Nuclear magnetic resonance imaging of water distribution in the trunk and scaffold roots of 'Valencia' orange trees with and without citrus blight.

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NUCLEAR MAGNETIC RESONANCE IMAGING OF WATER DISTRIBUTION IN THE TRUNK AND SCAFFOLD ROOTS OF 'VALENCIA' ORANGE TREES WITH AND WITHOUT CITRUS BLIGHT.

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Fruits, Jul.-Aug. 1991, vol. 46, n° 4, p. 471-475.

ABSTRACT - Nuclear magnetic resonance (NMR) images of the trunks and two scaffold roots of two 16-year-old 'Valencia' orange, *Citrus sinensis* (L.) Osbeck, trees on rough lemon, *C. limon* L. Burm. f., rootstock, one affected by citrus blight, the other healthy, were obtained by scanning them axially and longitudinally with 1.5 Tesla and 4.5 Tesla nuclear magnetic resonance imaging (MRI) instruments. Citrus blight is a tree decline of unknown cause. Uneven image intensity throughout the trunks gave detailed pictures of internal structures, such as annual rings, and mobile water distribution. The healthy trunk had more mobile water in the center than the blighted trunk. The roots also had an area of high water concentration in the center, but the difference was less pronounced than in the trunks. There were no visible differences between healthy and blighted wood in sections made after the NMR scans.

UTILISATION DE LA RESONANCE MAGNETIQUE NUCLEAIRE POUR L'ETUDE DE LA DISTRIBUTION DE L'EAU DANS LE TRONC ET LES RACINES PRINCIPALES D'ORANGER VALENCIA LATE. COMPARAISON ENTRE ORANGERS SAINS OU ATTEINTS DE BLIGHT.

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Fruits, Jul.-Aug. 1991, vol. 46, n° 4, p. 471-475.

RESUME - Les images de troncs et racines obtenues par résonance magnétique nucléaire (RMN) d'orangers Valencia late *Citrus sinensis* L. Osbeck âgés de 16 ans et greffés sur Rough Lemon *Citrus limon* L. Burm. f., comparant des arbres sains ou atteints de Blight ont été obtenues par balayage transversal et longitudinal avec des appareils de visualisation à résonance magnétique nucléaire Tesla 1,5 et Tesla 4,5.

Le Blight est une maladie d'étiologie inconnue. Les clichés montrant des gradients d'intensité variable rendent en détail la structure interne des organes par exemple le long des cernes du bois pour le caractère mobilité de l'eau. Les arbres sains disposent d'une plus grande quantité d'eau mobilisable dans le centre du tronc. Il en va de même des racines bien que la différence soit moins prononcée par rapport au tronc. Il n'y avait aucune différence visible entre arbres sains et malades sur des sections de bois préparées après l'observation par RMN.

Citrus blight, first described in the 1860s, remains one of the most serious citrus production problems in most of the humid citrus areas of the world (Muller and Prates, 1982 ; Smith, 1974 ; Timmer *et al.*, 1986). Its cause is still unknown and opinions are divided if blight is an infectious (Derrick *et al.*, 1990 ; Timmer, 1990) or an abiotic disease (Wutscher, 1989 a). It has been called a wilt disease resulting from xylem dysfunction (Young and Garnsey, 1977 ; Young *et al*, 1980 ; Timmer *et al.*, 1986) because of plugs in the vessels, but it is not clear if the plugs are a primary or secondary effect. The water transport in blight-affected trees is disturbed (Cohen, 1974), but plugs in the xylem do not explain the inhibition of lateral water movement when water is injected into the trunk. There are no reliable visual symptoms of blight, and the oldest test for distinguishing blight from other tree declines is the injection of water into the trunk, either by gravity flow (Cohen, 1974 ; Cohen and Wutscher, 1977) or more recently, injection with a syringe (Lee *et al.*, 1988). Low water uptake indicates citrus blight, but absorption varies from month to month and with the prevalence of blight in the orchard (Wutscher, 1990). Accumulation of zinc in the outer trunk wood and the bark is another diagnostic feature of blighted trees (Albrigo and Young, 1981 ; Wutscher *et al.*, 1977), and large numbers of amorphous plugs in the xylem are also characteristic of blight (Cohen *et al.*, 1982 ; Brlansky *et al.*, 1984). Blight-specific proteins in the leaves (Bausher, 1990) and the roots (Derrick *et al.*, 1990) have recently been described. Water conductivity and content are uniform throughout the trunks of healthy citrus trees ; except for a small inner core, the whole trunk participates in water transport (Cohen, 1979). The contention that

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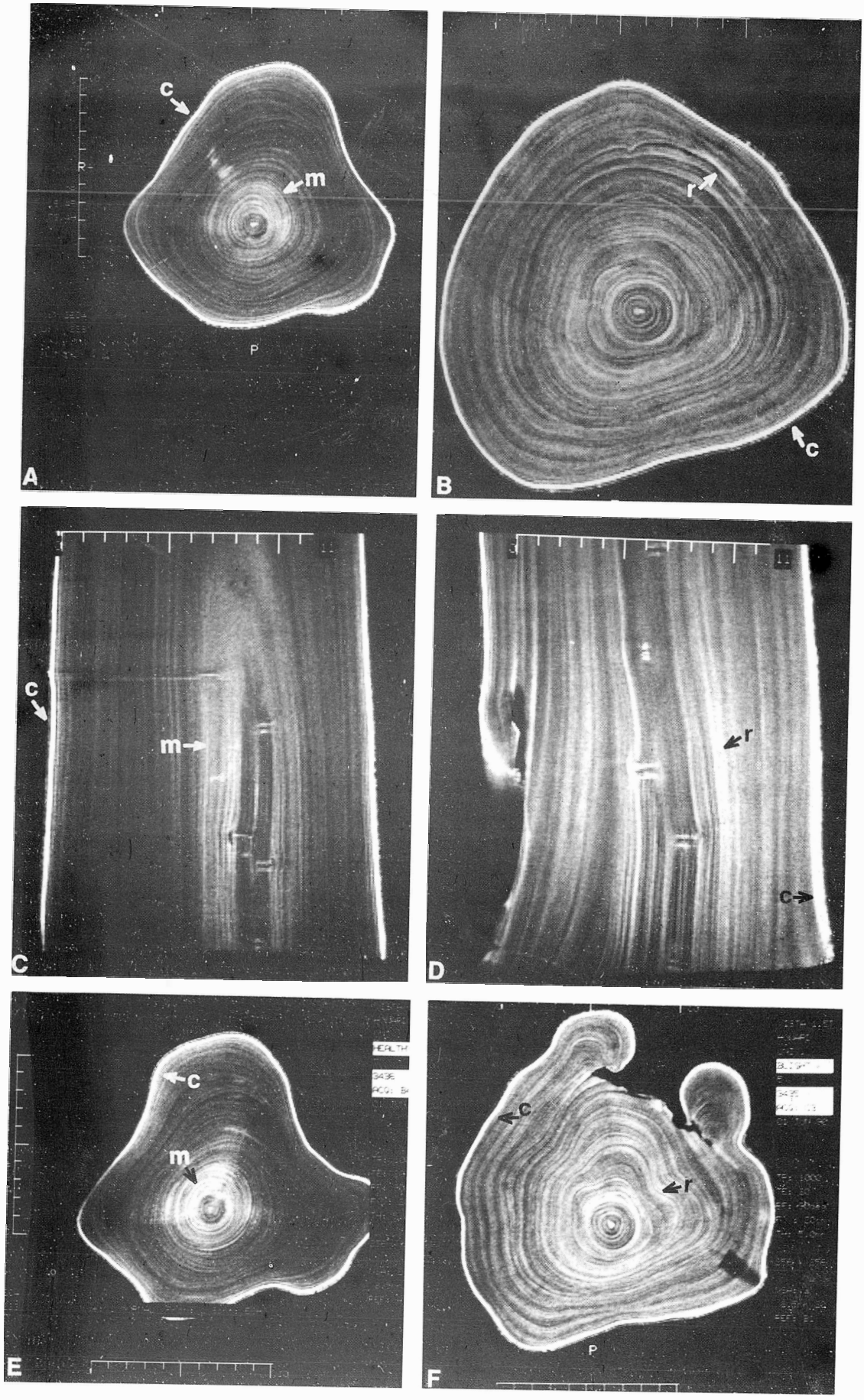

FIGURE 1.

A) Cross section, upper trunk, healthy.
B) Cross section, upper trunk, blighted.
C) Longitudinal image, mid-trunk, healthy.
D) Longitudinal image, mid-trunk, blighted.
E) Cross section, lower trunk, healthy.
F) Cross section, lower trunk, blighted.

m Area with high water concentration in the center of the trunk.
c Cambium.
r Annual rings with a high water content

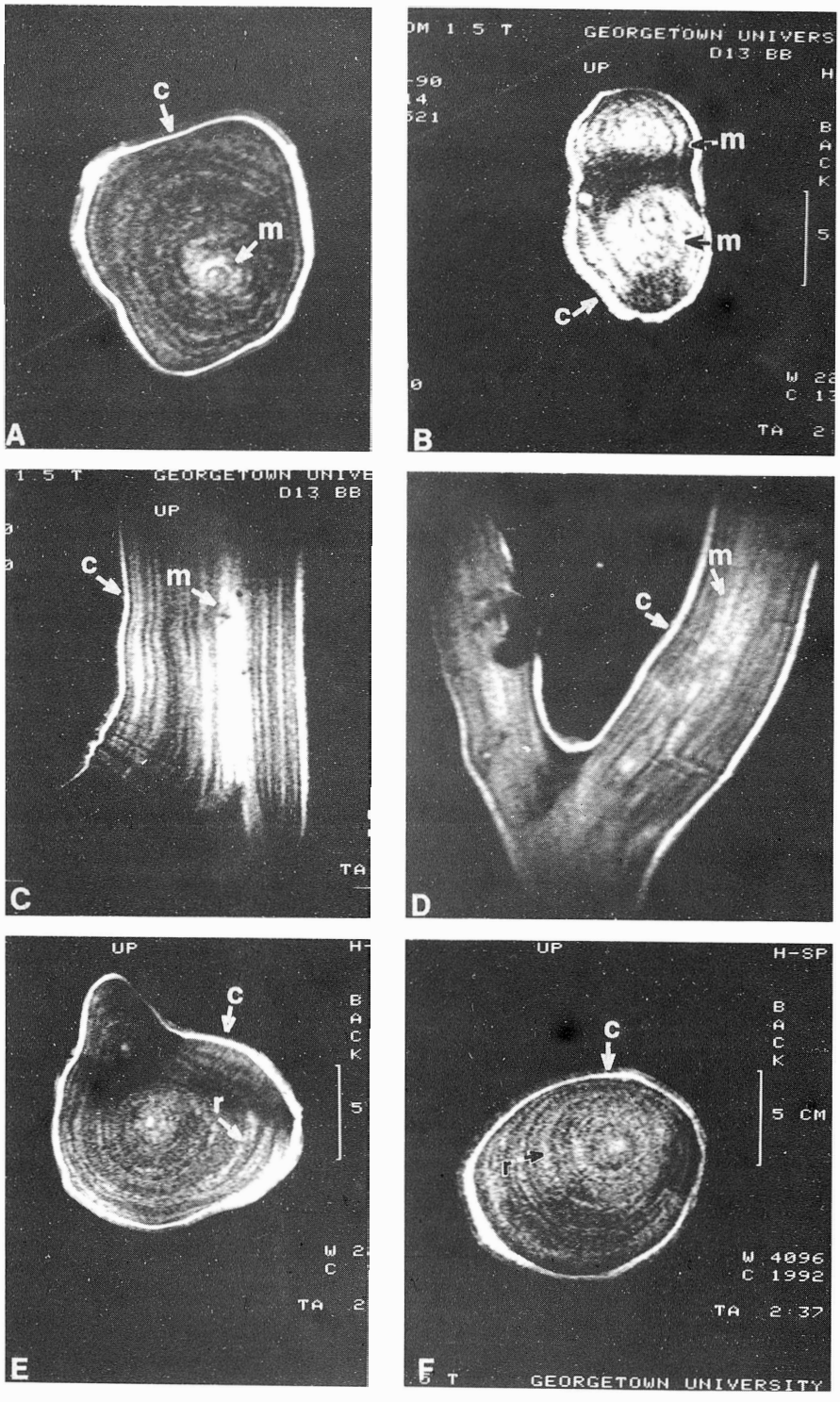

FIGURE 2.

A) Cross section, root, distal end, healthy.
B) Cross section, root, distal end, blighted.
C) Longitudinal image, root, healthy.
D) Longitudinal image, root, blighted.
E) Cross section, root, proximal end, healthy.
F) Cross section, root, proximal end, blighted.

m Area with high water concentration in the center of the trunk
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citrus, like many other trees, forms true heartwood (Nemec, 1975) is difficult to sustain. With blight, water conductivity of the inner wood is reduced and plugs are found throughout the cross sections, except in the outermost wood, which seems to have normal conductivity (Young and Garnsey, 1977 ; Cohen, 1979). The total water content of wood, determined by drying samples, is the same in blighted and healthy trees (Wutscher and Hardesty, 1979). The studies of water conductivity of the wood were done by destructive sampling, mostly extracted wood cores (Cohen, 1979 ; Young and Garnsey, 1977). Nuclear magnetic resonance imaging (NMR), which by means of static and radio-frequency magnetic fields permits mapping of mobile water distribution in entire parts of trees, has been shown to be feasible with forest trees (Chang *et al.*, 1989 a ; Chang *et al.*, 1989 b ; Wang and Chang, 1986 ; Wang *et al.*, 1989). It seemed appropriate to try this technique on blight-affected citrus trees.

One blighted and one healthy 'Valencia' orange, *Citrus sinensis* (L.) Osbeck, tree on rough lemon, *C. limon* Burm. f., rootstock, growing within 10 m of each other in a 16-year-old commercial citrus grove near St. Cloud, Florida, were selected, using water injection (Lee *et al.*, 1984) and zinc analysis of the wood (Wutscher *et al.*, 1977) to verify their status. The blighted tree absorbed 0.6 ml water/min in syringe injections and had 17 ppm Zn in the outer trunk wood ; the respective values for the healthy tree were 50 ml/min and 3 ppm. The trees were cut down with a chain saw at 11:30 a.m. on May 29, 1990. The trunks between the budunion and the scaffold limbs (blighted tree : 40 cm long, tapering from 19 to 15 cm diameter ; healthy tree : 45 cm long, tapering from 14 to 13 cm diameter) were removed and two of the main roots of each tree (blighted : 50 and 47 cm long, 3.2 and 2.7 cm diameter ; healthy : 58 and 46 cm long, 5.0 and 3.9 cm diameter) were cut off. All cut surfaces were immediately covered with paraffin to prevent drying out and they were subjected to nuclear magnetic resonance imaging less than 24 hours after they were collected.

The trunks were imaged with a 1.5 Tesla Picker MRI Scanner (Picker International, Highland Heights, OH) using a spin-echo technique (Wang *et al.*, 1989). The variation of the NMR signals is caused by the uneven distribution of water throughout the samples, differences in water mobility, and the NMR relaxation times. An image is reconstructed based on these differences and details of internal structure are revealed. In our experiments, the repetition time was 1000 μ sec and the echo time was 20 μ sec. The field-of-view was 20 cm, the spatial resolution 0.78 mm, and the slice thickness 5 mm.

The roots were imaged by a 4.7 Tesla SIS Co. system (Spectroscopy and Imaging Systems, Fremont, CA). A spin-echo imaging technique as described above was used, but this instrument has a stronger magnetic field and a stronger NMR signal and gives better image resolution. It cannot be used on large samples, such as tree trunks. The repetition time was 1.11 or 1.89 sec and echo time was 20 μ sec. The spatial resolution was 0.78 X 0.20 mm and the slice thickness 1.2 mm.

The NMR images clearly show details of the internal structure of the trees, such as annual rings and the cambium under the bark (Fig 1 A, B, C, D, E, F). In the healthy trunk, there was a high intensity region covering about one-third of the cross section, implying high water content, high water mobility, and a shorter T1 NMR relaxation time. In contrast, image intensity was rather homogenous and low in the blighted trunk, and the images of annual rings were more distinct, indicating a greater difference in the consistency of the wood laid down in the spring and in the fall. The trunk areas showing low water activity in blighted trunks were those where Cohen (1979 ; Cohen *et al.*, 1982) found reduced conductivity and where there are many xylem plugs (Brlansky *et al.*, 1984 ; Timmer *et al.*, 1986). Water appears to be distributed throughout blighted trunks in a striated pattern (Fig. 1 D). The total water content of the outer (2.5 cm) wood of blighted and healthy trees is similar (Wutscher and Hardesty, 1979), the NMR images show that the differences in water content appear to be limited to the inner wood. Longitudinal (sagittal) images (Fig. 1 C) show the high water concentration in the center of the healthy trunk very clearly.

The root images of the healthy and blighted trees were similar (Fig. 2 A, B, C, D, E, F). There was a region of high image intensity in the center of the roots that was more pronounced in the healthy roots, as in the healthy trunk. The image differences between the blighted and the healthy roots were more pronounced in the distal parts (Fig. 2 A, B) than in the proximal parts (Fig. 2 E, F). No visible differences between blighted and healthy wood were discernible when the wood was cut longitudinally after NMR imaging.

NMR imaging is the only method for showing actual water distribution in the wood *in vivo*. The images obtained in this experiment show abnormal distribution of mobile water throughout the trunk of the blighted tree, water concentrated in the annual rings, a pattern distinctly different from the largely dark image of the healthy trunk, indicating the lower moisture content of normal wood (Chang *et al.*, 1989 a). There could have been redistribution of water when xylem sap tension was released when the trees were cut. Water stress could be avoided by cutting the trees before sunrise and replication would be desirable, but the logistics of bringing the material to the instrument and the high cost involved are prohibitive. Similar experiments with forest trees have all been done with single logs (Wang and Chang, 1986 ; Wang *et al.*, 1989 a ; Chang *et al.*, 1989 b).

ACKNOWLEDGMENTS

The authors greatly appreciate the donation of trees by Mr. Orie Lee, St. Cloud, Florida, for the experiment described.

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UTILIZACION DE LA RESONANCIA MAGNETICA NUCLEAR PARA EL ESTUDIO DE LA DISTRIBUCION DEL AGUA EN EL TRONCO Y LAS RAICES PRINCIPALES DEL NARANJO VALENCIA LATE - COMPARACION ENTRE NARANJOS SANOS O AFECTADOS DE BLIGHT.

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Fruits, Jul.-Aug. 1991, vol. 46, n° 4, p. 471-475.

RESUMEN - Las imágenes de troncos y raíces obtenidos por resonancia magnética nuclear (RMN) de naranjos Valencia late *Citrus sinensis* L. Osbeck de 16 años de edad e injertados sobre Rough lemon, *Citrus limon* L. Burm. f., comparando árboles sanos o afectados de Blight fueron obtenidos por barrido transversal y longitudinal con aparatos de visualización de resonancia magnética nuclear Tesla 1,5 y Tesla 4,5.

El Blight es una enfermedad de etiología desconocida. Los cliches que muestran gradientes de intensidad variable presentan en detalle la estructura interna de los órganos por ejemplo a lo largo de los anillos de la madera para la característica mobilidad del agua. Los árboles sanos disponen de una mayor cantidad de agua mobilizable en el centro del tronco. Lo mismo acontece en las raíces, aun cuando la diferencia es menos pronunciada en relación al tronco. No había ninguna diferencia visible entre árboles sanos y enfermos en las secciones de madera preparadas después de la observación por RMN.