Communication

Xenobiotic Monitoring in Plants by ^{19}F and ^{1}H Nuclear Magnetic Resonance Imaging and Spectroscopy

Uptake of Trifluoroacetic Acid in *Lycopersicon esculentum*

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ABSTRACT

^{19}F and ^{1}H nuclear magnetic resonance imaging and spectroscopy have been used to monitor the uptake of trifluoroacetic acid in stems and leaves of *Lycopersicon esculentum*. The movement and location of a xenobiotic have been demonstrated *in vivo* by a noninvasive technique.

Contemporary methods used in the study of metabolism and uptake of xenobiotics, such as herbicides, by whole plants, generally involve destruction of the plant. Typically, radiolabeled xenobiotics are administered and are located following dissection of the plant. The products of metabolism of the administered compounds are identified by chromatography, ultraviolet or infrared spectroscopy, or mass spectrometry (9).

Short-term biochemical changes induced by the xenobiotic are studied using excised plant tissues, organs, and cells, and the results require careful extrapolation to whole plants because of, for example, senescence.

The increasing use of NMR spectroscopy and, particularly, NMR imaging in animal physiology and medicine (4, 7, 8), has led to the possibility of using this noninvasive technique in the study of uptake and metabolism in plants. Uptake of water in roots of *Vicia fabia* seedlings has been observed using water doped with a paramagnetic agent (copper sulfate) (2), and changing water content during relatively rapid transpiration has been studied in root systems of *Pelargonium hortorum* (3).

The purpose of the present study was to investigate the utility of NMR for observing uptake and localization of xenobiotics, such as herbicides, *in vivo* in plants. NMR is noninvasive, so the same plant can be used repeatedly, and successive measurements can be made at intervals of a few minutes. The xenobiotic can be located using imaging techniques, and any metabolism detected using chemical shift information.

Previous NMR studies in whole plants have made use of the ^{1}H nucleus (2, 3). We have used ^{19}F in the present experiments, however, for the following reasons: (a) many xenobiotics (especially herbicides and pesticides) contain fluorine and yet the background fluorine in plants is negligible; (b) ^{19}F is present at 100% natural abundance; (c) ^{19}F has a similar gyromagnetic ratio to ^{1}H; thus, the nucleus has 83% of the sensitivity of ^{1}H to the NMR experiment and, as an additional benefit, the same equipment can be used for both nuclei. By tuning the probe to the fluorine frequency it is then necessary to change only the transmitter frequency and preamplifier (11).

In this preliminary study, the uptake and localization of TFA, a mildly herbicidal compound which is known to be xylem mobile (5), was investigated in tomato plants.

MATERIALS AND METHODS

Lycopersicon esculentum var Ailsa Craig were grown from seeds under greenhouse conditions (25°C day, 20°C night) in John Innes No. 1 compost. Spectroscopy and imaging experiments were performed using 6 and 10 week old plants, respectively. The day before each experiment the plants were watered as usual to keep the soil just moist. Five min before the experiment was set up, the compost was carefully removed, and the plant roots were washed to remove residual soil which might contain magnetic components. The roots were placed in a glass vessel (2.5 cm diameter) containing the xenobiotic solution (5 mL) with the spectroscopy coil surrounding either the stem or one leaf from the first true leaves.

The TFA solutions were buffered in 4 mm sodium phosphate adjusted to pH 6.9 with 2 m KOH solution. The solutions used for spectroscopy and imaging were 0.45 and 1.8 m in TFA, respectively.

NMR was performed on an Oxford Research Systems 'BIOSPEC' with a 4.7 T/15 cm horizontal bore magnet. A small, 5 turn solenoidal, transmit/receive RF coil of diameter 8 mm, length 8 mm, positioned at the magnet center, was used for spectroscopy and imaging at both the ^{1}H and ^{19}F resonance frequencies (200.4 and 188.2 MHz, respectively). ^{19}F spectra were recorded at 15 min intervals, and each

spectrum was accumulated with 10 min of signal averaging (typically 120 signals averaged with 5 s repeat time). Each experiment was run for a total of 6 to 15 h, overnight at 25°C in constant artificial lighting conditions.

The approximate final concentrations of fluorine in leaves and stems were calculated at the end of each experiment. Fluorine content of tissue in the coil was estimated by comparing peak intensities with those of standards of buffered TFA of known concentration using the same acquisition parameters. The tissue enclosed in the coil was then excised and weighed, and concentrations of TFA (in μmol/g fresh tissue) were calculated.

RESULTS AND DISCUSSION

Figure 1 shows a typical series of ^{19}F spectra monitoring uptake of TFA in the plant stem. The concentration of TFA in the stem rises according to a sigmoidal curve leveling off at about 6 h. The results from repeated experiments under identical conditions are shown graphically in Figure 2. The final concentration of xenobiotic in the stem was 10 to 20 μmol/g of fresh tissue (Table I).

Figure 3 shows a typical series of ^{19}F spectra monitoring uptake of TFA in the youngest leaves (first 4 h). As expected, the leaves contained less fluorine than the stems in the early part of the experiment (first 2 h) but, whereas the concentration of fluorine in the stem reached a plateau, that in the leaves continued to rise over 12 h (Fig 4). The final concentration was over 80 μmol/g of tissue (Table I).
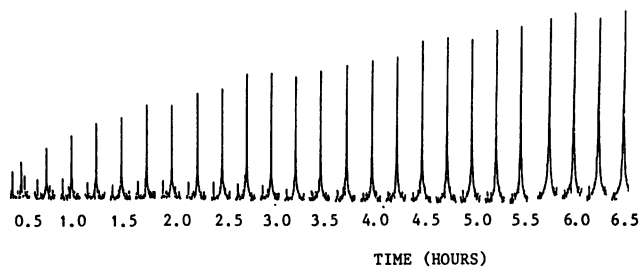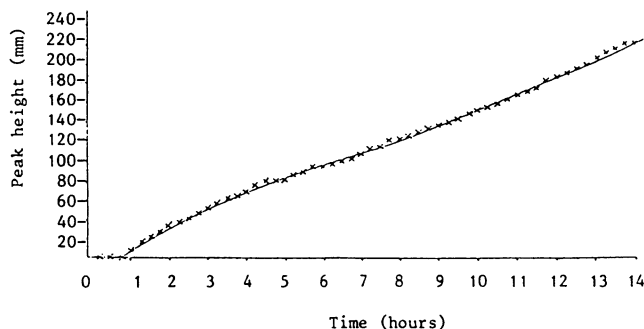

Figure 1. Typical results of ^{19}F NMR spectra monitoring uptake of TFA in the plant stem.
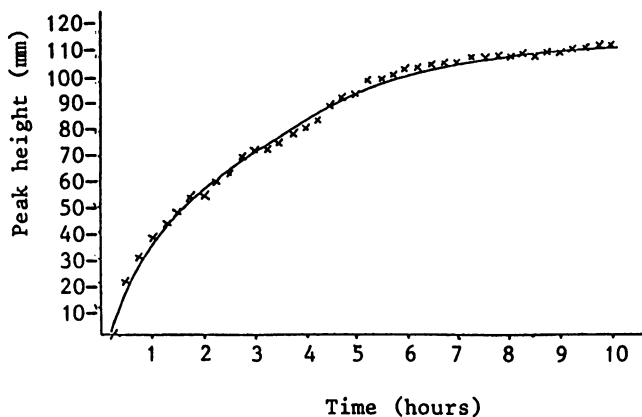
Figure 2. Averaged results of variations in peak heights of ^{19}F spectra with time, monitoring uptake of TFA in plants stem.
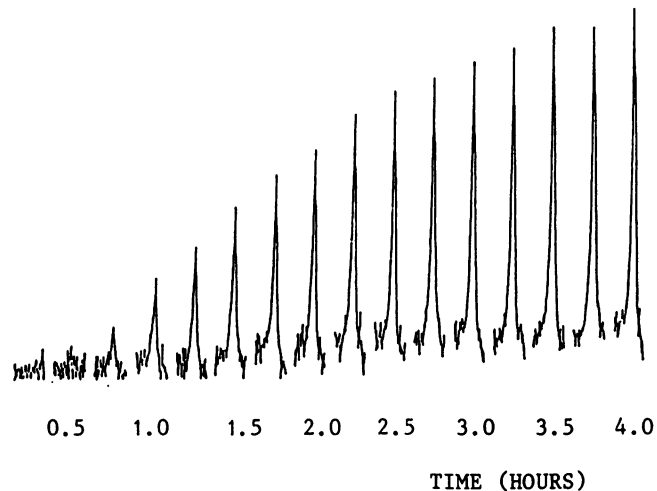
Figure 3. Typical results of ^{19}F NMR spectra monitoring uptake of TFA in youngest leaves.

Figure 4. Averaged results of variation in peak heights of ^{19}F spectra with time, monitoring uptake of TFA in youngest leaves.

These results show that TFA is transported through the stem and accumulates in the leaves, where it reaches very high concentrations. The leaves could be the target areas for herbicidal activity. The plants were very wilted after about 12 h, suggesting imminent plant death and explaining the slow increase in fluorine content of the leaves from 12 to 17 h. Plants which were transferred from the TFA solution to water after 12 h could not be revived.

It is notable that the chemical shift of the ^{19}F peak remained constant throughout the experiment and no additional peaks were observed. There was some evidence of line-broadening over the time period, but that may be accounted for by instrumental drifts. This shows that no extensive metabolism of the xenobiotic had occurred. However, the peaks are quite broad (line widths were about 850 Hz in leaves, 100 Hz in stems) and small changes in chemical shift, resulting from processes such as esterification, might not be observable. For example, the separation of ^{19}F lines due to TFA and its methyl ester is, at pH7, about 500 Hz. This would not easily be detected in leaves. It may be possible to reduce the line widths by controlling the plant growth medium (*e.g.* using hydroponics) so as to eliminate excess paramagnetic ions.

Three images of lower plant stems were obtained. Figure 5A shows a ^{1}H image of an untreated stem. The thin slice

Table I. *Concentrations of Trifluoroacetic Acid Accumulating in Stems and Leaves of Tomato Plants after Application of 0.45 M TFA*

All measurements were made immediately after application of TFA was completed.

Plant	Time of Application of TFA	Position of Coil	TFA Concentration
	h		*μmol/g fresh tissue*
1	4.00	Youngest leaves	11.80
	4.00	Stem (2 cm from root/stem interface)	7.63
	4.00	Branch of first true leaf	4.65
2	14.00	Youngest leaves	84.80
3	17.00	Youngest leaves	84.00
	2.50	Stem (2 cm from root/stem interface)	8.50
	8.50	Stem (2 cm from root/stem interface)	19.70
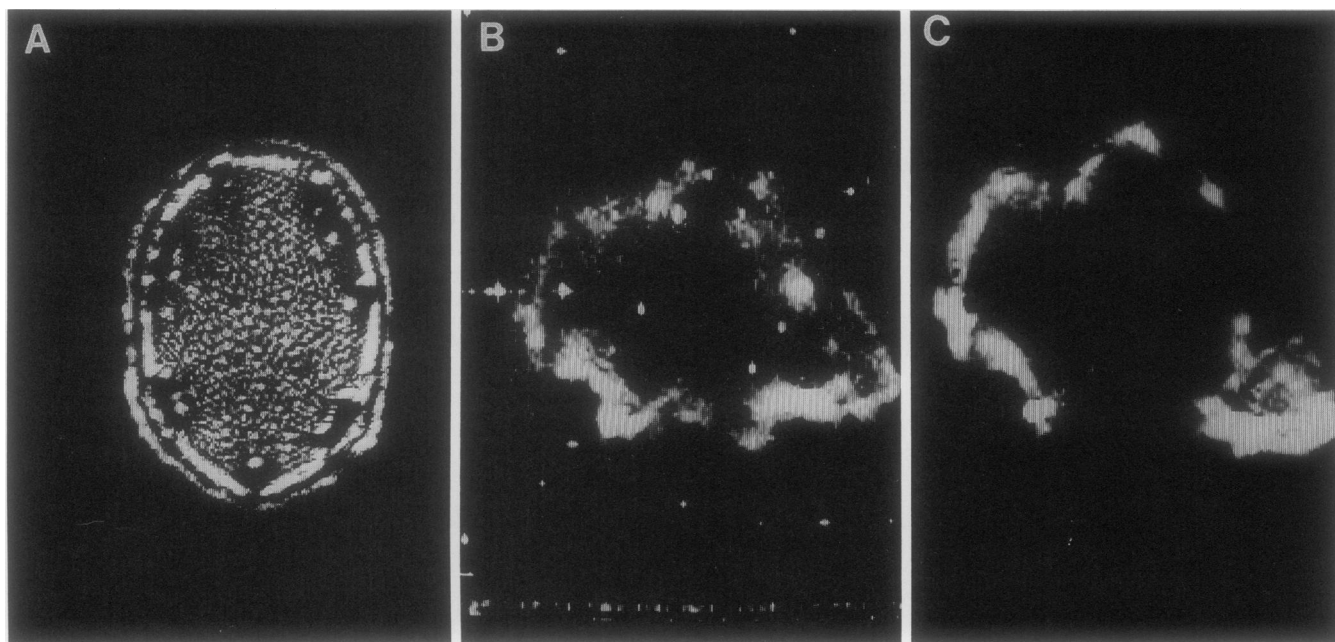
Figure 5. NMR images of plant stems: A, ^{1}H image of an untreated stem; B, ^{19}F image of a stem after treatment with TFA; C, ^{1}H image of the same stem taken a few minutes later.

selection (2 mm) allows the identification of several structures: epidermis, cortex, pith, and the vacuolar system as expected for dicotyledonous stem anatomy (10).

Figure 5B shows the ^{19}F image of the same part of the plant stem after treatment for 12 h with 1.8 M TFA. The star-shaped pattern shows distrubution of the fluorine in the vascular tissues, the brightest areas corresponding to the greatest concentrations of fluorine in this cross-section.

Figure 5C shows the same cross-section, observing ^{1}H immediately after the image shown in 5B was taken. The plant was wilting and only the water in the vascular tissues is observed (*cf.* image 5A). The same star-shaped pattern of vascular tissue is seen. This star-shaped image is a familar pattern seen in dicotyledenous vascular systems. Young stems are nearly circular in cross-section and contain three large bundles alternating with three small bundles of vascular tissue. In older plants and higher internodes these form a more continuous circle (10).

The ^{19}F image shows that the xenobiotic is located in discrete regions of the stem, as expected since TFA is a xylem mobile herbicide. The spectroscopy results show that the herbicide is transported through the stem and accumulates in the leaves. Despite the very high concentrations of herbicide used in both experiments, there seemed to be little or no lateral distribution into the phloem or the lumen of the stem. Any mass movement of TFA in the phloem would result in redistribution of the herbicide from the leaves throughout the plant—this was not observed.

CONCLUSION

The applications of NMR imaging and spectroscopy in the study of biological systems are increasing rapidly (6). In this paper we describe experiments which demonstrate the possibility of using these techniques to study the movement and

distributions of xenobiotics non-invasively in plants. In addition, successful differentiation between anatomical structures of the stem to a resolution of 40 μm has been demonstrated *in vivo*. NMR imaging of single cells is possible using somewhat more powerful instrumentation (1).

The utility of *in vivo* NMR in this type of work may ultimately be limited by the inherent insensitivity of the technique. Exceptionally high concentrations of xenobiotic were used in this preliminary study, and we expect to be able to obtain good results at much lower levels; nonetheless, it seems unlikely that NMR will, in the near future, be of use in detecting the very low concentrations of modern herbicides which are used in spraying. As this study demonstrated, however, high resolution NMR imaging can be used to confirm the distribution of xenobiotics, suggesting that it may be possible to develop NMR imaging methods to confirm the validity of NMR spectroscopy results.

The method particularly lends itself to the very important area of translocation studies of herbicides in whole plants. The techniques widely used are destructive, involving the use of radiolabeled herbicides. The plants are dissected and the herbicide located; it is particularly difficult to identify breakdown products. *In vivo* NMR has great potential in identifying the products of herbicide degradation.

ACKNOWLEDGMENTS

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