A NOVEL LABORATORY SYSTEM FOR DETERMINING FATE OF VOLATILE ORGANIC COMPOUNDS IN PLANTED SYSTEMS

BRADY J. ORCHARD,† WILLIAM J. DOUCETTE,*† JULIE K. CHARD,‡ and BRUCE BUGBEE‡

†Utah Water Research Laboratory, Department of Civil and Environmental Engineering, Utah State University, 8200 Old Main Hill,
Logan, Utah 84322-8200, USA

‡Crop Physiology Laboratory, Department of Plants, Soils, and Biometeorology, Utah State University, 4820 Old Main Hill,
Logan, Utah 84322-4820, USA

Abstract—Contradictory observations regarding the uptake and translocation of volatile organic compounds (VOCs) by plants have been reported, most notably for trichloroethylene (TCE). Experimental artifacts resulting from the use of semistatic or low-flow laboratory systems may account for part of the discrepancy. Innovative plant growth chambers are required to rigorously quantify the movement of VOCs through higher plants while maintaining a natural plant environment. The plant must be sealed in a chamber that allows rapid exchange of air to remove the water vapor lost in transpiration, to resupply the CO_2 consumed in photosynthesis, and to resupply the O_2 consumed in root-zone respiration. Inadequate airflow through the foliar region results in high humidity, which dramatically reduces transpiration and may reduce contaminant flux. Oxygen depletion in static root zones induces root stress, which can increase root membrane permeability. The root zone must be separated from the shoots to differentiate between plant uptake and foliar deposition. Here we describe the design, construction, and testing of a dual-vacuum, continuous high-flow chamber system for accurately determining the fate of VOCs in plants. The system provides a natural plant environment, complete root/shoot separation, the ability to quantify phytovolatilization and mineralization in both root and shoot compartments, continuous root-zone aeration, and high mass recovery.

Keywords—Phytoremediation   Hydroponics   Plant growth chamber   Vegetative uptake   Phytovolatilization

INTRODUCTION

Understanding plant uptake, translocation, and transformation is critical in assessing the effectiveness of phytoremediation and evaluating the potential for food chain contamination by organic chemicals. Because of their commercial interest, much of the experimental plant uptake data and related theory has been generated for pesticides [1]. Much less information is available for other industrial chemicals. The fate of volatile chlorinated solvents, such as trichloroethylene, in planted systems is of particular importance because of their widespread occurrence as groundwater contaminants.

Laboratory-scale studies are usually used to generate fate information prior to field-scale tests because they are cheaper, easier to control, and better enable investigators to elucidate fate mechanisms. Volatile compounds are particularly challenging to study in higher plants because the plants influx and efflux large volumes of gas as part of their normal metabolism. These gases must be continuously resupplied or removed to allow normal rates of plant metabolism. Inadequate gas transfer across shoot and root surfaces inhibits normal plant growth and can cause artifacts in experimental data.

The simplest approach to providing a realistic plant environment is to grow plants in open containers in a greenhouse or plant growth chamber where airflow across leaves can be easily maintained. However, mass recoveries in open systems are low for compounds that are volatile or readily transformed to volatile by-products, including carbon dioxide. This greatly complicates the data and the mechanistic interpretation. Sealed root zones can be used to prevent volatilization directly from the root zone, but they do not prevent translocated volatile compounds or volatile products generated in the foliar portion of the plant from escaping the system.

Plants must be enclosed in a chamber to capture volatile compounds. The chamber can be static, semistatic, or continuous flow. In completely static systems, the chemical is added to the system and samples are usually collected at the end of the study. However, photosynthesis quickly depletes CO_2 and humidity rises to saturation as a result of transpiration. Overheating of the plant chamber during the light period can also occur. Completely static systems can provide high mass recoveries but are only suitable for short duration experiments (minutes to hours). Static chambers do not facilitate detailed measurements of uptake kinetics.

Semistatic systems allow longer duration studies by intermittently replenishing the air. They are often used because of their low cost and operational simplicity. However, the intermittent flow causes unnatural fluctuations in CO_2 and humidity that alter normal plant metabolism. A continuous flow is necessary to fully simulate a natural environment.

The necessary flow rate is surprisingly rapid. The water vapor efflux from plants due to transpiration is 50 to 300 times greater than the CO_2 removal rate due to photosynthesis [2]. Thus, the minimum flow rate is determined by the transpiration rate of the plant in the chamber. Leaf area, incident photosynthetic photon flux (PPF), and the relative humidity in the chamber primarily determine transpiration rates [3]. The incident PPF must be high enough to support plant growth. A PPF of at least 300 μmol/m²·s (15% of full sunlight) is usually necessary to maintain reasonable plant growth [4]. Higher PPF

levels increase plant growth rates but also increase transpiration rates.

An adequate flow rate prevents condensation on the chamber walls and is determined by the water vapor efflux from transpiration. Maximum water vapor efflux usually occurs when all of the incoming shortwave radiation is converted from sensible to latent heat through transpiration. The energy input from lamp radiation can be measured or accurately estimated for most growth chambers from measurements of PPF and the radiation data presented in Bubenheim et al. [5]. The latent heat of evaporation of water is 2.45 kJ/g. A shortwave radiation input of 500 W/m^2 (0.5 kJ/m^2·s) would result in a water vapor efflux of 200 mg/m^2·s if all energy was used to evaporate water. The water-holding capacity of air depends on temperature and humidity and can be calculated from a psychrometric chart or water vapor tables. Air at 25°C holds approximately 7 g/m^3 (7 mg/L) of water between 40 and 70% relative humidity, so a flow rate of 28.6 L/m^2 of surface area per second (200 mg/m^2·s/7 g/m^3 ≈ 60 ft^3/m^2 of surface area per min) would be required to keep the chamber humidity at 70% [6]. Similar calculations are given in Garcia et al. [7]. As a rule of thumb, one air exchange of the chamber volume per minute is necessary to keep the relative humidity below 80%. Lower flow rates may be adequate with small plants in large chambers.

The exiting air can be sampled and analyzed to determine the amount of chemical or volatile transformation product(s) leaving the system. However, the continuous high flow rate needed to remove humidity results in effluent stream concentrations that are often too low to be measured directly. Solid or liquid traps are required to concentrate the sample prior to analysis. Higher flow rates provide better plant growth conditions, but they are more difficult to sample because breakthrough times and trapping efficiencies decrease as flow rates increase. Condensers placed inside the chamber can be used to reduce humidity and thus the required flow, but they increase system complexity and the condensate becomes an additional analytical consideration.

The duration of the experiment also significantly affects system design. The longer the duration, the larger the plants become and the greater the volume of effluent air that must be trapped. To eliminate the need for the large capacity traps required for continuous trapping, periodic short-term sampling is often used. However, this increases the number of samples to be analyzed, increases analytical costs, and makes the determination of mass recoveries more difficult.

For volatile organic compounds, distinguishing between root and foliar uptake is critical. Volatilization of the test chemical directly from the root container and subsequent sorption to the leaf surface can incorrectly infer root uptake and translocation. Indirect and direct approaches have been used to separate root and foliar uptake. The indirect approach is to grow control plants in covered, uncontaminated soil in the same chamber as plants grown in contaminated soil [8–10]. Any contaminant found in the control plant is attributed to foliar uptake and is used to correct the concentrations in the treated plant.

The direct approach attempts to isolate the upper and lower portions of the plant in separate chambers isolated by a physical seal [11–13]. The contaminant is added to the lower, root-zone chamber and any contaminant found in the upper, foliar chamber is attributed to root uptake and translocation. Generally, air is pulled through the foliar chamber, but the root-zone chamber is static. If the physical seal is not perfect, the induced negative pressure can cause leaks from the lower to the upper chamber, mistakenly inferring root uptake, translocation, and volatilization from the plant.

If the lower chamber is static, oxygen utilization by the roots during the course of the experiment can quickly drive the root zone anaerobic. This is especially important in hydroponic systems where oxygen transfer from the headspace into the solution may be rate limited. Plant stress induced by anaerobic root zones may result in increased uptake due to changes in root membrane permeability [14–17].

A closed-system exposure chamber that maintains a natural plant growth environment by providing CO_2 to the shoot compartment and oxygen to the root compartment through a set of computer-controlled pumps and valves was described by McFarlane and Pfleeger [18]. Normal plant functions such as photosynthesis, transpiration, and dark respiration were monitored and controlled, but the direct measurement of phytovolatilization or mineralization was not incorporated into the design. In a subsequent paper [19], low recoveries (~50%) were reported when the system was used to study a volatile organic (nitrobenzene).

Trichloroethylene (TCE) is a volatile organic compound (VOC) extensively used as a degreaser. This has resulted in its inappropriate disposal and subsequent soil and groundwater contamination at many sites. Phytoremediation is being promoted as a more cost-efficient alternative to conventional treatment technologies. Thus, the fate of TCE in planted systems has been examined in several laboratory studies using a variety of experimental designs (Table 1). The conclusions on the importance of TCE uptake and translocation are inconsistent but collectively have led to the widespread belief that plant uptake of TCE is a significant fate process. Experimental details are often insufficient to explain inconsistencies among studies. Artifacts associated with overly simple systems can result in low mass recoveries and lead to inaccurate conclusions. Leaks from the root to the foliar chamber can mistakenly infer root uptake and translocation.

This paper describes a high-rate, dual-vacuum, continuous flow-through chamber system for determining the uptake of VOCs that minimizes the experimental artifacts previously described. Here we describe our testing procedures and hydroponic validation experiments. The results from three studies using this system to evaluate the plant uptake of TCE are presented in a companion paper [20].

SYSTEM DESIGN

Design considerations

Accurate and realistic determination of the uptake and fate of volatile organics in planted systems requires a properly designed laboratory system. The system should provide a natural plant environment, the ability to distinguish between root and foliar uptake, the ability to quantify transpiration (phytovolatilization) of the test compound, the ability to measure transpiration rates, and the ability to quantify CO_2 resulting from the mineralization of the test compound by the plant and/or associated rhizosphere microorganisms. These criteria must be accomplished while maintaining high mass recoveries.

Ideally, the system should also be able to accommodate studies using either hydroponic or soil environments. In a soil system, the uptake of VOCs is influenced by a variety of processes including sorption to soil, mass transfer and diffusion to the roots via liquid and vapor phases, and plant-mediated rhizosphere changes. Hydroponic studies have long

Table 1. Summary of laboratory systems used to study the fate of trichloroethylene (TCE) in plants

| Reference	Exposure concentration	Exposure duration (weeks)	Plant type	System design	Mass recovery
Anderson and Walton [32]	Not reported	1–4.5	Legume, grass, composite, and loblolly pine	Direct separation of foliar and root sections of the plants Root-zone seal composed of a ground-glass stopper sealed with silicone rubber Plants grown in modified Erlenmeyer flasks Glass exposure chamber continuously evacuated Root compartment flushed with air at 24-h intervals	>70%
Schroll et al. [9]	0.175 mg/kg	1	Carrot and radish	10-L desiccator used as a closed, aerated growth chamber Plants grown in covered, uncontaminated soil were placed in the same chamber as plants grown in contaminated soil Air pushed into control dish to create a positive pressure	Not reported
Narayanan et al. [33]	75 mg/L 300 mg/L	14	Alfalfa	No physical separation between the foliar and soil environment U-shaped channel packed with a sandy-silt soil, enclosed with a glass and aluminum cover No control experiments conducted	Not reported
Gordon et al. [11]	5 mg/L	1	Poplar	Direct separation of the foliar and root portions of the plant Seal comprised of glass plates, aluminum foil, and plaster Air pulled from the void between the root and shoot compartments Static root-zone solution Reported 0.03% leakage of TCE into the foliar chamber	Not reported
Newman et al. [12]	50 mg/L	2–3.5	Poplar	No separation of the foliar and root sections of the plant Reactors constructed of PVC pipe Open reactors placed in a greenhouse	Not reported
Schnabel et al. [34]	140 µg/L 560 µg/L	3.5–15	Carrot, spinach, and tomatoes	No separation of the foliar and root portions of planted pots Vegetables grown in ceramic pots enclosed in modified aquariums Air continuously pulled through chambers	45–73%
Burken and Schnoor [13] Burken [35]	50 mg/L	1	Poplar	Direct separation of foliar and root portions of the plant Foliar/root seal comprised of teflon tape, a teflon-lined septum, and acrylic bathroom caulk Semistatic system consisting of two flasks Flow rate of 0.8–1.1 L/min induced by vacuum to foliar chamber Root-zone solution remained static	83%
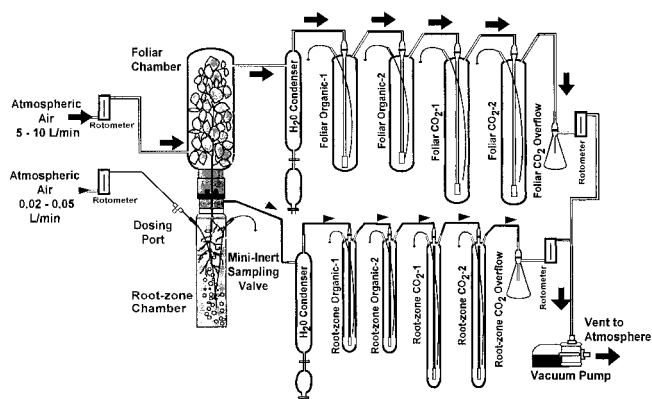
Fig. 1. Schematic representation of the dual-vacuum, continuous high-flow chamber system designed for determining the fate of VOCs in planted systems.

Fig. 2. Diagram of the foliar/root compartment seal.

been used to precisely define the rhizosphere environment and separate uptake mechanisms from the combined effects of plants and soil. While it might appear that the root-zone microbial activity in hydroponic culture would be decreased relative to that in soil, microbial activity in soil is limited by the availability of labile carbon as an energy source. This carbon comes from root exudates and root turnover, which have similar rates in both hydroponic and soil culture [21]. More than 97% of the microbial activity, in both soil and hydroponic culture, is on the root surfaces because of the steady supply of low molecular weight carbohydrates [22,23]. Although the respiration rate of a planted soil can be high, it asymptotically decreases to near zero as the soil is left unplanted and the easily degraded carbon is consumed. Therefore, little difference in root-zone microbial activity would be expected between soil and hydroponic plant growth environments. The system described here has been designed to be suitable for both hydroponic and soil studies but has only been tested for hydroponics. To date, four complete chamber systems have been constructed.

Although hydroponic studies enable the uptake of VOCs to be evaluated without the additional availability issues associated with a soil environment, they present additional design considerations. For compounds with high Henry's law constants, volatilization directly from the hydroponic solution is the major fate process that must be quantified to calculate mass balances. Due to transfer kinetics for oxygen, maintaining an adequate supply of oxygen at the root surface requires the addition of air. Large amounts of VOCs are purged from the solution even with a small root-zone air flow.

System construction

The system consists of separate foliar and root chambers (Fig. 1) with independent airflow control and appropriate traps for collecting CO_2 and VOCs from the exiting air stream. Air from outside the building is pulled through both the foliar and root chambers with a 1/3 hp vacuum pump (Model 72R645, Gast, Benton Harbor, MI, USA). The flow rate through the foliar chamber is 4 to 7 L/min to minimize humidity and potential build-up of volatilized contaminant, if transpired. This high flow also minimizes the potential for redeposition of transpired contaminant onto the leaf tissue and allows for normal evaporative cooling of leaf surfaces. Air enters at the bottom of the foliar chamber and exits at the top, minimizing the potential for transpired contaminant to be pulled back into the
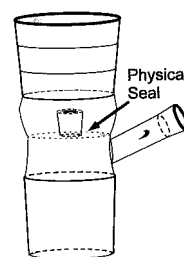
root compartment. Although the leaves are somewhat constrained by the chamber, the high flow rate provided turbulent air mixing throughout the column, as evidenced by the lack of condensation on the interior surfaces of the glass chamber. Flow rates through the root zone are maintained between 20 and 50 ml/min to ensure mixing and to control root-zone oxygen levels. Because the system is under slight negative pressure, flow rates are best monitored at the inlets of each chamber where the pressure is near ambient.

The root zone chamber (~2-L volume) is constructed of a 7.6- × 53-cm section of glass tubing with a 71/60-mm female ground-glass joint at the top. Inlets for the air line and sampling port are located opposite each other just below the ground-glass joint. The foliar chamber is constructed of 10- × 53-cm glass tubing with a 71/60-mm male ground-glass joint at the bottom and an air inlet near the bottom and an outlet near the top.

Both a physical seal and a pressure differential isolate the root and foliar chambers (Fig. 2). The physical seal is constructed of glass with two 71/60-mm ground-glass joints for connection to the foliar and root chambers and a molded glass cover that seals off most of the root-zone environment. A small ground-glass joint (19/22 mm) in the center of the seal accommodates the stem of the plant. The seal is completed with rope caulk (Frost King, Thermwell Products, Paterson, NJ, USA). Rope caulk provides a flexible, gas-tight, nontoxic seal. We found that commercially available silicone caulking (both latex and oil based) reduced plant growth. Wrapping the stem with teflon tape was a less effective seal than the rope caulk. A tight seal was required to maintain the vacuum necessary to sustain bubbling at the bottom of the root zones.

The pressure differential (air is drawn through a 60 cm long Swagelok® [Crawford Fittling, Solon, OH, USA] type 316 stainless steel [SS] needle to the bottom of the water column to ensure complete mixing) between the foliar chamber (−25 cm H_2O) and root chambers (−50 cm H_2O) is an approximately −25 cm H_2O column. This ensures that, if the seal became compromised, contaminant would not leak into the upper chamber and be mistaken for plant uptake, translocation, and phytovolatilization. Conversely, any contaminant volatilized from the leaves is quickly swept up and out of the foliar chamber by the high flow and is not drawn back into the root zone.

Each system includes 12 traps, including two water condensers, four organic traps, four CO_2 traps, and two CO_2 overflow traps. High flow rate and extended duration studies require the use of large volume traps to achieve adequate recoveries during the sampling interval.

Foliar organic and CO_2 traps are made of 7.6-cm diameter glass tubing with two 0.95-cm (95-mm) stems on either side of a 34/45-mm ground-glass joint. Large capacity, coarse-porosity gas dispersion tubes (Model CG-220-01, ChemGlass,

Vineland, NJ, USA) are inserted through the ground-glass joint to the bottom of the foliar traps. The smaller root-zone organic and CO_2 traps are constructed of 3.8-cm diameter glass tubing with two 0.95-cm (95-mm) stems on either side of a 24/40-mm ground-glass joint. Foliar organic traps are approximately 76 cm in length, root-zone organic traps are approximately 61 cm in length, and all CO_2 traps are approximately 107 cm in length. Regular capacity, medium-porosity gas dispersion tubes (Model CG-220-01, ChemGlass) are used in the root-zone traps. All traps are cooled to 4°C in a chilled water bath to minimize volatilization of the trapping solution. Additionally, a cooling coil inside the root-zone organic traps further improves cooling efficiency.

Sampling ports are constructed of an appropriate length of type 316 SS tubing welded through the center of a type 316 SS Swagelok cap. A 15-cm, 14-gauge laboratory pipetting needle (Fisher Scientific, Pittsburgh, PA, USA) is welded to the SS tubing. A Mininert® syringe valve (Fisher Scientific) is used to provide a gas-tight seal.

Connections from the plant chamber to the CO_2 traps are made with type 316 SS Swagelok unions (Crawford Fitting, Solon, OH, USA) and teflon (PTFE) tubing (Fisher Scientific). We initially used SS tubing throughout the system; however, its rigidity caused significant glass breakage. Teflon tubing showed minimal sorption losses and was more flexible. Subsequent connections between CO_2 traps are made with nylon or high-density polyethylene fittings and tubing (Consolidated Plastics, Twinsburg, OH, USA).

Volatile organic and CO_2 traps

Volatile organic traps. Efficiently trapping volatile organics at flow rates above 1 L/min is difficult using solid sorbent tubes because of kinetic and capacity limitations, flow channeling, and flow restrictions. Split sampling can overcome some of these difficulties but results in increased sample collection and analysis costs and requires the continuous, accurate measurement of flow rates to quantify phytovolatilization. Liquid traps containing ethylene glycol monomethyl ether have been widely used to trap volatile organics [8,9,24,25], and we scaled liquid ethylene glycol monomethyl ether traps to accommodate high flow rates and used these traps in the initial trials.

CO_2 traps. Ethanolamine was initially tested for trapping CO_2 because it had been used in similar applications [24]. However, at the high flow rates required in the foliar chambers, low efficiencies (<70%), significant foaming, and loss of trapping solution due to volatilization were observed.

Potassium hydroxide (KOH) solutions (>0.5 N) are much less volatile and were found to provide trapping efficiencies of greater than 98% at a variety of flow rates and trap configurations. A 2.0 N solution of KOH was used to maximize trap capacity. At the high flow rates used in this system, foaming still occurred. To prevent carryover of the solution, foliar traps are filled to only one-third volume (approximately 1,000 ml). In addition, an empty trap is located after the CO_2 traps to collect any overflow and to protect the vacuum pump. At 10 L/min, the calculated foliar trap capacity is 12 d. Additional KOH solution is added weekly to ensure sufficient capacity. Because of the lower flow rate in the root zone, the calculated trap capacity is greater than 600 d.

Trapping solution analysis

Trapping solution samples were analyzed for ^{14}C by liquid scintillation counting using a Beckman Model 1701 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) and Ready Gel® scintillation cocktail (Beckman Instruments). Organic trap samples were directly analyzed after adding 2 ml of sample to 5 ml of scintillation cocktail. Root-zone and water condenser samples were analyzed after adding 3 ml of sample and 2 ml of deionized water to 15 ml of scintillation cocktail. Samples were kept at 4°C until analysis to minimize TCE volatilization.

Samples with high ionic strength are difficult to analyze directly by liquid scintillation counting due to high background counts that vary with ionic strength and the formation of two phases in the scintillation cocktail. To avoid this problem and to minimize the potential carryover of [^{14}C]TCE, a barium chloride precipitation procedure was used to precipitate CO_2 from the KOH trapping solution. After the precipitate is separated from the solution, it is acidified and the $^{14}CO_2$ is re-evolved and collected in an amine-based trapping solution [24]. This method is based on procedures described for assessing aerobic biodegradation in soil [26]. Spike experiments, using [^{14}C]NaHCO$_3$, yielded recoveries of >90%. It is often assumed that the precipitate is solely CO_2; however, spike experiments with [^{14}C]TCE show that small amounts of TCE (<1%) can be carried through the precipitation process.

Method detection limits for the VOC and CO_2 trap samples, defined as the minimum activity (dpm) that can be measured and reported with 99% confidence as greater than zero, were determined based on the standard deviation of multiple matrix blanks for each given matrix type and the appropriate *t* statistic [27]. The minimum amount of ^{14}C that could be detected in a given trap (in terms of total ^{14}C or mg TCE) was conservatively calculated by assuming the ^{14}C concentration in the trapping solution was at the method detection limits (~2.5 dpm/ml for organic trapping solution; ~3 dpm/ml for CO_2 trapping solution).

TRIAL RUNS/CHAMBER VALIDATION

Three preliminary trials were performed to evaluate system performance and develop appropriate dosing and sample collection procedures prior to beginning full-scale studies. The complete system was assembled and enclosed within a walk-in plant growth room (Model PGW-132, Percival Scientific, Boone, IA, USA). Imperial Carolina hybrid poplar (*Populus deltoides × nigra,* DN34) cuttings (Hramor Nurseries, Manistee, MI, USA) were hydroponically rooted in the greenhouse for 1 to 2 weeks prior to being transplanted into the chamber system (5–7 d prior to dosing). With vigorous aeration, hydroponic rooting provides root initiation equal to that of solid media substrates like pearlite and minimizes residues on root surfaces. The size of the root system increased with increasing shoot growth. Although the entire 50-cm-deep column was aerobic, the root density was about 5 to 10 times higher in the top third of the column than in the bottom two thirds of the column. This root distribution is typical for both hydroponic and soil-grown plants [28]. At the end of the study, the longest roots had grown to the bottom of the root-zone column. A complete and appropriately dilute nutrient solution [29] was added to replace water lost to transpiration and sampling. This provides for vigorous growth and eliminates the need for periodic solution replacement. The nutrient solution also provided silica, which is absent from standard nutrient solution recipes but is ubiquitous in soils. Silica has been shown to regulate the uptake of micronutrients [30,31] and may play a role in the regulation of organic uptake.

During all trials, the growth room temperature was maintained at $29/24 \pm 1°C$ day/night with a 16-h photoperiod. Cool white fluorescent lamps provided a PPF of 600 $\mu mol/m^2 \cdot s$ at the tops of the chambers. The root-zone chambers were enclosed in a light-tight box to prevent algae growth in the nutrient solution. Daily transpiration rates are determined from the volume of nutrient solution added to the system, corrected for evaporation into the root-zone airflow (about 1% of the transpiration rate), and removed during sampling.

Trial 1: Testing the foliar/root seal

To evaluate the integrity of the foliar/root seal, a continuous dose of [^{14}C]TCE was supplied to the inlet air stream of the foliar chamber over a 3-d period. A glass rod was substituted for the poplar cutting and sealed in the same manner as the poplar cuttings. Flow rates were maintained at 4 to 7 and 0.02 to 0.05 L/min through the foliar and root chambers, respectively. No ^{14}C was found in any root-zone traps or root-zone solution, confirming the integrity of the foliar/root seal. Trapping efficiencies (3 d at 7 L/min) for the foliar VOC traps were 33%. This was confirmed during additional spike recovery studies. This is equivalent to the trapping efficiency predicted by using experimentally determined equilibrium distribution coefficients for TCE between air and the trapping solution ($\sim 2 \times 10^{-4}$). Sampling a fraction of the foliar chamber exit gas at a lower flow rate (split flow sampling) would improve sensitivity in the VOC traps but would increase sampling and analytical costs. Split flow sampling could allow an appropriate solid phase to be substituted for the liquid VOC traps.

Trial 2: Development of continuous dosing procedure

Because of the continuous flow, TCE must be continuously added to the root zone to maintain a constant concentration. To determine the dosing rate, a mixture of labeled and unlabeled TCE was spiked into the root-zone solution to yield an initial concentration of 1 mg/L. Root-zone solution samples, collected at 1-h intervals, showed that the TCE was almost completely purged from the solution within 24 h at a flow rate of 0.2 L/min. Manually spiking the root-zone solution on an hourly basis indicated that a constant concentration could be maintained with continuous dosing.

A continuous dosing procedure was developed based on the kinetic information obtained during this trial. A mixture of ^{14}C-labeled and unlabeled TCE is continuously delivered to the root-zone chamber via the inlet air stream using a multi-syringe pump (Fisher Scientific) equipped with a 25-μl teflon-tipped, gas-tight syringe (Hewlett Packard, Wilmington, DE, USA). A dosing rate of approximately 0.35 $\mu l/h$ (neat TCE solution) is needed to maintain a constant concentration of 1 mg/L. This dosing rate is dependent on the root-zone air flow rate. Any change in flow requires a corresponding change in the dosing rate to maintain constant TCE concentrations. Root-zone solution concentrations are monitored daily to ensure that a constant concentration is maintained.

This trial ran intermittently for 10 d. Recovery of ^{14}C was 96%, with 90% in the root-zone organic traps, 5% in the root-zone solution, 1% in the root-zone CO_2 traps, and less than 0.1% in the plant tissue. No ^{14}C was found in foliar organic or CO_2 traps. During this trial, it was observed that a significant amount of the ethylene glycol monomethyl ether in the foliar traps was lost due to volatilization. Spike recovery experiments showed that the addition of 20% ethylene glycol monobutyl ether could be used to reduce volatilization without reducing

trapping efficiency. An 80% ethylene glycol monomethyl ether/20% ethylene glycol monobutyl ether organic trapping solution was used in the third preliminary trial.

Trial 3: Testing of continuous dosing procedure

An additional trial was performed to test the ability of the continuous dosing system to maintain a constant root-zone concentration and determine the mass recovery under a continuous dosing scenario. Traps were sampled using 5-ml Hamilton Leur-Lock Gastight 1000 Series syringes (Fisher Scientific). All samples (3 ml) were taken in triplicate with triplicate methanol syringe washes between samples. Volatile organic traps were sampled at 3- to 4-d intervals to provide kinetic transpiration data. CO_2 traps were sampled at the end of each experiment. Water condensers were emptied and measured as needed.

Using the same flow rates as the second trial, the continuous dosing system maintained a root-zone solution concentration of 1.2 ± 0.2 mg/L during the dosing period. The mass recovery for this trial was 85%, with a distribution similar to that seen in the first trial.

CONCLUSIONS

The design, construction, and testing of a dual-vacuum, continuous high-flow chamber system for accurately determining the fate of VOCs in plants was described. The system is designed to provide a natural plant environment, complete root/shoot separation, the ability to quantify transpired VOCs and mineralization to CO_2 in both root and shoot compartments, continuous root-zone aeration, and high mass recovery. Split flow sampling of the foliar chamber exit gas could improve VOC trapping efficiencies but would significantly increase sampling and analytical costs and may not significantly improve overall mass recovery. While the performance of the system was evaluated using only TCE and hydroponically grown hybrid poplar, it was designed to accommodate soil studies and other volatile and nonvolatile chemicals. Results from several experiments using this system are presented in the companion paper [20].

REFERENCES

1. Nellessen JE, Fletcher JS. 1993. Assessment of published literature pertaining to the uptake/accumulation, translocation, adhesion, and biotransformation of organic chemicals by vascular plants. *Environ Toxicol Chem* 12:2045–2052.
2. Salisbury FB, Ross CW. 1992. *Plant Physiology,* 4th ed. Wadsworth, Belmont, CA, USA.
3. Thornley JHM, Johnson IR. 1990. *Plant and Crop Modeling: A Mathematical Approach to Plant and Crop Physiology*. Oxford University Press, New York, NY, USA.
4. Langhams RW, Tibbitts TW. 1997. Plant growth handbook. Special Report 99. Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames, IA, USA.
5. Bubenheim D, Bugbee B, Salisbury F. 1988. Radiation in controlled environments: Influence of lamp type and filter material. *J Am Soc Hortic Sci* 113:468–474.

6. Bugbee B. 1992. Steady-state canopy gas exchange: System design and operation. *Hortic Sci* 27:770–776.

7. Garcia R, Norman J, McDermitt D. 1990. Measurements of canopy gas exchange using an open chamber system. In Goel N, Norman J, eds, *Instrumentation for Studying Vegetation Canopies for Remote Sensing in Optical and Thermal Infrared Regions,* Vol 5—Remote Sensing Reviews. Harwood Academic, London, UK, pp 141–162.

8. Schroll R, Scheunert I. 1992. A laboratory system to determine separately the uptake of organic chemicals from soil by plant roots and by leaves after vaporization. *Chemosphere* 24:97–108.

9. Schroll R, Bierling B, Cao G, Dörfler U, Lahaniati M, Langenbach T, Scheunert I, Winkler R. 1994. Uptake pathways of organic chemicals from soil by agricultural plants. *Chemosphere* 28:297–303.

10. Ferro A, Kennedy J, Doucette WJ, Nelson S, Jauregi G, McFarland B, Bugbee B. 1997. Fate of benzene in soils planted with alfalfa: Uptake, volatilization, and degradation. In Kruger EL, Anderson TA, Coats JR, eds, *Phytoremediation of Soil and Water Contaminants.* ACS Symposium Series 664. American Chemical Society, Washington, DC, pp 223–237.

11. Gordon M, et al. 1997. Phytoremediation of trichloroethylene with hybrid poplars. In Kruger EL, Anderson TA, Coats JR, eds, *Phytoremediation of Soil and Water Contaminants.* ACS Symposium Series 664. American Chemical Society, Washington, DC, pp 177–185.

12. Newman LA, et al. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ Sci Technol* 31:1062–1067.

13. Burken JG, Schnoor JL. 1998. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. *Environ Sci Technol* 32:3379–3385.

14. Lee RB. 1977. Effects of organic acids on the loss of ions from barley roots. *J Exp Bot* 28:578–587.

15. Riveria CM, Penner D. 1978. Effect of calcium and nitrogen on soybean (*Glycine max*) root fatty acid composition and uptake of Linuron. *Weed Sci* 26:647–650.

16. Cakmak I, Marschner H. 1988. Increase in membrane permeability and exudation of roots of zinc deficient plants. *J Plant Physiol* 132:356–361.

17. Taiz L, Zeiger E. 1998. *Plant Physiology,* 2nd ed. Sinauer, Sunderland, MA, USA.

18. McFarlane JC, Pfleeger T. 1987. Plant exposure chambers for study of toxic chemical–plant interactions. *J Environ Qual* 16:361–371.

19. Fletcher JS, McFarlane JC, Pfleeger T, Wickliff C. 1990. Influence of root exposure concentration on the fate of nitrobenzene in soybean. *Chemosphere* 200:513–523.

20. Orchard BJ, Doucette WJ, Chard JK, Bugbee B. 2000. Uptake of trichloroethylene by hybrid poplar trees grown hydroponically in flow-through plant growth chambers. *Environ Toxicol Chem* 19:895–903.

21. Carson EW. 1974. *The Plant Root and Its Environment.* University Press of Virginia, Charlottesville, VA, USA.

22. Rovira AD, Davey CB. 1974. Biology of the rhizosphere. In Carson EW, ed, *The Plant Root and Its Environment.* University Press of Virginia, Charlottesville, VA, USA, pp 153–204.

23. Smart D, Ferro A, Ritchie K, Bugbee B. 1995. On the use of antibiotics to reduce rhizoplane microbial populations in root physiology and ecology investigations. *Physiologia Plantarum* 95:533–540.

24. Abbott CK, Sorensen DL, Sims RC. 1992. Use and efficiency of ethylene glycol monomethyl ether and monoethanolamine to trap volatilized [7-^{14}C]napthalene and ^{14}CO$_2$. *Environ Toxicol Chem* 11:181–185.

25. Topp E, Scheunert I, Attar A, Korte F. 1986. Factors affecting the uptake of ^{14}C-labeled organic chemicals by plants from soil. *Ecotoxicol Environ Saf* 11:219–228.

26. U.S. Food and Drug Administration. 1987. Environmental assessment technical assistance handbook. PB87-175345. National Technical Information Service, Springfield, VA.

27. U.S. Environmental Protection Agency. 1996. Test methods for evaluating solid waste. SW-846, Rev 4. Technical Report. Washington, DC.

28. Mian M, Nafziger E, Kolb F, Teyker R. 1994. Root size and distribution of field grown wheat genotypes. *Crop Sci* 34:810–812.

29. Grotenhuis T, Bugbee B. 1997. Super-optimal CO$_2$ reduces seed yield but not vegetative growth in wheat. *Crop Sci* 37:1215–1222.

30. Epstein E. 1994. Review: The anomaly of silicon in plant biology. *Proc Natl Acad Sci USA* 91:11–17.

31. Raf MM, Epstein E, Falk RH. 1997. Silicon deprivation causes physical abnormalities in wheat (*Triticum aestivum* L.) *J Plant Physiol* 151:497–501.

32. Anderson TA, Walton BT. 1995. Comparative fate of ^{14}C trichloroethylene in the root zone of plants from a former solvent disposal site. *Environ Toxicol Chem* 14:2041–2047.

33. Narayanan M, Davis LC, Erickson LE. 1995. Fate of volatile chlorinated organic compounds in a laboratory chamber with alfalfa plants. *Environ Sci Technol* 29:2437–2444.

34. Schnabel WE, Dietz AC, Burken JG, Schnoor JL, Alvarez PJ. 1997. Uptake and transformation of trichloroethylene by edible garden plants. *Water Res* 31:816–824.

35. Burken JG. 1996. Uptake and fate of organic contaminants by hybrid poplar trees. PhD thesis. University of Iowa, Iowa City, IA, USA.