THE MICROSCOPE PAST
50 YEARS AGO

Conifer Needles[1]

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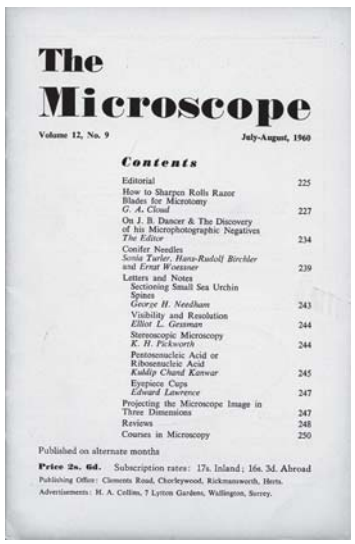
Conifer needles form an interesting and rewarding subject for microscopical study. The needles are easy to section, and after staining, make attractive permanent mounts. There is also the advantage that, with the exception of those of the larch, material can be found at any time of the year. This article is intended as an encouragement to others to try it for themselves, and as an introduction to the subject for those who may go further into the matter. We limited our investigations to the following conifers:

1. *Abies alba* — Silver fir
2. *Picea abies* — Norway spruce
3. *Pinus silvestris* — Scots pine
4. *Larix decidua* — European larch

All our material was collected from trees in the forest.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TECHNICAL NOTES ON PREPARATION

The needles can be fixed in 96% alcohol or in 10% formalin. As alcohol is preferable for delicate needles, such as those of the larch, we used alcohol throughout. Sectioning was carried out after a minimum period of 24 hours fixing. All our sections were cut by hand between elder pith. Then the sections were covered with Eau de Javelle (Liquor Kaliihypochlorosi) for 20 to 30 minutes to remove the cell contents. In the case of the larch needles, the caustic solution had to be diluted considerably, i.e., 3-4 drops to 2 cc water, otherwise the sections were destroyed; these sections had to be left in the diluted solution for 12 hours. After this, the sections were rinsed for about five minutes in 5% acetic acid and transferred to distilled water. The staining was carried out with Delafield's Haematoxylin and Safranin. The sections were then transferred through alcohol, terpineol to xylol, or through acetone to xylol, and finally mounted in Caedax (synthetic balsam).

The needles of conifers are nothing other than leaves in which, due to the fact that most conifers are evergreen, anatomical adaptations have taken place. These can be understood when one takes the living conditions of the trees into consideration. As these leaves are exposed to the winter cold, and the roots of the tree can only absorb a limited amount of water in the cold ground, transpiration must be cut to a minimum. This end is achieved through reduced surface expanse = needle shape. Apart from this surface reduction, a transverse section under the microscope shows other adaptations to limit transpiration.

NEEDLE OF THE SILVER FIR

The cross section of the needle of the *Abies alba* (Figure 1) is almost elliptical. The needle is curved-in slightly in the center of the upperside and correspondingly curved-out on the underside. The surface is smooth without protruding cells. The outer circumference is formed by the epidermis (Figure 1, E) which con-

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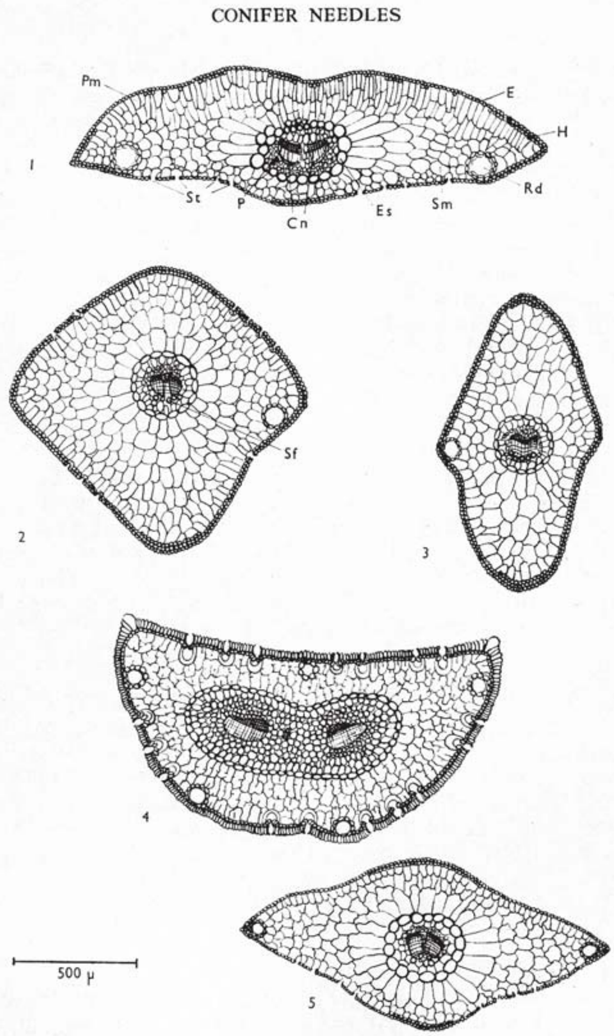

TRANSVERSE SECTIONS OF CONIFER NEEDLES

1, Abies alba. 2, Picea abies, vertical. 3, P. abies, lateral. 4, Pinus sylvestris. 5, Larix decidua.

Cn: Central nerve	Pm: Palisade mesophyll
E: Epidermis	Rd: Resin duct
Es: Endodermal sheath	Sf: Sclerotic fibres
H: Hypodermis	Sm: Spongy mesophyll
P: Pericycle	St: Stomata

sists of thickened cells. On the upperside and in the corners, immediately below the epidermis, there is an interrupted row of thick walled, sclerenchymatous cells; this is the sclerenchymatous hypodermis (Figure 1, H). On the underside, each section will show a few more or less exactly sectioned stomata (Figure 1 St); they lie to both sides of the curve, in two bands, which represent the well-known two white stripes on the underside of the living needle. Close observation shows that the stomata are sunken; they are usually closed by wax.

The hypodermis and the sunken stomata are added protection against too rapid transpiration. The endodermal sheath (Figure 1, Es) consists of a layer of large cells forming an unbroken line. Between the hypodermis and endodermal sheath lies the mesophyll, the upper part is the palisade mesophyll (Figure 1, Pm). On the underside, the cells are not so close, leaving intercellular spaces; this is the spongy mesophyll (Figure 1, Sm). Obvious in the corners are two large holes, the outer edges of which reach the epidermis; these are the resin ducts (Figure 1, Rd). They consist of a circle of cells which exude resin into the duct. In the center of the needle, there is a double central nerve (Figure 1, Cn), which contains, in the upper half, the xylem, and in the lower, the phloem. The tissue between the vascular bundles and the endodermal sheath is the pericycle (Figure 1 P).

NORWAY SPRUCE

Next, let us turn our attention to the needle of the *Picea abies* (Figures 2 and 3). Here we find two different kinds of needles, viz., lateral needles from side shoots and vertical needles from the tip of the tree. The vertical needle shows a distinctly square transverse section (Figure 2). This square section does not mean that the needle has four sides — there are still two, the upper and the lower — but they are very strongly curved. Stomata are to be found on both sides; sometimes they are more numerous on the upper side. The number of resin ducts is variable; usually there is only one but there may be two. The hypodermis is very strongly developed. The vascular bundle is undivided, and in the pericycle below the vascular bundle there is a band of sclerenchymatous tissue (Figure 2, Sf). The endodermal sheath is particularly well developed and consists of even oval cells.

On examining the lateral needle (Figure 3), one would hardly believe it to be from the same tree. Where the vertical needle is broad and square, the lateral needle in the same section is elliptical and compressed. Let us establish in which direction the compression has taken place. For this purpose we examine the resin ducts that are to be found in both sections. If we lay the two sections so that the positions of the resin ducts correspond (Figures 2 and 3), we will see that the compression is sideways. The horizontal diameter is greatly reduced, whereas the vertical remains practically the same. Besides this compression there is also a change in position of the needle as it grows on the tree; it lies flat with the upper and lower surface in a horizontal line. There is another interesting fact to be observed, which no doubt is connected with the position of the needle on the branch. In the case of the vertical needles, the hypodermis is frequently doubled on the underside; in the lateral needles, we find either no doubling of the hypodermis or then a double row in both corners. The vascular bundle of the lateral needle is of the same construction as in the vertical needle but somewhat thinner.

SCOTS PINE

The third needle under observation is that of the *Pinus silvestris* (Figure 4). Here the needles grow in pairs, the transverse section is half elliptical. The curved side is the underside. The needle is covered by a very strong cuticle; the stomata are distributed over the whole surface. The hypodermis forms an unbroken row, and in the case of older trees it is doubled. In the corners we find two resin ducts; sometimes there are more. The endodermal sheath is well developed and encloses two vascular bundles. With a higher magnification, many cells with circular bordered pits can be found in the pericycle.

EUROPEAN LARCH

Our last needle is that of the *Larix decidua* (Figure 5). The larch is a deciduous conifer, which no doubt accounts for the delicate structure of the needles. Again we find two different types of needle on each tree: long ones and short ones. The long ones grow singly on young shoots; the short ones in bunches on older shoots. For our studies, we used the long needles as these are somewhat stronger and easier to cut. The cross section has a certain similarity to that of *Abies alba*. The hypodermis is interrupted; we find it only in the corners and in the centers of the upper and lower sides. The stomata are in two rows on the underside, here again a similarity to *Abies alba*. The endodermal sheath is strongly developed, the vascular bundle undivided. In the corners are two very fine, narrow resin ducts.

This brings us to the end of our investigations, but opens a wide field for further microscopical work. The number of types of needles can be enlarged considerably. The preparation of permanent mounts of all the conifer needles to be found growing wild will provide the microscopist with interesting work for some time, quite apart from the possibility of obtaining cultivated types from the gardens of friends and acquaintances, or exotics from botanical gardens, not to mention collecting while on holidays abroad. Another interesting field would be comparative studies (measurements) in connection with the position of the trees, whether in low or high, sheltered or exposed places, etc. Those, finally, who are interested in microtechnique as such can experiment with stains, dehydrating methods and mounting media.

Eau de Javelle: Mix 20 g. chloride of lime with 100 ccm. water and add 15% solution of potassium carbonate until precipitation ceases. Filter and keep in a dark bottle. Solution and fumes are harmful to metals.

Note: The illustrations are all original and drawn from permanent mounts made specially for this purpose. They are all drawn to the same scale.