Microscopical Study of the Sedge

Carex oederi subsp. oedocarpa.

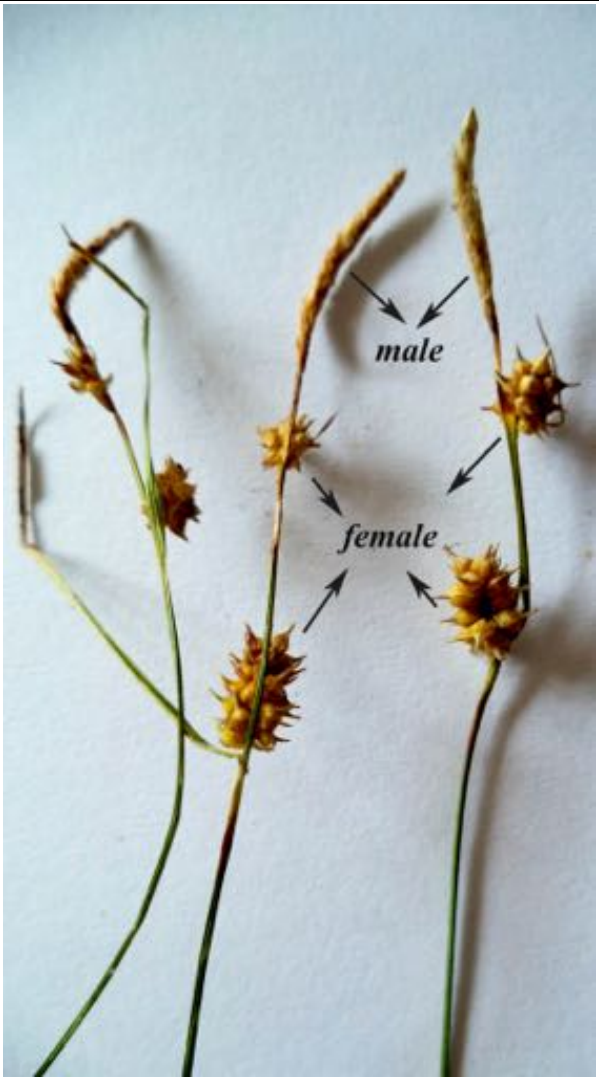
(also known as *Carex demissa*)

Roland Luts

The goal of this work was to microscopically investigate various parts of the plant (cross sections) and at the same time to look for convenient dyes/dye mixture to be used in later studies of other Carices. Pictures of some tests with other dyes will be shown at the end. Carex is a very rewarding subject for microscopy so this article perhaps can also be a stimulus for beginners in microscopy.

Carex oederi subsp. oedocarpa was chosen for this investigation because the leaves and other parts are not too wide and thus relatively good cross sections can be made by hand using a simple (double edged) razor blade, thus eliminating expensive microtomes and time-consuming embedding procedures. The manner of cutting will be explained.

This species belongs to the family of the CYPERACEA. More details of growth and habitus can be found in the literature and on the internet.



male and female 'flowers'


nearly ripe female 'flower'

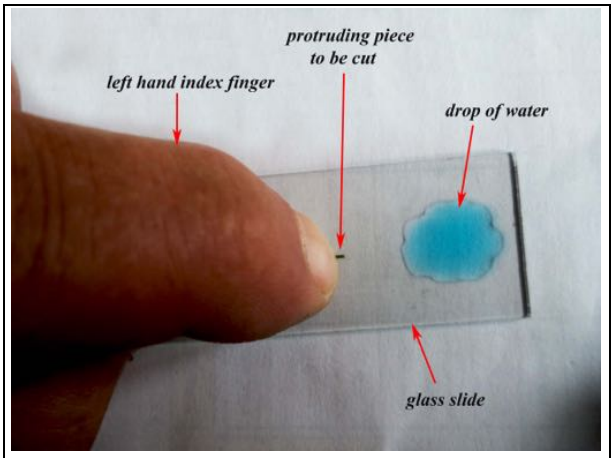
Parts of the plant investigated (6 sampling areas)

parts investigated

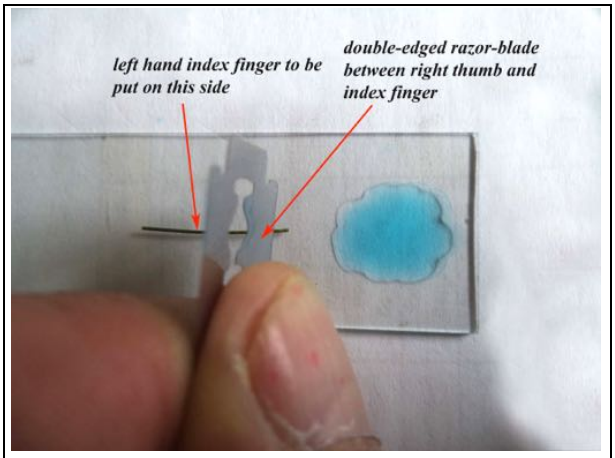
Procedure

Photographs were made with a SONY Cybershot DSC-W710, 16 Megapixels, hand-held above the ocular of the microscope KYOWA Medilux-12. The zoom function of the SONY is used in many instances. Almost any compact camera will do the job and 6 to 8 Megapixels is more than enough.

How I make the cross-sections by hand-cutting:



left-hand position



right-hand position

Take a plant that has been drying at room temperature for a few days. Cut pieces of 0.5 to 1.5 cm of the part to be investigated (see the picture 'parts investigated') with an old razor blade. Pay attention that the cut piece is not flying around as it will be lost .
The cutting is laid flat on a glass slide and with the index finger (or with the help of a glass slide or coverslip) hold the piece firmly in place. Under a dissecting microscope and with a **new and sharp** razor-blade in the other hand, pieces as thin as possible are being cut. Don't attempt to 'chop' quickly but direct the razor-blade edge accurately and vertically on the specimen in order to obtain good and thin cuttings. The razor-blade is guided by the index finger of the other hand. Bring the slices in the water drop that is on the same glass slide; they will expand in the water and will regain their natural shape. Do not wet the specimen to be cut with any water adhering to the razor because this will make cutting very difficult. A lot of procedures in the literature recommend wetting the piece to be sliced, but my own experience is that cutting dry (especially for Carices) is the best thing to do.

Slices in botanical samples must not be so thin (15 to 40 µm is good) as in histological samples (1 to 10 µm) so the use of microtomes of any kind can be eliminated if pieces to be cut are not too thick.

In order to see in what range of thickness my slices were, I did the following:
A dry piece of the Carex stem of exactly known length (about 4 cm) was cut into slices as thin as possible until there was about 1 cm left. This lasts about 1 hour. Slices were counted during the cutting. By a simple calculation, the mean thickness could be determined and that turned out to be 22µm. Good enough for microscopy. This means that there will be slices thinner and thicker than this mean value and the thinner slices can be separated from the bulk using a stereo microscope (best after the staining).

Picture-overview of the stained cross sections

All staining is carried out with Wacker triple stain W3Asim. This mixture contains Astrablue/Acriflavin/Acridin in the proportion 4:1:1. For more details on this stain (preparation and use) see (in German):

http://www.mikroskopie-bonn.de/bibliothek/botanische_mikrotechnik/157.html

A remark in staining procedures: Carices (and a lot of other plants) may contain substances that can react with dyes in forming a precipitate. This I found is especially the case with Astrablue. If this happens, cuttings should be washed a few times with water or with a mixture of water and alcohol (2:1 ratio) prior to staining.

Fig. 1a **Sample 1** 0.5 to 1 cm from the top

Fig. 1b **Sample 1** 1.5 to 2 cm from the top

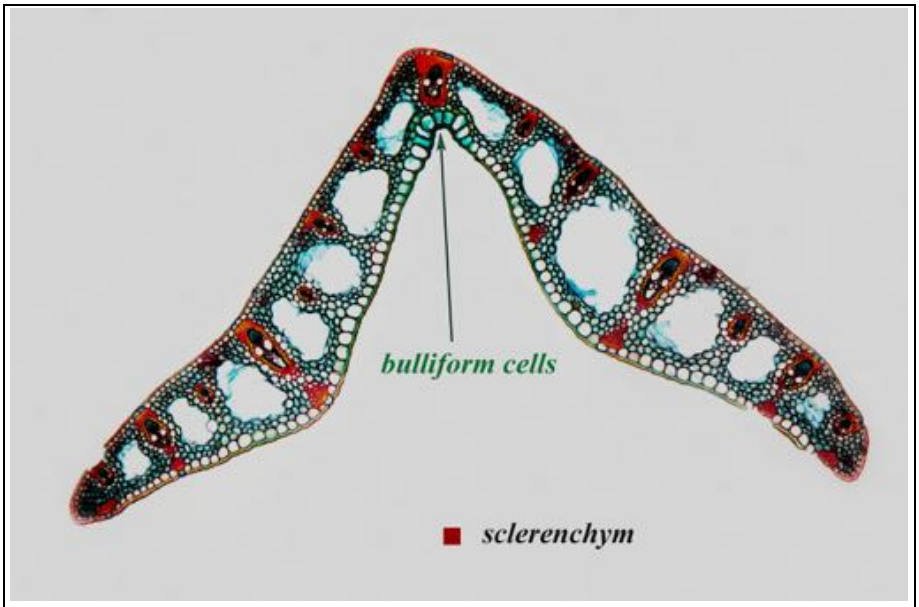


Fig. 2 **Sample 2** Middle of the leaf

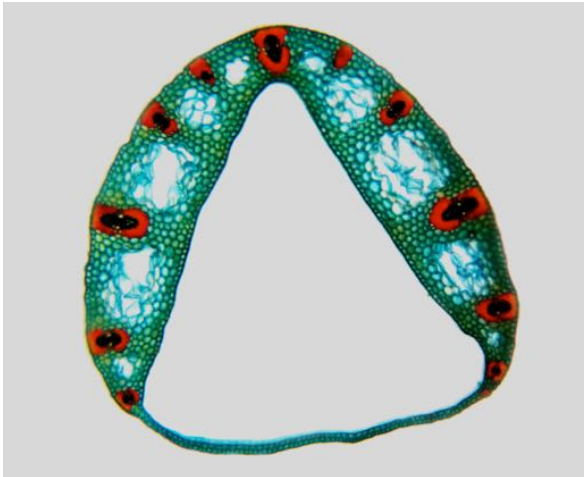


Fig. 3a **Sample 3** just above the beginning of the roots

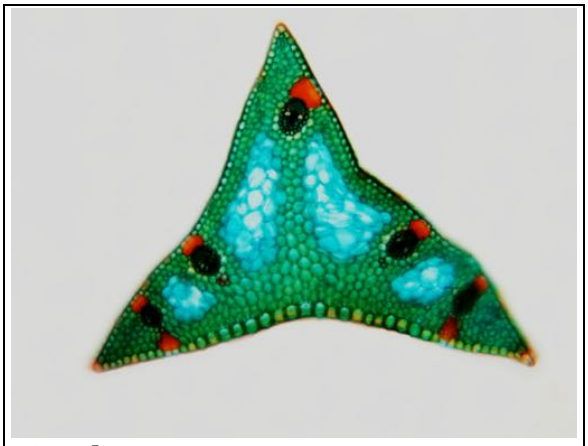


Fig. 3b **Sample 3** Core of fig. 3a

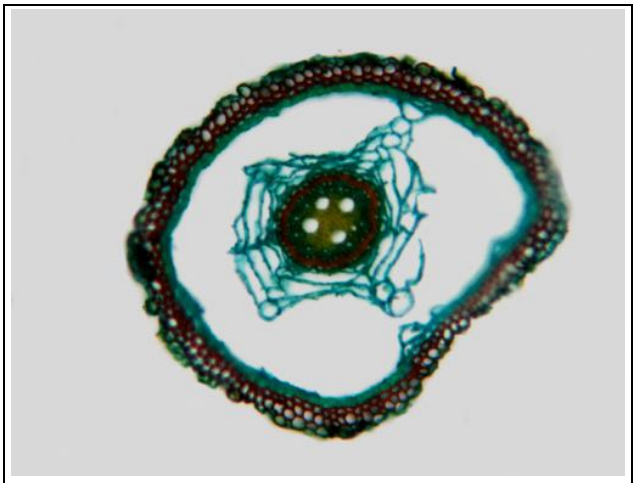


Fig. 4 **Sample 4** Thick root

Fig. 5 **Sample 5** Thin root

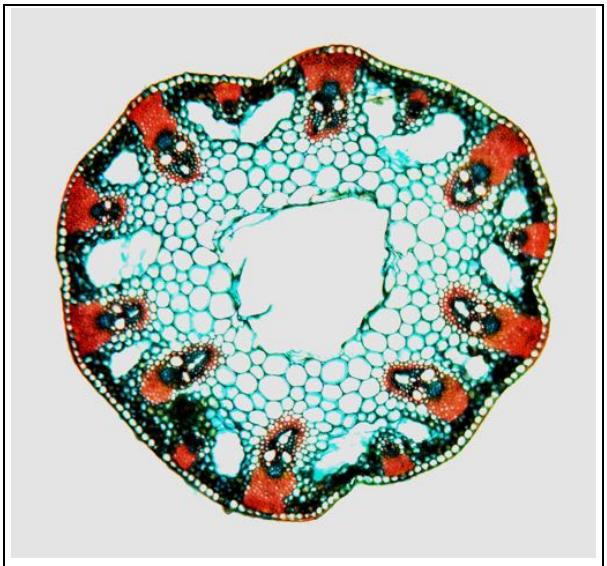


Fig. 6a **Sample 6** Stem

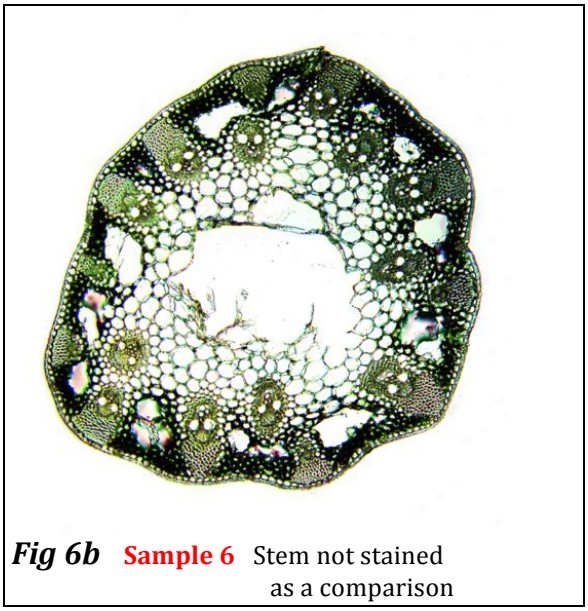


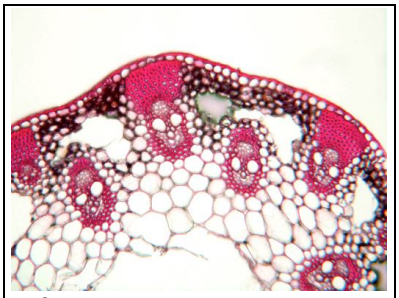
Fig 6b **Sample 6** Stem not stained as a comparison

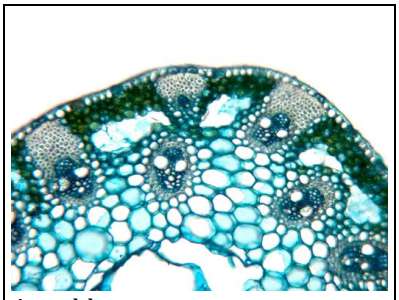
Some tests with other dyes/dye mixtures

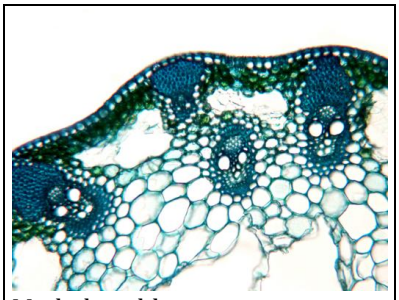
Samples to be stained are cross-sections from the above mentioned Carex stem.

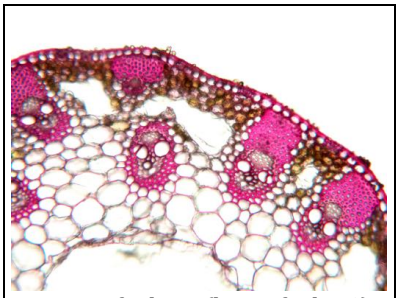
Note that the Astrablue in this test is the only dye that does not stain the sclerenchym cells and therefore will give the best results in double/triple staining. The other dyes, in combination, will give mixed colours as they compete with each other.

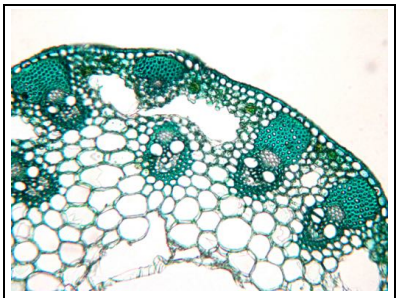
Single stain

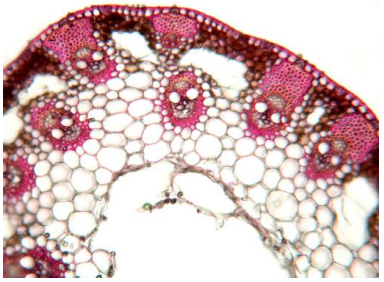

Safranin


Astrablue

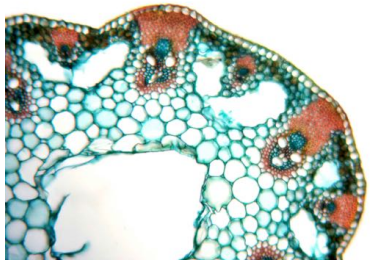

Methylene blue

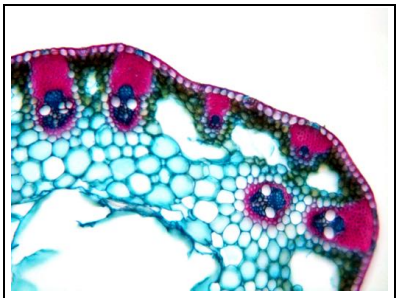

Diamant fuchsin (basic fuchs. ?)

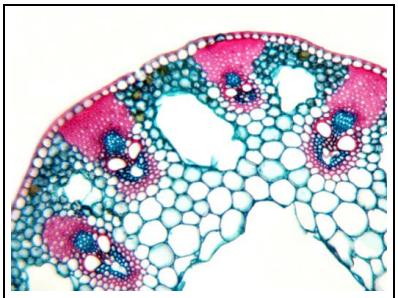

Brilliant green

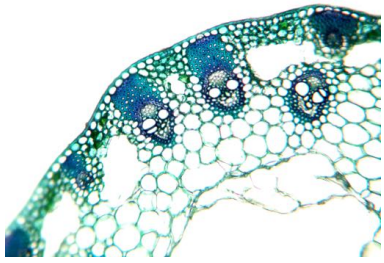

Acridin

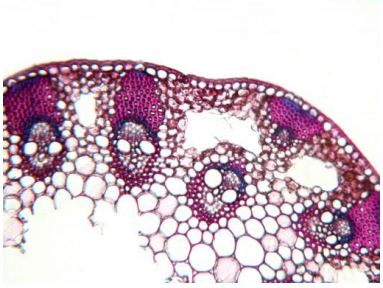
Double stain (the "W3Asim Wacker" is a triple stain)


W3Asim Wacker
(with Astrablue)


Diamant fuchsin_Astrablue


Safranin_Astrablue


Diamant fuchsin_Brilliant green


Methuylene blue_safranin

Comments to the author Roland Luts (Belgium) are welcomed,

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