Original articles

The induction of sun and shade leaves of the European beech (*Fagus sylvatica* L.): anatomical studies

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Summary. Primordia from buds of sun and shade twigs of European beech (*Fagus sylvatica* L.) were collected six times a year for anatomical investigations. Differentiation into sun-leaf and shade-leaf primordia was first observed in early August. Sun-leaf primordia had five, and shade-leaf primordia four layers of mesophyll meristem cells. With potted graft unions of beeches possible structural changes of leaf primordia were investigated. Trees adapted to shade develop sun-leaf primordia when put into full daylight, provided the transfer happened before July. Trees adapted to full daylight developed leaf primordia which remained structurally sun-leaf primordia when the plant was kept under shade conditions. Shade-leaf branches of young beech trees cut in February in order to expose the shade buds to full daylight developed either shade leaves or intermediate shade/sun leaves. These experiments show that the subtending leaf may provide the developing axillary bud with photoassimilates, but its character, whether sun or shade leaf, has no influence on the character of the developing leaf primordia.

Key words: Differentiation — Leaf primordia — *Fagus sylvatica* — Sun shade leaves

Introduction

Gäumann (1935) estimated that a 110-year-old beech tree has 64000 buds which give rise to 250000 leaves. The buds as well as the leaves of a single tree vary considerably in size and weight depending on their position on the twig, and on the position of the twig on the tree. The difference between sun and shade leaves is based primarily on anatomical features of the mature leaf. A leaf with two prominent layers of palisade parenchyma belongs to the category of sun leaf; a leaf with only one layer of palisade cells is regarded as a shade leaf. Accordingly, sun leaves have a thicker lamina than shade leaves. Although many intergrades can be recognized, the anatomical definition is commonly used as the basis for the evaluation of physiological characteristics. This procedure appears to be reliable because both morphological and physiological parameters of sun and shade leaves differ considerably between individual beech trees, especially when diseased trees are also taken into consideration.

There are no conclusive observations available to determine whether a change in external conditions from light to shade or vice versa can influence the structure of an expanding leaf. Since typical sun and shade structures can be found only in trees with crowns which differ visually between epi- and subcoronar regions – a prerequisite for an adult tree – changes in environmental light conditions are experimentally impossible. Because of the lack of experimental evidence, opinions differ on how early the sun- and shade-leaf structures are initiated, and at which stage of development a change in light conditions can still alter leaf structures. The question of whether physiological or environmental factors are responsible for the determination of sun and shade leaves has primarily been considered in connection with the timing of bud break (Jost 1983; Klebs 1914). The first publication dealing with the

timing of sun- and shade-leaf initiation claimed that leaf primordia are triggered inside the bud to develop either a sun leaf or a shade leaf (Nordhausen 1903). Magnus (1913) considered the autumnal leaf drop to be the time at which sun- or shade-leaf initiation occurs. More recently, Hansen (1959) stated that the structure of the mesophyll depends on the irradiation at the time of bud break. More recent experiments on American beech (*Fagus grandifolia* Ehrh.) have shown that shading buds of sun branches with aluminium dishes prior to bud break can reduce the sun-leaf character of the developing leaves (Goulet and Bellefleur 1986). None of the cited papers offers microphotographs which show at which time leaf primordia differ from each other. The aim of this study is to fill that gap.

Materials and methods

Crown branches of 120-year-old beech trees (*Fagus sylvatica* L.) were obtained by shooting with a rifle. The lower shaded branches were obtained with a small hacksaw mounted on an extendable pole. Light intensities at shade levels varied in winter from 58% to 82% of full daylight, and in summer (mid-June to end of October) from 6% to 12% of full daylight. For reversing experiments, transposable potted beeches were used which were grafted with crown twigs taken from 120-year-old beeches. Grafting was carried out in September by Kordes, (Bilsen, FRG). In January, the successfully grafted beeches were transferred to a cool cabin in the greenhouse of the Forest Botany Institute in Göttingen. The following spring, the graft unions were kept potted and placed outdoors. Some of them were kept in full daylight. The leaves that were checked had the structure of sun leaves. Another group of potted graft unions were shaded under a canopy of young birch trees. Daylight in this area was reduced to 12%–22% in summer and to 70%–78% in winter. After two seasons the shaded trees developed typical shade leaves. In the following spring, at the time of bud break (10 May), the two sets of potted beeches were exchanged. The sun trees were put in the shade and the shade trees were exposed to full daylight.

Light exposure experiments were carried out with 13- to 15-year-old beeches developing both sun leaves and shade leaves. The trees were from the plantation *"Fuchslöcher"* belonging to the Forest Botany Institute in Göttingen.

The following experiment was set up. Four beech trees were checked in summer for sun leaves and shade leaves. The branches with only shade leaves were labelled. In February of the following year the trees were cut approximately 25 cm above the ground to expose the labelled shade-leaf branches to full daylight. When leaves were mature, at the end of May, the labelled branches which had been exposed to full daylight since February were harvested. Their leaves were either fixed and embedded for anatomical investigations, or discs were analysed for the pattern of vein density and vein area percentage.

For anatomical investigations a great number of buds were opened under a stereomicroscope and, after most of the hairs had been removed, the recognizable leaf primordia were dissected with splinters of a razor blade mounted on a needle with a handle. Primordia as well as sections of leaf blades were fixed in 6% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0. After 6 h, the samples were washed in ice-cold 50 mM cacodylate buffer, pH 7.0, stained overnight at 4° C with 4% OsO_4 in the same buffer, washed with double-distilled (dd) water and dehydrated in a series of 5%, 10%, 20% ... ethanol, then transferred to propylene oxide and to diethyl ether (2 changes). When dehydrated, the samples were embedded in Spurr's (1969) epoxy resin. From the resin-embedded samples, semi-thin sections (0.5 µm) were cut with glass knives on a Reichert ultramicrotome type E. The sections were stretched on dd water and stained with 0.05% toluidine blue, pH 7.0, and mounted under a cover glass with 100% resin.

Photographs were taken with Agfa ortho-film, 25 ASA, by illumination with a green filter on a Zeiss photomicroscope using apochromatic oil objectives.

For determination of the percentage of leaf venation per leaf area, 7-mm diameter leaf discs were punched out from fresh leaves. The discs were gently heated on a slide together with a lump of glycerol-jelly (Kaiser's Glyceringelatine; Merck), covered with a flame-heated cover glass, and allowed to solidify under the weight of a lead cube. Using a ×6.3 objective, the vein pattern of the leaf blade was transmitted with a video camera (Panasonic CD 51/G) to a colour monitor (Hitachi HM-27198-C-11) with a computer program developed by Reinhold Meyer (Department of Biometry, Forest Faculty, University of Göttingen). For the determination of the area percentage of a certain grey value, mesh-density data of vein network were obtained.

Results

Timing of sun- or shade-leaf induction in buds

The investigation of buds from crown branches and low shade branches of 120-year-old beech trees started in November. Figure 1 shows cross-sections of leaf primordia. In L, the bud of a crown branch was dissected, and in S a leaf primordium from a low branch is shown. In both primordia leaf veins are still in the procambial stage; they are distinctly spaced, with small portions of interveinal tissue. There are five interveinal layers of meristematic mesophyll cells in the L- and four in the S-primordium. This difference shows that the structures of sun and shade leaves must have been initiated before November. Sun leaves are characterized by two layers of palisade meristem, while shade leaves have only one layer of palisade meristem.

The next collection from 17 January shows the same difference (Fig. 2 L and S), although primordia are thinner than those from November.

Fig. 1. Cross-sections of leaf primordia collected in November from 120-year-old beech trees. L Primordium dissected from a bud of a crown twig: sun-leaf structure with five layers of mesophyll meristem, marked by *black triangles* indicated by an *open triangle. S* Primordium dissected from a bud of a low inserted branch, showing four layers of mesophyll meristem (*black triangles*). The upper epidermis has dark inclusions of a phenolic nature. Scale of magnification below Fig. 3
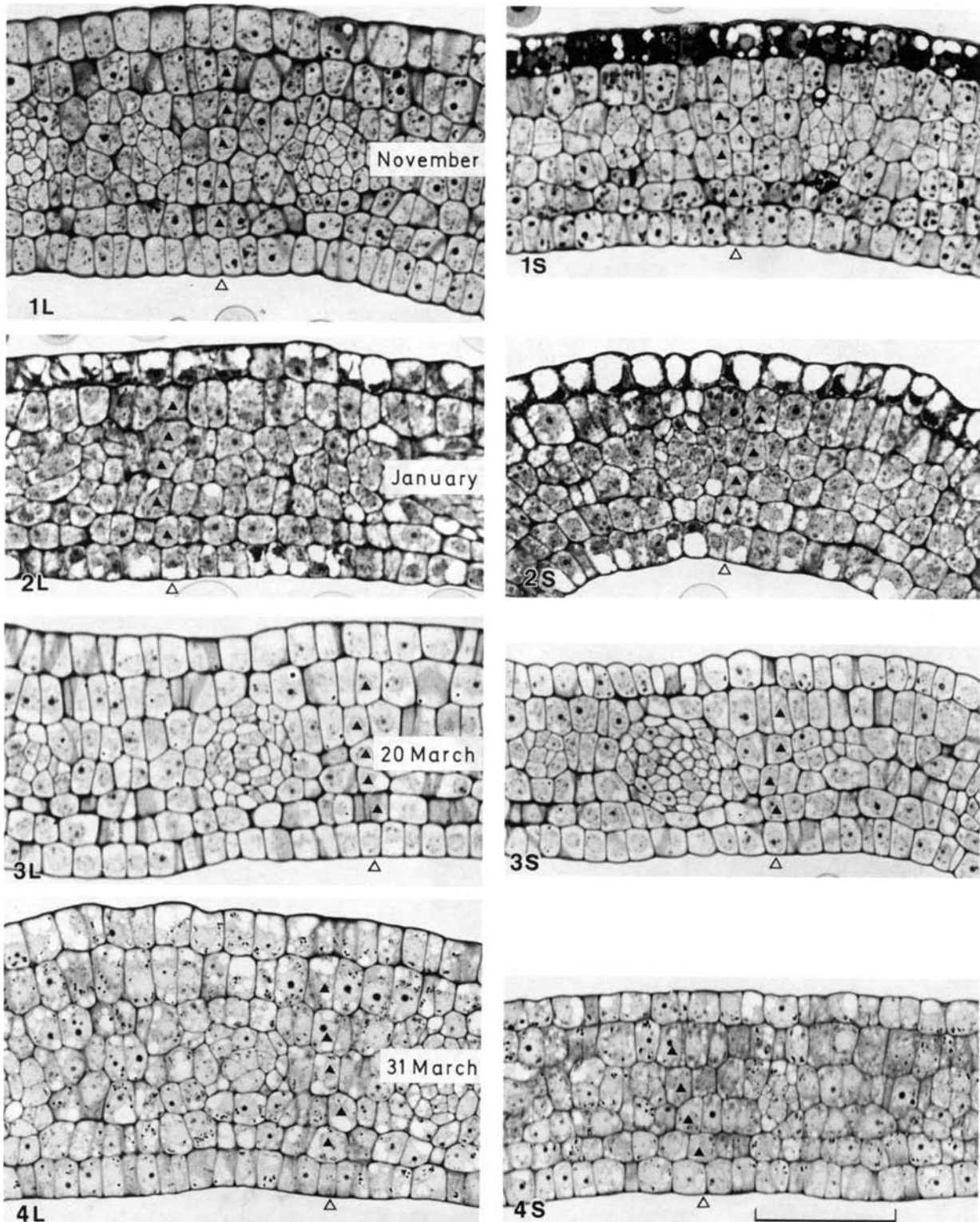
Fig. 2. Cross-sections of sun-leaf (*L*) and shade-leaf (*S*) primordia collected in January. Mesophyll meristem as described in Fig. 1

Fig. 3. Cross-sections of sun-leaf (*L*) and shade-leaf (*S*) primordia collected on 20 March. Explanations as in Fig. 1. Note that the dark inclusions in the upper epidermis have disappeared

Fig. 4. Cross-section of sun-leaf (*L*) and shade-leaf (*S*) primordia from buds collected on 31 March. Explanation as in Fig. 1
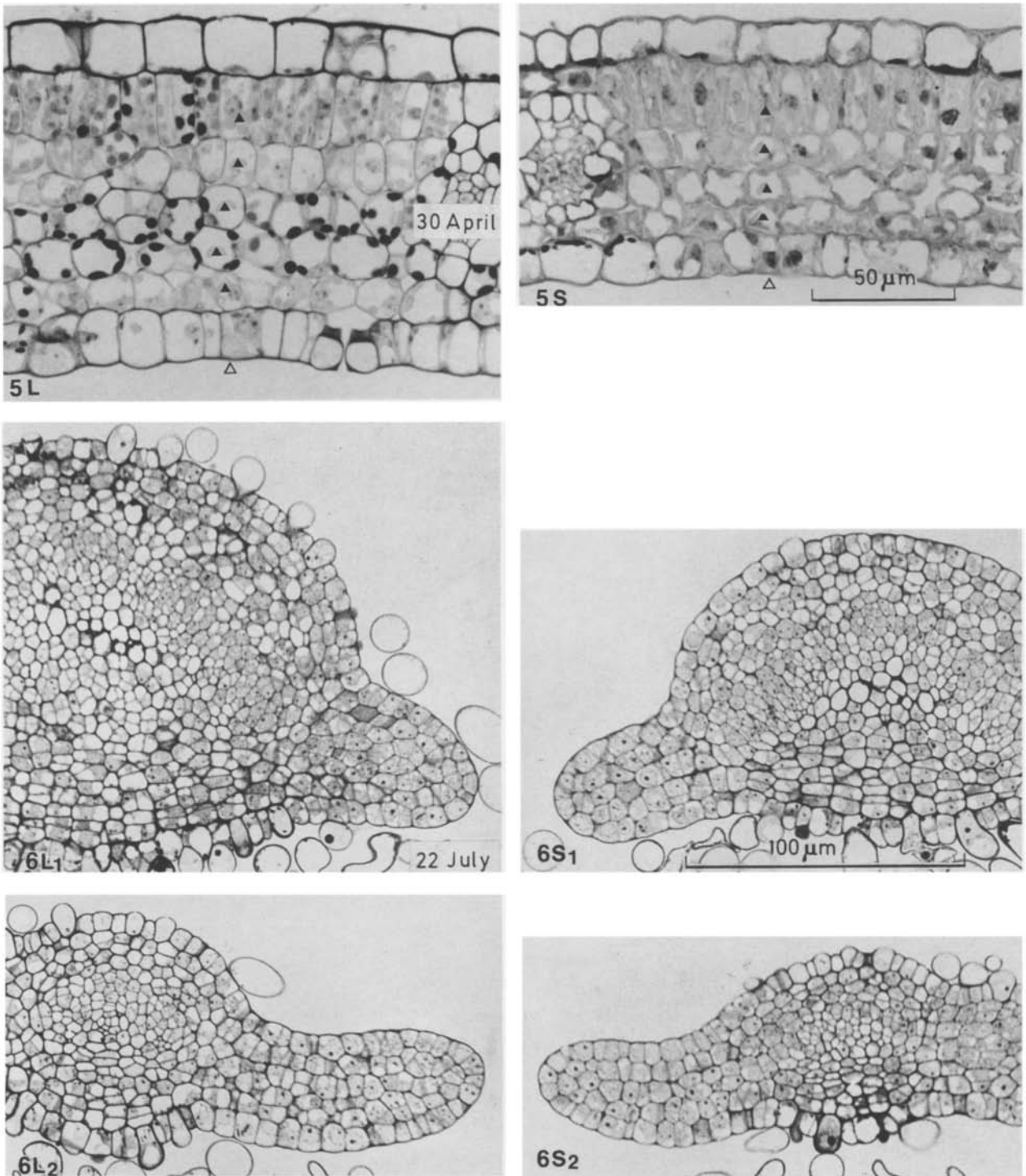
Fig. 5. Cross-sections of sun-leaf (*L*) and shade-leaf (*S*) primordia collected on 30 April during swelling of buds. Sun-leaf primordia contain plastids with starch in the mesophyll and bundle sheath cells; they have completed stomata. Shade-leaf primordia have a slimy consistency and are poorly fixed; they have no starch and show no completed stomata

Fig. 6. Cross-sections of the early stages leaf-primordium development from collections of 22 July. L_1 and S_1 show stages with the beginning of lamina formation and voluminous midribs. L_2 and S_2 show primordia with less prominent midribs and advanced lamina formation. Primordia indicated with L are from buds in the axils of sun leaves, those indicated with S are from shade-leaf buds. The number of mesophyll layers is 3 in both the L_2 and S_2-primordia
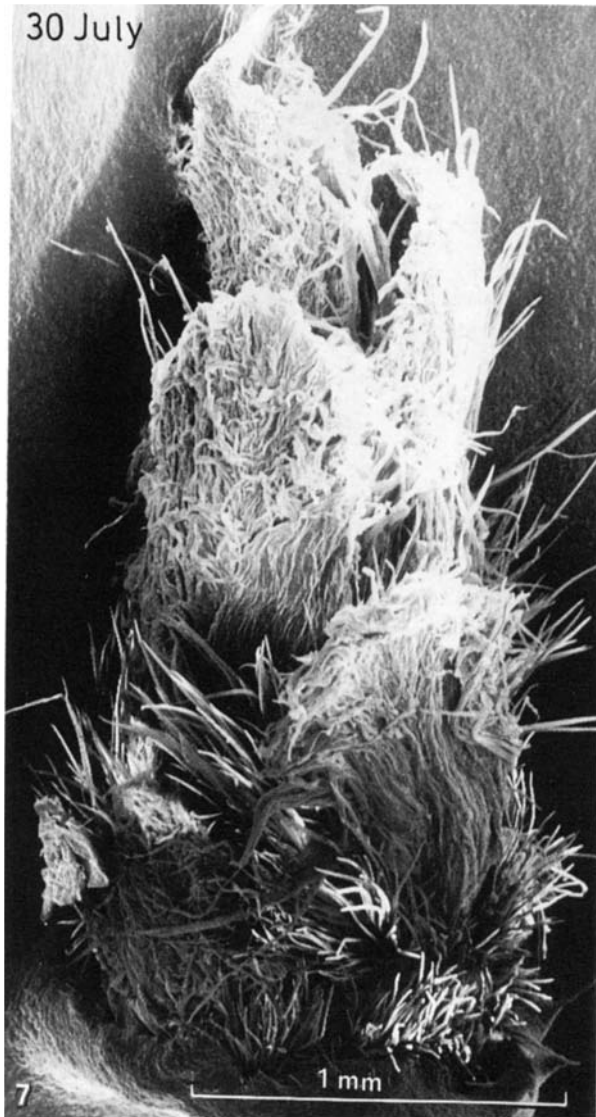
Fig. 7. Opened beech bud in which 4 leaf primordia are recognizable. It is the stage od development (30 July) which is also shown in Fig. 8. Scanning electron micrograph

from the bud axis, because their surface was slimy. After fixation, the conservation of cell shape was poor. Veins in both L-and S-primordia showed distinct vacuolated bundle sheath cells. Epidermal cells were enlarged and vacuolated. Stomatal guard cells were discernible only in L-primordia. Chloroplasts were easy to distinguish in both L- and S-mesophyll, but starch-containing plastids were restricted to L-primordia and appeared in mesophyll and bundle sheath cells. Again, mesophyll of L-primordia was five-layered, while that of S-primordia shoed four layers of mesophyll cells.

The first bud primordia of the new generation appeared in June in the axils of both sun and shade leaves, but leaf primordia could not be recognized in the buds before the middle of July. Figure 6 shows cross-sections of leaf primordia from buds collected on 22 July. Most primordia showed a prominent midrib and very small "wings" of the lamina as in Fig. 6 L_1 and S_1; other primordia had a less prominent midrib but more enlarged "wings" of the lamina (Fig. 6 L_2 and S_2). This is the first collection of the season in which the number of mesophyll layers is discernible: there are four layers in both L- and S-primordia. In this early stage of leaf development L- and S-primordia differ only in the thickness of the midrib.

The next collection was made on 30 July. At this stage of bud development, leaf primordia look like those shown in Fig. 7, a scanning electron micrograph. Such primordia are less than 1 mm in size. Fixed and embedded material of this stage is shown in Fig. 8. Here, L-primordia show four, and S-primordia three layers of mesophyll initials. It is probable that an additional layer of mesophyll initials is later added to each type of primordia.

This set of developmental stages (Figs. 1–8) shows that the anatomical differentiation of sun and shade leaves starts between 20 and 30 July.

Obviously, the number of meristematic mesophyll layers does not depend on the size of the primordium. When cambial sap became available on 20 March, buds were still unchanged, as were the prospective veins of the leaf primordia, which consisted solely of procambial cells (Fig. 3 L and S). In the swelling bud of 31 March (Fig. 4 L and S), procambial veins are clearly delimited. Again, interveinal mesophyll appears five-layered in buds of crown branches (L) and four-layered in primordia of shade branches (S).

The last collection before leaf expansion was made on 30 April (Fig. 5). In this developmental stage, the S-primordia were difficult to remove

Reversing experiments with grafted beech trees

The potted graft unions, all with scions from crown branches, primarily developed sun leaves in the first season. After 2 years, leaves of the shaded trees showed anatomically the structure of shade leaves with a single layer of palisade parenchyma and many intercellular spaces. The other group of potted trees, kept in full daylight, still had sun leaves. After exchanging the groups the next spring prior to bud break, the developing leaves were checked after maturation; they
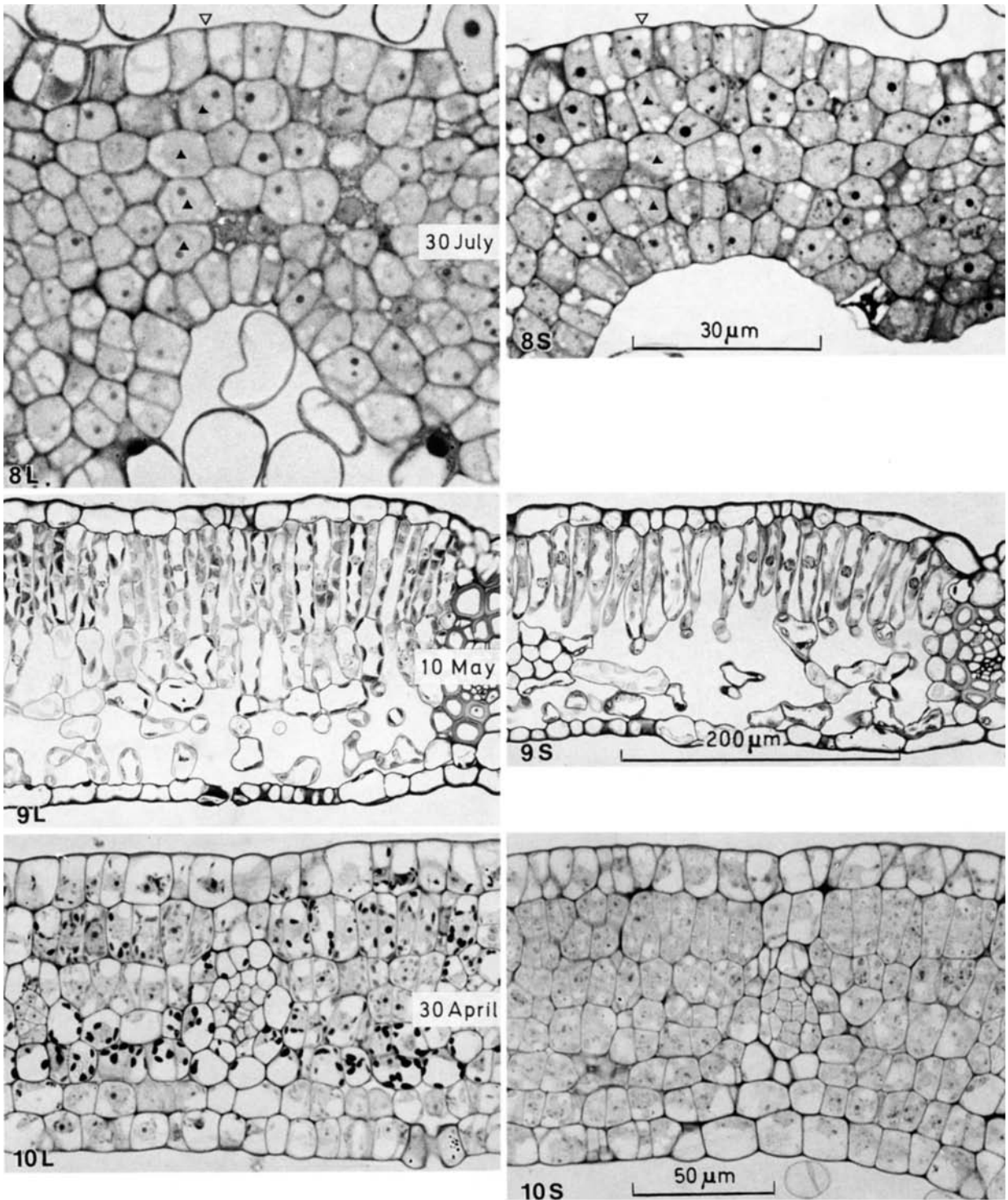
Fig. 8. Cross-sections of primordia of a sun-leaf bud (*L*) and a shade-leaf bud (*S*), collected on 30 July. There are four mesophyll layers in the sun-leaf primordium and three in the shade-leaf primordium

Fig. 9. Cross-sections of mature leaves of grafted beech trees. *L* shows sun-leaf structure with two layers of palisade parenchyma. This (potted) tree was transferred at bud break (10 May) of the previous year from shade to full daylight. *S* shows shade-leaf structure with one layer of palisade parenchyma. This tree was transferred at bud break (10 May) of the previous year from full daylight to a shady location

showed typical sun- and shade-leaf anatomy in accordance with the environment in which they had been kept the previous year (Fig. 9 L and S).

The new generation of buds was examined the following spring. The collection from 30 April shows leaf primordia as in Fig. 10 L and S. The primordia of L-buds constantly had five layers of mesophyll cells, narrow spacing of leaf bundles, stomata and starch-containing plastids in the mesophyll. Those of S-buds had five (occasionally four) layers of mesophyll cells, a broader spacing of bundles, but no stomata and no starch deposits. A month later it was found that expanded leaves from the shaded canopy varied in their anatomical structure between typical shade leaves with a single layer of palisade cells and leaves with two layers of palisade parenchyma.

This experiment shows that reversion of the light conditions in spring (10 May) can influence the structure of leaf primordia which later develop in the buds. This means that a sun-leaf primordium can develop in the axil of a shade leaf; and a shade-leaf primordium can develop in the axil of a sun leaf.

Light exposure experiments with young beech trees

Many observations have confirmed that shelterwood cutting, group felling or secondary felling in a forest can cause light damage to the beech underwood. The underwood beeches growing up in shade are suddenly exposed to bright light. In summer, such trees often react with discoloration of the leaves, or with anthocyanin formation. In the following spring such trees can react in two different ways. Either they develop normal leaves, or their leaves remain extremely small. These differences raise the suspicion that failing to develop normal foliage in the following season depends on the time of the incident, i.e., before or after induction of leaf primordia in the buds. With the experiment described in Materials and methods leaves were obtained deriving from shade buds which had been exposed to full daylight in February.

Examples of these leaves are shown in Fig. 11 A–D. The white spots in the areoles (intercostal fields) are intercellular spaces. The computer-aided measurements of the percentage area occupied by the veins were as follows (mean of five leaf discs; standard deviation not exceeding ±0.6%): 21.7% in A, 24.9% in B, 25.4% in C and 20.2% in D. The percentage of leaf area occupied by veins in typical sun leaves of beech is normally higher than 28%; extreme shade leaves have about 18% vein area.

In Fig. 11 A–D, right-hand side, leaf cross-sections representative of each group are shown. All four leaves have shade-leaf structures, although in Fig. 11 B and C some additional palisade cells are discernible.

This experiment clearly shows that alteration of light conditions after initiation of leaf primordia in July has little or no influence on the developing leaf structure.

Discussion

Leaf initiation and lamina inception have been expertly described by Maksymowych (1973). However, although periclinal divisions indicate leaf inception on a shoot tip, the procambial leaf trace is present in the shoot at an even earlier time. In *Populus deltoides*, procambial leaf traces were found belonging to −12 and −15 leaf plastochron (Larson 1975). This shows that the leaf is initiated a considerable time before its primordium is formed. Since our knowledge about signal transmission in trees is fragmentary, it seems unrealistic to expect the determination of sun or shade leaves at such early stages.

In some photographs taken during the present investigation, the cells are so small that the number and arrangement of meristematic cells can only be recognized when microscopic embedding and staining techniques are used. The results show that sun- and shade-leaf determination in beech is triggered towards the end of July. When applied before July, changes in light conditions influence the structure of the leaves of the next season. After the middle of August an increase in irradiation (by shelterwood cutting or similar procedures) has little or no influence on the structure of primordia of the next season's leaves. Such primordia are determined to form shade leaves, and since they

Fig. 10. Cross-sections of leaf primordia from buds of grafted beeches developed in the axils of leaves as shown in Fig. 9. The plant material was collected on 30 April (compare Fig. 5). The sun-leaf structure (*L*) was developed in the axil of a shade leaf (Fig. 9 *S*); it constantly shows five layers of mesophyll cells, starch containing mesophyll and bundle sheath plastids, and fully developed stomata. The primordium in Fig. 10 *S* was developing in the axil of a sun-leaf (Fig. 9 *L*). Its plant was transferred to the shade on 10 May of the previous year. The primordium shows five layers of mesophyll cells as that in Fig. 10 *L*, but starch and mature stomata are not discernible

A — 21.7 %

B — 24.9 %

C — 25.4 %

D — 20.2 %

200 μm
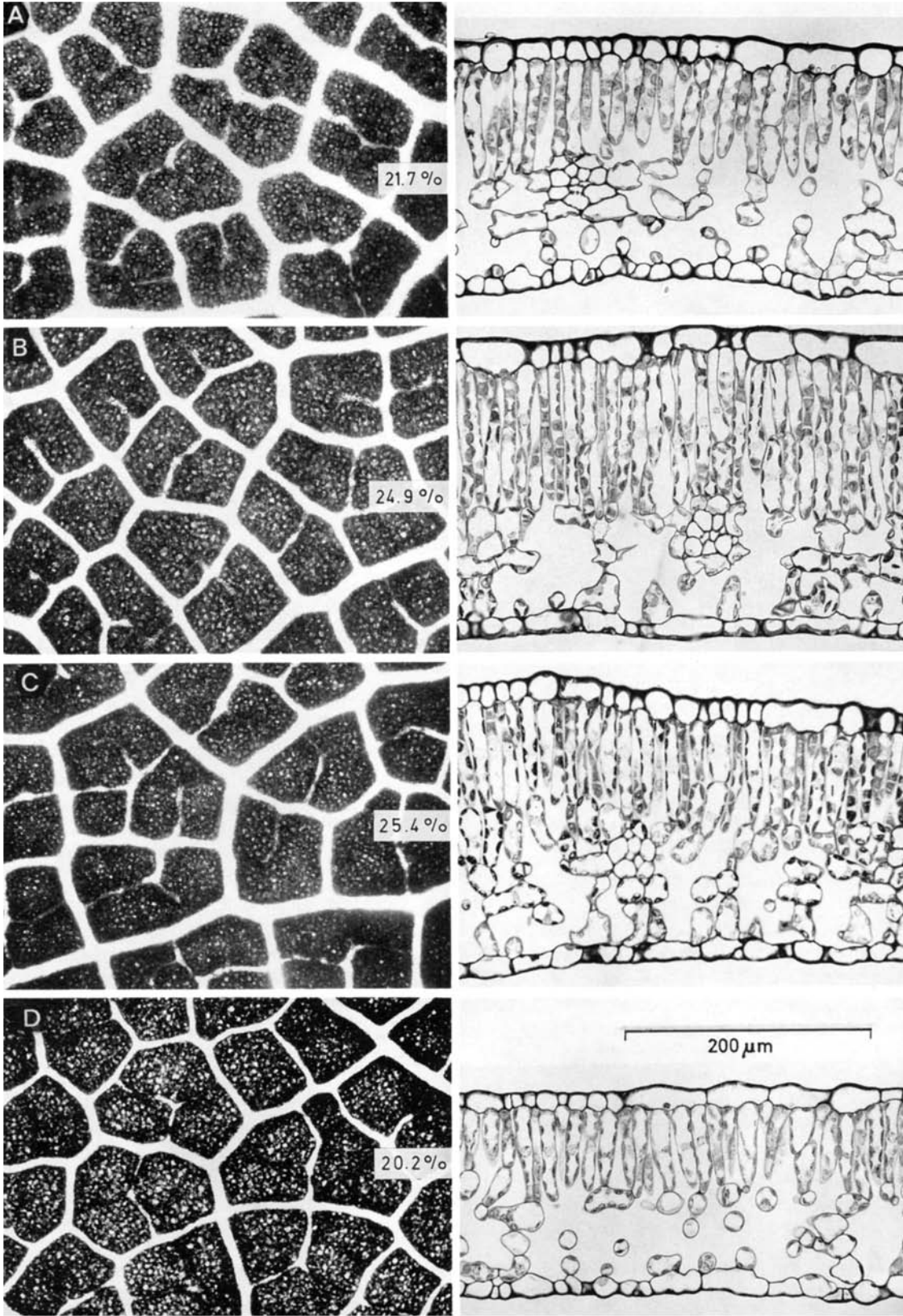
are initiated in the small casing of the bud, there is probably no space for additional growth.

Beeches show determined growth (Jost 1893); the number of leaves produced by a bud is limited. Sun buds usually have many more leaf primordia than shade buds. As a consequence, sun buds must have more leaf traces than shade buds, and the initiation of an additional leaf primordium depends on the initiation of its leaf trace. At the present time, it is not known whether a change of irradiation before July also has an influence on the number of leaf traces and leaf primordia in the bud. Since only 0.11% of the sun's irradiation (300–2000 nm) penetrates the scales of a beech bud, mainly as red and far red irradiation (Pukacki et al. 1980), it can be inferred that leaf initiation is triggered by phytochrome action.

Besides anatomical criteria, physiological phenomena have been investigated to characterize sun and shade leaves. Results obtained on herbaceous plants helped to distinguish sun plants from shade plants. This classification is based on light saturation measurements (Böhning and Burnside 1956), or on the capacity of the translocation system (Hoddinott and Hall 1982). It does not contribute to the processes of leaf differentiation in adult trees because in trees both sun and shade leaves are parts of the same organism, and the two are connected symplastically. Many investigations characterize sun and shade leaves on the basis of their pigment content (Seybold and Weissweiler 1942; Eller et al. 1981; Lichtenthaler 1985; Tanner and Eller 1986; Czeczuga 1987), leaf optics (Seybold and Weissweiler 1942; Lee and Graham 1986), photosynthesis ratios (McMillen and McClendon 1983), or fine structure of chloroplasts (Rühle and Wild 1985). A general survey of sun leaf characteristics was given by Lichtenthaler et al. (1981). These data were obtained from mature leaves which do not change their structure after alteration of the light conditions. Exposure of mature shade leaves to increased irradiation usually causes discoloration and probably other damage which is not visible. Such damaged leaves will supply their axillary bud less efficiently than will an undamaged leaf. It is, however, not known whether the development of a bud depends entirely on the supply from its subtending leaf. More important seems to be the fact that the sun- or shade-leaf character of the subtending leaf does not influence the developmental processes inside the axillary bud.

References

Böhning RH, Burnside CA (1956) The effect of light intensity on rate of apparent photosynthesis in leaves of sun and shade plants. Am J Bot 43: 557–561

Czeczuga B (1987) Carotenoid contents in leaves grown under various light intensities. Biochem Syst Ecol 15: 523–527

Eller BM, Glättli R, Flach B (1981) Optische Eigenschaften und Pigmente von Sonnen- und Schattenblättern der Rotbuche (*Fagus silvatica* L.) und der Blutbuche (*Fagus silvatica* cv *atropunicea*). Flora 171: 170–185

Gäumann E (1935) Der Stoffhaushalt der Buche (*Fagus silvatica* L.) im Laufe eines Jahres. Ber Schweiz Bot Ges 44: 157–334

Goulet F, Bellefleur P (1986) Leaf morphology plasticity in response to light environment in deciduous tree species and its implication on forest succession. Can J For Res 16: 1192–1195

Hansen HC (1959) Der Einfluß des Lichtes auf die Bildung von Licht- und Schattenblättern der Buche (*Fagus sylvatica*). Physiol Plant 12: 545–550

Hoddinott J, Hall LM (1982) The responses of photosynthesis and translocation rates to changes in the ζ-ratio of light. Can J Bot 60: 1285–1291

Jost L (1893) Ueber Beziehungen zwischen der Blattentwicklung und der Gefässbildung in der Pflanze. Bot Ztg 51: 89–138

Klebs G (1914) Über das Treiben der einheimischen Bäume, speziell der Buche. Abh Heidelb Akad Wiss Math Naturwiss Kl 3. Winter, Heidelberg

Larson PR (1975) Development and organization of the primary vascular system in *Populus deltoides* according to phyllotaxy. Am J Bot 62: 1084–1099

Lee DW, Graham R (1986) Leaf optical properties of rainforest sun and extreme shade plants. Am J Bot 73: 1100–1108

Lichtenthaler H (1985) Differences in morphology and chemical composition of leaves grown at different light intensities and qualitities. In: Baker NR, Davies WJ, Ong CK (eds) Control of leaf growth. Cambridge University Press, Cambridge, pp 201–221

Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf U (1981) Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynthesis Res 2: 115–141

Fig. 11. **A–D.** Leaf surface views (left) and leaf cross-sections (right) obtained from shade leaves of 13- to 15-year-old beech trees, which were cut above the lowest branches in February. The light-exposed shade buds developed leaves which anatomically showed both shade-leaf structure (**A, D**) and intermediate sun-shade leaf structure (**B, C**). Scale relates to cross-sections only. The surface views of leaf areas on the left-hand side show the pattern of minor veins. The indicated percentages relate to the areas the veins occupy. X 50.4

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Magnus W (1913) Der physiologische Atavismus unserer Eichen und Buche. Biol Centralbl 33: 309–337

Maksymowych R (1973) Analysis of leaf development. Cambridge University Press, Cambridge

McMillen GG, McClendon JH (1983) Dependence of photosynthetic rates on leaf density thickness in deciduous woody plants grown in sun and shade. Plant Physiol 72: 674–678

Nordhausen M (1903) Über Sonnen- und Schattenblätter. Ber Dtsch Bot Ges 21: 30–45

Pukacki P, Giertych M, Chalupka W (1980) Light filtering function of bud scales in woody plants. Planta 150: 132–133

Rühle W, Wild A (1985) Die Anpassung des Photosyntheseapparates höherer Pflanzen an die Lichtbedingungen. Naturwissenschaften 72: 10–16

Seybold A, Weissweiler A (1942) Spektrophotometrische Messungen an grünen Pflanzen und an Chlorophyll-Lösungen. Bot Arch 43: 252–290

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26: 31–43

Tanner V, Eller BM (1986) Veränderungen der spektralen Eigenschaften der Blätter der Buche (Fagus silvatica L.) von Laubaustrieb bis Laubfall. Allg Forst Jagdztg 157: 108–117