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ON THE OCCURRENCE OF AMPHISTOMATIC LEAVES IN GINKGO BILOBA L.

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ABSTRACT

In 1957 MAÁCZ reported the occurrence of cell configurations in the upper epidermis of the leaf of *Ginkgo biloba* L. suggesting the presence of rudimentary stomata. Maácz speaks, very cautiously, of "rudimentären Spuren der Spaltöffnungen", i.e. rudimentary traces of stomata. This observation is of interest because of the fact that *Ginkgo biloba* is regarded as a typically hypostomatic species.

An investigation of material obtained from four different specimens of this tree growing in the Leyden Botanical Garden revealed that the upper side of leaves from long shoots from a male tree and from a juvenile tree of still unknown sex exhibited normal stomata. The brachyblast leaves of the same trees, however, are distinctly hypostomatic. Examination of leaves from both long shoots and brachyblasts of our two female specimens showed both types of leaves to be hypostomatic. Furthermore, the leaves from a water shoot sprouting directly from the trunk of the male tree were also found, contrary to expectation, to be hypostomatic.

A possible correlation between the occurrence of stomata in the upper leaf epidermis and a more primitive type of leaf shape is discussed. This point is interesting because of the fact that fossil leaves of *Ginkgoaceae* from earlier geological periods are amphistomatic.

INTRODUCTION

Some years ago, MAÁCZ [1957] published the results of a study on the leaf epidermis of *Ginkgo biloba* L. In this publication he gave a description of the anatomical properties of the leaves and reported, among other things, the occurrence of cell patterns in the upper epidermis suggestive of the presence of rudimentary stomata, an interesting observation because this plant is generally regarded as a true hypostomatic species.

However, DALLIMORE and JACKSON [1954] state that "stomata mostly (occur) on the lower surface, rarely above", although they give neither details nor the origin of this knowledge.

The observations of Maácz are especially interesting because of the fact that, as shown by some *Ginkgoid* fossils, amphistomatic leaves did occur in the family of the *Ginkgoaceae* in earlier periods. OISHI [1939] even regarded some Tertiary leaves as belonging to the distinct and extinct genus *Ginkgoites* Sw. because of their amphistomatic character, even though their general appearance was almost similar to that of the leaves of recent *Ginkgo biloba*.

Since the material studied by Maácz may derive from only one specimen and because of the great variability of the species [KARSTENS, 1945], the present authors thought it desirable and attractive to reinvestigate this matter in more extensive material.

MATERIAL AND METHODS

Material

We had the opportunity to investigate leaves from four specimens of *Ginkgo* grown in the Leyden Botanical Garden. Some particulars concerning these specimens may be given here:

1. a male tree, planted in 1785 and flowering profusely every year [VEENDORP and BAAS BECKING, 1938];
2. a small tree planted at the beginning of this century and growing in a stunted, more or less weeping form. This specimen is still sterile;
3. a female tree of the forma *fastigiata* Henry, planted in 1820 (not in 1850 as mentioned erroneously by KARSTENS [1945]). During the last half-century this tree has annually produced large quantities of exclusively unfertilized seeds;
4. a branch from a female tree growing at Slikkerveer (Holland) [KARSTENS, 1945] grafted on 23 February 1935 upon a branch of the above-mentioned male tree. As yet no macrosporangiophores have appeared.

Although all the leaves of *Ginkgo biloba* are fan-shaped, differences can be observed between the leaves growing on long shoots and those from the short shoots or brachyblasts. In general, leaves originating from long shoots are smaller, relatively narrower, and more and more deeply laciniated than those from the brachyblasts (See Fig. 1a-f).

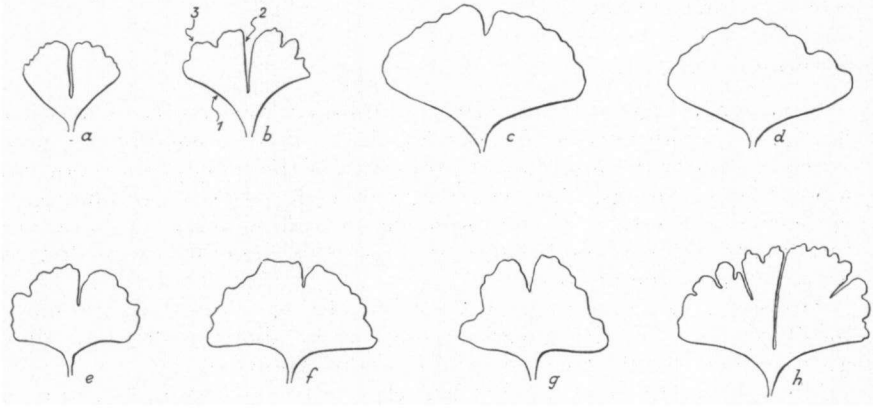

Fig. 1. *Ginkgo biloba* L. Contours of leaves: a and b from long shoots (male tree); c and d from brachyblasts (male tree); e from long shoot (female tree); f from brachyblast (female tree); g from trunk brachyblast (male tree); h from water shoot (male tree). 1, lateral margin of the leaf; 2, margin of the incision; 3, upper margin of the leaf.

The four specimens mentioned were sampled during August when the leaves had reached maturity. In order to be able to compare leaves from long and short shoots, the samples were collected separately (Samples 1a and b, 2a and b, 5a and b, 6a and b, 7a and b). Leaves

were taken from the periphery of the trees, at about 1 m from the ground. Samples 1 and 2 were taken from opposite sides of the same tree in order to obtain some information on the influence of exposure on the structure of the epidermis. Samples 1, 5, and 6 were collected from comparable positions on the trees in order to avoid a possible influence of exposure. Sample 3 comprised leaves of two brachyblasts sprouting directly from the trunk. These leaves closely resemble those from ordinary brachyblasts, but are relatively smaller. Sample 4 was taken from the top part of a heavy shoot (a so-called water shoot) growing out of the lower part of the trunk. The leaves on this shoot more or less resemble those from normal long shoots, but are distinctly larger, more strongly laciniated, and rather thin. This may be due to the fact that Sample 4, like Sample 3, was collected from heavily shaded parts of the tree, in contrast to the leaves of the other samples which were fully exposed. Sample 7 contains leaves from the grafted female branch. Table 1 gives some particulars concerning the various samples.

TABLE 1

Source of Leaf Samples

Tree	Sample	Exposure	Height	Origin of sample	Illustration
male	1a	S	1 m	long shoot	Fig. 1, a, b
	1b	S	1 m	brachyblast	Fig. 1, c, d
	2a	NNW	1.5 m	long shoot	
	2b	NNW	1.5 m	brachyblast	
	3	NE	1 m	brachyblast (trunk)	Fig. 1, g
	4	N	0.75 m	water shoot (trunk)	Fig. 1, h
juvenile	5a	S	1 m	long shoot	
	5b	S	1 m	brachyblast	
female	6a	S	1 m	long shoot	Fig. 1, e
	6b	S	1 m	brachyblast	Fig. 1, f
female (graft)	7a	E	1.5 m	long shoot	
	7b	E	1.5 m	brachyblast	

Fixation

Good results were obtained with F.A.P.A., a fixative containing aethyl alcohol, formalin, propionic acid, and acetic acid in the following proportions:

50 parts of 96 % aethyl alcohol,
10 parts of 40 % formalin,
3 parts of concentrated propionic acid,
3 parts of concentrated acetic acid,
34 parts of distilled water.

After fixation, the leaves were held in the fixative.

Maceration

In order to permit isolation of the epidermis, the leaves were macerated. Schultze's method proved satisfactory. The leaves were kept for 2 or 3 days in the maceration fluid, composed of 20 parts by weight of 35 % nitric acid and 1 part by weight of potassium chlorate. At the end of this time the now colourless leaves were treated with a 1 % aqueous solution of ammonia. After this treatment, only parts of the vascular system and the epidermis remain intact.

Preparation

Before taking the pre-treated leaves from the ammonia solution, a small hole was pierced in the blade close to the petiole. When the leaf is carefully lifted out of the liquid it collapses, and the macerated internal tissues flow away through this opening. Brought into water, the leaf can easily be stretched and the desired epidermal pieces cut out for the preparation of microscopic slides. For this purpose, the fragments are first carefully cleaned with a brush of any mesophyll cells still adhering to them, then mounted in distilled water under a cover glass and, finally, sealed in with Venetian terpentine.

Very interesting preparations can be made, as Maácz also found, by cutting and unfolding fragments taken from the margin of the leaf. (Fig. 1b, 1 and 2). In this way a single preparation includes the epidermis of the upper side, of the margin proper, and that of the lower side, situated side by side in one strip. (See Plates 3a and b).

No staining is required because in slides produced according to this method the pattern of the epidermal cells and stomata is very distinct.

Observations

Investigation of the slides containing epidermal preparations of the leaves from long shoots (Table 1, Sample 1a), and from brachyblasts (Sample 1b) obtained from the male *Ginkgo* tree revealed that Maácz's observations correspond well with our findings only for the brachyblast leaves. This applies not only to the leaf shape but also to the anatomical features of the epidermis. As in Maácz's material, stomata were found in the epidermis of the smooth lateral margins ("Kante der Blattspreite", See our Fig. 1b, 1) and, furthermore, along the margin of the incisions (Fig. 1b, 2) and of the upper margin of the leaf (Fig. 1b, 3). However, contrary to Maácz's findings, these stomata did not appear to be distinctly smaller than those present in the lower epidermis, and no traces of rudimentary stomata were found in the upper epidermis.

Quite an interesting phenomenon was observed in the leaves of the long shoots of the same male tree: wholly normal stomata were encountered all over the epidermis of these leaves. Actually, such leaves are distinctly amphistomatic, the stomatal density in the upper epidermis being 9–10 stomata per mm^2 against as much as 110–140

per mm² in the lower epidermis. These values apply to the areas between the veins.

The two types of leaves are similar in respect of the presence of stomata along the sides.

The same results were obtained with the leaves of Samples 1a and 2a and with those of Samples 1b, 2b and 3. Therefore, it was contrary to expectation that the leaves from the water shoot (Sample 4), actually a long shoot sprouting from the base of the trunk of the same male tree, did not exhibit stomata in the upper leaf epidermis.

Leaves from long shoots of the juvenile tree (Sample 5a) were similar to those from the male tree (Samples 1a and 2a) in that they all contain fullgrown stomata in their upper epidermis. The brachyblast leaves of the juvenile tree (Sample 5b) proved to be identical to those from the male tree (Samples 1b, 2b and 3) in respect of the absence of stomata in the upper epidermis.

With the exception of the leaves from the above-mentioned water shoot, it is obvious that the male tree and the juvenile tree are similar with respect to the amphistomatic and hypostomatic character of the long shoot and the brachyblast leaves respectively.

Quite a different situation was encountered in both female specimens. In these, all leaves derived from either long shoots (Samples 6a and 7a) or from brachyblasts (Samples 6b and 7b), are distinctly of the hypostomatic type.

The results are summarized in Table 2.

TABLE 2
Occurrence of Stomata in the Various Types of Leaves

Tree	Sample	Leaves of Long Shoots			Leaves of Brachyblasts		
		lower epidermis	lateral leaf margin	upper epidermis	lower epidermis	lateral leaf margin	upper epidermis
male	1a	+	+	+			
	1b				+	+	—
	2a	+	+	+			
	2b				+	+	—
	3				+	+	—
	4	+	+	—			
juvenile	5a	+	+	+			
	5b				+	+	—
female	6a	+	+	—			
	6b				+	+	—
female (graft)	7a	+	+	—			
	7b				+	+	—

DISCUSSION

The most important result of this study is the establishment of the occurrence of normally-shaped stomata in the upper epidermis

of certain leaf-types of *Ginkgo biloba* L. This is in contradistinction to the observation of Maácz, who could only demonstrate patterns of epidermal cells suggesting the presence of rudimentary traces of stomata.

One is tempted to try to establish a positive correlation between the presence of stomata on both sides of the leaves of *Ginkgo* and a leaf-shape which might be considered to be primitive. In the material we had at our disposal, the leaves of the long shoots are more laciniate than those from the brachyblasts. This is especially the case for the leaves from the long shoots of the male tree (Samples 1a, 2a and 4) and of the juvenile tree (Sample 5a). Much less difference is present between the leaves from long shoots and brachyblasts of the two female specimens (Samples 6a and 7a compared to 6b and 7b). Here there is little question of leaf dimorphy since both leaf types show hardly any incision at the upper margin.

Because fossil members of the family of the *Ginkgoaceae* exhibit more or less laciniate leaves, such leaves can be considered to represent a more primitive type. The leaves of the long shoots of the male tree and the juvenile tree are indeed more laciniate than those of the brachyblasts and might, therefore, be considered to represent a primitive type. In addition, these primitively-shaped leaves are indeed amphistomatic. Furthermore, the non-primitive leaf-types from long shoots and brachyblasts of both female specimens do not show the occurrence of stomata on their upper side.

Thus far, a positive correlation between an amphistomatic leaf-type and a primitive leaf-shape seems to be present. However, the deeply-incised leaves of the water shoot of the male tree do not possess stomata on both sides. Here the correlation is absent.

It would be of considerable interest to investigate a more ample material for the occurrence of amphistomatic leaves, especially from trees of known sex. After all, it may be that male and female specimens differ in this respect.

ACKNOWLEDGEMENTS

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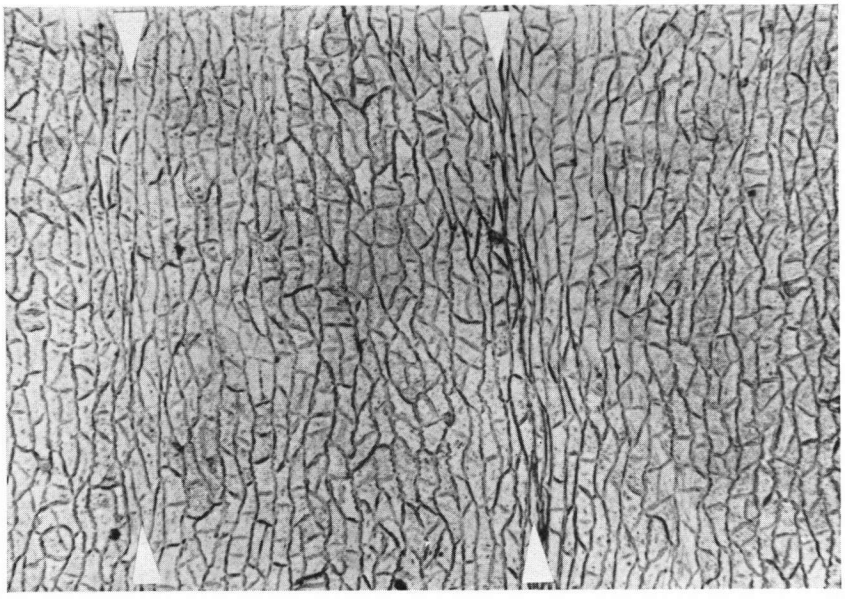

Plate 1a. *Ginkgo biloba* L. Upper epidermis of a brachyblast leaf. Female tree (Sample 6b). Stomata absent. The arrows indicate the position of two underlying veins. About 36 ×.

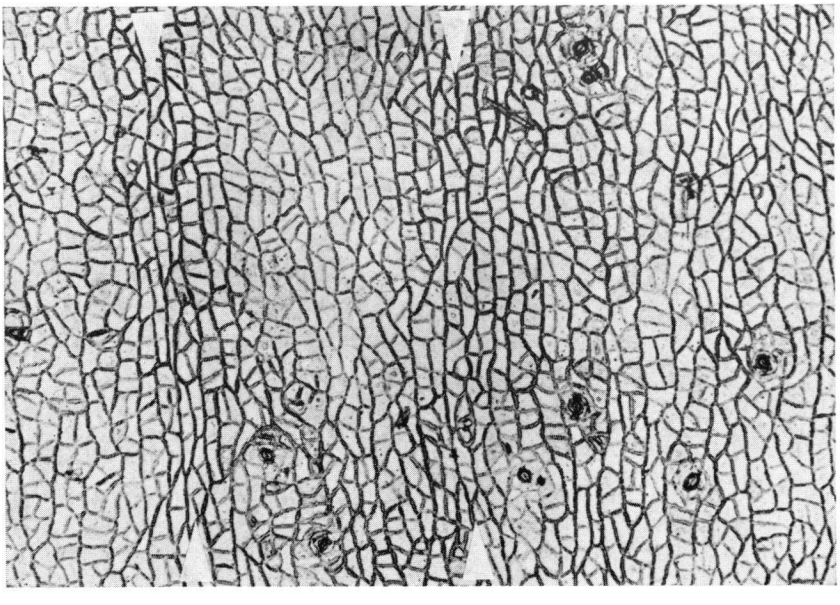

Plate 1b. *Ginkgo biloba* L. Upper epidermis of a long-shoot leaf. Male tree (Sample 1a). A small number of stomata is present. The arrows indicate the position of two veins. About 36 ×.

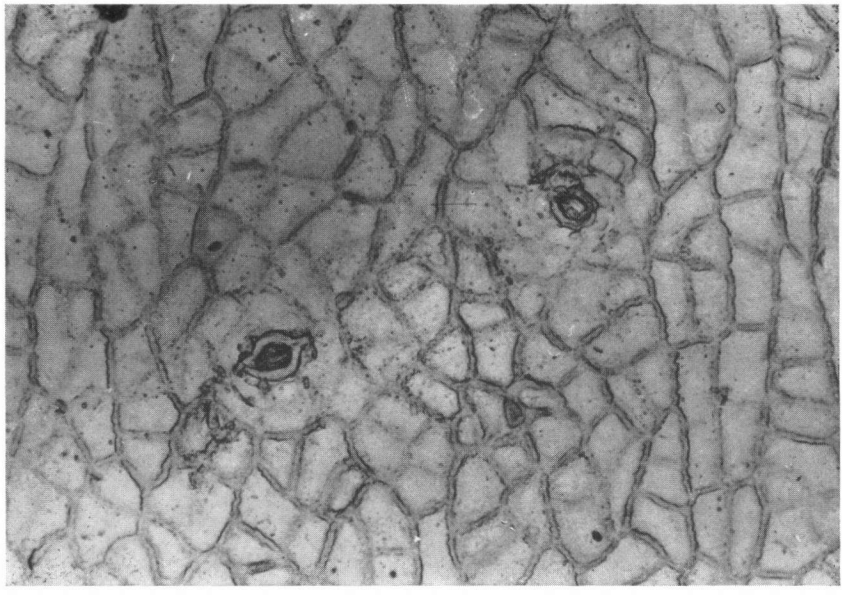

Plate 2a. *Ginkgo biloba* L. Magnification of another part of the epidermis shown in Plate 1b. Two stomata are clearly visible. 109 ×.

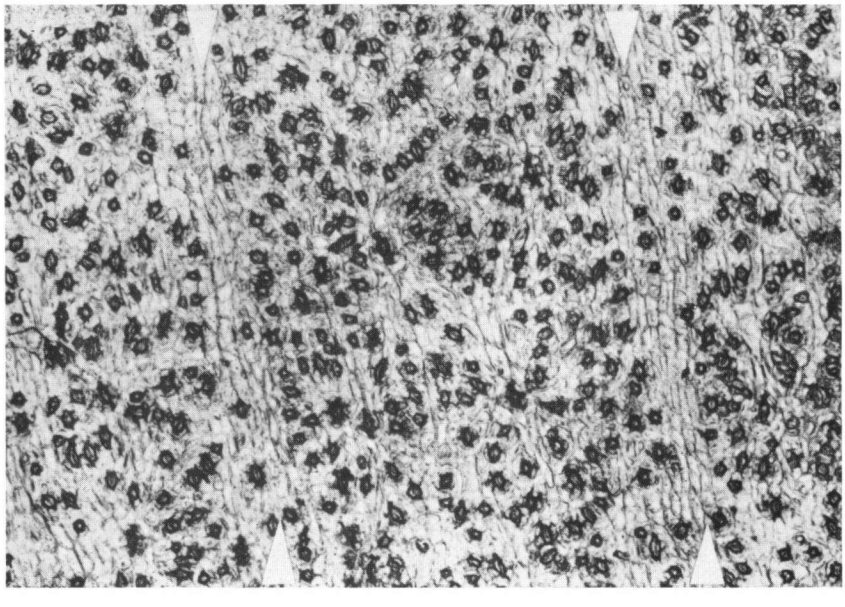

Plate 2b. *Ginkgo biloba* L. Lower epidermis of a long-shoot leaf. Male tree (Sample 1a). A multitude of stomata can be seen. The arrows indicate the position of two veins. About 36 ×.

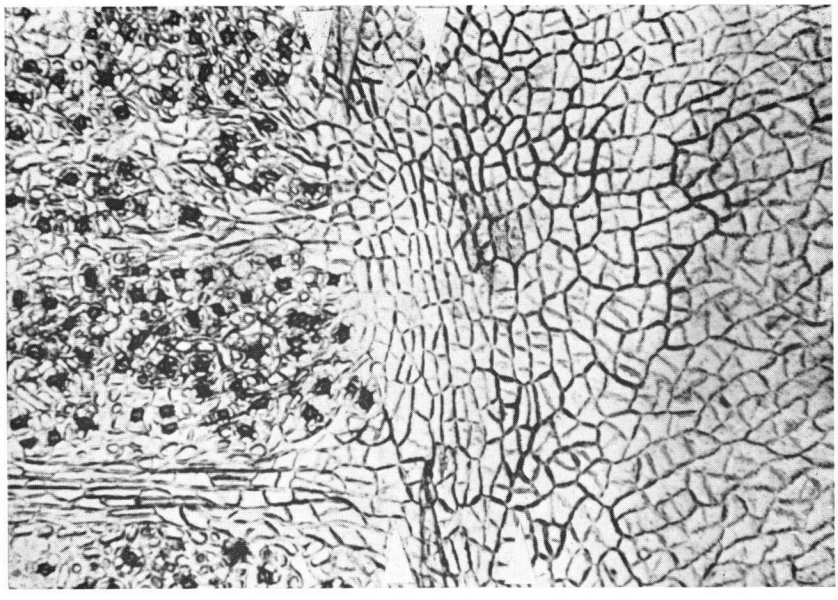

Plate 3a. *Ginkgo biloba* L. Part of the upper margin of a brachyblast leaf. Male tree (Sample 1b.). To the left, the lower epidermis, with stomata; to the right, the upper epidermis, without stomata. The arrows indicate the limits of the strips of epidermis of the margin proper. About 36 ×.

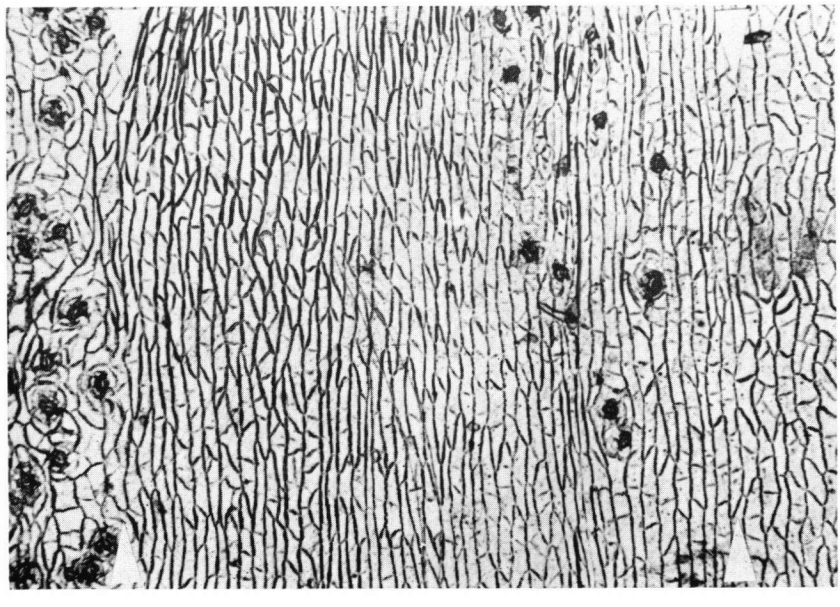

Plate 3b. *Ginkgo biloba* L. Part of the lateral margin of a long-shoot leaf. Female graft (Sample 7a). To the left, the lower epidermis, with stomata; to the extreme right, the upper epidermis, without stomata. The arrows indicate the limits of the margin; to the right, the narrow strip with stomata. About 36 ×.