, L. H. 1897. The survival of the unlike. 2d ed. Macmillan, New York. 515 pp.

BOND, T. E. T. 1945. Studies in the vegetative growth and anatomy of the tea plant (*Camellia thea* Link.) with special reference to the phloem. II. Further analysis of flushing behaviour. Ann. Bot. N.S., **9**:183–216.

CLAUSEN, J. JOHANNA, and THEODORE T. KOZLOWSKI. 1965. Heterophyllous shoots in *Betula papyrifera*. Nature **205**: 1030–1031.

CLOWES, F. A. L. 1961. Apical meristems. Blackwell, Oxford. 217 pp.

CRITCHFIELD, WILLIAM B. 1960. Leaf dimorphism in *Populus trichocarpa*. Amer. J. Bot. **47**:699–711.

FANKHAUSER, J. 1882. Die Entwicklung des Stengels und des Blattes von *Ginkgo biloba*, L. Stampfli'sche Buchdruckerei, Bern. 11 pp.

FLORIN, RUDOLF. 1936. Die fossilen Ginkgophyten von Franz-Joseph-Land II. Allgemeiner Teil. Palaeontographica B, **82**:1–72.

FOSTER, ADRIANCE S. 1938. Structure and growth of the shoot apex in *Ginkgo biloba*. Torrey Bot. Club Bull. **65**:531–556.

FULFORD, R. M., J. D. QUINLAN, H. J. LACEY, and H. W. B. BARLOW. 1968. The acropetal movement of growth substances from young leaves on woody shoots. Pp. 1187–1203 *in* F. WIGHTMAN and G. SETTERFIELD [ed.], Biochemistry and physiology of plant growth substances. Runge, Ottawa.

GOEBEL, K. 1900. Organography of plants. Part I. General organography. English ed. Clarendon, Oxford. 270 pp.

GUNCKEL, JAMES E., and RALPH H. WETMORE. 1946*a*. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. I. The origin and pattern of development of the cortex, pith, and procambium. Amer. J. Bot. **33**:285–295.

———, and ———. 1946*b*. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. II. Phyllotaxis and the organization of the primary vascular system; primary phloem and primary xylem. Ibid. **33**:532–543.

GUNCKEL, JAMES E., and KENNETH V. THIMANN. 1949. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. III. Auxin production in shoot growth. Amer. J. Bot. **36**:145–151.

GUNCKEL, JAMES E., KENNETH V. THIMANN, and RALPH H. WETMORE. 1949. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. IV. Growth habit, shoot expression and the mechanism of its control. Amer. J. Bot. **36**:309–316.

HATCHER, E. S. J. 1959. Auxin relations of the woody shoot. The distribution of diffusible auxin in shoots of apple and plum rootstock varieties. Ann. Bot. N.S., **23**:409–423.

JACOBS, WILLIAM P., and IELENE B. MORROW. 1957. A quantitative study of xylem development in the vegetative shoot apex of *Coleus*. Amer. J. Bot. **44**:823–842.

PEARL, RAYMOND. 1907. Variation and differentiation in *Ceratophyllum*. Carnegie Inst. Washington Pub. 58. 136 pp.

PRAY, THOMAS R. 1963. Origin of vein endings in angiosperm leaves. Phytomorphology **13**:60–81.

ROMBERGER, J. A. 1963. Meristems, growth, and development in woody plants. U.S. Dep. Agr. Tech. Bull. 1293. 214 pp.

SACHS, JULIUS. 1893. Physiologische Notizen VII. Über Wachstumsperioden und Bildungsreize. Flora **77**:217–253.

SEWARD, A. C., and J. GOWAN. 1900. The maidenhair tree (*Ginkgo biloba*, L.). Ann. Bot. **14**:109–154.

SMITH, ROBERT F. 1967. The leaf dimorphism of *Liquidambar styraciflua*. Amer. Midland Natur. **77**:42–50.

SPRECHER, ANDREAS. 1907. Le *Ginkgo biloba* L. Imprimerie Atar, Geneva. 207 pp.

TAKHTAJAN, A. L. 1959. Essays on the evolutionary morphology of plants. [Transl. O. H. GANKIN.] Amer. Inst. Biol. Sci., Washington, D.C. 139 pp.

TITMAN, PAUL W., and RALPH H. WETMORE. 1955. The growth of long and short shoots in *Cercidiphyllum*. Amer. J. Bot. **42**:364–372.

WYLIE, ROBERT B. 1939. Relations between tissue organization and vein distribution in dicotyledon leaves. Amer. J. Bot. **26**:219–225.

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