Environ. Sci. Technol. 2005, 39, 3695–3702

Direct Observation of Organic Contaminant Uptake, Storage, and Metabolism within Plant Roots

EDWARD WILD,* JOHN DENT, GARETH O. THOMAS, AND KEVIN C. JONES

Departments of Environmental and Biological Sciences, Lancaster University, Lancaster, LA1 4YQ, UK

Two-photon excitation microscopy (TPEM) is used to visualize and track the uptake and movement of anthracene and phenanthrene from a contaminated growth medium into living unmodified roots of maize and wheat over a 56-day period. The degradation of anthracene was also directly observed within the cortex cells of both species. The power of this technique is that neither the plant nor the compound require altering (staining or sectioning) to visualize them, meaning they are in their natural form throughout the experiment. Initially both compounds bound to the epidermis along the zone of elongation, passing through the epidermal cells to reach the cortex within the root hair, and branching zones of the root. The PAHs entered the epidermis radially; however, once within the cortex cells this movement was dominated by slow lateral movement of both compounds toward the shoot. Highly focused "streams" of compound were observed to form over time; zones where phenanthrene concentrated extended up to 1500 μm in length over a 56-day period, for example, passing through several adjoining cells, and were detectable in cell walls and cell vacuoles. Radial movement was not observed to extend beyond the cortex cells to reach the vascular tissues of the plant. The longitudinal movement of both compounds was not observed to extend beyond the root base into the stem or vegetative parts of the plant. The lateral movement of both compounds within the cortex cells was dominated by movement within the cell walls, suggesting apoplastic flow through multiple cell walls, but with a low level of symplastic movement to transport compound into the cellular vacuoles. Degradation of anthracene to the partial breakdown products anthrone, anthraquinone, and hydroxyanthraquinone was observed directly in the zones of root elongation and branching. The technique and observations have important applications to the fields of agrochemistry and phytoremediation.

Introduction

Understanding the uptake of soil-bound xenobiotics by plants and discerning the behavior of these chemicals within the root, shoot, and leaf have key implications for exposure pathways, contaminated land assessment, and phytoremediation (*1*–*3*). Food consumption represents a major source of xenobiotic contamination for humans, with plant-based foods being a major proportion of dietary exposure (*4*). It is therefore important to understand exactly how chemicals are transported into or bind to the plant root system to enter the human food chain. Phytoremediation aims to utilize plants to cleanse or amend polluted soils, to achieve complete mineralization of the compound to nontoxic end products, through plant-initiated biochemical processes (*3*). This is considered particularly important for relatively recalcitrant or persistent organic pollutants (POPs), such as the polynuclear aromatic hydrocarbons (PAHs), which are resistant to degradation in soils (*5*). The complexity of plant physiology and biochemistry may give certain plants a greater potential for phytoremediation (*3*).

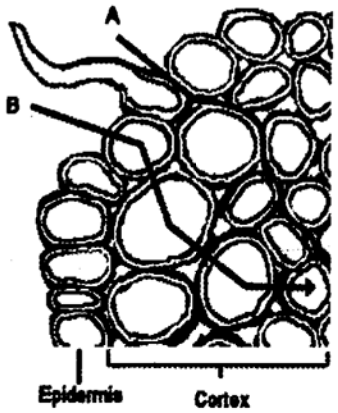


FIGURE 1. Pathways of water movement across the root epidermis and cortex. Apoplastic movement (A) can occur through cell walls and intercellular spaces. Symplastic movement (B) can occur through cell cytoplasm and plasmodesmata, or transcellularly across cell membranes.

Organic compound uptake by roots has been widely studied (*6*–*11*). However, the fundamentals of how specific chemicals, such as various families of POPs, move into root systems through the apoplastic and symplastic pathways (see Figure 1) and the mechanisms involved in these movements are little understood (*6*). Apoplastic water movement involves diffusion between cell walls, not entry into the cells, while symplastic movement is through the cell cytoplasm or vacuoles and to interconnected cells via the plasmodesmata. Indeed, our basic knowledge of where/how organic contaminants are stored or degraded within plants is still very limited (*7*, *9*, *10*). For these reasons, it is often not clear whether phytoremediation is a viable approach to soil cleanup (*3*) and—if it is—whether enhanced rates of compound loss occur *directly*, because of plant uptake/degradation, or *indirectly*, because of enhanced soil microbial activity, which has been stimulated by the presence of plants (*12*). Hence, it is important to understand exactly how and where POPs uptake/storage/degradation occurs: is it within the root, bound at its surface, or within the dynamic region of the rhizophere?

Conventional contaminant extraction of plant tissues or carbon-14-labeling techniques have led to the following general observations about POP uptake/processing. (i) Compound uptake is broadly determined by the physicochemical properties of the chemical and the structure/properties of the plant (*8*, *10*). (ii) Soil-bound POPs, such as PAHs, are strongly associated with soil organic matter and inefficiently transferred to plant roots (*5*, *8*, *10*). (iii) POPs are poorly translocated, remaining in below-ground plant parts (*8*–*10*). PAHs and PCDD/Fs have been found primarily in the peel of carrots grown in contaminated soil, for example, rather than the inner core (*6*, *8*, *9*). However, it is not known

* Corresponding author phone: 44 01524 593300; fax: 44 01524 593985; e-mail: e.wild@lancaster.ac.uk.

10.1021/es048136a CCC: $30.25
Published on Web 04/09/2005

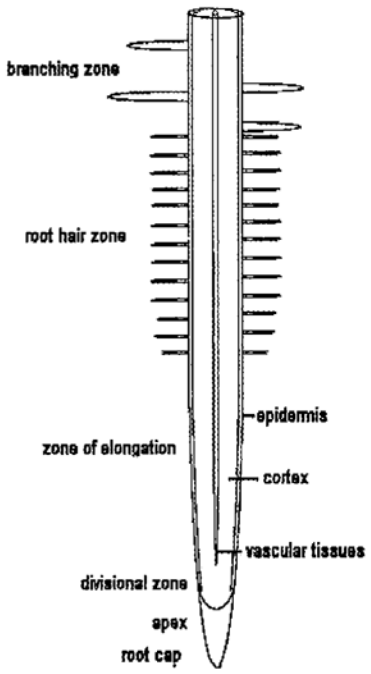


FIGURE 2. Schematic of a root showing the root cap, apex, divisional zone, zone of elongation, root hair zone branching zone, epidermis, and cortex.

from existing studies if these compounds are adsorbed to the surface or bound within the root cell structure. (iv) The main pathway for POP accumulation in leaves is believed to be from the atmosphere (*13*, *14*). (v) the plant lipid content may have a significant effect on the storage capacity of plants for POPs (*1*).

The growing root represents a linear sequence of cell functional differentiation—from the root cap to the branching zone—with respect to substrate uptake, cell growth, and cell structure and function (see Figure 2). Key structural components of root cells, such as cellulose, proteins, hemicellulose, lignin, and pectin, differ in proportion/volume along a developing root. It is hypothesised that the progressional change in cell structure from the root tip to the branching zone is likely to affect the uptake and processing of POPs.

A new technique is now available that can help shed light on these issues. We have recently pioneered the use of plant and chemical autofluorescence and two-photon excitation microscopy (TPEM) techniques that enable direct in situ visualization of the movement of compounds that autofluoresce inside living plant tissues. Our previous work reported on the diffusion of PAHs into living plant leaves and their localization within the leaf structure (*15*) and monitored PAH photodegradation on and within living leaves in real time (*16*). This study extends the application of TPEM to monitor the uptake, location, storage, and degradation of anthracene and phenanthrene in the roots of maize and wheat, during a 56-day growth study.

Materials and Methods

Maize (*Zea mays*) and wheat (*Triticum aestivum*) plants were grown for 1−56 days in a sand culture spiked with either phenanthrene or anthracene, to assess their uptake, storage location, and metabolism within the root system.

Compound Generation. Anthracene and phenanthrene were obtained from Aldrich Chemical Co. at a purity of 99.9%. Some of the breakdown products of anthracene (see Figure 3)—anthrone, anthraquinone, 1,4-dihydroxyanthraquinone, phthalide, and phthalic anhydride—were obtained from Acros Organics at a purity of 98% p.a, 96%, 99%, and 99%, respectively. Each compound (2.5 mg) was placed in a 10 mL

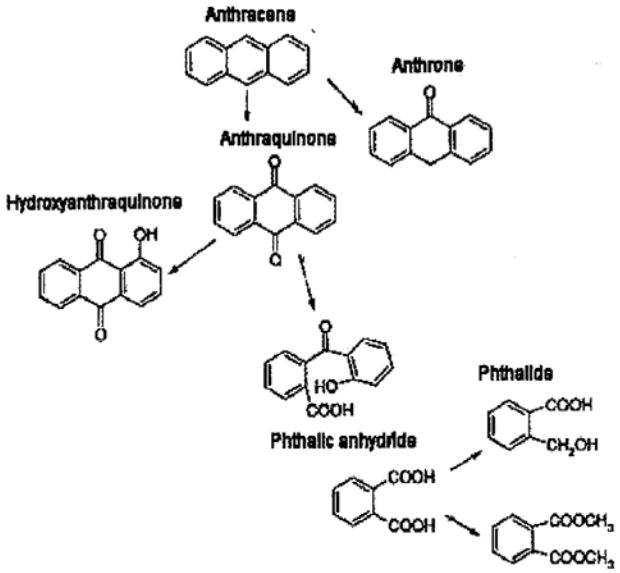


FIGURE 3. Pathways proposed for anthracene degradation. Adapted from ref 17.

vial with 10 mL of acetone. The vial was placed in an amber container, to reduce any risk of photodegradation. This was placed in a Branson 3210 sonic bath for 45 min at radio frequency 47 kHz ± 6% to aid solublisation.

Plants and Medium Preparation. Maize and wheat seeds were obtained from Unwins Seeds Ltd, Cambridge. They were germinated on moist cotton wool in a darkened environment at 25 °C for 2 days and then placed individually onto moist contaminated growth medium within growth containers and covered with a moist cotton wool cap. HydroTops Bioponic, Bioponic Grow & Bloom, and Essentials Hydroponic Oxygen Increase were added, in the proportions specified by the manufacturer. Plants were watered with ~20−40 mL of hydroponic solution twice daily under a 16 h photoperiod, illuminated by a 400 W Na solar light. The growth room was maintained at ~25 °C for the duration of the experiment. Plants were analyzed after 1, 7, 14, 21, 28, 35, 42, 49, and 56 days postplanting by removing samples from the growth medium and rinsing the roots with distilled water to remove any remaining growth medium before immediate analysis using TPEM. Fine white sand was washed in distilled water and allowed to dry before being solvent rinsed with hexane and acetone. Sand (60 g) was contaminated with 10 mL of 0.25 mg/mL anthracene or phenanthrene solution. The acetone was allowed to evaporate; the dry contaminated sand was then placed in a 20 mL sealed glass amber growth container. Glass was used to ensure the PAHs did not bind into plastic containers.

Preparation of Control Slides and Control Plants. Compounds were applied in solution as a homogeneous layer to individual solvent-rinsed BDH super premium microscope slides using a micropipet, typically at ~0.3 μg cm^{-2}. These were "controls" to ascertain compound fluorescence using TPEM. Compound was also directly applied to uncontaminated roots, typically at ~0.3 μg cm^{-2}, to ascertain the compound fluorescent characteristics in situ within or on roots.

Instrumentation. A Bio-Rad Radiance 2000 MP scanning system was used with a Spectra Physics Tsunami/millennia laser (690−1050 nm) and a Nikon Eclipse TE300 inverted microscope. The laser wavelength was set to 700 nm. Images were collected and processed using the Bio-Rad Lasersharp 2000 imaging software Confocal assistant 4.02 and Amira 3.1. Fluorophore-containing compounds fluoresce if excited by a range of wavelengths. Individual compounds show

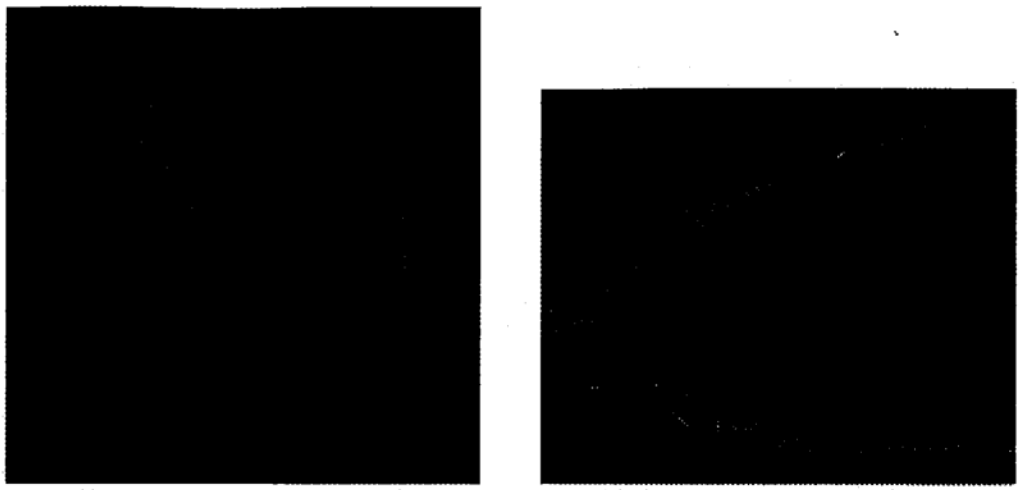


FIGURE 4. Uncontaminated root tip, representative of the findings through the experiment.

unique fluorescence emission spectra, allowing their unambiguous identification. Anthracene and phenanthrene and some of the products of anthracene metabolism—anthrone, anthraquinone and hydroxyanthraquinone (see Figure 3; (*17*))—were independently identified in living roots of maize and wheat using their autofluorescence spectra. Plant-specific autofluorescence excitation and emission profiles were determined when excited at 350 nm using TPEM, allowing the visualization of the compounds of interest within the root. Emission detection filters were set to detect anthracene and phenanthrene at 390 ± 35 nm (HQ390/70) using blue as a pseudo-color-channel. Cell walls (HQ528/50), chloroplasts, and cell organelles were detected at 590 ± 35 nm (HQ590/70) using green and red as pseudo-color-channels, respectively. All studies were performed with either a Nikon ×20/0.75 Plan Fluor D.I.C. water immersion lens or a Nikon 60x/1.20 Plan Apo D.I.C. water immersion lens. 1,4-Dihydroxyanthraquinone was used as a test substitute for hydroxyanthraquinone, which could not be obtained for this experiment. The addition of an OH substitution is likely to cause a slight decrease in the frequency of the emission and an increase in intensity of fluorescence. 1,4-Dihydroxyanthraquinone and hydroxyanthraquinone are detected using the same filters at an almost identical wavelength, and dihydroxyanthraquinone was therefore believed to be a suitable substitute for determining the fluorescence characteristics of hydroxyanthraquinone (*18*). Anthrone, anthraquinone, and hydroxyanthraquinone (1,4-dihydroxyanthraquinone) were detected using one or two filters simultaneously. Anthrone (HQ390/70) (HQ528/50) was readily identified using fluorescent green as the pseudo-color-channel, where it fluoresced at a different wavelength and at a higher intensity than the plant constituents. Anthraquinone (HQ528/50) (HQ590/70) and hydroxyanthraquinone (1,4-dihydroxyanthraquinone) (HQ590/70) were detected using orange and red as the pseudo-color-channel, respectively. The intensity of fluorescence produced by the degradation products meant they were easily detectable within the plant, corresponding to their exact fluorescence profiles as determined using compound applied to control glass slides and roots.

Experimentation. TPEM was used to study the uptake of anthracene and phenanthrene from a contaminated growth medium into roots of maize and wheat over a 56-day growth period. Compound was also applied to uncontaminated roots to discern the fluorescence profiles in situ. Once the fluorescence characteristics of the various chemicals had been identified using excitation at a specific wavelength, the sample plants were analyzed. During each study, control samples were also analyzed. Plants were analyzed after 1, 7, 14, 21, 28, 35, 42, 49, and 56 days. At least three plants were analyzed at each occasion, for each chemical, and the observed results were correlated and averaged. At least three roots on each plant were analyzed. The metabolism of anthracene was visualized as it was converted from anthracene to anthrone, anthraquinone, and hydroxyanthraquinone. Initially compound fluorescence profiles were identified using pure compound applied to glass slides. 1,4-Dihydroxyanthraquinone was used as a substitute for hydroxyanthraquinone. Leaves and shoots were also studied, although no compound was ever found within them. Images were made using *xz* and *xy* scans, and 3D reconstructions of the samples were also produced using Amira 3.1 software.

Results and Discussion

Initial Observations on Compound Uptake and Movement into Roots. A generalized root can be divided into five regions, differing in structure and function. These are depicted in Figure 2 and are (i) the root cap and growing apex, (ii) the divisional zone, (iii) the zone of elongation, (iv) the root hair zone, and (v) the branching zone (*19*, *20*). The root cap, growing apex, divisional zone, and zone of elongation represent regions of intense growth, through continual root exploration into previously unoccupied soil. The cells are in continual transition, but essentially remaining the same age for each growth zone throughout the experimental period. Monitoring uptake and location of organic compounds in roots is therefore complicated, because this is likely to vary along the root, and with time, as the root grows/elongates. Presumably more uptake takes place over time, *and* the compound can also move further radially and/or longitudinally in the root with increasing time.

The following initial and general observations can be made on the results:

(1) No detectable levels of anthracene or phenanthrene were observed in the root cap, apex, or divisional zones of maize or wheat over the 56-day duration of the experiment (see Figure 4).

(2) Anthracene and phenanthrene were observed at the outer surface of the epidermal cells along the remaining length of the root (i.e. the elongation, root hair, and branching zones). This is illustrated by Figure 5.

(3) Anthracene and phenanthrene behaved in a similar manner, being located only at the root surface (i.e. on or in the epidermal cells) throughout much of the zone of elongation. As just noted, they were located at the outer surface of the epidermal cells, but also up to 6 μm within the epidermal cell walls for both wheat and maize, as also illustrated by Figure 5.

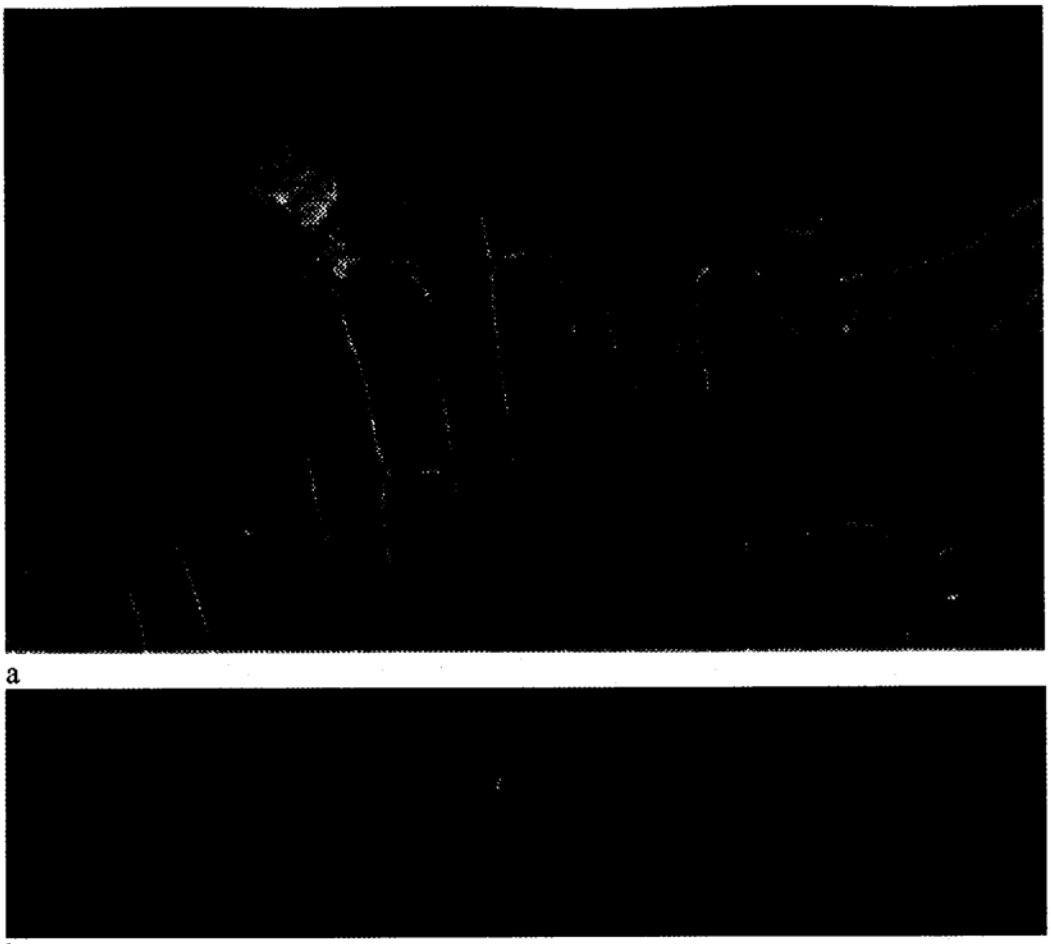


FIGURE 5. Phenanthrene cluster within an epidermal cell within the root hair zone of a maize root. Phenanthrene is shown as blue, the cell walls and hairs as green. Image a is an *xy* cross section taken at 5 μm from the root surface. Image b is an *xz* cross section showing the compound to be at the surface of the epidermal cells.

(4) Uptake and radial movement of both compounds was observed in the root hair and branching zones of both wheat and maize. Figure 6 shows anthracene and phenanthrene that has moved radially through the epidermal cells to enter the cortex cells. Both compounds entered the cell walls of the cortex cells (see Figure 6) and, to a lesser extent, cell vacuoles. Within the cortex cells both compounds were visualized to concentrate and "stream" longitudinally toward the shoot, as shown in Figure 6. The compounds formed distinct bands/streams within the cell walls and, to a lesser extent, cellular vacuoles. Movement within the cell walls and vacuoles and associated longitudinal streaming increased with time.

(5) The longitudinal "streaming" of both compounds was not inhibited by cell wall boundaries, with compound streams extending through two or more cells, for compound located within both the cell vacuole and cell wall. The streaming of these compounds and their behavior suggested that over time they become located within distinct regions of the root, from where they move in a shootward direction, as represented by Figure 6. Compound uptake, localization, and metabolism were studied most intensively within these regions, as will be described below.

(6) The radial and longitudinal movement of both compounds could be monitored and the rates of penetration measured directly throughout the study. The uptake and movement of anthracene within maize cell walls was approximately 3 times slower than that of phenanthrene over the duration of the experiment, an observation that may reflect differences in their solubility. Both compounds were observed to move approximately 3 times slower in wheat than maize (see Table 1).

(7) No detectable levels of either compound were found in the shoots or above ground foliage of the plants throughout the duration of the experiment.

Migration into the Roots and Observation of a New Phenomenon—Strong Localization within Certain Root Cells/Zones. Anthracene in maize is used in this section for initial detailed discussion.

Radial and longitudinal rates of compound movement into the roots were observed directly and are summarized in Table 1. Within the root hair and branching zones of the maize roots, anthracene was observed to enter the epidermal cells radially, to a depth of ~16 μm after 7 days. By 14 days, anthracene extended to 45 μm and was approaching the primary cells of the cortex. After 21 days it had moved ~100 μm radially into the root cortex, entering the cell walls of the second and third cortex cells. At this stage, it also started to exhibit the streaming phenomenon, which, as far as we are aware, has not been noted previously. As Figure 6 shows, the compound became much more concentrated in certain zones, focusing and streaming longitudinally toward the shoot. At this time, the streaming anthracene formed distinct bands, up to 20 μm in length within the cell walls. Thereafter, the number and length of these bands of focused anthracene increased within the cortex cell walls. They had reached 50–70 μm in length after 28 days, for example, and extended up to ~185 μm within the root. After 35 days, anthracene was observed not only to be within the cell walls but also, to a lesser extent, within cell vacuoles. By this stage it was forming

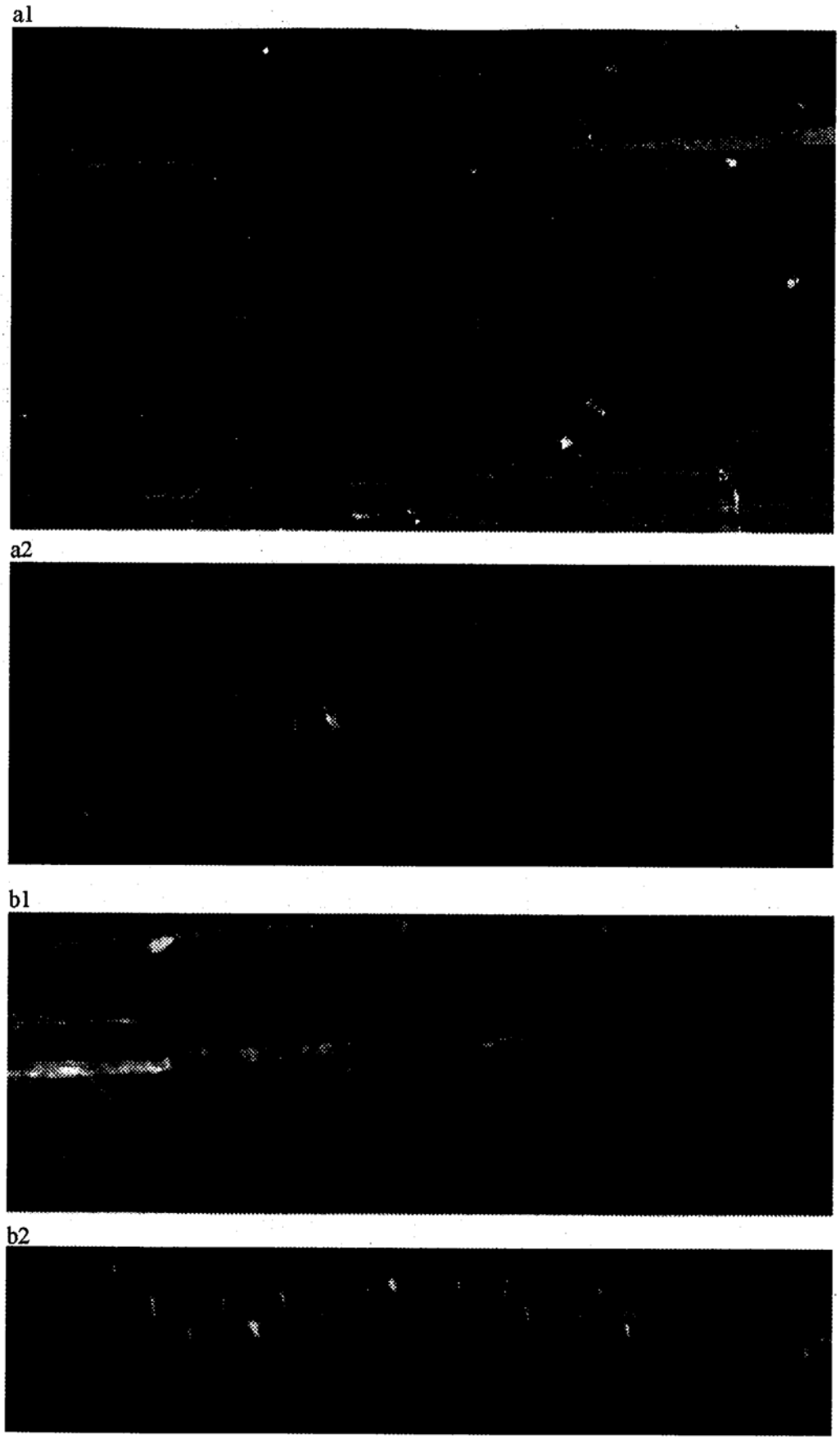


FIGURE 6. Phenanthrene (a) and anthracene (b) forming streams within the cortex cells of maize roots, at a depth of 140 and 155 μm, respectively. Phenanthrene and anthracene are shown as blue in their respective images. Anthraquinone, a degradation product of anthracene, is shown as orange. Parts a1 and b1 are *xy* images taken at 140 and 155 μm within the cortex cells for phenanthrene and anthracene, respectively. Parts a2 and b2 are *xz* images showing phenanthrene and anthracene within the cortex cells.

TABLE 1. Radial and Longitudinal Movement of Anthracene and Phenanthrene within Roots of Maize and Wheat over a 56-day Time Period

			movement (μm)							
compound	species	location	day 7	day 14	day 21	day 28	day 35	day 42	day 49	day 56
anthracene	maize	radial	16	45	100	185	190	190	200	220
	wheat	radial		14	42	96	115	140	155	180
phenanthrene	maize	radial	43	76	110	230	225	235	230	240
	wheat	radial		30	60	150	170	180	185	200
anthracene	maize	lateral			20	70	200	330	400	490
	wheat	lateral				50	90	185	300	300
phenanthrene	maize	lateral		50	320	450	520	580	750	1500
	wheat	lateral			7	48		220	380	465

bands within the cell walls up to 200 μm in length, extending laterally through two or three cells. Focused anthracene within the cell vacuoles was <10 μm length and appeared more diffuse—and limited to single cells—than that associated with the cell walls. After 49 days, the bands were more concentrated within the branching zone of the root; those within the cell walls and the cell vacuoles were ~200–330 μm and up to 20 μm long, respectively. Finally, after 56 days they reached 490 μm in length. Approximately 5% of the anthracene detected within the root by this stage was within the cell vacuoles and 95% the cell walls.

Direct Observations of Anthracene Metabolism. In addition to the processes just discussed, where anthracene became more concentrated in certain regions of the root over time, it was also possible to see direct evidence of the partial breakdown/metabolism of anthracene occurring. Figure 7a shows the appearance of new colorations, detectable after 21 days. This corresponded to the primary and secondary products of anthracene degradation, anthrone and anthraquinone (Figure 3). At this time, this was ~1–2% of the total mass of streaming compound within the root (see Figure 7b). After 28 days, the proportion of degraded compound remained <10%, with similar amounts of anthrone and anthraquinone. However, it constituted ~20–30% by day 49, when anthraquinone and hydroxyanthraquinone dominated; they were detected both within the cell walls and cell vacuoles (see Figure 7c). By day 56 approximately 50% of the streaming anthracene showed signs of degradation; anthracene, anthrone, anthraquinone, and hydroxyanthraquinone were all detected by this stage, with anthraquinone (~60%) and hydroxyanthraquinone (~40%) dominating (see Figure 7d).

Comparison with Wheat. Data on the migration rates for wheat are available in Table 1. In summary, anthracene uptake rates into wheat were slower than for maize. After 28 days, it had reached 96 μm in wheat primary and secondary cortex cell walls, for example, compared to 185 μm in maize. By this time it had also begun to stream laterally within the cell walls, forming bands up to ~50 μm toward the shoots, with initial compound degradation being visualized and representing ~1–2% of the localized chemical burden. The initial degradation product observed in wheat was anthrone. Similar to maize, after 35 days, the streaming compound extended up to 90 μm in length, with compound metabolism representing ~5%. Anthracene was also observed to enter the cell vacuoles, but to a much lesser extent than for maize. Between 42 and 56 days, the streaming of anthracene extended from 96 to 300 μm in length, with degradation increasing from 10 to 40% of the streaming compound and being dominated by anthraquinone and hydorxyanthraquinone. Less than 5% of streaming compound was observed within the cortex cell cellular cytoplasm.

Comparisons with Phenanthrene. Phenanthrene's behavior was broadly similar to that of anthracene, although it migrated more rapidly than anthracene into both plant species. It also formed localized "hot-spots" in the root cortex. After 21 days, phenanthrene had penetrated ~110 μm into the second and third cortex cells of maize. It formed discrete bands up to 320 μm in length, within both the root hair and branching zones of the root; movement into the cellular vacuole was also clearly seen. However, the compound was predominantly streaming within the cell walls. The movement of phenanthrene was not restricted to single cells; rather, bands passed through two or more cells within the cell walls (see Figure 6). After 56 days, phenanthrene had formed substantial bands, up to ~1500 μm within the root cell walls of the cortex, and to a maximum of ~100 μm within the cellular vacuoles. These extended through a number of different cells.

For each chemical/plant combination, movement within the cell walls and vacuoles and the associated longitudinal 'streaming' increased with time. The streaming was not inhibited by cell wall boundaries but extended through two or more cells for compound located within both the cell vacuole and cell wall. The streaming indicated that the compounds became focused within distinct regions of the root, from where they move slowly in a shootward direction.

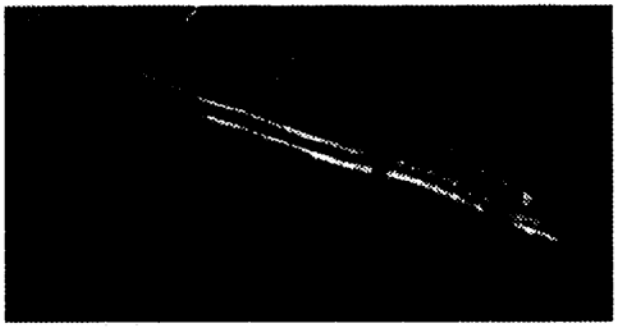
The results of the whole study are summarized schematically in Figure 8.

Water in the root is continually in motion—in cells, between cells, in tissue, and between tissues. Water and dissolved constituents entering a plant are subjected to the gradients created by the evapotranspiration stream (*20*). However, plant water also forms a part of the structure in which it is moving (*19*). In the epidermis, where initial uptake occurs, water flow is radial (*19, 21*). The epidermis constitutes a barrier to diffusion and slows the radial movement of anthracene and phenanthrene into roots, compared to that of water. The predominant flow of water becomes longitudinal—as opposed to radial—once it moves further into the cortex. Apoplastic transport is through the apparent free space, which comprises intracellular spaces, cell walls, and spaces between the cell walls and cytoplasm, totalling ~5% of the root volume (*19, 20*) (Figure 1). The movement of water and solutes through the apoplast is dominated by diffusion into the root cell walls and motion through cell walls from cell to cell. The cell wall is more permeable to water and its solutes than the protoplast (*19, 20*). This would appear to provide the dominant pathway for the movement of anthracene and phenanthrene through the roots. However, in addition to the movement through the apoplast, there is probably a small loss to the symplast, suggested by the occurrence of smaller amounts of compound within the cell vacuole (*19, 20*).

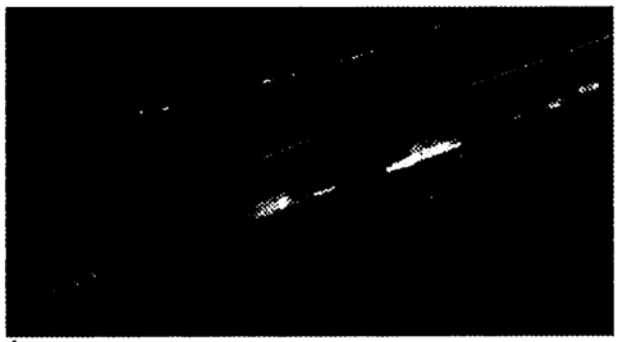
Radial diffusion through the epidermis is succeeded by longitudinal flow within the cell walls of the cortex, where radial forces have diminished. The cortex contains several layers of highly vacuolated parenchyma cells, with large intracellular spaces (*19*). The cortical intercellular spaces and walls of the epidermis represent the apoplast of the outer

a

b



c



d

FIGURE 7. Four *xy* images showing the degradation of anthracene within the cell walls of the cortex cells of maize: (a) 21 days of grown, with anthracene in blue and anthrone in bright green; (b) 28 days of grown, with anthracene in blue/white, anthrone in bright green, and anthraquinone in orange; (c) 35 days of growth, with anthracene as blue and anthrquinone as orange; and (d) 49 days of growth, with anthracene in blue, anthrone in green, and hydroxyanthraquinone in red.

root tissue. The function of the cortex is to transport compounds taken up through the epidermis to the vascular tissues of the root, through apoplastic and symplastic transport (*19*, *21*). It is thus interesting that, once within the cortex, anthracene and phenanthrene do not migrate radially through the cortex to the stele and vascular tissues but move slowly longitudinally through the apoplast of the cortex cells. Here they are found predominantly within the cell wall, where the focused streaming of compound was observed (*22*).

The uptake of anthracene and phenanthrene is clearly likely to be linked to the uptake of water, where compound

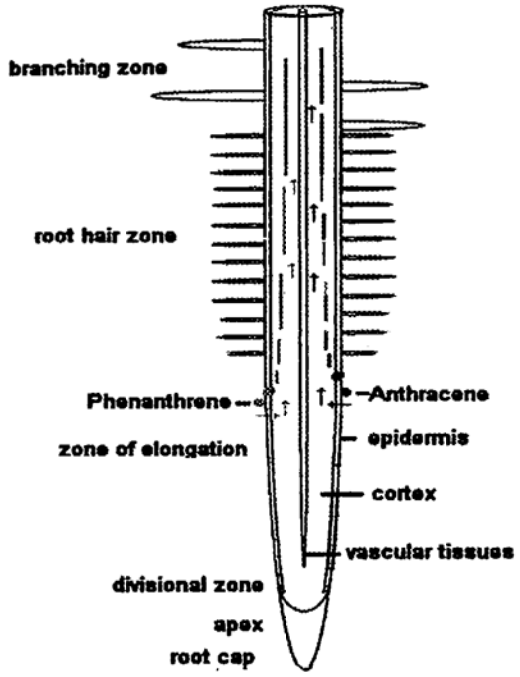


FIGURE 8. Schematic summarizing the movement, location, and degradation of anthracene and phenanthrene after 56 days. Note that, for convenience, anthracene is shown as entering one side of the root and phenanthrene the other. The color coding is as follows: blue, anthracene right/phenanthrene left; green, anthrone; orange, anthraquinone; red, hydroxyanthraquinone.

solubility plays a role. However, their solubilities at 25 °C are 0.075 and 1.65 mg/L for anthracene and phenanthrene respectively, a difference of a factor of ~20 (*23*). The uptake and movement of phenanthrene was faster in both wheat and maize than anthracene, but only by a factor of ~3 (Table 1). Other factors must therefore also affect the rate of movement, most likely the degree and nature of compound interactions with the cell walls and other structures. Growing roots synthesize mainly structural polysaccharides required for the formation of the primary cell walls. Cellulose is the simplest and most frequent structural polymer, forming ridged structural microfibrils. Hemicellulose and pectic compounds, together with glycoprotein, form a gel-like matrix. In addition, water is the most variable component of the cell wall (*19*). The cell wall is a complex medium and can be regarded as a hydroscopic system of cellulose micelles within intramicellular spaces with a radius of 1−10 nm, making the cell wall more permeable than the protoplast and thereby allowing apoplastic transport through cell walls (*19*). It may thus be that anthracene and phenanthrene are residing within this structure in solution, binding to the polysacharide components, and moving longitudinally in the direction of least resistance through the cell walls through the apoplast. Essentially, once compound has begun to undergo apoplastic movement within the cortex cells, we envisage it as being "dragged" laterally by the flow of water. Here the compound may become partially bound to the cellulose microfibrals of the cell wall structure, further slowing its progress through the root.

As noted earlier, a proportion of both compounds was located within the cell vacuoles. To have entered/reached such a location, they are likely to have been transported through the symplast. For the compound to have crossed cell boundaries from vacuole to vacuole, there will need to have been a degree of symplastic transport, although this was probably limited, due to the compounds having to pass through the plasmodesmata.

It is important to stress that both compounds only migrated as far as the cortex cells and did not reach the vascular transport system, the phloem/xylem. This suggests that such chemicals move through and become located within the cortex of the root and that the vascular transport system is not a likely transport route for such compounds to the above-ground parts of the plant.

In summary, the slow lateral movement of both anthracene and phenanthrene and the low level of compound entering the vacuole of the cortex cells suggest that apoplastic transport is the dominant pathway through which these compounds are travelling within the root, being integrally linked to the flow of water but being severely retarded relative to it.

Metabolism. Although anthracene and phenanthrene behaved similarly in both plants, only anthracene was observed to degrade in this study. This occurred specifically within the metabolically active cell walls of the mature cortex cells. The suggested degradation of anthracene to anthrone, anthraquinone, and hydroxyanthraquinone may be attributed to metabolism performed by a number of nonspecific enzymes within the cell vacuole, plasmalemma, and cell walls. Harms and co-workers (*7*) used wheat cell cultures and carbon-14-labeled anthracene to study its metabolism. They reported anthracene and its metabolites bound to various cell wall components, including pectin, lignin, hemicellulose, and cellulose. This supports the findings here, where anthracene was located predominantly within the cell walls.

Extensive investigations have suggested that the activity of most enzymes and enzymes systems change in different growth zones of the root (*19*). The compound degradation may be specifically related to degradation in mature cells, as it is the mature cells that synthesize compounds of primary metabolism derived from respiratory pathways and it is where the respiration rate and enzymatic activity are relatively high. The cells in the zone of elongation possess a complete profile of enzyme systems, with the activity of some enzymes increasing after elongation, including those associated with uptake and compound assimilation (*19*). It may therefore be that compound degradation only/principally occurs in the mature region of the root, where the enzymes involved in degradation are sufficiently developed.

Degradation of organics or their sequestration in vacuoles can presumably be seen as a protective mechanism, by isolating toxic xenobiotics from the metabolic powerhouse of the cytoplasm (*3*).

This study utilizes new methodologies to visualize and track how organic pollutants are taken up into roots and how they behave once within the root. It provides insights into the complex processes involved in plant POP processing. However, it has also raised questions regarding the complexity of plants and plant uptake in response to organic chemicals. The technique may be of particular interest to the agrochemical industry, aiding the development of new chemicals and more efficient/targeted use of existing ones. It is also likely to aid phytoremediation studies, where understanding the behavior of chemicals in specific plants can help to optimize phytoremediation strategies for soil cleanup.

Acknowledgments

We are grateful to the UK Natural Environment Research Council (NERC) for provision of a Ph.D. studentship (NER/S/A2002/10394) to E.W.

Literature Cited

(1) McLachlan, M. S. Bioaccumulation of hydrophobic chemicals in agricultural food chains. *Environ. Sci. Technol.* **1996**, *1*, 252–259.

(2) Alkorata, I.; Garbisu, C. Phytoremediation of organic contaminants in soils. *Bioresource Technol.* **2001**, *79*, 273–276.

(3) Meagher, R. B. Phytoremediation of toxic elemental and organic pollutants. *Curr. Opin. Plant Biol.* **2000**, *3*, 153–162.

(4) Alcock, R. E.; Sweetman, A. J.; Juan, C.; Jones, K. C. A generic model of human lifetime exposure to persistent organic contaminants: Development and application to PCB-101. *Environ. Pollut.* **2000**, *110*, 253–265.

(5) Jones, K. C.; de Voogt, P. Persistent organic pollutants (POPs): State of the science. *Environ. Pollut.* **1999**, *100*, 209–221.

(6) Muller, J. F.; Hulster, A.; Papke, O.; Ball, M.; Marschner, H. Transfer of PCDD/PCDF from contaminated soils into carrots lettuce and peas. *Chemosphere* **1994**, *29*, 2175–2181.

(7) Harms, H. H. Bioaccumulation and metabolic fate of sewage derived organic xenobiotics in plants. *Sci. Total Environ.* **1996**, *185*, 83–92.

(8) Wild, S. R.; Jones, K. C. Organic chemicals in the environment. Polynuclear aromatic hydrocarbons uptake by carrots grown in sludge-amended soil. *J. Environ. Qual.* **1992**, *21*, 217–225.

(9) Wild, S. R.; Jones, K. C. The significance of polynuclear aromatic hydrocarbons applied to agricultural soils in sewage sludges in the U.K. *Waste Management Res.* **1994**, *12*, 49–59.

(10) Wild S. R.; Berrow, M. L.; McGrath, S. P.; Jones, K. C. Polynuclear aromatic hydrocarbons in crops from long-term field experiments amended with sewage sludge. *Environ. Pollut.* **1992**, *76*, 25–32.

(11) Hulster, A.; Muller, J. F.; Marschner, H. Soil-plant transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans to vegetables of the cucumber family (*Cucurbitaceae*). *Environ. Sci. Technol.* **1994**, *28*, 1110–1115.

(12) Singer, A. C.; Crowley, D. E.; Thompson, I. P. Secondary plant metabolites in phytoremediation and biotransformation. *Trends Biotechnol.* **2003**, *21*, 123–130.

(13) Thomas, G.; Sweetman, A. J.; Ockenden, W. A.; Mackay, D.; Jones, K. C. Air-pasture transfere of PCBs. *Environ. Sci. Technol.* **1998**, *32*, 936–942.

(14) Schreiber, L.; Schonherr, J. Uptake of organic chemicals in conifer needles: Surface adsorption and permeability of cuticles. *Environ. Sci. Technol.* **1992**, *26*, 153–159.

(15) Wild, E.; Dent, J.; Barber, J. L.; Thomas, G. O.; Jones, K. C. A novel analytical approach for visualizing and tracking organic chemicals in plants. *Environ. Sci. Technol.* **2004**, *38*, 4195–4199.

(16) Wild, E.; Dent, J.; Barber, J. L.; Thomas, G. O.; Jones, K. C. Real-time visualisation and quantification of PAH photodegradation on and within plant leaves *Environ. Sci. Technol.* **2005**, *39* (1), 268–273.

(17) Cajthaml, T.; Moder, M.; Kacer, P.; Sasek, V.; Popp, P. Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection. *J. Chromatography A.* **2002**, *974*, 213–222.

(18) Guilbault, G. G. *Practical Fluorescence—Theory methods and techniques.* Marcell Dekker Inc.: New York, 1973.

(19) Kolek, J.; Kozinka, V. *Physiology of the plant root system.* Developments in Plant and Soil Sciences volume 46, Kluwer Academic Publishers: Dordrecht/Boston/London, 1991.

(20) Waisel, Y.; Eshel, A.; Kafkafi, U. *Plant Roots: The hidden half,* 3rd ed.; Marcel Dekker: New York/Basel, 2002.

(21) Kramer, P. J.; Boyer, J. S. *Water Relations of Plants and Soils.* Academic Press: San Diego, 1995.

(22) de Kroon, H.; Visser, E. J. W. *Root Ecology,* Ecology studies 168; Springer Verlag: Berlin, Heidelberg, 2003.

(23) Mackay, D.; Shiu, W. Y.; Ma, K. C. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. PAHs and PCDD/Fs;* Lewis Publishers: Ann Arbor, MI, 1992; Vol. 2.

Received for review November 26, 2004. Revised manuscript received March 10, 2005. Accepted March 11, 2005.

ES048136A