Transparent plants: an NMR case-study of blackcurrants

S.M. Glidewell, B. Williamson & G.H. Duncan

Imagine the advantages of being able to view a living, growing plant in 3D, with the freedom to select contrast to reflect different physico-chemical states of tissues within the specimen. NMR microimaging provides a non-invasive method to look at the internal structure of plants and therefore to study living tissues as they function, change, grow, age, or become affected by stress or diseases.

Until recently, the changes taking place during internal plant development could be studied only by histological methods based on approaches devised last century. Destructive sampling of a population of individuals was followed by chemical fixation of specimens to stabilise proteins, desiccation in fluids and embedding in wax or resin. With the rigidity provided by the infiltrated wax or resin, the specimen could then be sectioned thinly and stained to add contrast before examination by transmitted light in conventional microscopy. Despite important advances in microtomy and microscopy (see Ann. Rep. 1994, 172), only relatively small specimens can be handled by most of these methods. Unfortunately, at the end of tissue processing, the specimen has changed its dimensions markedly and many important plant constituents (e.g. oils, waxes, gums and resins) have been extracted unintentionally. If a 3D depiction of the internal structures is required, the sections must then be photographed and the images reconfigured to the original shape.

NMR imaging provides a powerful non-invasive means to look at the internal structure of plants. In addition, the technique circumvents problems encountered with attempting to section extremely tough specimens (e.g. woody stems, nuts, and ripe fruits containing hard seeds). Since no light is involved in the imaging process, there is no size limitation on the samples, other than the bore of the super-conducting magnet. Although NMR imaging is recognised as a powerful technique which reveals many anatomical features unambiguously, at this stage in its development for uses in botany, the identity of many tissue features needs to be corroborated and the origin of the contrast in the image understood.

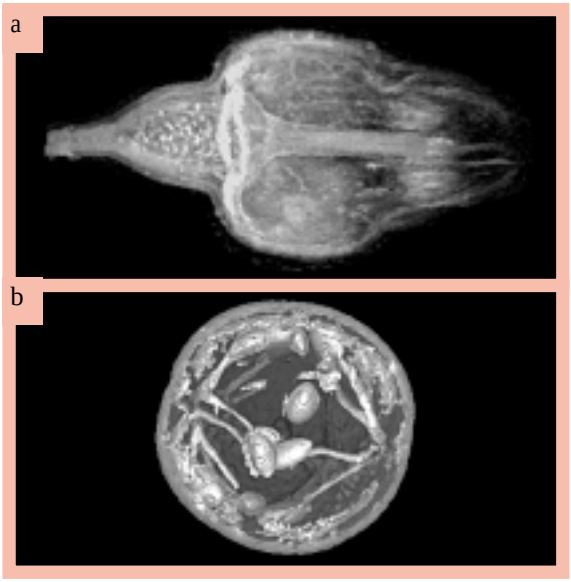

Figure 1 a) Maximum intensity projection of a closed blackcurrant flower b) Surface rendered image of an entire ripe blackcurrant fruit – 'electronically cut' to reveal interior detail

For instance, a recent study[1] of blackcurrant fruits from flower (*Fig. 1a*) to maturity (*Fig.1b*) revealed very clearly the vascular bundles in the periphery of the flower (*Fig. 2a*) and the ovary (*Fig. 2b*). These bundles were equally clear in the mature fruits (*Fig. 2c*). However, the relative intensities have reversed. This is not an artefact, as images acquired with a wide range of parameters (which can lead to contrast reversals depending on relative relaxation times) consistently produced images of flowers where the vascular bundles had high (bright) intensity in a dark field and mature fruits where the reverse was true. Only with the

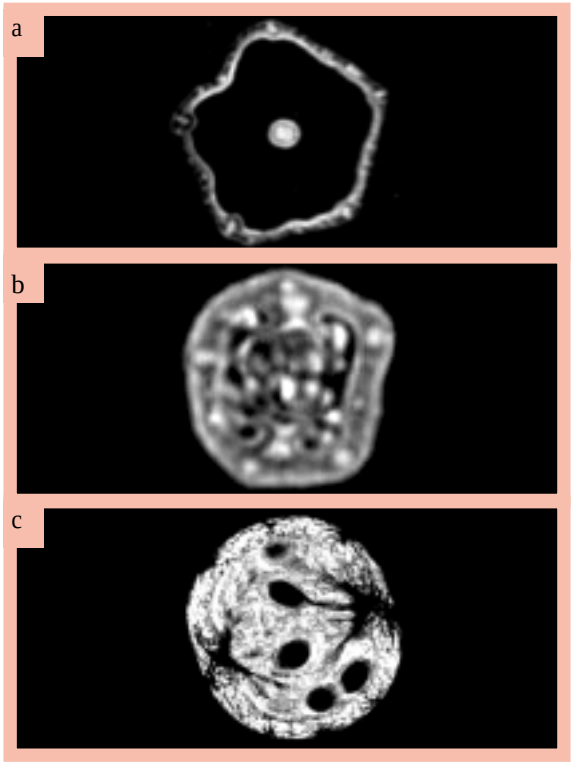

Figure 2 a) Transverse plane of closed blackcurrant flower b) Median transverse plane of ovary of blackcurrant flower c) Transverse plane of ripe blackcurrant fruit.

use of other microscopical techniques in tandem with the NMR was the apparent contradiction resolved.

SEM pictures of freeze-fractured ovaries (*Fig. 3a)* showed that the dark areas around the ovules in the NMR images (*Figs. 3b, 2b)* were indeed empty, whereas resin-embedded sections viewed by light

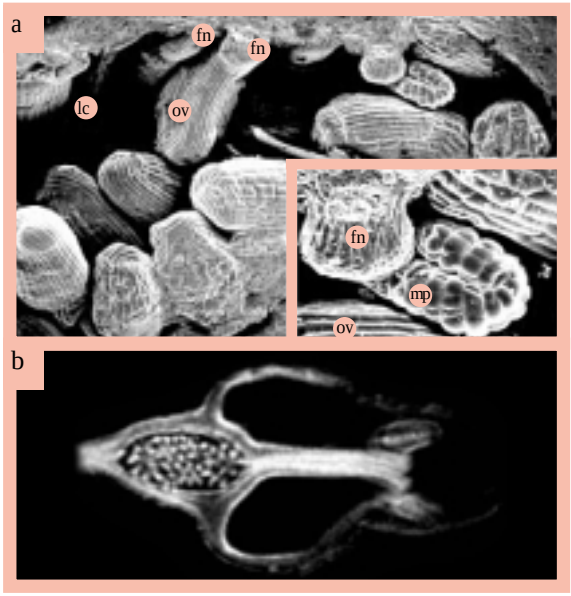

Figure 3 a) LTSEM of ovary of blackcurrant flower. Inset – close up of micropylar region. *lc – locular cavity, fn – funiculus, mp - micropyle, ov – ovule* b) NMR median longitudinal plane of closed blackcurrant flower.

microscopy of green fruits showed that the black vascular bundles in the NMR images corresponded to parenchyma cells separated by air spaces around a small vascular bundle which corresponded in size with the bright core at the centre of the NMR images of the 'bundle'. The phenomenon of

Nuclear Magnetic Resonance (NMR) imaging works by placing the sample in a strong magnetic field (inside a large magnet (shown in the picture)). This causes a tiny proportion of certain atomic nuclei to move to a higher energy level. A pulse of radiofrequency energy upsets this equilibrium and causes the nuclei to precess in phase. After the pulse is over, the system relaxes back to equilibrium, emitting radiofrequency energy. The amplitude of this emitted energy and time at which it is detected depend on the number of nuclei present and the rate at which they relax.

Most imaging experiments use hydrogen nuclei (protons) and the commonest proton-containing molecule is water. So NMR is imaging mostly water, but also other mobile protons such as those in lipids. In addition to the mobile proton distribution in a plant tissue, the signal intensity depends very strongly on the relative relaxation times of the protons which in turn are determined by the environment in which they find themselves. This can, for example, be water closely bound to large molecules such as polysaccharides or proteins, water in cell vacuoles which contain dilute solutions of many species, water in viscous gums or lipids in seed endosperms. In addition, factors such as diffusion, compartment size and tissue inhomogeneities can also affect the relaxation rates.

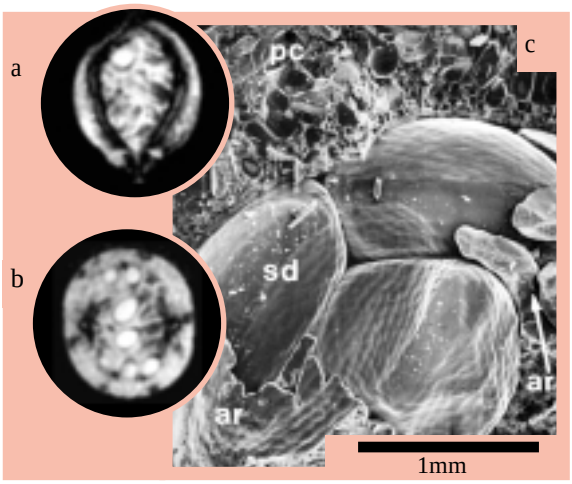

Figure 4 These two NMR images (a, b) are planes of the same small green fruit attached to a bush. As well as showing the aril network, the seeds can be seen as bright images and the bright vascular traces supplying the placentae are clearly evident in the longitudinal plane (a). c) LTSEM of small green fruit. *pc – pericarp, sd - seed, ar – aril.*

reduced intensity in NMR images due to interfaces between gas spaces and tissues is well understood. The interpretation of these botanical specimens therefore is confirmed and the NMR images validated for further work

The pulpy tissues around the maturing seeds constitute the aril, finger-like parenchyma tissues growing

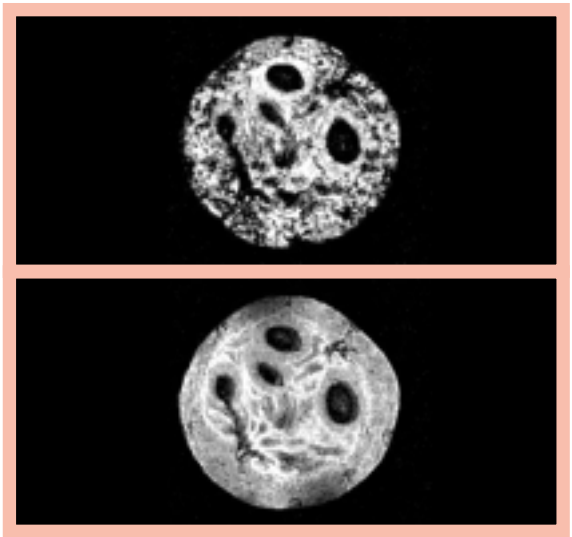

Figure 5 NMR images, using different protocols, of a large green fruit. By this stage, the seeds have hardened and appear dark. The gelatinous sheaths can be seen around them and the funiculi connecting them to the placentae which, along with the peripheral vascular bundles, are still bright.

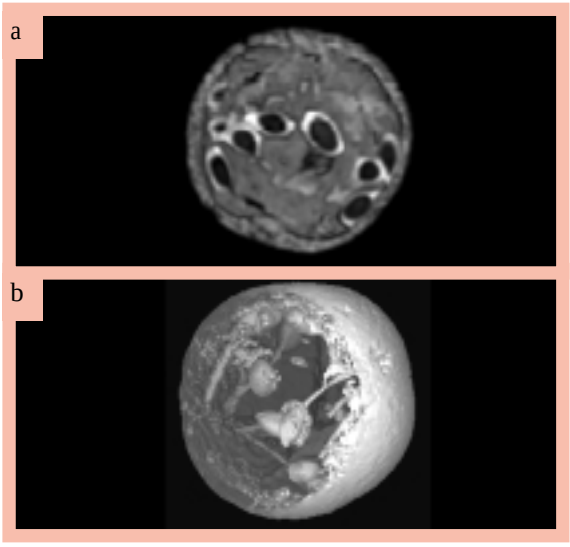

Figure 6 a) Median transverse plane of a ripe blackcurrant fruit. b) Surface rendered image of ripe fruit.

from the placentae to fill the spaces between seeds in the locular cavity. In NMR images (*Figs. 4a,b)*, the aril was displayed as a 3D network of bright strands, an interpretation confirmed by LTSEM (*Fig. 4c).*

Seed maturation involves the growth of a thick hard seed coat, deposition of lipid reserves in the endosperm and retention of a gelatinous sheath around each seed. All these features were resolved and studied non-invasively by NMR imaging (*Fig. 5)* and confirmed by conventional histological methods. By the time full ripeness is achieved, the fruit is too squashy and the seeds too hard for conventional sectioning. NMR images show very marked contrast between the dark seeds and bright gelatinous sheaths surrounding them (*Fig. 6a)* to such an extent that a surface-rendered 3D image of the inside of the fruit can be derived (*Fig. 6b*).

To appreciate the full 3D effect of these images, visit our website at *http://www.scri.sari.ac.uk* and click on 'Special Topics' where you can journey through a blackcurrant fruit or see right inside a closed flower or ripe fruit as it rotates.

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Reference

1 Glidewell, S.M., Williamson, B., Duncan, G.H., Chudek, J.A. & Hunter, G. (1999) *New Phytologist* 141, 85-91.