NON-DESTRUCTIVE EXAMINATION OF HERBARIUM MATERIAL FOR TAXONOMIC STUDIES USING NMR IMAGING

D. MASSON*†, S. M. GLIDEWELL*‡, M. MÖLLER§, R. R. MILL§, B. WILLIAMSON* & R. M. BATEMAN¶

Many taxonomic distinctions are made or refined on the basis of herbarium material that is either dried or preserved in spirit medium. Hitherto, examination of internal structure has only been possible by the destructive sectioning of the preserved material. In this paper, the use of nuclear magnetic resonance (NMR) imaging for the non-destructive, non-invasive, complete three-dimensional structural examination of herbarium material is demonstrated for the first time. The experimental materials were the fruiting structures of two species of Southern Hemisphere *Podocarpaceae*: *Acmopyle pancheri* and *Podocarpus nivalis*. Material dried in accordance with standard herbarium techniques was used, as well as material preserved in spirit and freshly gathered fruits. The dried material was subsequently rehydrated using standard techniques, and protocols established for the specimens. Appropriate selection of NMR imaging parameters allowed a variety of anatomical features to be highlighted on a single specimen. Fresh specimens from living material gave the best NMR signals. Dry specimens gave no signal except from the lipid in the seed, but when rehydrated the images yielded almost as much information about internal structure as did a fresh specimen of the same taxon. Thus, NMR imaging has great potential value as a non-invasive method for obtaining details of the internal structure of fruits and seeds and is particularly useful when, as in the case of *Acmopyle,* the sclerotesta of the seed is too lignified for sectioning by conventional methods.

Keywords. Acmopyle, MRI, *Podocarpus*, preservation.

INTRODUCTION

Much plant taxonomy depends on the comparative anatomy and morphology of reproductive organs. The present paper examines the potential of nuclear magnetic resonance (NMR) imaging techniques for non-invasive examination of the internal structure of fruits and seeds derived from specimens previously dried for preservation in herbaria. Herbaria contain large numbers of specimens that are routinely used in taxonomic research, including the all-important types that are the universally recognized reference points for taxonomic studies. However, such research is frequently restricted to purely external examination of specimens and, even then, the friability

* Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.
† Present address: Department of Chemistry, University Science Laboratories, South Road, Durham DH1 3EL, UK.
‡ Correspondence: s.glidewell@scri.sari.ac.uk.
§ Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK.
¶ Department of Botany, Natural History Museum, Cromwell Road, London SW7 5BD, UK.

and fragility of old or delicate material often precludes rigorous examination. The investigation of internal structures is normally a destructive process involving dissection, which limits the number of investigations possible before the material is exhausted. For type material, such investigations would be prohibited. Moreover, examination of internal anatomy and morphology by conventional methods requires the rehydration of tissue before conventional dissection techniques are applied, with the concomitant uncertainty as to the congruence of the detailed structure of rehydrated material with that of the original fresh specimen.

NMR imaging provides a powerful non-invasive means for studying the internal anatomy of living organisms in three dimensions, a property that has made it an invaluable diagnostic tool in clinical medicine. Because the technique has proved equally applicable to plant tissues (e.g. Ratcliffe, 1994; Chudek & Hunter, 1997) it was decided to explore its potential use in the examination of rare herbarium material. This paper reports experiments in which NMR images of fresh, dried, rehydrated and spirit-preserved material were compared, investigating for the first time, the applicability of this technique to taxonomic studies of herbarium material.

The chosen model systems are drawn from the *Podocarpaceae*, a family of gymnosperms distributed chiefly in the Southern Hemisphere. Unlike most conifers (but like the almost exclusively Northern Hemisphere family *Taxaceae*), nearly all podocarp species possess partially soft fruits. Many species are in cultivation at the Royal Botanic Garden Edinburgh (RBGE), where the family is currently the subject of monographic studies. Two species were chosen for the experiments reported here: *Acmopyle pancheri* (Brongn. & Gris) Pilg. and *Podocarpus nivalis* Hook. One of only two living members of *Acmopyle, A. pancheri* is endemic to the island of New Caledonia. Within the large and widespread genus *Podocarpus* L'Hérit. ex Pers. (over 100 species), *P. nivalis* belongs to *P.* subgen. *Podocarpus* sect. *Australis* de Laub. and is native to New Zealand.

Rehydration experiments aimed to establish the relationship between the NMR images of freshly picked fruits and those obtained from rehydrated dried material. Thus, it was considered crucial to maintain tissue integrity as far as possible during the rehydration process. Herbarium specimens of these two species bearing ripe fruits are very scarce, so selected fresh fruits from plants growing in the RBGE Living Collection were subjected to the same drying and rehydration techniques that would be used routinely on herbarium material. This approach allowed experimental examination of far more fruits than could legitimately be removed from a herbarium specimen.

Both species have firm seeds which are borne on a fleshy, soft structure, traditionally termed a 'receptacle', which is formed by the fusion of two or more modified sterile bracts; this 'receptacle' is in turn attached to the main axis by a peduncle. Strictly, it is not homologous with the true receptacle of angiosperms, but the term has become so entrenched in the *Podocarpaceae* literature that it is used here rather than the neglected alternative of podocarpium. The female reproductive structures (receptacle plus seed) of *Podocarpus nivalis* are small; the seed and receptacle are

4–6mm and 3–7mm long respectively (Gray, 1956). A greenish, inverted seed is attached to the top of a fleshy 'receptacle' which is bright red at maturity, after changing from a dark red initial receptive stage through dull grey-green, lime green, yellow, orange and salmon colours. This structure is composed typically of two sterile bracts and has a smooth, non-warty surface. In contrast, the fruiting structure of *Acmopyle* is one of the largest in the *Podocarpaceae*. The approximately spherical seed, measuring 10–12mm in diameter, is obliquely inserted on a fleshy receptacle that can be more than 20mm long when fully turgid. This receptacle is composed of four to six sterile bracts and is unusual within the family in having a warty surface texture, a characteristic shared only by *Dacrycarpus* (Endl.) de Laub. In *Dacrycarpus* species, both receptacle and seed are bright red when ripe, but in *Acmopyle pancheri* the receptacles are dark violet at maturity and emit an odour similar to that of raspberries; the seed is a greyish violet colour. The reproductive biology of *A. pancheri* was recently outlined by Möller *et al.* (1999a).

All herbarium material is dry and often very brittle, so before dissection or histological examination it must be rehydrated. The time required for rehydration depends on the characteristics of the tissue and the rehydration protocol. Delicate tissues such as flowers and soft fruits generally require a shorter period to rehydrate than firmer tissues such as many leaves and 'dry' fruits such as nuts. Rehydration occurs more rapidly at higher temperatures, although very high temperatures can compromise maintenance of tissue characteristics and integrity. For reconstitution of the original shape and size of each organ studied, the standard practice in herbaria, for soft tissues such as flowers or fleshy fruits, is to boil the material in tap water (with or without added detergent to assist in tissue softening) for a short period (usually one or two minutes for the softest tissues). Vigorous boiling can, however, soften tissues too much so that specimens disintegrate if dissection is attempted. Thus, it is important to establish the optimal protocols for rehydrating a given tissue, so that damage is minimized.

Materials and Methods

Living materials of *Podocarpus nivalis* (accession 19850452 from New Zealand) and *Acmopyle pancheri* (accession 19842681 from New Caledonia), maintained in cultivation at RBGE, were used. Both these accessions have been employed in other NMR experiments investigating reproductive biology (Möller *et al.*, 1999b, 2000). Specimens of ripe *P. nivalis* and 11-month-old *A. pancheri* fruits were selected for the experiments reported here.

Dehydration

Fruits were dried to constant weight in open Petri dishes in a drying room at 30–35°C. Linear dimensions were recorded before and after dehydration.

Rehydration

Previously dried fruits were rehydrated by immersion in tap water at temperatures of 60°C, 75°C and 95°C. Weights were recorded until constant weight was attained and linear dimensions were recorded.

Spirit preservation

Ripe fruits of both species were also preserved by immersion in 'spirit', specifically Copenhagen mixture, a modified 'Kew cocktail' consisting of 58% water, 37% methanol and 5% glycerol v/v.

NMR imaging

NMR images were acquired using a Bruker AMX 300 micro-imaging system at a magnetic field of 7.1 T (300 MHz for ^{1}H). Samples were dried and rehydrated using the protocols described above. Specimens, with the exception of the dry fruits, were supported on a piece of plastic tubing and placed in a capped sample tube containing a pad of moist tissue to maintain humidity. Dried specimens were also supported on a piece of plastic tubing but were placed in a dry sample tube. The specimens were then imaged in coils of 5 or 10mm diam. for *Podocarpus* and 15 or 20mm diam. for *Acmopyle* with the longitudinal axis of the fruit parallel to the static magnetic field vector.

NMR image intensity is a function of the concentration of mobile protons (principally those in water) in a given voxel (volume element). Additional contrast is generated by selection of experimental parameters which differentiate protons with differing relaxation rates. Certain atomic nuclei (in this case hydrogen nuclei or protons) have a magnetic moment; in simple terms this means that they will preferentially align and spin around the axis of an applied magnetic field. A pulse of energy of the correct radio frequency (RF) and duration will cause the magnetic moments to spin in phase with each other and 'flip' them so that they are spinning antiparallel to the magnetic field. When the RF pulse ceases, the spins then relax back to an equilibrium state parallel to the applied field at a rate $1/T_1$ and lose phase coherence at a rate $1/T_2$. In spin echo experiments, there are two RF pulses: the first inverts the magnetic moment and the second, applied at a time TE/2 after the first, refocuses the spins to form an echo at time TE. The sequence is repeated at a time TR after the first RF pulse. T_1-weighted images are generated by using a short repetition time (TR) so that only those areas with short T_1s will have relaxed back to equilibrium before the next excitation pulse. Therefore, these areas will have the highest intensity and appear brighter. Areas with long T_2s have high intensity in T_2-weighted images as the signal from areas with short T_2s loses phase coherence before the refocusing pulse, appearing darker in the image. Detailed explanations of NMR imaging are given by Callaghan (1991), Chudek & Hunter (1997), and Glidewell *et al.* (1997).

Three-dimensional spin-echo images according to three different weighting regimes

were acquired from the same fruit: fresh, dried and rehydrated for *Podocarpus* and *Acmopyle*. Images with the same weightings were also produced from a different specimen of each of the two species after being preserved in spirit (see above). Parameters for the differently weighted images, which were chosen to give realistic experimental duration, are shown below. Regime A gave the least weighting with small amounts of T_1- and T_2-weighting, regime B gave T_1-weighted images and regime C, images with T_2-weighting.

Podocarpus

Regime A: TE 6.56ms; TR 500ms or 250ms
Regime B: TE 6.56ms or 5.28ms; TR 50ms or100ms
Regime C: TE 30ms; TR 1000ms or 500ms

Acmopyle

Regime A: TE 6.56ms; TR 1000ms
Regime B: TE 6.56ms; TR 100ms
Regime C: TE 30ms; TR 500ms

Data matrices were $128 \times 128 \times 256$ or $64 \times 64 \times 128$ for *Podocarpus* giving voxel sizes from $(63–156\mu m)^3$, and 128^3 for *Acmopyle* giving voxel sizes of $(94–313\mu m)^3$. Data were processed using software supplied by Bruker in the UXNMR package to generate three-dimensional datasets which could be visualized as series of two-dimensional images ('slices') of any orientation or, after maximum intensity projection, as three-dimensional images.

Results

The results are divided into two sections, the first dealing with dehydration and rehydration protocols for the two species and the second comparing the NMR images obtained from fresh, dehydrated, and rehydrated material.

Establishment of protocols

Dehydration. Weight losses in *Podocarpus* fruits were around 80% of fresh weight. The seeds exhibited little change in size during dehydration but there was considerable shrinkage of 40–60% in the receptacle. The fruits reached constant weight after 24h of drying. Weight losses of *Acmopyle* fruits ranged from 25–60% of fresh weight, the variability probably reflecting different initial hydration levels. There was a slight reduction of, on average, less than 10% in seed diameters and a shrinkage of the receptacle varying from 10–40% in length and 20–40% in diameter. Weight equilibrium, irrespective of initial moisture levels, was reached after 72h of drying.

Rehydration. Constant weight of *Podocarpus* fruits was attained after 15min at 95°C, 2h at 75°C and 4h at 60°C. *Acmopyle* fruits heated at near boiling point rehydrated very quickly, full weight being reached after only 45min, but both seed and receptacle

turned black. Fruit placed in cold water showed no significant rehydration; after brief immersion in hot water, slight rehydration occurred. Fruit heated at 75°C rehydrated and a constant weight, representing ~90% of original fresh weight, was attained after 4h.

For NMR imaging, samples were dried for 24h (*Podocarpus*) and 72h (*Acmopyle*) and rehydrated for 2h (*Podocarpus*) and 6h (*Acmopyle*) by immersion in water at 75°C. These conditions were found to give the greatest retention of structure in the respective samples.

NMR imaging

Images of fresh, dried, rehydrated and spirit-preserved specimens of *Podocarpus* are shown in Fig. 1 and of *Acmopyle* in Fig. 2. Each image is displayed with an intensity scale to maximize the anatomical detail visible. Image contrast between the different tissue types depends on the weightings used as described in Materials and Methods. The images shown are of median longitudinal orthogonal electronic 'slices' of the whole fruit and a transverse 'slice' through the seed. Fig. 3(a,b) shows 'slices' through the seeds of specimens of both species arranged so that the frequency-encoded direction of the image is from left to right across the page. The image of the prothallus is clearly displaced to the right irrespective of its geometry within the seed indicating a chemical shift effect (see Discussion).

Podocarpus. Images of the fresh specimen show the vascular system clearly within the fruit both in the receptacle as a branched system going to the bracts and in the sarcotesta of the seed (Fig. 1). The termination of the vasculature at the sterile bract on the left-hand shoulder is particularly prominent. In the T_2-weighted image (C), the brightest region is the fleshy receptacle containing cells with a high cell volume-to-wall ratio. The signal from the vascular system is less intense, although still discernible from the air spaces, which appear as dark areas around the vascular traces and are most discernible under regime C in fresh material.

The images from the dried specimen contain signal only from the prothallus of the seed; the receptacle and sarcotesta gave no signal. The receptacle and sarcotesta are, however, again observed in the images of the fruit after rehydration. The vascular system is evident in the images of the rehydrated and spirit-preserved specimens. In images of rehydrated fruit, the receptacle differs from that of the fresh fruit in that the flesh appears to be of a more homogeneous nature and the dark areas around the vascular traces have disappeared. Specimens preserved in spirit appear similar to the rehydrated specimens although the receptacle is smaller in size.

The embryo is visible under all treatments, usually as a brighter spot in or near the centre of the prothallus in transverse sections, except for spirit preserved material under weighting regimes B and C (Fig. 1).

Acmopyle. Images of the fresh specimen show clearly the vascular system within the receptacle forming a central trace with two side branches (Fig. 2). The greatest

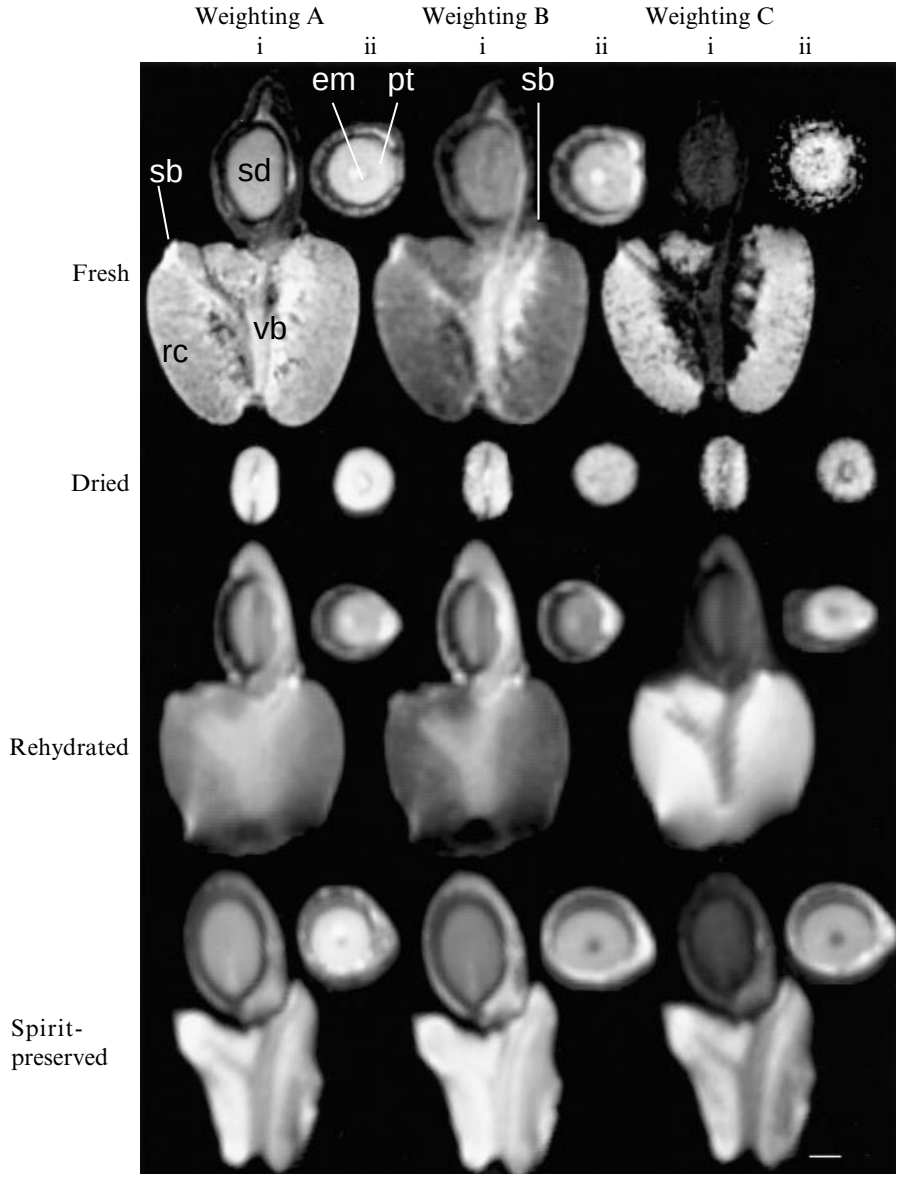

FIG. 1. NMR images of fruit of *Podocarpus nivalis*. From top to bottom the rows are: fresh; dried; rehydrated; spirit-preserved. The top three rows are all of the same specimen. Each treatment shows a median (through the receptacle) longitudinal slice (i) imaged according to three different weighting regimes. Alongside each longitudinal slice is a median transverse 'slice' (ii) through the seed displayed with an expanded intensity scale. Abbreviations: em, embryo; pt, prothallus; rc, receptacle; sb, sterile bract; sd, seed; vb, vascular bundles. Scale bar: 1mm.

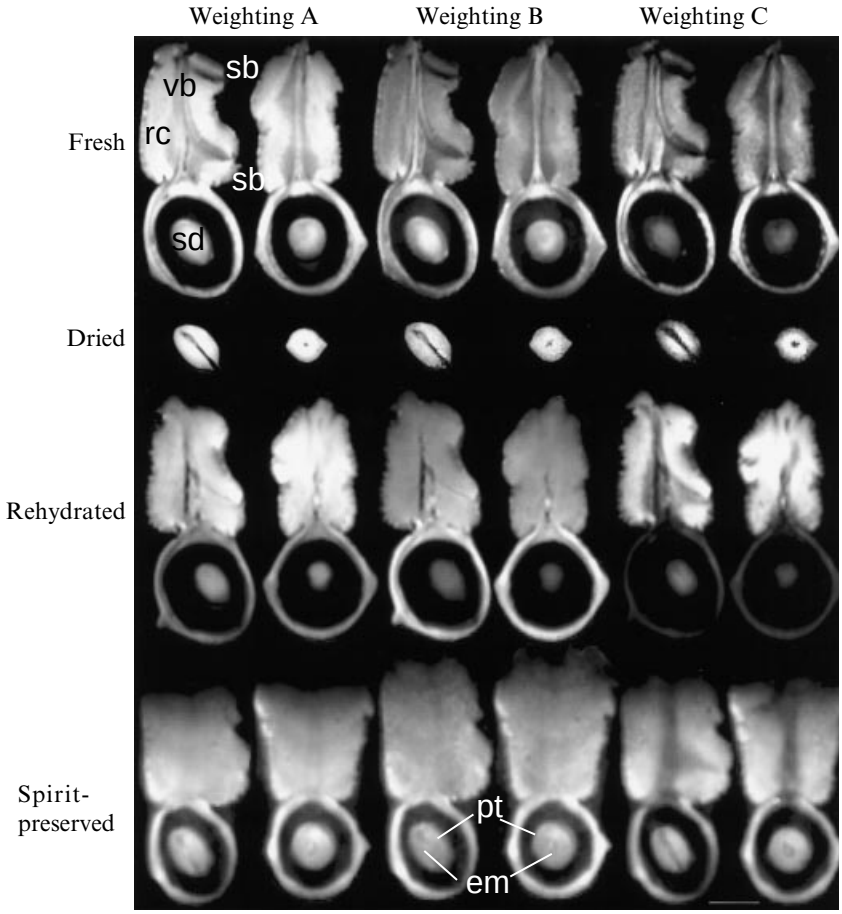

FIG. 2. NMR images of fruit of *Acmopyle pancheri.* From top to bottom the rows are: fresh; dried; rehydrated; spirit-preserved. The top three rows are all of the same specimen. Each treatment shows two orthogonal median (through the receptacle) longitudinal 'slices' imaged according to three different weighting regimes. The images of the dried fruits are of median longitudinal and transverse 'slices' through the prothallus of the seed. Each image is individually intensity scaled. Abbreviations: em, embryo; pt, prothallus; rc, receptacle; sb, sterile bract; sd, seed; vb, vascular bundles. Scale bar: 5mm.

intensity and least contrast are visible in the least relaxation-weighted images (regime A). The sclerotesta of the seed is dark. Images with more T_1-weighting (B) are broadly similar but show slightly more contrast around the vascular traces. Images with T_2-weighting (C) show the sarcotesta and prothallus less clearly.

As with P*odocarpus*, only the prothallus appears in the images of the dehydrated specimen. On rehydration, the image of the receptacle is restored but shows less fine detail and contrast between tissues than when fresh; the prothallus and endotesta appear smaller than in the fresh specimen. The greater detail visible in the enlarged

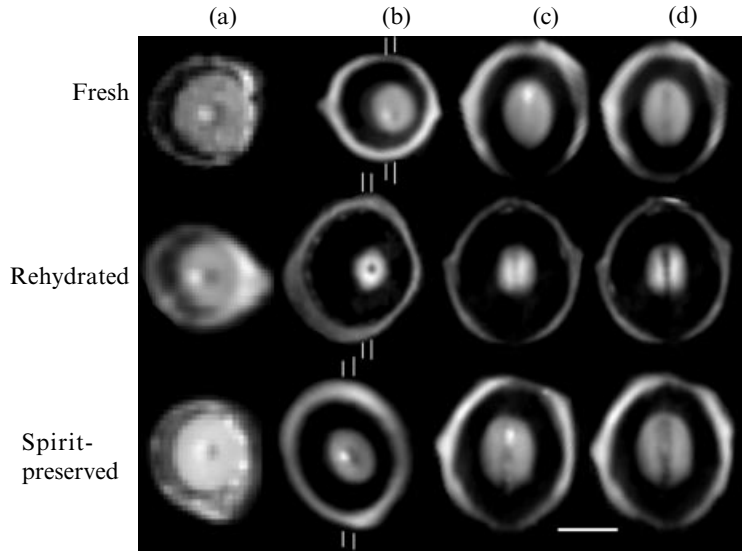

FIG. 3. NMR images illustrating the chemical shift effect in the seeds of (a) *Podocarpus nivalis* and (b–d) *Acmopyle pancheri.* From top to bottom the rows are fresh, rehydrated and spirit-preserved. Columns (a) and (b) show transverse 'slices' through the seeds The frequency-encoded direction is from left to right in all cases and is the direction in which chemical shift effect manifests itself. The orientation of the seeds relative to the frequency-encoded direction was different in different experiments and thus demonstrates that the effect is real as the displacement is always in the frequency-encoded direction and not constant relative to the geometry of the seed. Columns (c) and (d) show longitudinal 'slices' through the seed of *A. pancheri* in the planes indicated by the vertical lines; through the embryo in (c) and through the adjacent 'space' in (d). Scale bar: 5mm.

images of the seed in Fig. 3 shows that the bright features visible in the sarcotesta in Fig. 2(c) fresh are also visible after rehydration.

In the spirit-preserved specimen, there is much less detail and contrast than in the fresh or rehydrated specimens (Fig. 2). The location of the vascular traces in the receptacle is discernible only using regime C and no detail is visible in the sarcotesta under any regime.

Figure 3 illustrates the chemical shift effect in which the lipid-rich prothallus of the seeds of both species is shifted in the frequency-encoded direction of the experiment (see Discussion for further details). This effect was observed with all three relaxation weightings but is most clearly seen in the T_2-weighted (C) images. In *Acmopyle*, the embryo can be seen in the central cleft of the prothallus in longitudinal section in Fig. 3(c) in fresh and spirit-preserved fruits. It was not visible in the rehydrated specimen.

None of the specimens of either species regained its original size, shape or colour after rehydration.

Discussion

Establishment of protocols

Dehydration. The optimum drying time was longer for *Acmopyle pancheri* than for *Podocarpus nivalis* because *A. pancheri* fruits are much larger than those of *P. nivalis*.

Rehydration. Under all heating regimes, the fruits of *Podocarpus* did not fully regain fresh size or weight. The receptacle was less turgid than when fresh and had lost much of its bright red ripe colour, becoming flesh-pink, suggesting that some of the anthocyanins in the receptacle had been destroyed or leached out. In contrast, the original shape was nearly restored. The fruits rehydrated rapidly when boiled (15min were sufficient), but more gentle heating for 2h at 75°C resulted in almost full rehydration; this process was considered less injurious to the fruit and more likely to maintain tissue integrity.

Acmopyle pancheri fruits (both seed and receptacle) are covered in a waxy bloom thus rendering them impermeable to cold water on which they floated. Full rehydration required immersion in water warm enough to render the wax coating water-permeable. Immersion at both 75° and 95°C resulted in immediate sinking and swelling of the fruit, particularly the receptacle. At 95°C, some blackening of the lowest sterile bracts of the receptacle was observed. This effect was much less marked or absent at the lower temperatures. Hence, although rehydration at 75°C took longer than at 95°C, the cooler temperature subjected the fruit to less deterioration.

NMR imaging

Podocarpus. The least weighted image (A) tends towards a map of mobile water in the specimen. Dark areas around the central vascular bundle in the receptacle correspond to air spaces, as corroborated by dissection of other specimens and by comparison with the rehydrated specimen in which these spaces were infiltrated with water. These dark areas are absent in the spirit-preserved specimen as the protons in the methanol/glycerol/water mixture contribute to the NMR signal.

In the T_1-weighted image (B) of the fresh specimen, the vascular tissue is brighter than the remainder, indicating a shorter T_1 for these tissues than for the fleshy receptacle. The same pertains in the rehydrated fruit but not in the spirit-preserved specimen where the receptacle is infiltrated additionally with methanol and glycerol from the spirit cocktail. Both these molecules have shorter proton T_1s than pure water and will also serve to reduce the water relaxation time. Water infiltration in the rehydrated specimen has removed the contrast between the bright vascular trace around the sarcotesta so clearly visible in the fresh fruit imaged under regimes A and B. The relative darkness of the seed compared with the receptacle in images with T_2-weighting (C) indicates its shorter T_2 compared with the softer receptacle and sarcotesta. Because each image is individually intensity-scaled, the median trans-

verse view without the bright receptacle (Fig. 1c,ii), demonstrates that there is some signal from the seed.

The chemical shift effect (Glidewell *et al.*, 1997) illustrated in Fig. 3 is particularly marked in the images of *P. nivalis,* where the prothallus can be seen shifted in the upfield direction relative to the outer layers of the seed and central embryo. The shift occurs because the prothallus is rich in lipids which contain mobile protons; these resonate at higher frequencies than water protons. The signal observed from the dried specimens thus originates largely from lipids. In the spirit-preserved samples, methyl protons are present in the methanol preservative as well as in the natural prothallus lipids. The observed shift effect should therefore be less pronounced, as there would be a high concentration of methyl protons in the aqueous regions of the fruit as well as in the prothallus. The observed effect suggests that the preservation medium is partitioned between the different tissues of the fruit with the methanol preferentially concentrated in the lipid-rich prothallus.

Acmopyle. The receptacle of *A. pancheri* is less fleshy and juicy than that of *P. nivalis*. It would therefore be expected to have a shorter T_2, a fact reflected in the relative darkness of the receptacle in the T_2-weighted images (C) of *A. pancheri* (Fig. 2) compared with those of *Podocarpus* (Fig. 1). This lack of fleshiness is also the reason for the more similar image intensities of the vasculature and receptacle parenchyma in the T_1-weighted images (B), because the parenchyma has a shorter T_1 than in *P. nivalis*, closer to that of the vascular traces. As with *Podocarpus*, the reduced darker areas around the vascular traces in the receptacle of rehydrated specimens compared with fresh ones (Fig. 2) result from water infiltration.

The lipid-rich portions of the *A. pancheri* fruits, notably the prothallus, also exhibit a chemical shift effect (Fig. 3) similar to *P. nivalis.* In this case, the shift effect is not seen in the rehydrated sample, suggesting the possibility that there is incomplete rehydration of the seed and that the signal from that region is predominantly that of lipids. In Fig. 4 the three-dimensional nature of the images is emphasized by depicting the data as maximum intensity projections. These are best seen as animations rotating in space at http://www.rbge.org.uk (via home page) and http://www.scri.sari.ac.uk/Special/NMR. When the relaxation weighting is such that the internal structures, like the vasculature, are brighter than the outer layers, the three-dimensional effect is particularly marked. This is seen around the seed of *P. nivalis* imaged with least weighting (A), and in the T_1-weighted (B) image of its receptacle. In *A. pancheri* the vasculature is less well defined, but in the rehydrated sample the epimatial ridge is seen in all the images and the seed prothallus in the T_2-weighted (C) image.

Conclusions

This study demonstrates that NMR imaging reveals considerable three-dimensional structure in rehydrated and spirit-preserved herbarium specimens. The non-destructive nature of the technique means that many measurements can, in theory,

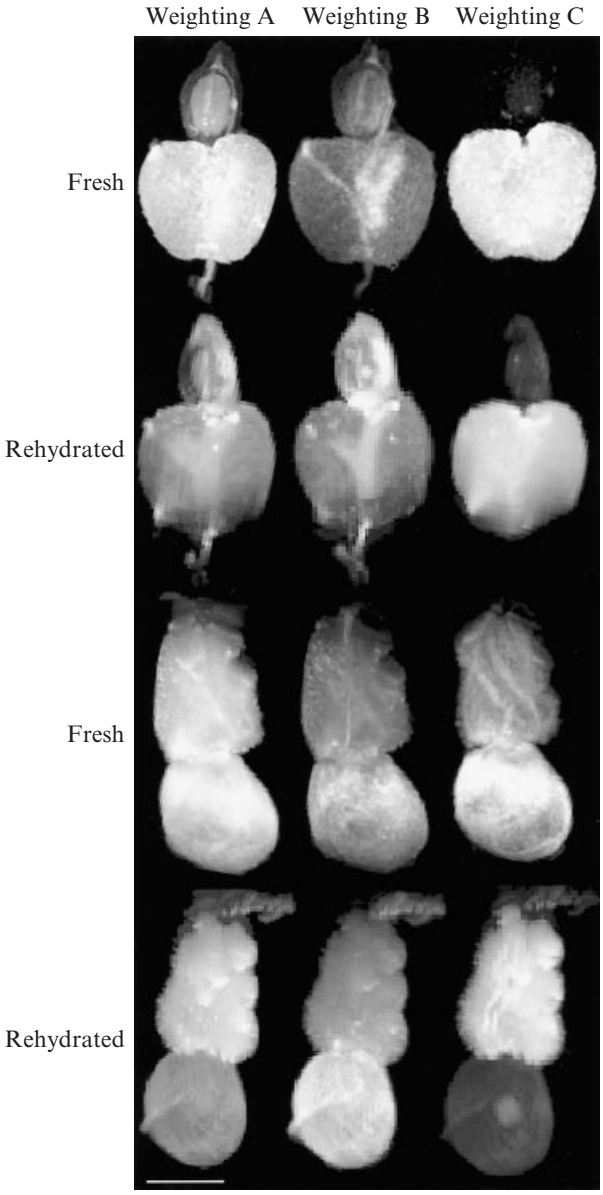

FIG. 4. Maximum intensity projections of three-dimensional images of *Podocarpus nivalis* (upper) and *Acmopyle pancheri* (lower) both fresh and rehydrated for each of the three relaxation weightings. Scale bar: 5mm.

be made on a single rehydrated specimen which can then be dehydrated and returned to storage. Although detail and contrast are poorer than obtained from fresh material, much useful information for taxonomic analysis can be gathered with minimal sample preparation and handling. This approach could be particularly useful in the

case of fragile specimens. For the specimens investigated here, which have fleshy sarcotestae and extremely hard seeds, examination by conventional sectioning would have been well-nigh impossible.

Different relaxation weightings reveal anatomical detail in various parts of the fruits. The large degree of liquid infiltration into the receptacles in the rehydrated and spirit-preserved specimens gave high relative signal in these regions in T_2-weighted images, thus favouring such weighting for this kind of material. The advantage of the technique, however, is that an infinite number of different regimes may be applied, each with the potential to reveal certain aspects of the structure. This versatility contrasts with conventional histochemistry in which serial sectioning and staining destroy the specimen and allow only one plane of view.

Because this study is the first to attempt NMR imaging of herbarium material, it was deemed appropriate to determine the optimum conditions for rehydration by conventional means. More detailed information on which to base a judgement would be available, however, by using NMR imaging to follow the progress of rehydration under different protocols. Such experiments could lead to optimization of the entire preservation process by allowing determination of the best method of preserving herbarium material to recover anatomy, for example, the optimum speed and intensity of drying, the use of drying vs spirit preservation or the nature of the most appropriate spirit mixture.

In addition to serving as a tool to facilitate the improvement of preservation methods in the future, NMR imaging has great potential for the non-destructive investigation of the vast store of material already in herbaria around the world.

Acknowledgements

The Royal Botanic Garden Edinburgh and the Scottish Crop Research Institute are supported by the Scottish Executive Rural Affairs Department (SERAD) This paper is a product of RBGE Project FFL/99/060, funded by the Flexible Fund of SERAD. The NMR imager at SCRI was purchased by Mylnefield Research Services Ltd. We wish to thank the horticultural staff at RBGE, particularly A. Ensoll, for maintaining the Living Collections.

References

Callaghan, P. T. (1991). *Principles of Nuclear Magnetic Resonance Microscopy*. Oxford: Oxford University Press.

Chudek, J. A. & Hunter, G. (1997). Magnetic resonance imaging of plants. *Progress in Nuclear Magnetic Resonance Spectroscopy* 31: 43–62.

Glidewell, S. M., Williamson, B., Goodman, B. A., Chudek, J. A. & Hunter, G. (1997). An NMR microscopic study of grape (*Vitis vinifera* L.). *Protoplasma* 198: 27–35.

Gray, N. E. (1956). A taxonomic revision of *Podocarpus* X. The South Pacific species of section *Eupodocarpus*, subsection D. *J. Arnold Arbor. Harvard Univ.* 37: 160–172.

MÖLLER, M., MILL, R. R., GLIDEWELL, S. M., WILLIAMSON, B. & MASSON, D. (1999a). Pollination drop mechanism and cone development of *Acmopyle pancheri* – present state of knowledge. *Proc. 4th Int. Conif. Conf. Wye, Kent, 23–27 August 1999*, pp. 35–36 (abstract).

MÖLLER, M., MASSON, D., MILL, R. R. *et al.* (1999b). Nuclear magnetic resonance imaging: a novel tool for systematics? *2nd Bienn. Conf. Syst. Assoc., The University of Glasgow, 23–27 August 1999*, p. 44 (abstract).

MÖLLER, M., MILL, R. R., GLIDEWELL, S. M., MASSON, D., WILLIAMSON, B. & BATEMAN, R. M. (2000). Comparative biology of the pollination mechanisms in *Acmopyle pancheri* and *Phyllocladus hypophyllus* (Podocarpaceae *s. l.*). *Annals of Botany* 86 (1): 149–158.

RATCLIFFE, R. G. (1994). *In vivo* NMR-studies of higher plants and algae. *Adv. Bot. Res.* 20: 43–123.

Received 15 March 2000; accepted with revision 16 June 2000