# RESEARCH NOTES

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## In Situ Observations of Root-gall Formation Using Nuclear Magnetic Resonance Imaging[1]

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The study of root disease is a time consuming and laborious process. Reliable data on the dynamic, temporal, and spatial variation of this process are often unobtainable because of the inaccessibility of roots during disease development. As a result, speculation is often necessary in the description of pathogen activity in the rhizosphere. Because of these difficulties, progress in ecology and epidemiology of root disease has been slow.

Nuclear magnetic resonance imaging (NMR) is a new, nondestructive and noninvasive technique used to investigate metabolic processes and anatomical structure in biological material. NMR is based on the interaction between magnetic moments of atomic nuclei and magnetic fields and radiowave pulses generated in the laboratory. Protons placed in a strong magnetic field will align themselves parallel to that field. A radiowave pulse (rf) of a specific frequency can displace proton orientation to a degree proportional to the strength and duration of the pulse. The protons emit a unique rf pulse as they regain their original orientation. The time necessary to recover the undisturbed state is called the relaxation time. The excited nuclei interact with their surroundings or with the spin of adjacent protons. These interactions result in relaxation times which yield qualitatively different types of information and are called T1 and T2 relaxation times. This technology has been used to image sequential changes in plant structure and water flow and distribution in plant roots without disturbing the potting medium (2,3,9); however, NMR has not been applied to the study of disease in plants.

The purpose of this study was to evaluate NMR for observing the development of galls caused by *Meloidogyne incognita* without removing the roots from the potting medium or disturbing the rhizosphere.

Tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) seeds were planted in washed sand and grown in a greenhouse at 22 C. After 17 days, plants were transplanted to 3-cm-d conical, 11-cm-tall plastic tubes and filled with 600–850-μm-d sand. Plants were inoculated with 10,000 eggs of *M. incognita* (Kofoid & White) Chitwood. Eggs were recovered from tomato roots (7) and applied with a pipet. Inoculated and control plants were maintained in the laboratory under fluorescent lamps. NMR images using T1 and T2 relaxation times were acquired after 2, 4, 5, and 6 weeks of growth. Permanent, external markings on each tube maintained the exact orientation of the root system for imaging.

NMR images were obtained at 2.0 telsa using a General Electric NMR Instruments CSI system (Fremont, CA) modified for NMR microscopy (1,8). For this work, a 3.5-cm-d rf coil was built to provide cou-

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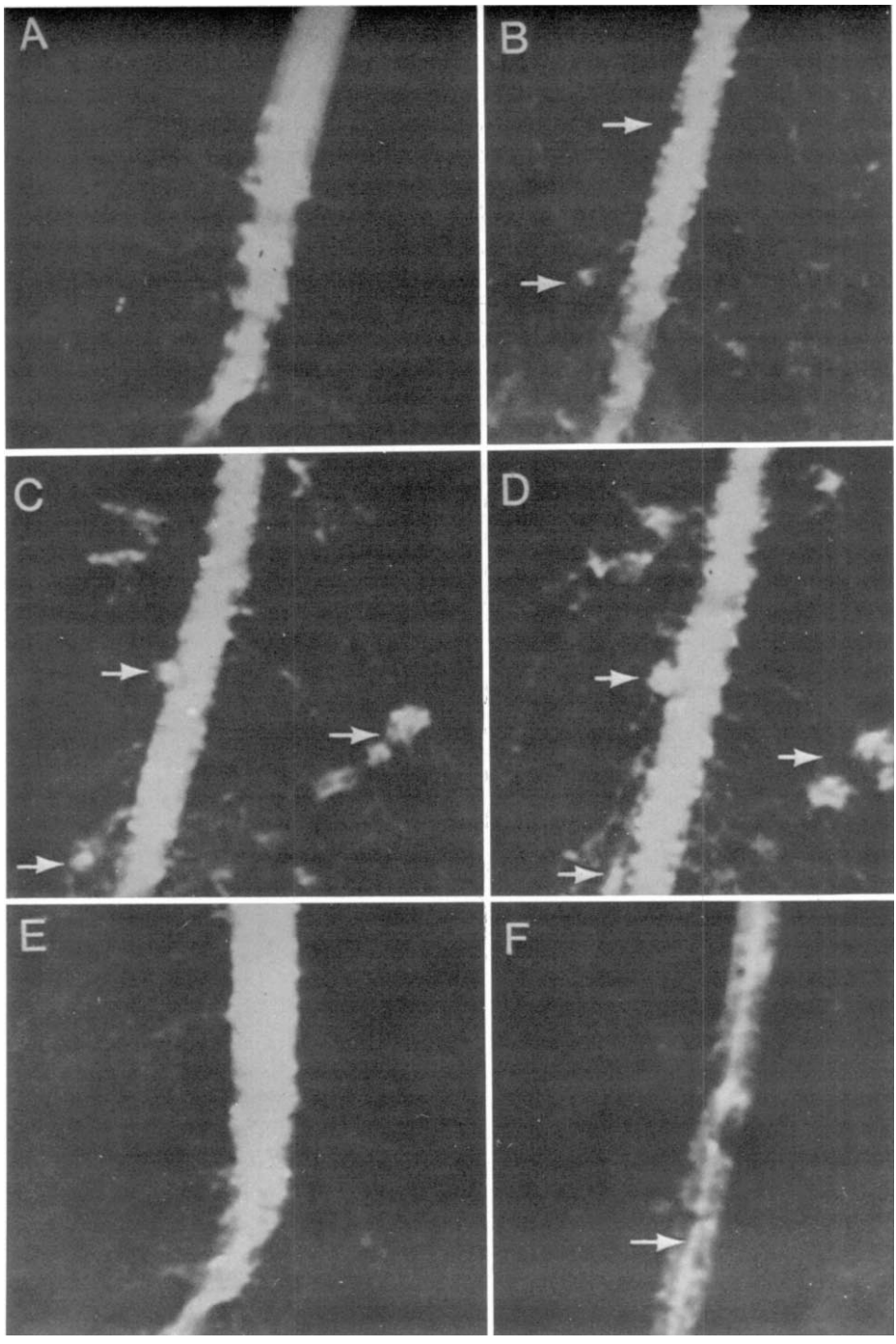


FIG. 1. Time series of nuclear magnetic resonance images of roots of *Lycopersicon esculentum* inoculated with eggs of *Meloidogyne incognita*. A) T1 image 1 week after inoculation. B) T1 image showing initiation of gall formation (arrows) occurring 2 weeks after inoculation. C, D) T1 images showing gall formation (arrows) at 4 and 6 weeks after inoculation. E) T1 image of uninoculated plant after 6 weeks. F) T2 image of the same root as in D showing vascular cylinder (arrow) (×1.07).

pling to the small samples and thereby improve the signal-to-noise ratio in the images. All images were acquired using the spin echo technique (4). Slice selection gradients normally used to define the 1–10-mm slices of most NMR tomographic images were combined. The resulting "thick slice" images were a projection of the entire diameter (3.5 cm) and length (5.0 cm) of the coil in a two-dimensional plane. A sagittal projection (i.e., a plane parallel to the axis of the plant) was used to produce an image of the entire root system. Images were reconstructed on a 256 × 256 matrix yielding individual picture elements (pixels) of 200 × 200 $\mu$m.

Plants were imaged using two different pulse sequences. The first, a T1 weighted or partial saturation sequence, used a repetition time (TR) of 400 msec between the rf pulse used for spatial encoding. The second timing parameter of the sequence, the echo time (TE), is the time between the 90° rf pulse and the spin echo formed by a subsequent 180° rf pulse. Increasing this time weights the image contrast with greater dependence on T2. In the partial saturation images TE was 25 msec. The second pulse sequence was a Carr-Purcell-Meiboom-Gill (CPMG) sequence. It employs an initial 90° rf pulse followed by a train of 180° refocusing pulses forming images with TE of 20, 40, 60, and 80 msec. The signal (S) in a pixel for the partial saturation of the CPMG sequence is given by the equation:

$$S = N(H)(1 - \exp(-TR/T1) * \exp(-TE/T2)$$

where N(H) is the proton density and the other terms have already been defined. Thus a short TE, or a short TR as in the partial saturation sequence, produces an image with contrast determined primarily by T1 and spin density. For this work, a TR of 2.0 was used in the CPMG sequence; this is sufficiently long to remove most of the T1 dependence. Thus the image formed at TE = 20 msec reflects the changes in relative spin density. Subsequent images at longer TE reflect increasing dependence on T2. Since many pathologies in clinical NMR have prolonged T1 or T2 (6), the developing galls might reflect similar behavior.

Images using T1 relaxation times after 2 weeks showed the initiation of gall formation (Fig. 1B). Subsequent (T1) images at 4 and 6 weeks (Fig. 1C, D) provided evidence of gall formation at these sites. Other galls, not observed until week 4, showed progressive development through week 6. Gall formation was confirmed by examination of the roots at the end of 6 weeks. NMR images did not exhibit morphological changes in the uninoculated plant (Fig. 1E), and no galls were found upon examination of those roots at the end of the incubation period. T2 images (Fig. 1F) resulted in visualization of vascular tissues.

NMR imaging can serve as a noninvasive method of observing root gall formation caused by *M. incognita*. T1 and T2 images allowed visualization of different morphological features. T1 images allowed visualization of morphology probably by showing water bound to membranes (Fig. 1A–D), whereas T2 images (Fig. 1F) had little surface detail but showed nonbound water, which would be the case in the plant's vascular elements. Other studies (5) demonstrate the use of $^{13}C$ and $^{31}P$ enrichment techniques which allowed the quantification and location of small inorganic molecules or organic macromolecules in intact organelles and membranes. Thus, proton and other types of NMR appear to have tremendous potential as noninvasive tools in the study of root pathology.

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