Non-invasive and non-destructive imaging of flower initiation and development of dormancy in red raspberry by NMR microimaging and *en bloc* optical sectioning

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1 Introduction

- In order to manipulate fruiting of raspberry outwith the main summer production season the developmental stage of the flower primordia and the state of dormancy within the overwintering bud must be accurately assessed
- Flower initiation and development of dormancy occur concurrently but independently in response to the lower temperatures and shortening daylengths of autumn.
- When buds become endodormant their water content is reduced as free water becomes bound to membranes (Faust et al, 1991). A period of chilling is then required to break dormancy, leading to an increase in free water.
- At SCRI NMR microimaging is being used to non-invasively monitor changes in water status and development of flower primordia in raspberry buds. To complement this and understand the structural changes within buds at both the macro and micro level buds are being optically imaged using a confocal laser scanning microscope (CLSM).

2 Materials & Methods

En bloc optical sectioning:

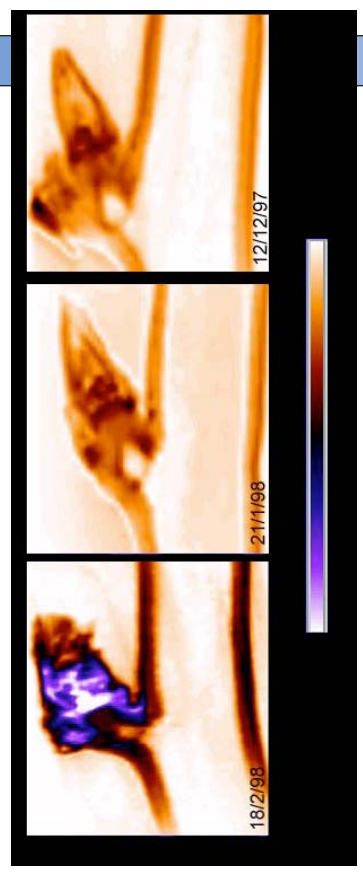
- Excised raspberry buds were fixed using 5% glutaraldehyde in PIPES fixative.
- The buds were stained using Safranin-O (0.01%) and embedded in Araldite resin (Prior et al, 1998).
- A Bio-Rad MRC 1000 CLSM was used to optically section the embedded raspberry buds.

NMR microimaging

- Measurements were performed with a Bruker AM300/WBFT spectrometer (7.1T).
- A 20mm coil was modified to accept a woody plant specimen by drilling out the base, so that the same bud and stem could be imaged through the winter.
- A series of 2D and unweighted 3D spin echo images were acquired using standard Bruker pulse sequences.

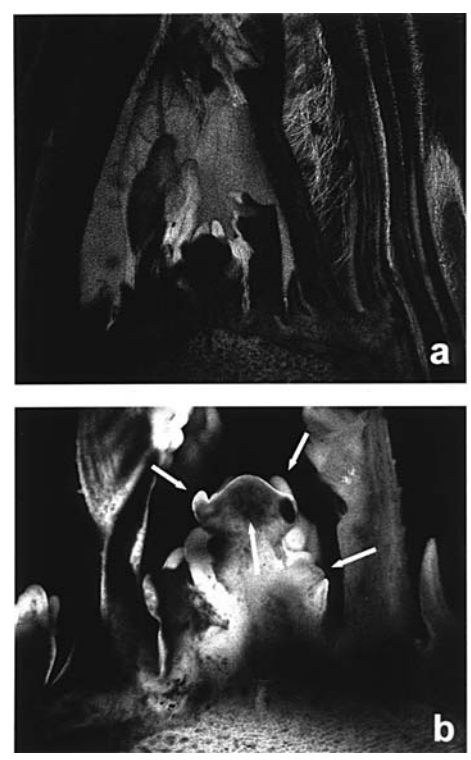
3 Results

Median longitudinal section of a bud taken with a Zeiss Tessovar macro camera. Terminal and axillary flowers can be seen developing at the centre of the image.



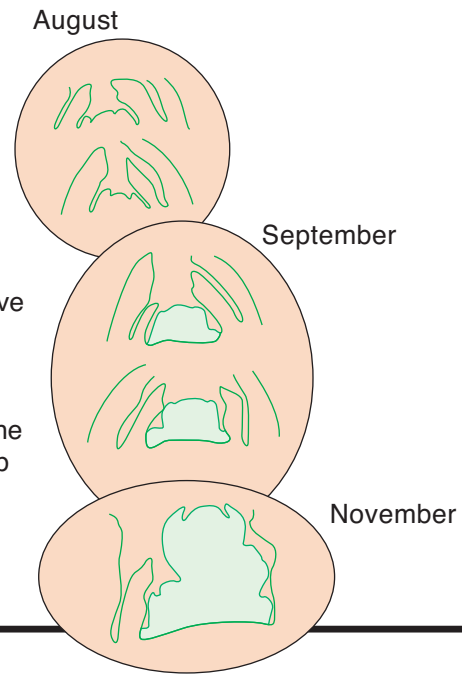
Sequence of NMR images of the same raspberry bud taken through the winter. A longitudinal slice taken from each 3-D data set is shown and has been colour coded on an arbitrary scale to show the increase in signal intensity as the chilling requirement of the bud is fulfilled and bound water in the bud is freed.

CLSM optical section through a resin embedded bud stained with safranin-O (a. x4 objective b. x10 objective). a) the terminal flower primordia can be seen in the centre, with developing leaves and protective bud scales to the right and left. b) terminal flower: arrows indicating the rounded torus and sepal rudiments, a secondary flower stem can be seen on the right of the terminal flower.



Flower initiation

Schematic representation of floral development in raspberry. The apical meristem changes from vegetative to reproductive as days shorten. The shape of the apex is modified and becomes domed and conical as the flower primordia develop



NMR images of a raspberry bud visualised using 3-D maximum intensity projections. Top left panel shows a 3-D image of bud, orthogonal views taken from the same data set in transverse and longitudinal planes are shown in the other panels.

4 Conclusions

- CLSM optical sectioning was found to be a simple and reliable method to visualise flower initiation and differentiation in raspberry buds. Clearly defined images at both the macro and micro level were obtained. This technique enabled flower primordia to be visualised even when obscured by overlaying structures, without the need for mechanical sectioning.
- Dormancy in raspberries is a dynamic process, and the use of NMR microimaging of water status in buds presents a powerful technique for direct, non-invasive, observation of developmental and temporal changes of internal structures in raspberry buds.

5 References

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6 Acknowledgements

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