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Plant Uptake of Organic Chemicals

UPTAKE AND TRANSLOCATION OF *p,p′*-DICHLORODIPHENYLDICHLOROETHYLENE SUPPLIED IN HYDROPONICS SOLUTION TO *CUCURBITA*

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Abstract—Field studies show shoots of zucchini (*Cucurbita pepo* L.) accumulate various hydrophobic contaminants from soil, although many other plants do not, including cucumber (*Cucumis sativus* L.). To investigate the mechanism for this uptake, we presented *p,p′*-dichlorodiphenyldichloroethylene (DDE) to these two species in hydroponics solution. A mixture of DDE bound to Tenax™ beads stirred with a solution of water passing through a reservoir provided a flowing solution containing DDE at approximately 2 μg/L for many weeks duration. Approximately 90% of the DDE supplied in solution was adsorbed on the roots of both cucumber and zucchini. Less than 10% of the sorbed DDE was released subsequently when clean solution flowed past these contaminated roots for 9 d. The shoots of both species accumulated DDE, but the fraction that moved from the roots to the shoot in zucchini, ranging from 6 to 27% in various trials, was 10-fold greater than that in cucumber, 0.7 to 2%. The gradient in DDE concentration in zucchini tissues was in the order root $\gg$ stem $>$ petiole $>$ leaf blade, indicating the movement was through the xylem in the transpiration stream. Some DDE in leaf blades might have been absorbed from the air, because the concentration in this tissue varied less with time, position in trough, or species, than did DDE in stems and petioles. The remarkable ability of zucchini to translocate DDE could not be attributed to differences in tissue composition, growth rate, distribution of weight among plant parts, or in the leaf area and rate of transpiration of water from leaves. Some other factor enables efficient translocation of hydrophobic organic contaminants in the xylem of zucchini.

Keywords—Cucumber Phytoremediation Transpiration Transport Zucchini

INTRODUCTION

Polychlorinated biphenyls, dibenzodioxins/dibenzofurans (dioxins), and pesticides such as *p,p′*-dichlorodiphenyltrichloroethane (DDT), *p,p′*-dichlorodiphenyldichloroethylene (DDE), and chlordane, are extremely hydrophobic compounds with octanol-water partition coefficients $\log(K_{OW})$ ranging from 5.0 to 8.3 [1]. These compounds have no natural analogs and enzyme systems have not evolved to mediate their degradation, resulting in contaminant half-lives measured in years [2,3]. These chemicals are members of a group of compounds called persistent organic pollutants (POPs) that have long-term persistence in the environment, potential for bioaccumulation and toxicity, and global distribution.

Phytoremediation, the use of vegetation to remediate soils and sediments contaminated with heavy metals or organic chemicals, has been the subject of several comprehensive reviews [1,4,5]. The benefit of phytoremediation is that it is relatively inexpensive and can be applied on a large scale to moderately polluted lands. Several mechanisms may remediate soils contaminated with organic pollutants. Exudation of enzymes from roots or stimulation of microbial communities are mechanisms to degrade organic compounds in the root-zone. However, only low–molecular weight polycyclic aromatic hydrocarbons [6,7], certain pesticides [8,9], and relatively less chlorinated biphenyls [10,11] are degraded in the rhizosphere. Over time, POPs bind strongly to soil or sediment organic matter [12,13], and they become highly sequestered, as shown by the lack of metabolism by soil fauna and microbes [14]. Another mechanism of phytoremediation is movement of chemicals into plants with the transpiration stream, where they could be degraded, volatilized, or sequestered in plant tissues. Moderately hydrophobic compounds, with a $\log(K_{OW})$ of two to four, are taken up efficiently by plants [15,16]. More hydrophilic contaminants are unable to cross the plant membrane barrier, and more hydrophobic contaminants adsorb too strongly to soil or external membranes [17]. Due to their recalcitrant and hydrophobic nature, POPs are not expected to be susceptible to the physiological processes driving plant-based remediation.

However, recent studies have shown that certain plant species accumulate significant quantities of weathered POPs from soil. Pumpkin and zucchini (both *Cucurbita pepo* L.) accumulated dioxins and furans in leaves and fruit, with congener profiles that indicated their source was from soil, rather than from air [18]. Hexachlorobenzene was found in oil from pumpkin seeds and correlated to the concentration in soil. Movement was shown to occur through roots into shoots, both in hydroponics and in field soil [19]. Zucchini also takes up DDE

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through a soil-to-plant exposure pathway [20,21]. It is likely that a similar mechanism allows several plant species to accumulate chlordane in shoot tissues. Chlordane was detected in shoot tissue of spinach, lettuce, dandelion, and zucchini. The pattern of chlordane enantiomers in soil, air, and shoot indicated uptake was from the soil [22,23].

A better understanding of the mechanism by which POPs from soil are taken up by zucchini and translocated to the shoot could lead to a selection of genotypes and growth conditions to optimize the phytoremediation of contaminated soil. We have investigated one such mechanism, in which organic acids exuded from roots chelate and dissolve nutrients bound in soil, thereby disturbing soil structure on a microscopic scale, and subsequently releasing sequestered organic compounds [21]. We found correlations across genotypes between uptake of DDE, acquisition of nutrients [24], and exudation of organic acids [25,26]. However, other physiological features of zucchini are likely to be involved in its phytoremediation potential. In particular, plant species may differ in their ability to translocate hydrophobic compounds from the root to the shoot in the transpiration stream. To examine these features independent of the release of compounds from soil, we developed a technique to present DDE in low concentration in hydroponics solution to plant roots. We used this technique to compare the uptake of DDE by zucchini, a species that accumulates DDE in the shoot when grown in the field, to that of cucumber, a plant of similar morphology but with a negligible ability to take up DDE when grown in the field. These experiments investigated the time course of uptake and distribution of DDE among plant tissues in vegetative plants.

METHODS

System to supply DDE in hydroponics solution

Ten grams of Tenax™ TA (2,6-diphenyleneoxide polymer) porous beads of 80 to 100 mesh (Sigma Aldrich, St. Louis, MO, USA) were placed into a 250-ml flask with 1.0 g DDE (Sigma Aldrich) and 50 ml hexane, and the flask was stirred for several hours. The hexane was removed with a rotating evaporator under aspiration at 50°C. The dry Tenax mixture was transferred to a 1-L high-density polyethylene mobile phase reservoir (Alltech, Deerfield, IL, USA) equipped with pressure relief, gas-tight inlet and outlet ports, and a magnetic stirrer. Approximately 0.5 L of water solution was maintained in the reservoir with the 10 g of Tenax. The Tenax beads floated on the surface of the gently stirred solution, and solution flowing through the reservoir first percolated through this layer of Tenax before leaving through a steel frit filter (Hastalloy™ C Mobile phase filter, Alltech) just above the stirrer at the bottom of the reservoir. Water was passed through the mixing chamber at approximately 20 ml min^{-1}, and distributed to troughs of plants through an 8-channel peristaltic pump (Masterflex model 751905, Cole Parmer, Vernon Hills, IL, USA) using 1.6 mm inside × 3.2 mm outside diameter Tygon™ tubing (polyurethane formulation 3606, Fisher Scientific, Pittsburgh, PA, USA). A schematic of the mixing chamber is shown in Figure 1. This configuration ensured that all troughs received equal flow rates of solution at equal inlet concentrations of DDE. This system was used to supply plants with solution containing DDE for intervals of up to 14 d per trial.

Extraction of DDE from air and hydroponics solutions

The concentration of DDE was assayed in samples of solution collected after the peristaltic pump and before addition

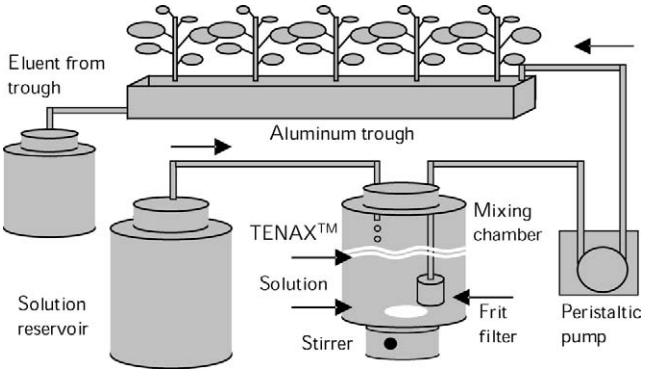

Fig. 1. Schematic diagram of the system used to supply *p,p′*-dichlorodiphenyldichloroethylene in hydroponics solution to plants. Tenax™ (Sigma Aldrich, St. Louis, MO, USA).

to the troughs. Samples of solution leaving the troughs were analyzed to determine the efficiency of plant uptake and to permit a mass balance calculation of DDE added to the system. Solution was collected in a glass Erlenmeyer flask for a fixed duration. The volume was measured to determine flow rate. A 100-ml aliquot was spiked with trans-nonachlor as an internal standard to give 100 ng L^{-1} in the final solution. Solutions were stored at 4°C until analysis.

Water solutions were extracted through a C18 filter cartridge (Bakerbond 6 ml solid phase extraction column, Malinkrodt Baker, Phillipsburg, NJ, USA). Filter cartridges were prepared by elution with 20 ml of methanol and then with 20 ml of deionized water. A 100-ml aliquot of the solution to be extracted was added and filtered under a partial vacuum over a period of 30 min. Care was taken to prevent drying of the cartridge after preparation and before the entire sample was filtered. After extraction, the filter was dried by passage of air using a vacuum and then by centrifugation at 5,000 rpm for 15 min. A 2.5-ml volume of hexane was added to the dry cartridge and eluted by gravity into a Target™ vial (National Scientific, Rockwood, TN, USA) for gas chromatography analysis. The apparent recovery of DDE was determined from clean water spiked with known amounts of DDE. The recovery from four replicate samples was 86 ± 7%.

Air in the growth room or greenhouse was sampled at a rate of approximately 350 L min^{-1} over a 24-h period. The air was drawn through a glass fiber filter and then a polyurethane foam plug to collect DDE in the vapor phase. The DDE in the foam plug was extracted and analyzed as described previously [27]. Air was assayed on three dates.

Growth and harvest of plants in hydroponics

Zucchini summer squash (*Cucurbita pepo* L. cv Black Beauty) and cucumber (*Cucumis sativus* L. cv Marketmore) seeds were obtained from Seedway (Hall, NY, USA). Seeds were germinated on moist filter paper in clear plastic boxes until the cotyledons emerged and then were transferred to aluminum troughs containing nutrient solution. Plants were grown until they had three true leaves in a growth room maintained at 25°C with a 12-h photoperiod of 200 μmol m^{-2} s^{-1} photosynthetic active radiation. The stock solutions for preparing nutrient solutions contained (per L): 27.2 g KH_2PO_4, 101.1 g KNO_3, 189 g $Ca(NO_3)_2{\cdot}4H2O$, 98.6 g $MgSO_4{\cdot}7H_2O$, 2.0 g Fe-ethylenediaminetetraacetic acid, 1.24 g H_3BO_3, 0.676 g $MnSO_4{\cdot}H_2O$, 0.115 g $ZnSO_4{\cdot}7H2O$, 0.100 g $CuSO_4{\cdot}5H_2O$, and 0.0484 g $Na_2MoO_4{\cdot}5H_2O$. The standard nutrient solution was

a 2.5-ml aliquot of each stock solution diluted to 1 L in deionized water. To deplete phosphorus and slow growth of plants, KH_2PO_4 was replaced by KCl at 19 g L^{-1}.

Plants grew in troughs of 40 × 40 × 760 mm interior dimensions constructed of 3.1-mm U-shaped aluminum extrusions developed for architectural purposes. Aluminum plates sealed the ends of each trough. The trough was covered with a thinner U-shaped extrusion with sides that fitted tightly within the trough. This cover had nine 19-mm diameter holes at 76-mm spacing. Plants were placed through these holes so the roots were immersed in the nutrient solution in the trough and the stem was held in place with a polyurethane foam rubber plug, which prevented air movement through the cover. Before DDE was supplied, the plants were thinned to five zucchini or nine cucumber per trough. The flow rate was set to 2.5 ml min^{-1} per trough in the light period. Nutrient solution was conserved by using intermittent flow in the dark. Solution flowed for a total of 16 h per day.

Plants were harvested around noon on several days during each trial. At each harvest, the roots, including stem below the foam plug, were separated from the aerial parts and handled separately to prevent contamination of the shoot by nutrient solution. Roots were partially dried by placing between paper towels until no excess water was soaked up by the towels. The area of detached leaves was measured with a leaf area meter (model LI3000A, LI-COR, Lincoln, NE, USA). A short segment at the base of the stem with adventitious roots was cut free of the roots and discarded. The shoot was cut into stem, petiole, and leaf blade fractions. Cotyledons and any flowers were included with the stem. These parts were weighed and tissues stored in the freezer. A subsample of each tissue type was freeze-dried to determine dry weight. Relative growth rate was determined from a plot of logarithm fresh weight versus days of growth.

Ground dried material was analyzed for crude lipid, protein, and carbohydrate using established protocols. Crude lipid was extracted from a 250-mg subsample using chloroform:methanol:water fractionation [28]. The chloroform phase was dried at 50°C under a stream of air to determine the weight of lipid. The water phase was dried at 50°C under a stream of air. The dried extract was dissolved in 10 ml of distilled water and reducing sugars determined by reduction of $K_3Fe(CN)_6$ [29]. Starch in the residue from the extraction was digested with amyloglucosidase (Boehringer Mannheim, Amsterdam, The Netherlands), and reducing sugars determined by reduction of $K_3Fe(CN)_6$. Another 250-mg tissue sample was added to a 100-ml Kjeldahl flask. After 4 ml of H_2SO_4 was added, the flask was heated to boiling for 6 min, and then 10 ml of 30% H_2O_2 was added drop-wise with heating. The clear solution was cooled, diluted to 100 ml, and filtered. Total reduced N was determined in the tissue digest by colorimetric reaction with Nessler reagent [30]. Total reduced N was converted to protein using a conversion factor of 6.25.

Uptake of DDE was measured in three trials or plantings. Before DDE was supplied to plants in trials one and two, they were moved to a greenhouse under ambient sunlight, in May and October, respectively. Temperatures in the greenhouse varied from 15°C at night to 30°C during the day. High-intensity discharge lights supplemented sunlight in the greenhouse for a 14-h photoperiod. Maximum light intensity was 1,400 μmol m^{-2} s^{-1} photosynthetic active radiation. During trial three in December, the plants remained in the growth room under the conditions described for early growth of seedlings. In each trial, the DDE mixing chamber was placed in line between the nutrient reservoir and the peristaltic pump to start the DDE supply to the plants. This occurred at noon and the supply was maintained for up to 14 d.

In trial one, plants were removed from one position in each trough on a harvest date. In the other two trials, all plants in each trough were harvested simultaneously. Plants were grouped at the inlet and outlet end of each trough to minimize problems due to root entanglement. Each group was two plants of zucchini or four of cucumber, because zucchini had approximately twice the biomass of cucumber on a per plant basis. The plant in the middle of the trough was used to determine tissue composition, but was not analyzed for DDE. The plants from each location were combined for analysis. All tissues were analyzed twice, either with samples from duplicate troughs, or from duplicate subsampling of tissue from each group of plants.

Measurement of transpiration

Gas exchange was measured in an open system by using an infrared gas analyzer operating in the differential mode to compare the gas concentration returned from a leaf chamber to that from a reference flow of air [31]. The infrared gas analyzer was both a CO_2 and H_2O analyzer (LI-COR Model 6262). The leaf chamber consisted of two identical frames of acrylic covered with polypropylene film that were held closed with a spring clamp and sealed with 0.3-cm-thick closed-cell foam rubber. The chamber enclosing the leaf had inner dimensions of 5 cm × 10 cm × 2 cm deep. The chamber was oriented so the upper surface of the leaf was perpendicular to solar radiation. The leaf was held in this position in unobstructed sunlight for 1 min, until the infrared gas analyzer reading stabilized. The leaf gas exchange rates per unit area were calculated from the change in concentration of H_2O multiplied by the air-flow rate and divided by the leaf area in the chamber.

Extraction and analysis of DDE in vegetation

A wet extraction method was used to recover DDE from vegetation [32]. A tissue subsample of 10 to 30 g wet weight was added to a 1-L blender jar with 30 ml of 2-propanol and 3 μg of trans-nonachlor as an internal standard. After blending for 30 s, 60 ml of petroleum ether was added and the sample was blended for 5 min. The extract was decanted through a funnel packed with glass wool and collected in a 500-ml glass separatory funnel with Teflon® stopcock. After draining for 20 min, 100 ml of deionized water and 10 ml of saturated sodium sulfate solution were added. The funnel was capped and shaken gently for 10 s, and the solvent phases were allowed to separate for 20 min. After drawing off the water layer, the petroleum ether was rinsed two additional times with 100 ml of deionized water and once with 10 ml of saturated sodium sulfate solution. The petroleum ether was collected and stored with 10 g of anhydrous sodium sulfate. A 2-ml aliquot was filtered through a glass fiber filter (0.2 μm, Laboratory Science, Sparks, NV, USA) prior to chromatography. The apparent recovery of DDE was determined from clean leaf blade samples spiked with known amounts of DDE. The apparent recovery from four replicate samples was 185 ± 13%. The reason for this apparent concentration of DDE during extraction is not known.

The DDE content in these extracts was determined on a gas chromatograph with an electron capture detector (model HP 6890 Hewlett-Packard, Avondale, PA, USA). The 30 m ×

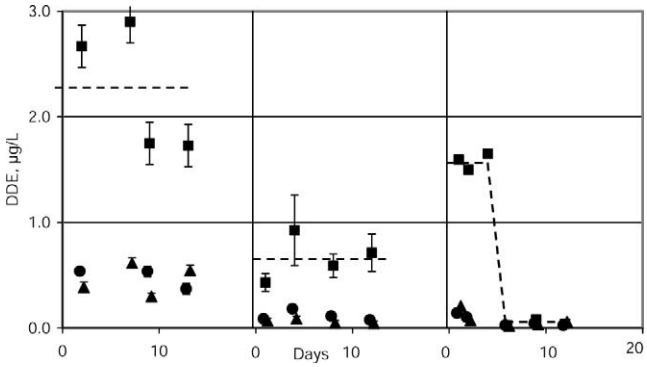

Fig. 2. Concentrations of *p,p′*-dichlorodiphenyldichloroethylene (DDE) measured in the nutrient solution during three trials. Symbols represent the solution supplied to the plants (■) or solution drained from troughs in which cucumber (●) or zucchini (▲) plants were growing; error bars represent the standard deviation.

0.25 mm × 0.25 µm column contained a dimethylsiloxane/5% diphenyl film (Supelco 24034, Bellefonte, PA, USA). The oven was held at the initial temperature of 175°C for 1 min, heated at 3.5°C/min to 225°C, heated at 25°C/min to 280°C, and held at this temperature for 7.5 min. The total run time was 25 min. A 1-µl splitless injection was used, and the injection port was maintained at 250°C. The carrier gas was He flowing at 1 ml min^{-1}, and the make-up gas was 5% CH_4 in Ar at 59 ml min^{-1}. The electron capture detector was maintained at 300°C. Under these conditions, the retention times of trans-nonachlor and DDE were 15.6 and 16.1 min, respectively.

Calibration standards were made from stocks of crystalline DDE and trans-nonachlor (U.S. Environmental Protection Agency National Pesticide Standard Repository, Fort Meade, MD) dissolved in petroleum ether (vegetation) or hexane (water solutions). Calibration standards were 5, 20, 100, 250, and 1,000 µg/L and each calibration level was amended with 100 µg/L trans-nonachlor as an internal standard.

Data analysis

At least two samples of solution were collected from inlet and outlet positions of different troughs on each date. Analysis of variance by date and species determined the error of these measurements. Measurements of DDE in plant tissue were either replicated by plants under similar conditions in different troughs or by duplicate analysis of plant samples that were grouped during harvest. Analysis of variance by day of harvest, species, and position in the troughs determined the error of these measurements. Amounts are reported per one plant of zucchini or two of cucumber. All amounts or concentrations are corrected for the apparent recovery of DDE from spiked samples of solutions or leaf tissue.

RESULTS

Concentration of DDE in solution

In the first trial, plants were supplied with DDE in solution at a constant rate for 14 d. For a few hours after flow initiation and prior to applying it to plants, DDE was in excess of 20 µg L^{-1} in the solution leaving the Tenax DDE mixing chamber. However after 2 d and during the remainder of the trial, the concentration of DDE in solution from the mixing chamber remained relatively constant at 1.7 to 3.0 µg L^{-1}. Figure 2 summarizes for the three trials the DDE concentrations in solution supplied to the plants and in solution leaving the troughs. The concentration of DDE produced by the Tenax DDE mixing chamber changed during the three trials. It averaged 2.3, 0.67, and 1.6 µg L^{-1}, in May, October, and December, respectively. However within each trial, we assumed the concentration supplied to the plants was relatively constant. The roots adsorbed most of the DDE in solution. The concentration in the solution leaving the troughs was approximately 20% of that supplied in May, and approximately 10% of that supplied in October (Fig. 2).

Table 1. Growth parameters for cucumber and zucchini for each of three trials

Species	Trial	Month	Relative growth rate (g^{-1} day^{-1})	Leaf area ratio (cm^2 g^{-1})	Final fresh weight (g)
Cucumber	One	May	0.210	38	64
	Two	Oct	0.085	44	44
	Three	Dec	0.095	—	26
Zucchini	One	May	0.210	37	49
	Two	Oct	0.120	40	73
	Three	Dec	0.095	—	41

Concentration of DDE in air

Air in the growth chamber had a DDE concentration of 2.5 ± 0.2 ng m^{-3} when plants were supplied with solution containing DDE and 1.5 ng m^{-3} at other times. The concentration of DDE in air in the greenhouse was lower, approximately 1.0 ng m^{-3}, largely because ventilation was required to control air temperature in the greenhouse.

Growth and transpiration

The growth rate and fresh weight of plants varied among the trials (Table 1). Plants grew fastest in a greenhouse in May and at approximately half this rate in October in a greenhouse or in December in a growth room. The two plant species did not differ in leaf area–to–weight ratio or in growth rate except in October. The distribution of fresh weight among plant parts, and composition of the plants, did not differ greatly among trials, as seen by the low standard deviation when averaged over all trials (Table 2). Cucumber had a greater mass fraction in roots and zucchini had a greater mass fraction in petioles. Cucumber had less dry matter per unit fresh weight in root and stem, but more in leaf blades than did zucchini. No significant differences were found between plant species in lipid or protein concentration expressed on a dry weight basis.

The rate of transpiration per unit area of leaf blade did not vary between plant species. This characteristic was measured during each trial. Figure 3 is a plot of the light response of transpiration for plants growing in a greenhouse in May. Different light levels correspond to different times of day. The light response could be predicted for both species using a single saturation response curve with a maximum rate of transpiration of water per unit leaf area of 7 mmol m^{-2} s^{-1}.

Accumulation of DDE in plant tissue

In the first trial, both date of harvest and position in the trough influenced the amount of DDE accumulated by plants (Table 3). When considering only those plants near the inlet for the solution supplying each trough, the amount of DDE per plant increased at an approximately constant rate over time. A similar trend was found for plants near the outlet. However,

Table 2. Physical parameters for cucumber and zucchini averaged over three trials. All values expressed as ratios ± standard deviation

Factor Species	Root	Stem	Petiole	Leaf blade
Fresh weight ratio		Fraction of total plant weight		
Cucumber	0.50 ± 0.02	0.17 ± 0.02	0.09 ± 0.03	0.25 ± 0.02
Zucchini	0.34 ± 0.01	0.16 ± 0.03	0.25 ± 0.04	0.25 ± 0.02
Dry matter fraction		Fraction of fresh weight		
Cucumber	0.038 ± 0.002	0.070 ± 0.005	0.062 ± 0.005	0.163 ± 0.002
Zucchini	0.045 ± 0.002	0.096 ± 0.001	0.064 ± 0.003	0.141 ± 0.014
Lipid fraction		Fraction of dry weight		
Cucumber	0.053 ± 0.015	0.053 ± 0.004	0.049 ± 0.007	0.092 ± 0.040
Zucchini	0.059 ± 0.016	0.057 ± 0.003	0.048 ± 0.005	0.109 ± 0.040
Protein fraction		Fraction of dry weight		
Cucumber	0.207 ± 0.018	0.154 ± 0.016	0.075 ± 0.009	0.212 ± 0.004
Zucchini	0.230 ± 0.022	0.127 ± 0.014	0.060 ± 0.010	0.191 ± 0.027

plants near the inlet had far more DDE than did those near the outlet when harvested on the same day. Analysis of variance indicated that position in the trough was significant ($p < 0.002$) and, within position, the amount of DDE per plant was linearly related to days of supply ($p < 0.01$). The amount of DDE per plant sample, one zucchini or two cucumber plants, did not differ between species. At 14 d, the remaining plants contained approximately 80% of the DDE that had been supplied to them in solution.

The distribution of DDE among plant tissues varied much less due to time or position in the trough than did the amount of DDE per plant. In Table 4, the distribution was averaged over both inlet and outlet positions and the harvests at 11 and 14 d after the start of feeding DDE. One trend was evident. Averaged over these samples, the fraction of DDE in aerial parts of zucchini was 10-fold higher than that for cucumber.

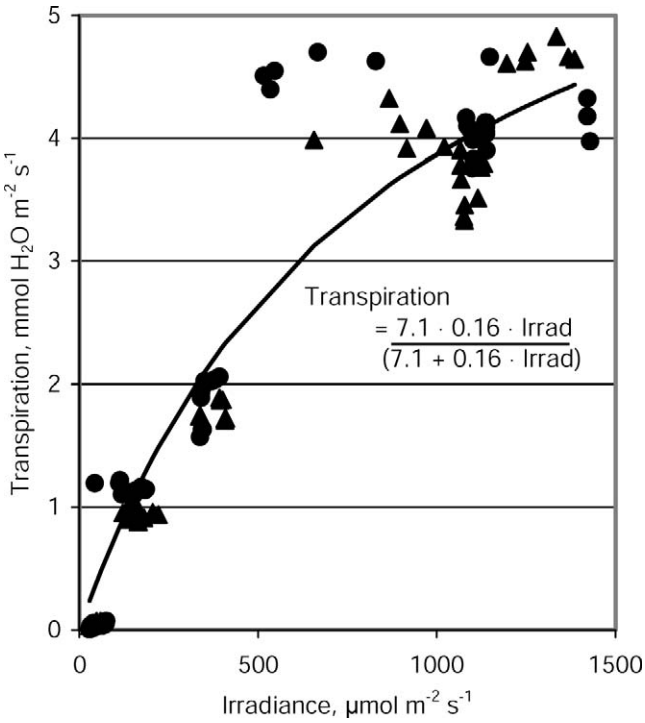

Fig. 3. Rate of transpiration of water from leaf blades of zucchini (●) and cucumber (▲) measured as a function of light intensity in a greenhouse. The fitted line is given by the equation on the graph.

The shoot of zucchini contained 5.7% of the DDE in the plant, compared to only 0.5% in cucumber. After 14 d of feeding, the actual amounts in the shoots were 1.01 and 0.071 μg, per plant sample of zucchini and cucumber, respectively.

In the second trial, stem and petiole tissues of zucchini accumulated five- to 10-fold higher concentrations and amounts of DDE than did those of cucumber (Table 5). For both species, roots near the inlet of the troughs accumulated more DDE than those near the outlet. The concentration and amount of DDE in roots did not vary between species; however, again, zucchini stems and petioles contained at least 10-fold greater levels of the contaminant than was present in cucumber tissues. For zucchini, stem and petiole tissues of plants near the inlet also had more DDE than for those near the outlet. Among leaf samples, only zucchini near the inlet had significantly more DDE. This pattern, taken with the low and constant amount of DDE in the air, indicate that uptake through the root was the primary path for entry of DDE into the root and stem tissues of both cucumber and zucchini; it is likely that a significant portion of the leaf DDE content results from aerial deposition.

Both species accumulated and retained nearly all the DDE supplied in hydroponics solution, within experimental error of the tissue determinations. Assuming that the DDE content was an average of the concentrations measured for plants near the inlet and outlet, the five plants of zucchini per trough contained 16 and 26 μg DDE, after 8 and 14 d of DDE supply, respectively, and the nine plants of cucumber contained 11 and 32 μg of DDE. The amount supplied to each trough was 13 and

Table 3. Amounts of *p,p′*-dichlorodiphenyldichloroethylene (DDE) accumulated in whole plants by time and location in the trough in the first trial. Amounts are expressed per plant sample, two plants of cucumber or one of zucchini ± standard deviation. No replication of positions on days 2 and 5

Species	Total DDE accumulated (μg)				
	Cucumber		Zucchini		DDE supplied
Location	Inlet	Outlet	Inlet	Outlet	
Days					
2	4.7	1.8	4.4	2.5	8
5	10.3	3.2	14.8	3.7	14
11	—	13.6 ± 2.9	—	5.4 ± 0.7	21
14	22.1 ± 3.8	—	23.6 ± 2.6	—	29

Table 4. Distribution of *p,p′*-dichlorodiphenyldichloroethylene (DDE) among plant parts in the first trial. Values are averages over plants at inlet and outlet positions ± standard deviation when harvested after 11 or 14 d of feeding DDE

	Distribution, % of total DDE in plant			
Species	Root	Stem	Petiole	Leaf blade
Cucumber	99.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Zucchini	94.3 ± 1.5	2.2 ± 0.5	2.3 ± 0.4	1.2 ± 0.7

26 μg DDE, respectively, at 8 and 14 d, calculated from the flow rate and DDE concentration in solution.

The gradient in movement of DDE within the shoot of zucchini was refined by separating the lower from the upper petioles. When divided this way, the stem, lower petiole, and upper petiole tissue compartments had similar biomass, and similar lengths, or transport paths of approximately 12 cm for each segment. There was a gradient in DDE concentration among these tissues. The stem had two- to threefold more DDE than the lower petiole, which had approximately twice the DDE of the upper petiole (Table 5). This gradient in concentration also extended to the leaf blades of zucchini near the inlet. Such a concentration gradient is consistent with movement of DDE into plant tissues via the transpiration stream.

During the third trial, cucumber and zucchini growing in a growth room were supplied with DDE for 5 d, immediately followed by 9 d of growth in DDE-free solution. The amount of DDE in roots did not change significantly over time once the supply of DDE ceased (Fig. 4). The DDE that was absorbed by roots was not released into the solution flowing from the troughs (Fig. 2). The amount of DDE in the eluent from each trough summed over 9 d was 0.7 μg DDE, for both cucumber and zucchini. This was an order of magnitude less than the amount of DDE in all the roots in each trough; 9 and 13 μg, for roots of cucumber and zucchini, respectively.

Some DDE continued to move to the shoot of zucchini after DDE was no longer supplied in solution (Fig. 4). However, the fraction of DDE in stems increased with time more slowly than in the previous trials when the DDE was supplied continuously. Zucchini had more DDE in stem than cucumber at each harvest (Table 6), and this difference increased with time. Petioles of cucumber were not analyzed for DDE in this trial; they lacked sufficient tissue for accurate determination.

Table 5. Concentration, amount, and distribution of *p,p′*-dichlorodiphenyldichloroethylene (DDE) in cucumber and zucchini after 14 d supplying DDE in hydroponics solution in the second trial

		Part				
				Petiole		
Species	Trough location	Root	Stem	Lower	Upper	Leaf blade
		Concentration (ng g^{-1} fresh wt)				
Cucumber	Inlet	465	3.3	1.6	—	3.0
Cucumber	Outlet	132	2.0	0.8	—	3.2
Zucchini	Inlet	343	47.6	15.7	7.7	4.0
Zucchini	Outlet	76	10.5	4.6	3.1	2.1
LSD[a]		109	14	3.3	—	1.1
		Amount (μg per plant sample)				
Cucumber	Inlet	10.8	0.031	0.006	—	0.035
Cucumber	Outlet	2.8	0.021	0.003	—	0.040
Zucchini	Inlet	7.5	0.449	0.158	0.051	0.058
Zucchini	Outlet	2.0	0.107	0.059	0.033	0.038
LSD		2.4	0.15	0.06	—	0.014
		Distribution (% of total in plant)				
Cucumber	Inlet	99.3	0.3	0.1	—	0.3
Cucumber	Outlet	97.8	0.7	0.1	—	1.4
Zucchini	Inlet	91.3	5.5	1.9	0.6	0.7
Zucchini	Outlet	89.5	4.7	2.6	1.4	1.7
LSD		1.4	0.9	0.9	—	0.2

[a] LSD = least significant difference from analysis of variance with species and location as factors.

DISCUSSION

Method of supply

We used Tenax water partitioning to provide a relatively constant concentration of DDE in flowing solution. This system avoided experimental artifacts and difficulties encountered

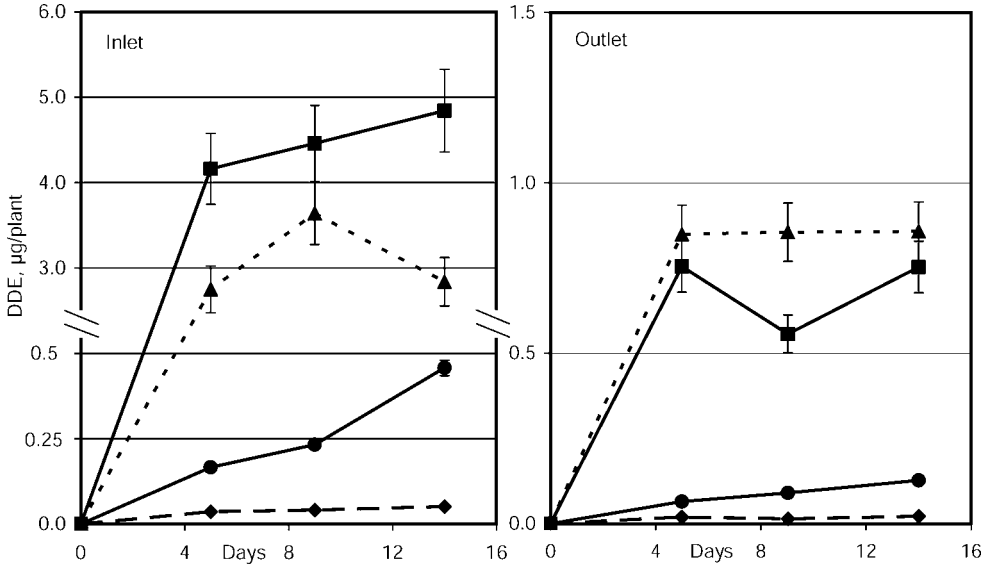

Fig. 4. Amounts of *p,p′*-dichlorodiphenyldichloroethylene (DDE) accumulated in zucchini and cucumber tissues in a trial in which it was supplied in solution for 5 d, and then clean solution was supplied for another 9 d. Panels are for plants at inlet and outlet positions along the trough; symbols represent mean values for zucchini shoot (●) and root (■) and cucumber shoot (◆) and root (▲); error bars are standard deviations.

Table 6. Distribution of *p,p′*-dichlorodiphenyldichloroethylene in cucumber and zucchini as a function of time during the third trial in which it was supplied in solution for 5 d, and then clean hydroponic solution was supplied for another 9 d

		Distribution (% of total in plant)			
Species	Days	Root	Stem	Petiole	Leaf
Cucumber	5	98.2 ± 0.7	0.8 ± 0.4	—	1.0 ± 1.0
	9	98.6 ± 0.4	0.8 ± 0.2	—	0.6 ± 0.6
	13	97.8 ± 0.5	0.7 ± 0.3	—	1.5 ± 0.9
Zucchini	5	95.0 ± 0.2	3.1 ± 0.5	1.0 ± 0.5	0.9 ± 0.5
	9	92.7 ± 0.5	3.5 ± 1.2	2.5 ± 1.2	1.3 ± 0.7
	13	90.7 ± 0.8	5.3 ± 0.8	2.1 ± 0.8	1.8 ± 0.7

with other methods of supplying a hydrophobic chemicals in water. For example, plants typically are grown in static solution in which the concentration of chemical is depleted over time [33,34] and either an organic solvent or a detergent are needed to disperse or solvate the organic chemical. These dispersal agents easily could affect the root surface and uptake of such chemicals into plants. Our method generated a low but relatively constant concentration of DDE in solution without other added chemicals. One experimental weakness to our method was that the proximity of plants to the DDE solution inlet was a significant factor in contaminant uptake per plant. The plants near the inlet accumulated approximately 80% of the DDE supplied, although plants near the outlet accumulated only 20%.

Binding to roots

Most of the DDE supplied in solution was adsorbed onto the roots and the amount associated with roots increased in proportion to the amount of DDE supplied. Cucumber and zucchini were similar in the quantity of DDE adsorbed to the roots and in the time course of accumulation of DDE by roots. This sorption process was not reversed when DDE-free solution flowed past the roots; less than 10% of the DDE adsorbed on the roots was released over 9 d. This rapid sorption has been observed in other hydroponics studies of POPs [34]. Although DDE was adsorbed rapidly to roots, the distribution of DDE to the rest of the plant was far from equilibrium. The lipid phase is the likely reservoir for hydrophobic contaminants in plants. The lipid content was actually less in roots than other plant tissues (Table 2). Thus, at equilibrium, the shoot should have more DDE than the root. Cucumber and zucchini did not differ in distribution of lipids among tissues, so differences in composition do not explain the difference between these two plant species in partitioning DDE between root and shoot. Comparison of a series of hydrophobic chemicals showed the time to reach steady state in the xylem transpiration stream increased exponentially with K_{OW} [15]. Extrapolating this behavior to a chemical such as DDE, with $\log(K_{OW})$ of seven, suggests the time to reach a steady state is much longer than the time course of our experiments.

Movement to stem and petiole

The concentration gradient of DDE in a plant should indicate the direction of transport until equilibrium is reached, as the chemical will move from a region of high concentration to one of low concentration. In both cucumber and zucchini, the gradient in DDE concentration was in the order, roots $\gg$ stem $>$ petiole. Thus, in both species, DDE movement was from root to stem and then petiole, rather than from leaf to petiole and stem. These species appeared to differ in the rate of movement of DDE in the transpiration stream rather than in the pathway of movement. In our hydroponic experiments, both zucchini and cucumber moved some DDE to the shoot, but the rate of movement for zucchini was 10-fold greater than that for cucumber. Similar results were found comparing cucumber and zucchini grown in soil in the field or in pots with soil; zucchini took up DDE into the stem, leaves, and fruit, although cucumber did not [35,36]. A comparison of various summer squashes showed zucchini has a greater ability than yellow summer squash, a very similar subspecies within *Cucurbita pepo* L., to translocate DDE to the shoot [24,25].

Mechanistic models suggest movement of DDE among tissues should be slow, because a large volume of water is necessary to move hydrophobic compounds between compartments containing lipids [17]. The rate of transpiration was similar for zucchini and cucumber (Fig. 3), so the rate or volume of water flowing from roots to leaves could not explain the difference in movement of DDE. An increased mobility of DDE per unit volume of water moving in the transpiration stream appeared to distinguish zucchini from cucumber.

Zucchini and pumpkin have been considered unusual plant species, in that extremely hydrophobic chemicals move from the soil through roots into the shoot [18,19]. Careful analysis of a dozen other plant species showed that, depending on species, 22 to 95% of phenanthrene and 32 to 96% of pyrene in the shoots moved from soil to shoots in the transpiration stream [37], the remainder was due to transfer from air. Thus all plants are likely to translocate some fraction of a hydrophobic chemical in the transpiration stream. However, there seems to be a wide variation among species in the rate of this movement. Zucchini and pumpkin are exceptionally adept at this process. Our hydroponic study showed that the ability to transport DDE from root to shoot is one aspect in which the physiology of zucchini differs from that of cucumber. This inherent ability to transport DDE within the plant may be as important as other physiological factors. For instance, we showed that zucchini exuded greater quantities of organic acids from their roots than did cucumber. These organic acids disrupted soil structure and released DDE from the soil matrix so it was more available for uptake by plants [25,26].

Movement to leaves

The DDE in leaf blades did not vary with time, position in trough, or species as much as did the contaminant content in the stems and petioles. This suggests movement to stems and petioles was in the transpiration stream from roots, although much of the DDE in leaves may have been absorbed from the air. However, movement from air to leaves was a poor mechanism for transfer of DDE to other plant tissues. Although we measured DDE in leaf blades at the earliest harvest date of each trial, the amount in leaves of cucumber did not increase during growth, except in the December trial, and other shoot tissues had negligible DDE. Only after stem and petiole tissues of zucchini accumulated DDE did we detect an amount in leaves that was greater than in cucumber. In all our trials, the concentration per unit tissue dry weight of leaves decreased with time (data not shown). Some of our reports of movement of DDE in field-grown plants included the petiole with leaves, and thus registered a substantial accumulation of DDE in leaves [24,35].

Comparison with plants grown in soil

The results of our study of young vegetative plants grown in hydroponics differed from those of studies of the same species grown in soil into fruit production. In our study, the majority of chemical consistently was found in the roots, although a much greater fraction of POPs was found in leaves or fruit of plants after a period of fruit production under field conditions. In pumpkin or zucchini grown in field-soil contaminated with dioxins, the contamination of fruit was as great as the leaves [18]. The concentration of chemical in other parts of the plant was not reported, and it was unclear whether leaves included the petioles or only the blades. In field studies of DDE uptake in zucchini [24,35], the petiole and leaf blade accounted for approximately 40% of DDE moved to the shoot after a period of fruit production. Fruit had a similar amount of DDE, and roots only accounted for a small fraction of the total DDE in the plant at the time of final harvest. The majority of DDE was found in roots only in plants such as cucumber and summer squash other than zucchini, which were less able to take up DDE [24,35]. When grown in soil in pots, a substantial fraction of DDT was found in shoots of zucchini and pumpkin, while species such as fescue and alfalfa accumulated DDT almost exclusively in roots [38]. Although roots of pumpkin had a greater concentration of DDT than any other tissue, the upper stem tissue, e.g., petiole, had the greatest amount of DDT, 56 to 62% of the total.

At least two factors might explain why the movement of DDE in vegetative plants grown in hydroponics differed from that in plants grown into fruit production in the field. First, plants in hydroponics have a greater fraction of biomass in roots. In our study, roots corresponded to 34 to 50% of biomass on a fresh weight basis, although roots typically accounted for only 2 to 8% of biomass of fruiting plants harvested from soil [35,38]. Thus in hydroponics, a greater fraction of chemical is likely to be retained in the roots. During fruit growth, the volume of vegetation increases relatively slowly compared the flow of water due to transpiration. The rate of volume increase due to growth, relative to the rate of transpiration, is much greater in young and rapidly growing plants. To some extent, movement of DDE in the transpiration stream could not keep up with rapid growth and increase in biomass of young plants. Even for field-grown zucchini, roots of rapidly growing plants 12 d after transplanting contained 50 to 60% of the DDE in the plant, although roots had only 3 to 8% the DDE after a period of fruit production [36].

CONCLUSION

Essentially all the DDE supplied to plant roots in flowing nutrient solution was adsorbed to the roots of cucumber and zucchini. Plants near the solution inlet accumulated more DDE than those downstream. The two plant species did not differ in the sorption of DDE to roots. However, zucchini moved tenfold more of the DDE to the shoot than did cucumber. This ability to move DDE within the plant is an important feature for phytoremediation strategies, because harvesting shoots is far easier than harvesting roots. Growth rate and biomass distribution have an effect on the distribution of DDE. The concentration gradient among plant tissues indicated that movement of DDE in zucchini was in the transpiration stream. Further studies are required to identify more precisely the characteristics of zucchini that promote movement of DDE and other persistent organic pollutants from the root to the shoot in the transpiration stream.

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