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Root uptake of organic contaminants into plants: Species differences

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ROOT UPTAKE OF ORGANIC CONTAMINANTS INTO PLANTS:

SPECIES DIFFERENCES

by

Naho Orita

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ABSTRACT

Root Uptake of Organic Contaminants into Plants:

Species Differences

by

Naho Orita, Master of Science

Utah State University, 2012

Major Professor: Dr. William J. Doucette
Department: Civil and Environmental Engineering

Trace amounts of xenobiotic organic contaminants have been frequently identified in the environment, including surface water and wastewater streams, and some are even in drinking water. The concern of unintended ingestion by humans or wildlife of such compounds resulting from the uptake by plants has risen in recent years. Although the uptake of a variety of xenobiotic organic contaminants by plants has been reported and the contaminants are found in the fruits in some cases, the differences between plant species are not fully understood. The emphasis of this research is to investigate the unique uptake ability of zucchini that has been reported repeatedly in recent years.

Xylem saps, collected using a pressure chamber technique, were used to determine the values of Transpiration Stream Concentration Factor (TSCF), the ratio of the contaminant concentration in the xylem to that in the solution. Soybean "hoyt," squash "zephyr," and zucchini "gold rush" were used to compare the uptake ability of each plant. The root tissue was analyzed for total carbon and lipid content. Xylem sap

was analyzed for total organic carbon and protein contents. The solubilities of the compounds in the xylem sap and deionized water were also determined using a modified shake flask method.

From the measurement of TSCF, the uptake of hydrophobic contaminants in zucchini "gold rush" was found to be three-to tenfold of the other two plant species. The lipid content of the root tissue from zucchini "gold rush" was twice as much of that in soybean and squash "zephyr," indicating enhanced adsorption of the hydrophobic compounds. The solubility of triclocarban in the xylem sap of zucchini "gold rush" was also twice the amount of that in soybean xylem sap. The enhanced solubility could be a result of high protein content measured in zucchini "gold rush" xylem sap, which may be increasing the facilitated transport of the hydrophobic compounds.

The data generated in this study will be used to better understand the mechanistic differences associated with the plant uptake of organic contaminants by different species. This information can also be used in the selection of the plant species used in risk assessment studies and phytoremediation studies.

(81 pages)

PUBLIC ABSTRACT

Root Uptake of Organic Contaminants into Plants:

Species Differences

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Utah State University, 2012

Major Professor: Dr. William J. Doucette
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Xenobiotic organic contaminants are widely found in the environment, including soils, sediments, surface waters, wastewater streams, and even in drinking water. Food chain contamination resulting from the uptake of these contaminants by plants is a concern. Although the uptake of a variety of xenobiotic organic contaminants by plants has been reported but the differences between plant species are not fully understood. The emphasis of this thesis research is to further investigate the unique root to shoot transfer ability of "gold rush" zucchini that has been reported repeatedly in recent years.

A pressure chamber technique was used to measure transpiration stream concentration factor (TSCF) values, a descriptor used to quantify root to shoot transfer for several organic chemicals of varying hydrophobicity in soybean "hoyt," squash "zephyr," and zucchini "gold rush." Root tissue was analyzed for total carbon and lipid content. Xylem sap was analyzed for total organic carbon and protein content. The

solubilities of the compounds in the xylem sap and deionized water were also determined using a modified shake flask method.

The measured TSCF values showed that the uptake of hydrophobic contaminants in zucchini "gold rush" was three to tenfold greater than soybean and squash "zephyr." The lipid content of the zucchini "gold rush" root tissue was twice that of soybean and squash "zephyr" and showed greater sorption of the hydrophobic compounds. The solubility of triclocarban in zucchini "gold rush" xylem sap was also twice that in soybean xylem sap. The enhanced solubility could be associated with the high protein content measured in zucchini "gold rush" xylem sap.

The data generated in this study will be used to better understand the mechanic differences associated with the plant uptake of organic contaminants by different species. This information can also be used in the selection of the plant species used in risk assessment studies and phytoremediation studies.

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CHAPTER 1

INTRODUCTION

The uptake of xenobiotic organic contaminants by plants and the resulting ingestion of these plants by humans or wildlife is a potential public health and environmental safety concern. In addition to risk assessment, understanding plant uptake of organic contaminants is important for evaluating the potential effectiveness of phytoremediation and in the development and management of herbicides.

The uptake of a variety of organic contaminants by plants has been reported for numbers of organic pollutants including organic solvents, chlorinated solvents, pesticides, persistent organic pollutants (POPs) including polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCB), and more recently pharmaceutical and personal care products (PPCPs) [e.g., 1-14]. In some cases, contaminants have been detected in parts of the plants intended for human consumption [e.g., 15].

The transpiration stream concentration factor (TSCF), the ratio of chemical concentration in the xylem to that in the water for transpiration, is often used as a descriptor for the transfer of contaminants from roots to shoots. Compounds that enter plant roots at the same rate as water have a TSCF value of one. For nutrients that are actively taken up by plants, like nitrogen, phosphorus and potassium [16], TSCF values can be greater than one. Values of TSCF should be less than one for xenobiotic organic compounds. TSCF values are used in models along with transpiration rate to predict the concentration of contaminants in the shoots and edible tissues of plants [17]. Transpiration rates depend mainly on environmental factors such as sun light, humidity,

wind speed and temperature [18,19], while the root uptake of xenobiotic organic compounds depends mainly on the hydrophobicity of the compound [5,20].

Relatively few experimental TSCF values have been reported for organic contaminants due to the experimental costs, lack of standard method for TSCF determination, and the absence of a regulatory agency mandate for the generation of TSCF values. Also, for the few compounds that have more than one reported value, the variation is generally quite large likely due to methodology differences but also potentially associated with differences in the plant species used.

Using relatively small sets of experimental plant uptake data, usually for a single plant species, several relationships between TSCF and chemical hydrophobicity, expressed as the logarithm of octanol water partition coefficient (Log K_{ow}) [e.g., 5,20] have been reported. These relationships have been used to predict TSCF values for organic chemicals lacking experimental plant uptake data. The bell-shaped relationship between log K_{ow} and TSCF reported by Briggs et al. in 1982 [21], suggested that both highly hydrophobic and highly water soluble compounds would not be significantly taken up by plants. However, more recent studies examining the relationship between log K_{ow} and TSCF indicate that root uptake is most important for highly water soluble, non-ionized, low log K_{ow} compounds [e.g., 5,22].

These relationships between TSCF and log K_{ow} do not consider plant species as a variable although differences in root lipid contents have been suggested as a potential factor that can influence uptake [5,21-26]. Although the soybean plant seems to be most frequently used in the uptake studies of organic contaminants, a variety of food crops has been used in similar studies including cabbages, carrots, corns, cucumbers, potatoes,

wheat, squash, and zucchini [27-33]. While most demonstrate a similar uptake behavior, zucchini "gold rush" (*cucurbita pepo ssp pepo)* has been repeatedly reported to show higher than expected translocation of hydrophobic organic compounds [10,34-36]. This unique ability for translocation of hydrophobic compounds is not fully understood but could be the result of unique root exudates or xylem sap properties [e.g., 8,10] or the proteinic substances that plays a role of solubilization in xylem sap [35,37].

To further investigate the influence of chemical hydrophobicity and plant species on root uptake and translocation, the TSCF values were measured for a series of xenobiotic organic compounds ranging from -0.07 to 4.90 in log K_{ow} using soybean "hoyt," squash "zephyr," and zucchini "gold rush." The selection of the plant species were based on the popularity of soybeans in risk assessment studies, uniqueness of zucchini "gold rush" reported repeatedly, and another species from the *cucurbita* family squash "zephyr" that has reported not to transfer hydrophobic compounds from root to shoot as readily as the zucchini.

To measure plant root uptake and translocation, a pressure chamber technique was used. Commonly used by plant physiologists to determine water potential and root hydraulic conductivity, Dettenmaier et al. [5] used this technique to determine TSCF values, because it has several advantages over traditional intact plant uptake studies including shorter experimental durations, minimal losses due to volatilization and metabolism, and the direct measurement of xylem sap.

The main focus of this research is to investigate the unique root to shoot transfer ability of zucchini "gold rush" that has been reported repeatedly in the literature for hydrophobic organic chemicals when compared to other plant species. A pressure

chamber technique was adapted from previous studies [e.g., 5] and used to measure TSCF values for a series of organic chemicals of varying log K_{ow} for plant species including soybean, squash "zephyr," and zucchini "gold rush." The root tissues were analyzed for total organic carbon and the lipid. Xylem saps were analyzed for total carbon and protein contents. The solubilities of the compounds in the xylem saps and deionized water were also determined using a modified shake flask method.

The data generated in this thesis research will be used to better understand the mechanic differences associated with the plant uptake of organic compounds by different species. This information can also be used in the selection of the plant species used in risk assessment studies and phytoremediation studies.

CHAPTER 2

LITERATURE REVIEW

Xenobiotic organic compounds uptake by plants

Numerous studies have shown the potential for organic contaminants to be taken up from solid or liquid media by food crop's plant roots and transferred into above ground tissues [5,15,27-29,31,35,36,38]. The contaminants were found in the edible parts of the plants in some cases. Thus, uptake and transfer into edible plant tissues is a potential public and environmental health and safety concern when plants are growing in contaminated environments.

Transpiration stream concentration factors (TSCF) and bioconcentration factors (BCF), or concentration in above ground tissues (e.g., micrograms of chemical compound per gram of wet plant) have been used to describe the extent of chemical transfer. The difference between those descriptors will be described later on this chapter.

Uptake by roots

The root uptake of organic compounds is thought to depend on: (i) physical/chemical properties of the compound, (ii) environmental conditions including sun light, humidity, wind speed and temperature [18], and (iii) plant physiological characteristics such as plant species [39]. The physical/chemical properties of the compound have been studied numerous times and their hydrophobicity, usually expressed as an octanol water partition coefficient (K_{ow}) is believed to be the key factor [17,40,41]. Relatively few experimental data for the physiological differences among plant species have been reported and the variety of the plant species used is limited.

For ionizable compounds, the acid dissociation constant value (pK_a) and the pH of the environment determine the relative hydrophobicity of the compound. Generally, it is believed that the ionized organic compounds are not taken up by plants as well as neutral organic compounds because charged compounds need to be transported using proton pumps which use energy supplied from ATP-ADP reaction [42] although hydrophobic ion pairing (HIP) has been suggested to increase hydrophobic compound's solubility in organic solvents and root membranes [43]. Due to the high transport activation energy requirement, charged organic compounds are unlikely to be transported across hydrophobic membrane.

The uptake of water from roots surface to xylem is believed to be following one of three different pathways: the apoplast, symplast and transmembrane [44]. The transport of xenobiotic organic compounds from roots to xylem is thought to follow the same pathway as water. The amount of the contaminant uptake has been shown to be proportional to water [16,45], indicating passive uptake. The roots transport pathways to xylem are illustrated in Fig. 1.

In the apoplast pathway, water enters the cell wall in the hydrophilic root's hair, then, moves through the continuous system of cell walls as it travels through the epidermis and cortex. In the symplastic pathway, water enters the symplast at the root's hair passing through plasma membrane.

Transpiration across a plasma membrane can occur either by diffusion or through specialized transmembrane proteins. One of the most common water channel proteins is the aquapolin that can transport water 20 times faster than any other proteins [19,46]. The aquaporins are believed to be used exclusively on water uptake by plant roots with the

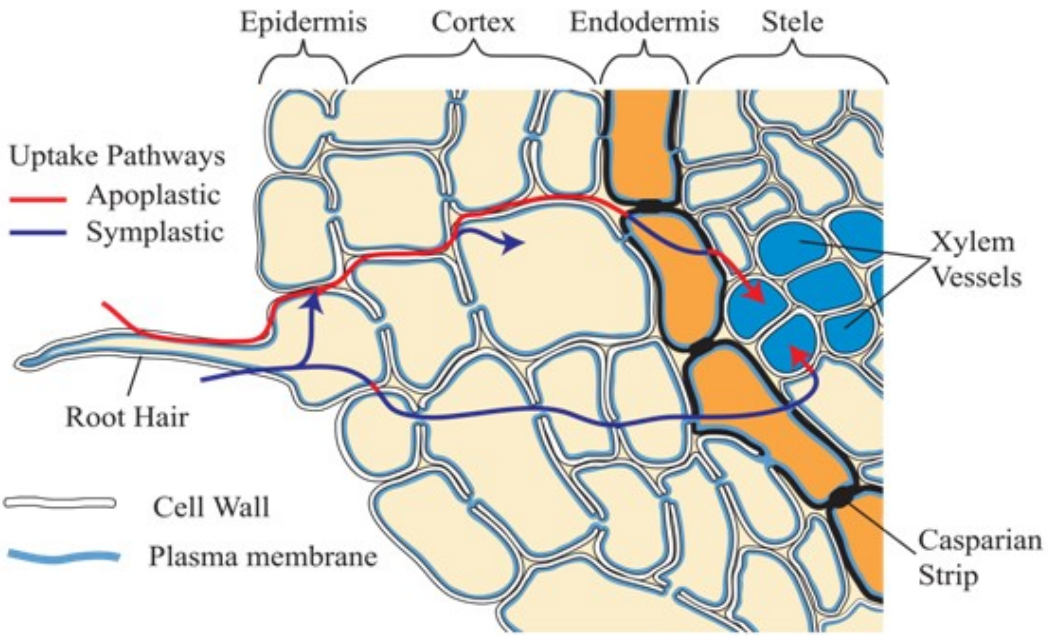

Fig. 1. Diagrams of transport pathway to xylem (Adapted from Campbell et al., 1999[44]. Biology. Harlow, UK: Pearson Benjamin Cummings. with modifications)

minimal resistance [46,47] especially under low water transpiration conditions [48], where water travels apoplascically under high water transpiration conditions [49].

Water travels from one symplast to another through plasmodesmata [44]. Lipophilic compounds favor the symplastic pathway, partitioning to tissue as they cross the membrane [50]. The apoplastic pathway is blocked by the hydrophobic Casparian strip as the water and reaches the endodermis. The water then is forced to pass through the plasma membrane to go into the symplast of the endodermal cell and transported to vascular cylinder symplastically. It implies that the rate limiting step associated with the transport of organic chemicals is the release from the root membrane into the xylem vessels in either apoplastic or symplastic pathways.

Relationship between plant tissue and exposure concentration

The soil plant bioconcentration factor (BCF) and transpiration stream concentration factor (TSCF) are the two descriptors most often used to quantitatively describe the relationship between plant tissue and exposure concentrations. Both BCF and TSCF are generally assumed to be constants for a particular chemical compound in risk assessment studies. Values of BCF are the ratios of the contaminant concentration in the plant tissue (e.g., shoots, roots, fruits) relative to that in the exposure medium in which the plant is growing [51]. Values of TSCF are the ratio of the contaminant's concentration in the xylem to that in the exposure solution [16] and are used along with the amount of water transpired by a plant to predict the amount of contaminants in the above ground tissues [21]. Although both descriptors are widely used, the values of TSCF could be more useful in plant uptake models used in phytoremediation because of its direct relationship to transpiration.

The BCF is commonly measured using intact plants growing in contaminated soil or hydroponics. Values of BCF are calculated simply-- concentration in the target plant compartment (e.g., shoots, roots, fruits) divided by concentration in the media used. The values of BCF for the roots are often referred to as root concentration factor (RCF). The approach does not measure xylem concentration or account for passive uptake which is a function of the water transpired by the plant [51]. Losses of the compounds due to volatilization and metabolism within the plant are not directly accounted for. There is little information regarding the impact of plant age on BCF or RCF values and it is possible that values measured using young plants in a laboratory setting may be different than older plants harvested in the field. The values of TSCF are typically used in

modeling efforts since they can be used to relate directly to transpiration and other factors that are related to transpiration including plant age, plant size, and climate.

As previously mentioned, the TSCF is a ratio of chemical concentration in the xylem to that in the exposure solution. Compounds that enter plant roots at the same rate as water are assigned a TSCF value of one. The value can be greater than one for nutrients such as nitrogen, phosphorus and potassium if they are actively taken up by plants [16]. Values of TSCF are less than one for organic compounds that are passively translocated from roots to shoots along with water used for transpiration. Generally, transpiration rates depend on environmental factors such as sun light, humidity, wind speed and temperature, while the root uptake of xenobiotic organic compounds depends mainly on hydrophobicity of the compound [16,20]. The hydrophobicity, expressed as the logarithm of octanol water partition coefficient, is believed to be a key property especially for predicting the root uptake of organic compound [20] especially in the uptake prediction modeling.

Values of TSCF have typically been measured using two general approaches, one using intact plants and the other using a detopped plant in a pressure chamber. In the intact plant method, plants are usually grown hydroponically with constant root-zone chemical concentration in the solution. This intact plant approach does not allow direct collection of xylem sap from the plants and can be difficult to account for losses due to volatilization and metabolism within the plant [51].

In the pressure chamber method, a hydroponically-grown plant is detopped just below the first cotyledonary node and inserted to a chamber that contains known concentration of a chemical. As pressure increases in the chamber, the xylem is forced

through the roots and can be analyzed directly as it exits the cut stem. The main advantages of the pressure chamber method are the shorter duration of the experiment it that enables the direct correction and measurement of compounds in xylem sap.

The TSCF values are often used to predict the total amount of contaminant uptake by plants as a function of transpiration in phytoremediation studies. For example, one simple way to calculate the plant uptake using the TSCF values is expressed in Equation 1.

$$\text{Plant Uptake}=(\text{TSCF})(C_c)(T)(f) \quad \text{(Equation 1)}$$

Values of TSCF (unitless) are multiplied by the concentration of contaminants (e.g., mg/L) in the water used by plants, volume of water transpirated (e.g., L), and the fraction of the contaminated water used (≤1, unitless) to calculate the mass of chemical (e.g., mg) taken up by a single plant or a group of plants.

Relatively few experimental values of BCF and TSCF have been reported for organic contaminants due to the experimental costs, lack of standard method for determinations, and the absence of a regulatory agency mandate for the generation of TSCF, BCF values. While a few compounds have more than one reported value, the variation is generally quite significant likely due to methodology differences but also potentially associated with differences in the plant species used.

A wide variety of food crops has been used in the plant uptake studies [27-33] which has demonstrated similar uptake behavior. Consequently, the plant species is often neglected to be an influential factor on the plant uptake studies. Several recent studies have reported the higher than expected translocation of hydrophobic organics by zucchini "gold rush" (*cucurbita pepo ssp pepo*) [10,34-36]. This unique ability of zucchini "gold

rush" has not been fully understood, although some suggest that the differences in root lipid contents can be a potential factor that can influence uptake [5,21-26] in addition to the unique root character of exudates and/or xylem sap [e.g., 8,10]

Prediction of transpiration stream concentration factor

To predict values of TSCF, several quantitative structure-activity relationships (QSAR) have been developed. A bell shaped relationship between TSCF vs log K_{ow} was first observed by Briggs [21] and it has been frequently used in plant uptake models [16,50,52]. Based on this relationship, expressed in Equation 2, highly water-soluble polar compounds with low log K_{ow} are not expected to be readily taken up by plants due to the lipophilic character of the roots. The highly hydrophobic compounds with high log K_{ow} do not reach the xylem because of their strong sorption to roots [21]. That implies an intermediate hydrophobicity is necessary for significant uptake and transport of organic compounds and neither very polar nor hydrophobic compounds are expected to be significantly translocated.

Even though Briggs' bell shaped relationship is widely used, there have been several more recent studies that indicate that the root uptake of non-ionized, polar, and hydrophilic compounds, including MTBE [12], sulfolane [13], 1,4-dioxane [14], is more likely [e.g., 5,53]. One of the models developed accordingly is expressed in Equation 3. When the relationships are compared, as illustrated as Fig. 2, it can be seen that both relationships predict minimal root uptake for hydrophobic compounds with high log K_{ow} values; however, the two relationships are diametrically opposed for hydrophilic compounds with low log K_{ow} values. The difference between the two relationships makes a significant change in the potential of phytoremediation. For example, a polar compound

such as caffeine, the relationship established by Briggs et al. [21] suggests that the chance of the compound to be remediated is minimal, including phytovolatilization, metabolism, or sequestration. In contrast, the relationship developed by Dettenmaier et al. [5] indicates that the polar, hydrophobic compounds have the highest potential for successful phytoremediation.

$$TSCF = 0.784 * \exp\left(\frac{-(\log K_{ow} - 1.78)^2}{2.44}\right) \quad \text{(Equation 2)}$$

$$TSCF = \frac{11}{11 + 2.6^{\log Kow}} \quad \text{(Equation 3)}$$

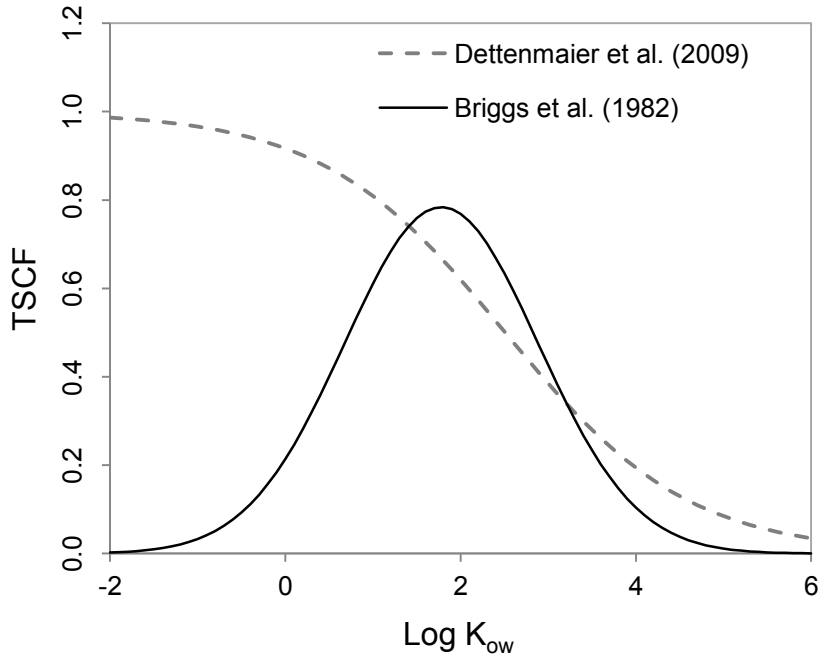

Fig. 2. Comparison of two relationships established by Briggs et al. (1982) and Dettenmaier et al. (2009) [5]

Potential differences among plant species

Determination of the potential differences in the uptake of organic compounds between plant species is valuable for risk assessment as well as phytoremediation. A

plant with higher capability for taking up organic contaminants from groundwater will have higher potential for food chain contamination but will also have a higher potential to remove the target compound from contaminated environments in phytoremediation applications.

Among the wide variety of the food crops examined for the uptake studies, the *cucurbitae* family has been reported to accumulate higher levels of organic pollutants including polychlorinated dibenzo-p-doxins (PCDD) and polychlorinated dibenzofurans (PCDF) [54], p,p-dichlorodiphenyldichloroethylene (pp'-DDE) [10], chlordane [9], dieldrin [8,55], and polychlorinated biphenyl (PCB) [11] when compared with other plant species.

White et al. have studied extensively on the root exudates that may be involved in the process associated with solubilization of hydrophobic compounds [10,56-58]. It was suggested that the low molecular weight organic acids such as citric acid found in the root exudates of zucchini "gold rush" plants (*cucurbita pepo ssp pepo*) [58] solubilize pp'-DDE [57] resulting in enhanced desorption from the soil [56] and the 10x higher uptake of the compound than a squash "zephyr" plant (*cucurbita pepo ssp ovifila*) [10]. The difference between the root uptake abilities of two subspecies within the *cucurbitae* family could be also due to significant difference in genetic mechanisms [59,60].The adsorption onto the root surface also has been studied numerous times and it is believed to be proportional to the lipid content of the root tissue [5,24-26].

The mechanism of the translocation of the compound from the roots to above ground tissue in zucchini plants has been recently studied Murano et al. [35] where he reported the significant uptake of dieldrin by the plant when compared to other species

[8]. It has been suggested by Murano et al. [35] and Campanella and Paul [37] that the proteins found in zucchini xylem sap enhances the solubility of dieldrin resulting in higher translocation of hydrophobic compounds.

Although it is thought that only a few proteins are synthesized in roots [61], more recent study indicates that the xylem sap contain small molecular weight inorganic compounds and organic substances including hormones, amino acids, sugar, and proteins [62,63] and the xylem sap proteins are synthesized by the stele cells and transported to the xylem vessels by the flow of water [62]. Information on xylem sap proteins is available for several different plant species including oilseed rape [64], green cauliflower [65], cucumber [65], squash [65], soybean [66], a hybrid poplar [67], peach [68], tomato [69], and corn [70]. The studies show different sets of proteins for different plant species and the information is generally quite different from one study to another. Also, it was found that the composition of xylem sap proteins could alter significantly by pathogen infection [65], indicating that the xylem sap is species specific and depends partially on environmental conditions.

Most of those previous studies investigating the six steps involved only a single compound and the relationship between the uptake and the compound's hydrophobicity was hardly discussed. Furthermore, it still remains unclear which factor, root exudates, root tissue, or xylem sap, makes the zucchini unique on with respect to hydrophobic chemical uptake.

CHAPTER 3

MATERIALS AND METHODS

Values of TSCF were determined for several organic chemicals ranging log K_{ow} from -0.07 to 4.90 using a pressure chamber method with hydroponically grown plants including soybean, zucchini "gold rush" and squash "zephyr." Each experiment was run in triplicate. Tritiated water was used as a conservative tracer in experiments where ^{14}C-labeled compounds were used. To understand differences in uptake by "gold rush" zucchini, xylem saps from all three species were analyzed for total organic carbon (TOC), protein concentration and solubility. Lipid and total carbon contents were also determined for their root tissue.

Study compounds

Values of TSCF were determined for ^{14}C-labeled caffeine, endosulfan and triclocarban. Table 1 below shows a description of relevant chemical properties for the study compounds. Detailed environmentally relevant parameters including a structure of each compound can be found in Appendix A-1.

Only compounds that are non-ionized under the experimental conditions were evaluated in this study. For example, caffeine is an ionizable compound; however, under experimental conditions (pH =5.6) caffeine would be essentially neutral.

The concentration of the^{14}C-labeled compounds in the xylem sap samples, was measured directly by Liquid Scintillation Counter (LSC) (Beckman Counter LS6500) after each sample was mixed with 5 mL of scintillation cocktail (Beckman Ready Safe).

Table 1. Select chemical properties of the study compound

	Compounds	Common Use	Log K_{ow}	TSCF from Literature	SPARC calculated pKa (25C)
$^{14}C/^{3}H$-labeled	$^{3}H_2O$	-	-1.38[A]	1[B]	N/A
	caffeine	stimulant	-0.07[C]	0.83[B]	0.05
	Endosulfan	pesticide	3.83[A]	N/A	N/A
	Triclocarban	additive in antibacterial soaps	4.9[A]	N/A	N/A

(N/A: Not Applicable, A: Hansch and Leo, 1995[71], B: Dettenmaier et al., 2009 [5] C: Hansch et al., 1989 [72])

Plant preparation

Plants used in this study were: dwarf soybean "Hoyt" (*glycine max L.)*, zucchini "gold rush" (*cucurbita pepo ssp pepo)*, and straight neck bi-colored squash “zephyr” *(cucurbita pepo ssp ovifera)*. Soybean is one of the most common plants used in similar studies and was previously used by Dettenmaier [5] to generate TSCF values for a wide range of compounds using the same pressure chamber approach. The zucchini “gold rush” and the squash “zephyr” were selected based on literature indicating significant differences in root to shoot transfer between two similar species [10]. Seeds for the soybean were obtained from the Crop Physiology Laboratory (CPL) at Utah State University and the zucchini “gold rush” and squash “zephyr” seeds were obtained from Dr. Jason C. White (The Connecticut Agricultural Experiment Station), as well as Park Seed Company. The seeds from each species were incubated separately in a germination box at a temperature of 25 ±1 ºC (Fig. 3).

After germination, the approximately 3 to 4 day-old seedlings were rolled in a damp paper towel. The wrapped seedlings were then inserted in a 200 mL beaker filled 1/3 with tap water (Fig. 4). This step is important to enhance uniform vertical stem

growth to the first cotyledonary node. The pressure chamber method works the best for the plants that have relatively straight stems.

When the plants reached between 3 and 4 inches tall and/or 7 to 10 days from germination, they were transferred to 30 L plastic containers for hydroponic cultivation in starter nutrient solution [73] described in Appendix B. After 10 days in the starter solution, they were transferred to another 30 L container filled with a vegetation growth nutrient solution [73] (composition listed in Appendix B) until they were ready to be sampled. All plants were kept in a greenhouse in the CPL and grown for 5 to 8 weeks prior to pressure chamber experiments (Fig. 5). The plants were transferred to the Utah Water Research Laboratory in a glass container when their roots were big enough to be sampled (Fig. 6).

Fig. 3. Germination of zucchini seeds, Gold Rush

Fig. 4. Vertical growth of squash, zephyr

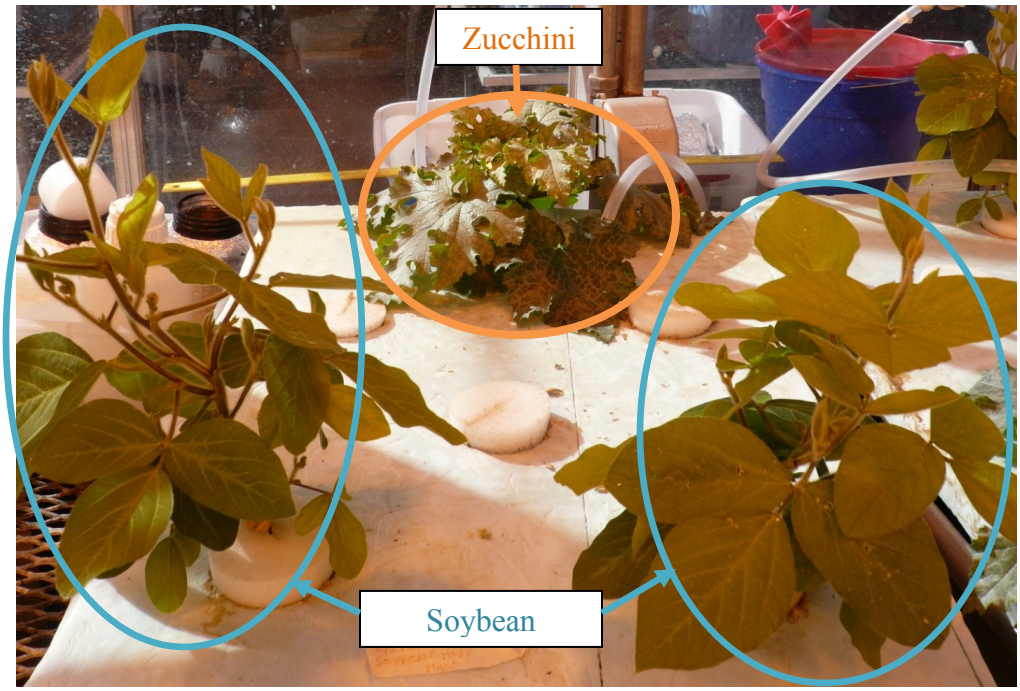

Fig. 5. Soybean and zucchini “gold rush” plants in hydroponic cultivation

Fig. 6. Zucchini “gold rush” roots before the pressure chamber experiment

TSCF determination using pressure chamber approach

The pressure chamber technique, first established by Scholander [74], is one of the most common techniques used by plant physiologists to determine water potential and root hydraulic conductivity. The pressure chamber technique enables sufficient volumes of xylem sap to be generated for the direct measurement of compound concentrations. The technique generally followed the approach used by Dettenmaier et al. [5].

A schematic of the pressure chamber system is shown in Fig. 7. A de-topped plant is sealed in the pressure chamber that is connected to a compressed oxygen tank to pressurize the system. The xylem sap produced by the chamber is then directly collected using a fraction collector.

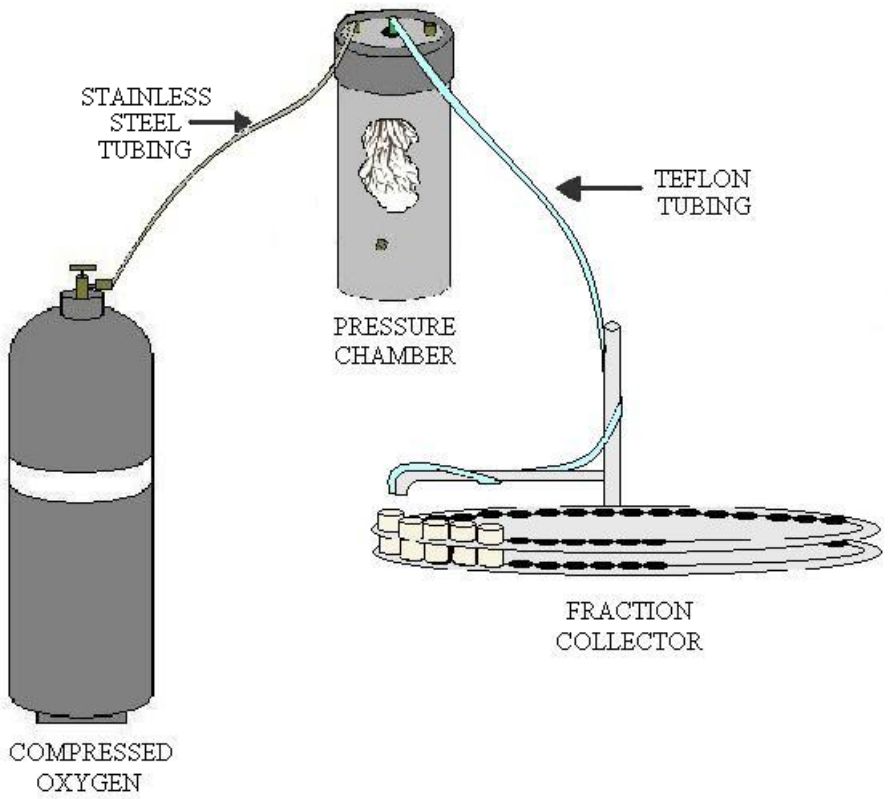

Fig. 7. Pressure chamber schematic

First, the nutrient solution was poured into the chamber. Then, the compound(s) of interest, if any, were added to the nutrient solution in the pressure chamber. No compounds were added to the solution for the xylem sap comparison analysis. The plant was detopped just below the first cotyledonary node with a pair of pruning shears and an inch length of rubber tubing was immediately attached to the stump. The assembly was then inserted into the center of the inner lid of the chamber. An inverted rubber stopper was used for the soybean plant with the tubing assembly to minimize the gap between the assembly and the inner lid of the chamber. A detailed diagram of the pressure chamber is shown in Fig. 8, followed by Fig. 9 illustrating the stem attachment to the Teflon tubing assembly. Dental adhesive was used to seal the gap between the rubber stopper and the stump to minimize the gas leakage from the system.

The outer lid of the pressure chamber was tightly screwed onto the chamber before pressurizing the system with compressed oxygen gas. Compressed air has been used in similar studies [22,50], but oxygen transfer limitations could cause the root to become anoxic due to continued metabolism. Root tips are very sensitive to oxygen deficiency [75] and an anoxic condition in the root zone would decrease the respiration rate of the plant, which is one of the most critical properties associated with the plant's transpiration. Therefore, compressed oxygen was used in this project to saturate the root zone and prevent the root zone from becoming anoxic due to continued root metabolism.

The pressure was gradually increased until a sap flow rate of approximately 70% of the intact plant transpiration rate was reached (usually around 20 psi). The pressure difference between the roots and xylem used in the chamber typically falls within the range of reported measurements for pressure differences in intact plant roots and xylem [76]. The pressure was kept constant in the chamber by frequently monitoring and adjusting the pressure gage. The compressed oxygen introduced to the system continuously mixed the nutrient solution.

Samples of xylem sap were collected directly using a fraction collector (ISCO, CYGNET) programmed to sample approximately 2 mL into a 7 mL high-density polyethylene (HDPE) scintillation vial purchased from Fisher Scientific (Fig. 10). Sampling duration for each sample varied between 30 seconds to 9 minutes depending on plant species and size of the roots used in an experiment. Xylem sap was collected for 60 to 300 minutes, depending on the physical/chemical properties of each compound.

Paired root-zone nutrient solution samples were collected through a septum sealed port on the bottom of the chamber using a syringe every 30 minutes to monitor the

fluctuation of the solution concentration (Fig. 11). The experiments were carried out at 25 ±1 ºC. The experiments were run in triplicate for all of the studied compounds.

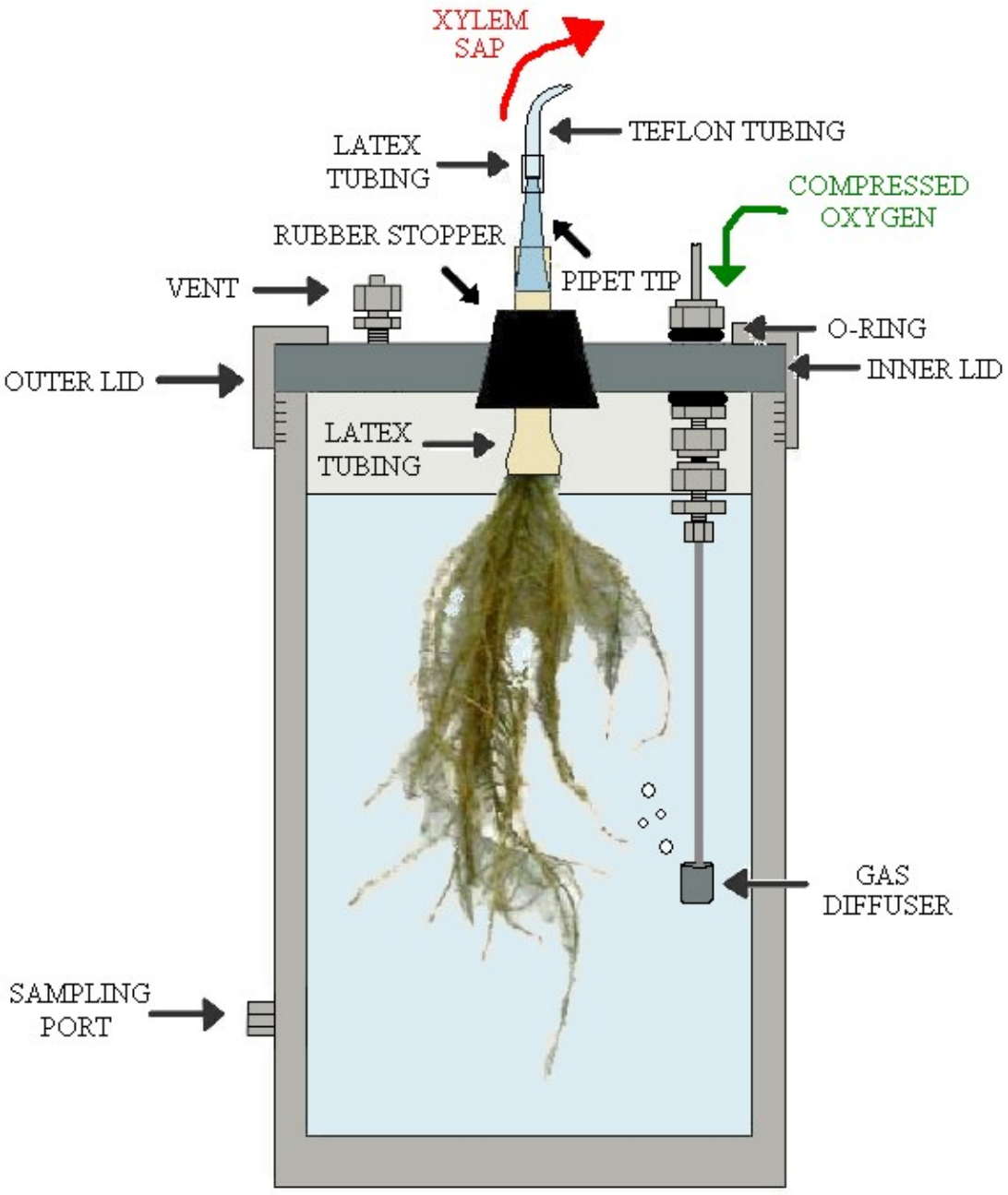

Fig. 8. Pressure chamber detailed diagram

Fig. 9. Top of the chamber connecting stem and Teflon tubing

Fig. 10. Fraction collector collecting xylem sap

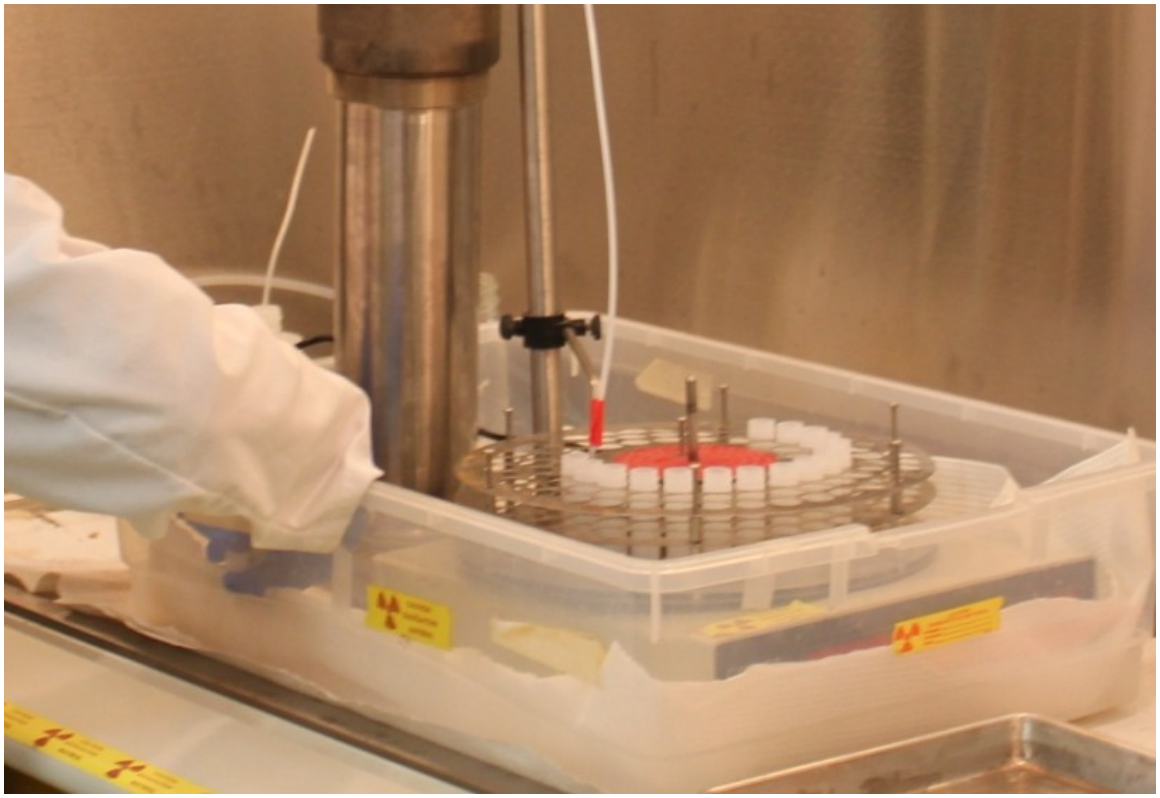

Fig. 11. Sampling exposure solution from bottom port of chamber

Transpiration stream concentration factor calculation

Each plant was first exposed to the compound of interest as it was inserted into the pressure chamber system. Therefore, the initial concentration of the compound in the xylem was zero. The concentration, then, gradually increased with time until it reached equilibrium.

The TSCF value was calculated as shown in Equation 3 below, where C_x is the steady state xylem concentration and C_{RZ} is the concentration in the root zone solution.

$$\text{TSCF}=C_x/C_{RZ} \qquad \text{(Equation 3)}$$

Root tissue comparison analysis

Root tissues from the three species were collected and analyzed for their carbon and lipid contents. Prior to analysis, the root tissues were air dried on an aluminum sheet in a fume hood for seven days at room temperature. The air-dried root tissues were shredded using a coffee bean grinder and then ground into smaller more uniform pieces using mortar and pestle (Fig. 12).

Lipid analysis

The lipid content of the root tissue was determined by extracting 2 g dry root tissue with ethyl ether for 24 hours in a Soxhlet apparatus (Fig. 13). Lipid content was calculated by dividing the extracted lipid weight by the dry tissue weight added to the thimble. Fresh tissue lipid content was then calculated by multiplying the dry lipid content by the fractional water content.

Fig. 12. Dry roots preparation diagram

Fig. 13. Soxhlet lipid extraction

Root tissue total carbon/inorganic carbon analysis

The carbon contents of the three plant species root tissues were determined using PRIMAX^{SLC} TOC Analyzer (Model CS22) by SKALAR (Fig. 14). The instrument analyzes total carbon (TC) by catalytic combustion method at 1050 ºC using cobalt oxide. Carbon was oxidized in the flow of pure oxygen into gaseous carbon dioxide, and the flow of the oxygen transported the carbon dioxide to the IR detector at 4.2 micrometer.

Inorganic carbon (IC) was measured by analyzing the evolved carbon dioxide upon acidification and purging of the sample. First the sample was purged with nitrogen to remove carbon dioxide. Then phosphoric acid was added to convert the inorganically bound carbon to the carbon dioxide gas. Total organic carbon was calculated by the difference between TC and IC.

Fig. 14. Total carbon analyzer PRIMAX^{SLC}

The average proportions of major elements in algal biomass are described in the Redfield Formula, expressed in Equation 5. The elemental composition is often used to look at the differences between types of organic matter. Ratios of carbon to oxygen, carbon to hydrogen, and carbon to nitrogen give information of the organic matter, which may increase solubility of organic compounds; however, without knowing the ratios it is difficult to distinguish the difference based on just carbon content of the root tissue.

$$Protoplasm = C_{106}H_{263}O_{110}N_{16}P_1 \qquad \text{(Equation 5)}$$

Calculating the percentage of the carbon based on the formula above, it contained 35.8 % carbon in dry weight (DW). Even though the formula is for the algal biomass, a recent study conducted at USU confirms that the carbon content of the plants average out

as 36.8 % DW [77] and others have reported up to 45 % DW [18]. The proportion of IC in the plant roots is expected to be none or very low due to the plants' biological origin.

For the TC analysis, between 50 to 100 mg of the ground tissues were weighed and set into a cuvette that was inserted directly into the instrument for the carbon content analysis. For the IC analysis, 50 to 100 mg of samples were delivered in a test tube, then 5 to 10 drops of distilled water were added to saturate the sample. Finally the prepared sample was directly inserted into the instrument for the analysis.

Xylem sap protein analysis

Protein content of the xylem sap produced by the three plants was analyzed using a bicinchoninic acid (BCA) protein assay kit by Sigma Aldrich. It is a colorimetric method similar to the Lowry Procedure; Cu^{2+} -protein is formed under alkaline conditions and then Cu^{2+} is reduced to Cu^{1+}. The purple color is developed by BCA with Cu^{1+} in an alkaline environment that provides the amount of Cu^{2+} reduced by proteins. It has a linear range of concentration between 100 to 1000 mg/L.

Because the BCA protein assay could be interfered with high concentration of amino acids including systeine, cyctine, tryptophan, and tyrosine, a trichloroacetic acid (TCA) precipitation method was used to remove the interfering substances prior to the BCA assay. After the TCA precipitation method, 50 parts of reagent A containing BCA, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 N NaOH were mixed with 1 part of reagent B, containing 4% copper (II) and sulfate pentahydrate. Then 20 parts of the BCA working reagent are mixed with 1part of a protein sample. Samples were mixed well using vortex. Then the samples were incubated in a 60 ºC bath for 15 minutes. After the samples were cooled to room temperature, the absorbance of the

solutions was measured using a NanoDrop spectrophotometer at 562 nm. Standard curve was made accordingly and unknown samples were measured in a similar manner. Bovine Serum Albumin (BSA) was used as the protein standard. A set of protein standards ranging from 50 mg/L to 500 mg/L was prepared simply by diluting the standard stock solution.

Xylem sap total organic carbon analysis

The total organic carbon (TOC) content in xylem saps was analyzed using Apollo 9000 TOC Analyzer by Teledyne Tekmar. The instrument analyzes TOC by combustion with a patented platinum catalyst.

Carbon in the sample is first converted to carbon dioxide by the combustion, then a career gas sweeps the derived carbon dioxide through a non-dispersive infrared (NDIR) detector. The NDIR generates a signal that is proportional to the amount of carbon dioxide in the sample that is compared with calibration data to calculate the sample concentration.

Xylem sap solubility analysis

The solubilities of ^{14}C-caffeine and ^{14}C-triclocarban in xylem sap extracted from soybean and zucchini "gold rush" were determined using a modified shake flask method OPPTS 830. 7840 [78]. In this procedure, 10 times the reported literature solubility of ^{14}C-caffeine and ^{14}C-triclocarban were weighed into nine plastic vials. Deionized water, xylem sap from soybeans and zucchini "gold rush" were added to three vials each and securely sealed. The sealed vials were then shaken for 24 hr (Fig. 15). After the 24 hr period, all of the vials were centrifuged and 20 μL of the supernatant were taken out. The

concentration of the compound was analyzed using LSC. The procedure was repeated periodically until the concentration reached the compound's equilibrium.

Statistical analysis

The CRAN R (version 2.13.1) and Sigma plot (version 10.0) were used for the statistical analysis of data obtained from this project and plotting the data points. The residual sum of square (RSS) is used to determine how well the data points fit the model developed by Dettenmaier [5]. The Tukey's significant difference test was performed to determine which modes of the factors affect the value of the TSCF the most significantly.

Fig. 15. Solubility analysis- shaking the vials

CHAPTER 4

RESULTS AND DISCUSSION

Pressure Chamber Technique: Operational Considerations

The first step, securely sealing the plant in the chamber, was one of the most difficult challenges of the procedure. For example, it was found that soybean plants are easier to seal than zucchini "gold rush" or squash "zephyr" plants because of the soybean's rigid, woody nature, and more uniform sizes of the stem. In addition, it was found that older plants are more difficult to seal within the pressure chamber system than younger plants. All of the plants utilized in the pressure chamber technique were between 5 to 8 weeks in age. When the plants are older than about ten weeks the outer skin of the stem gets more brittle which makes it harder to seal.

When the plant was not properly sealed, the nutrient solution from the root zone moved directly into the pressure chamber system without passing through roots. This short-circuiting could be visually detected by a red tinted sample in the collection vials instead of a clear xylem sap due to the presence of iron-EDDHA in the nutrient solution used in this study (Fig. 16). Because of the large size of the molecule, iron-EDDHA is thought to be filtered through the membrane of the roots.

Another operational concern is that the xylem sap flow rate (transpiration rate) gradually decreases over the course of the experiment. This could be due to the change in the oxygen water volume ratio within the chamber or decreases in the root membrane permeability. The xylem sap flow rate was kept relatively constant by increasing the pressure of the chamber periodically.

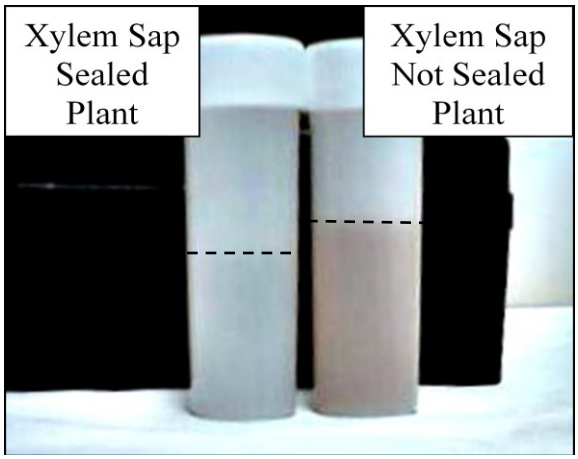

Fig. 16. Xylem sap samples

The final measured concentrations of the target compounds in the root zone were less than the initial concentrations even for ^{14}C-caffeine and tritiated water (up to 50 % less than the initial concentrations). While it was anticipated that significant sorption onto the roots would lower the root zone concentration of the more hydrophobic compounds it was somewhat surprising to observe a significant decline in concentration for tritiated water and caffeine.

To further investigate this observation, a study was performed to determine the potential sorption on the inner surface of the stainless steel chamber. A 4" length of 1/8" diameter stainless steel pipe was used instead of the plant roots to establish the sorption onto the stainless steel surfaces or the Teflon tubing used in this project.

As shown in Fig. 17, the concentration in the root zone did not change more than 5 % in either tritiated water or ^{14}C-caffeine, suggesting that there is minimal sorption onto any surface of the equipment as expected. The stainless steel roots study indicates that the decrease in chamber concentration of caffeine and tritiated water is not due to sorption to any of the equipment.

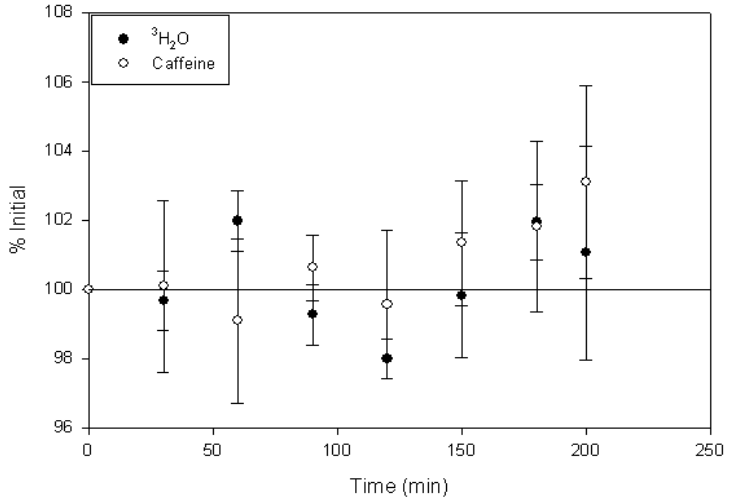

Fig. 17. Stainless steel roots study root zone concentration
(Error bar represents 95 % confidence interval from the experiments)

The change of the root zone concentration of the tritiated water and caffeine with saturated roots is illustrated in Fig. 18. The results illustrate the identical decrease pattern for both tritiated water and caffeine reaching equilibrium concentration after 60 minutes. The recovery of the concentration varied in range of 55 % to 75 % at the end, proportional to the size of the roots indicating that the compound loss in the chamber could be due to dilution, especially for hydrophilic compounds such as tritiated water and caffeine. The potential of the dilution can be explained by osmosis, as a result of fluid exchange between the roots and spiked root zone solution in the chamber.

To confirm the prediction of dilution theory, the same procedure was performed using air-dried roots instead of damp roots. If the loss of the compounds is due to dilution, the concentration of the compounds should stay the same throughout the time period since there is no fluid in the roots to exchange with the root zone solution in the chamber. The results are shown in Fig. 19.

The results of the dry root study illustrates very similar trends as the stainless steel roots study, the concentration of the two compounds stayed constant throughout the study, confirming that the decrease of the hydrophilic compounds within the chamber is caused by dilution of the exchange of water in the roots with the spiked root zone solution in the chamber.

Based on the results observed in this study using tritiated water and ^{14}C-caffeine, it could be said that the dilution of the target compound is likely to happen not only for those two tested compounds but other hydrophilic compounds as well. Another important fact to note from this study is that the concentration of hydrophilic compounds reaches its equilibrium after 60 minutes, therefore, the average of paired samples taken after 60 minutes should be considered as root zone concentration when calculating values of TSCF.

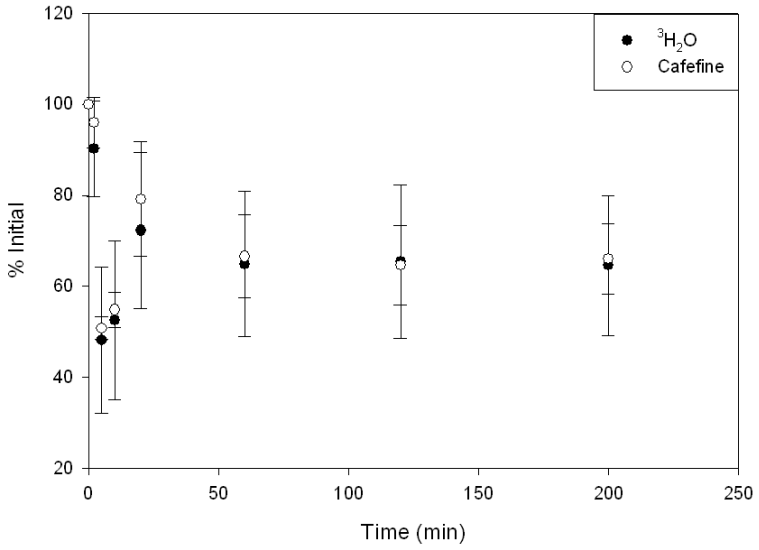

Fig. 18. Saturated roots exposure solution concentration
(Error bar represents 95 % confidence interval from the experiments)

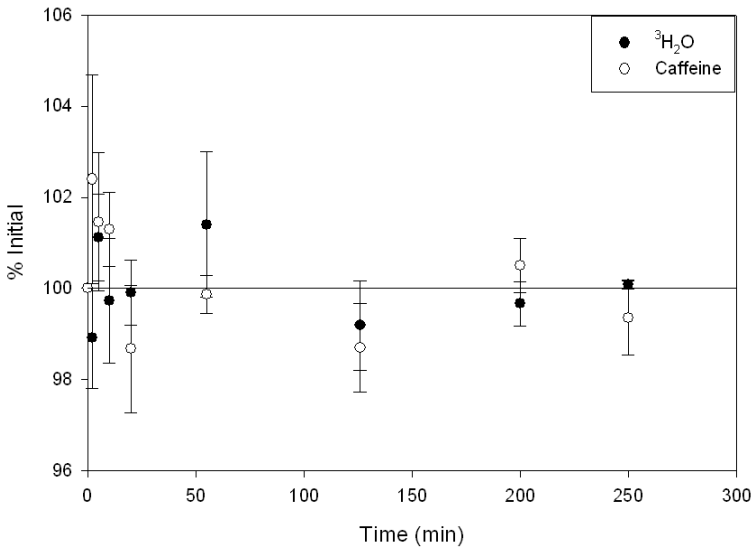

Fig. 19. Dried roots exposure solution concentration
(Error bar represents 95 % confidence interval from the experiments**)**

Root concentration factor (RCF)

Briggs et al. [21] showed that the root concentration factor (RCF), the ratio between the chemical concentration in the roots and that in the exposure media (water or soil) contacting the roots was directly related to the log K_{ow} of the chemical and the lipid content of the roots. Thus, it was expected that the measured pressure chamber root zone concentrations at steady state would be lowest for the most hydrophobic compound and with the plant species having the highest root lipid content. To illustrate this, the root zone triclocarban concentrations monitored for three species used in this project are shown in Fig. 20. The steady state root zone concentration of triclocarbon was lowest for zucchini "gold rush."

Based on the results illustrated in Fig. 20, the root concentration factor (RCF) was calculated for triclocarban by subtracting the final exposure solution concentration from the known spike solution concentration. This assumes that there is no significant sorption

to the stainless steel root chamber and that all the mass added to the chamber that is not in solution was sorbed to the roots.

The RCF values calculated for triclocarban (Log K_{ow}=4.90) were 26.1 ±0.29, 5.93 ±0.19, and 4.74± 0.19 for zucchini "gold rush," squash "zephyr," and soybean, respectively. The calculated RCF values fall into the similar range of the experimental data with DDE (log K_{ow} = 5.69 [79]) from White [36]. This result indicates the lipid content of the "gold rush" zucchini root may be higher than the other two species, which lead to the conduction of lipid extraction of the root tissues discussed later on in this chapter.

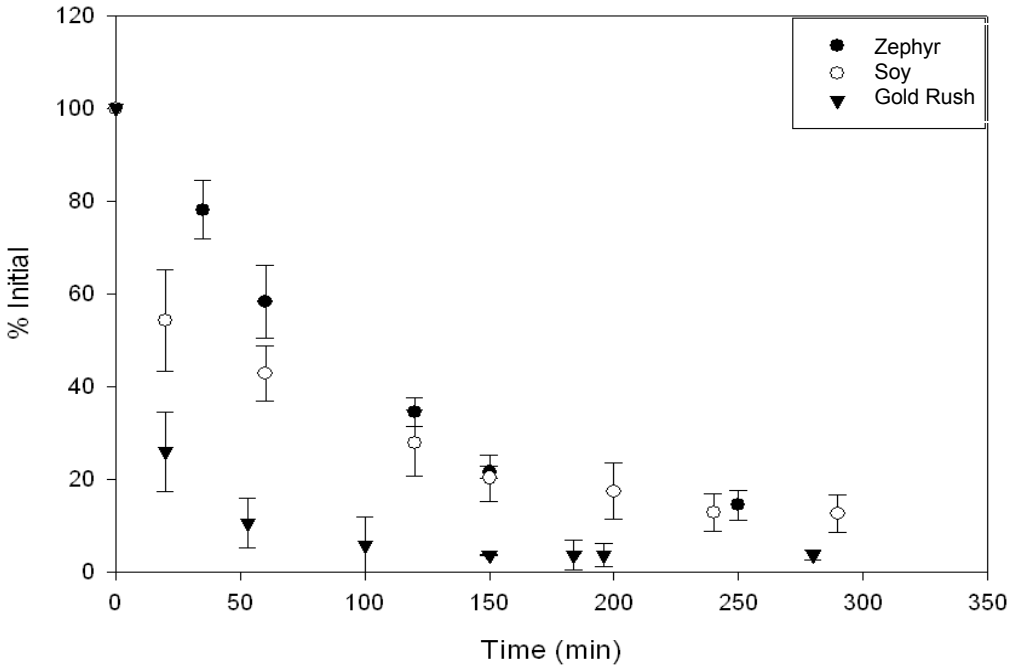

Fig. 20. Comparison of species on triclocarban concentration in the exposure solution (Error bar represents 95 % confidence interval from the experiments**)**

Transpiration stream concentration factor (TSCF)

The measured TSCF values for the four compounds are summarized in Table 2 with the corresponding log K_{ow} values, as well as the TSCF values found in a literature published by Dettenmaier et al. [5]. The TSCF values ranged from 0.04 to 1.02 while the log K_{ow} values ranged from -1.38 to 4.9. The four compounds were tested on each plant at least three times and the conservative tracer, tritiated water's TSCF values and the shape of the steady state TSCF calculation curve suggests that there were no significant problems with the data quality.

Table 2. Average measured TSCF 95% C.I. and corresponding log K_{ow}

		Measured TSCF Values			**Literature Values**	
	Log K_{ow}	Soy	Zephyr	Gold Rush	Soy & Tomato	Predicted
Tritiated water (^3H_2O)	-1.38[A]	**1.03**	**1.03**	**1.01**	**1.00**[C]	**0.98**[C]
95 % C.I. (n=9+)		±0.0215	±0.0236	±0.0153	±0.01	-
^{14}C-Caffeine	-0.07[B]	**0.783**	**0.830**	**0.813**	**0.83**[C]	**0.92**[C]
95 % C.I. (n=3)		±0.0558	±0.0299	±0.0173	±0.018	-
^{14}C-Endosulfan	3.83[A]	**0.215**	**0.194**	**0.617**	**-**	**0.22**[C]
95 % C.I. (n=3)		±0.0112	±0.00691	±0.0141	-	-
^{14}C-Triclocarban (TCC)	4.90[A]	**0.0437**	**0.0617**	**0.400**	**-**	**0.09**[C]
95 % C.I. (n=3)		±0.00728	±0..00976	±0.0299	-	-

(C.I.: Confidence Interval, A: Hansch and Leo 1995 [71], B: Hansch et al., 1989 [72] C: Dettenmaier et al., 2009 [5])

The steady state TSCF value of tritiated water was sampled a total of 27 times and the observed mean was 1.02 ±0.02, very similar to the expected value of 1.0 reported by Dettenmaier et al. [5]. ^{14}C-caffeine was sampled three times for each plant, a total of nine times and the mean was 0.81 ±0.02, which was also the anticipated value from a previous study done by Dettenmaier et al. (2009) [5]. The TSCF values of tritiated water and ^{14}C-caffeine were statistically identical for all soy, squash "zephyr," and zucchini "gold rush" as expected (see Appendix C-1 for details). The high TSCF values for caffeine indicates

that non-ionized, polar compounds seem to be favored by roots uptake and likely to be transferred to shoots as Dettenmaier et al. [5] suggested.

The calculated steady state TSCF of ^{14}C-endosulfan was almost 0.20 ±0.01 for soybean and squash "zephyr" and 0.62 ±0.01 for zucchini "gold rush." Similarly, the TSCF value of ^{14}C-triclocarban was about 0.053 ±0.01 for soybean and squash "zephyr" plants and 0.40 ±0.03 for zucchini "gold rush." The TSCF values ^{14}C-endosulfan and ^{14}C-triclocarban obtained for soybean and squash "zephyr" are statistically identical and fit the model of Dettenmaier et al. [5]. However, the values for ^{14}C-endosulfan and ^{14}C-triclocarban obtained using zucchini "gold rush," are significantly higher than for soybean and squash "zephyr" indicating the higher root to shoot transfer potential for zucchini "gold rush" for hydrophobic compounds. Statistic analysis illustrated in Fig. 21 provides the evidence that the TSCF values of zucchini "gold rush" on hydrophobic compounds cannot be explained using the existing model where all of the other values can be explained using Dettenmaier's model [5].

Based on the measured TSCF values, a new fit was created for the zucchini "gold rush," using the model developed by Dettenmaier et al. [5], shown in equation 6 followed by Fig. 22. The approach used for the non linear regression analysis found in Appendix E.

This new curve fit suggests that the difference between the two models increases with the compounds hydrophobicity indicating the high potential of translocation on hydrophobic compounds. Further investigation on the new curve fit should be conducted using compounds with broader range of log K_{ow}.

$$TSCF = \frac{11}{11 + 1.73^{\log Kow}} \qquad \text{(Equation 6)}$$

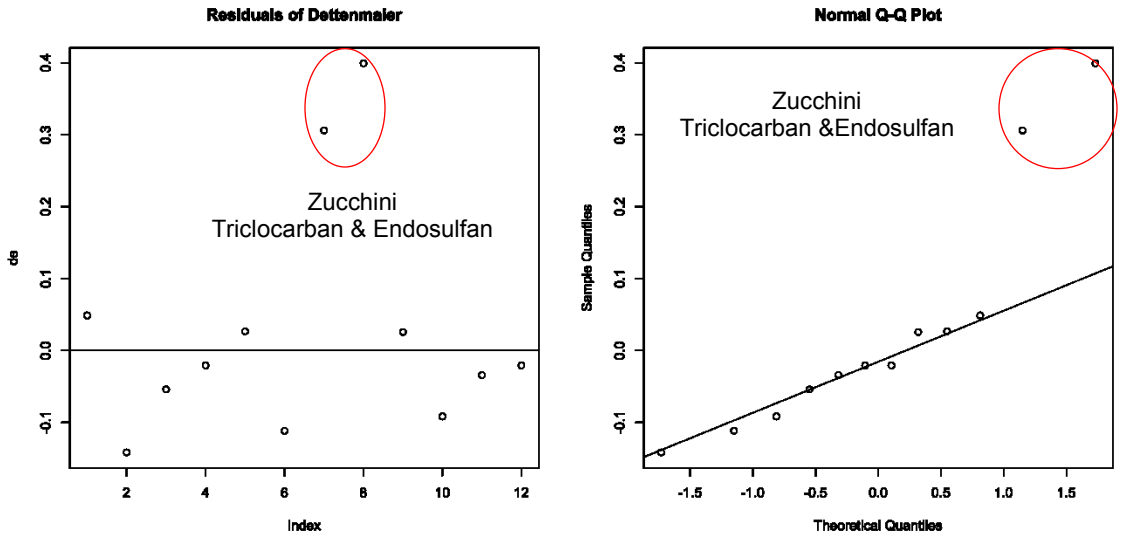

Fig. 21. (Left) Residuals of experimental data to Dettenmaier's model [5] and (Right) Quantile-Quantile plot for the residuals of experimental data

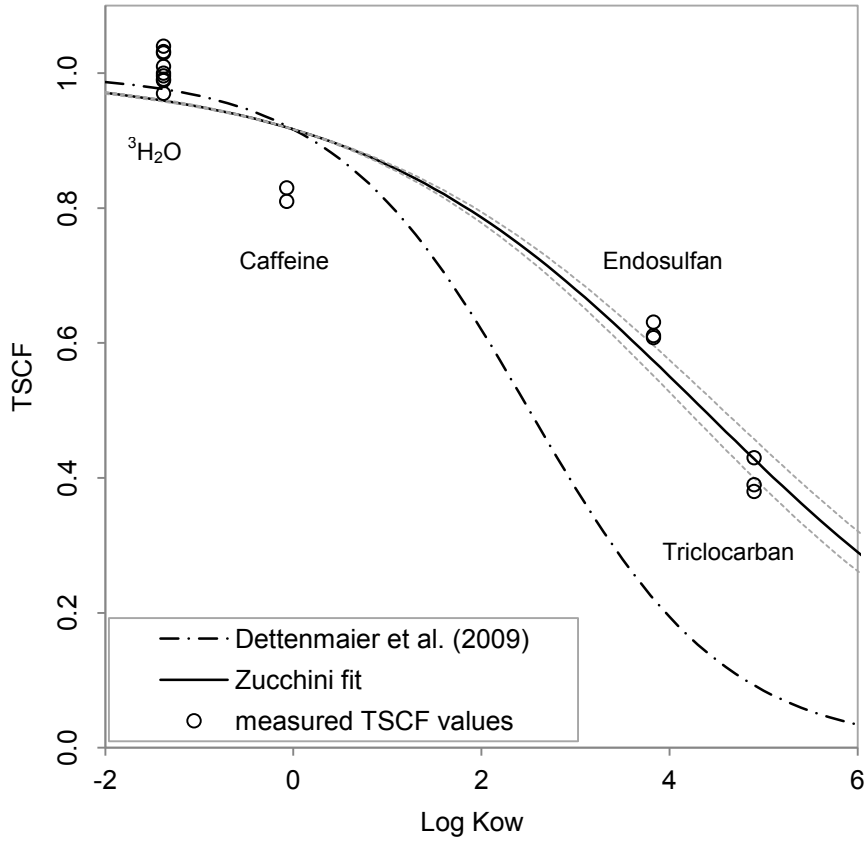

Fig. 22. Measured TSCF values for "gold rush" zucchini and corresponding log K_{ow} values compared to existing prediction methods (Dotted lines represent error associated with the curve fit)

Comparison of plant species

As it was mentioned previously, the sorption of triclocarban differs among plant species. Also, the values of TSCF differs significantly only on hydrophobic compounds (log K_{ow} >2.5) including endosulfan and triclocarban. Here is an example of the TSCF vs. Time plot of ^{14}C-trichlocarban and ^{14}C-endosulfan on the three species (Fig. 23). All of the TSCF vs. Time plot can be found in Appendix D. Each point represents the ratio of the xylem concentration to the root zone concentration. The steady state TSCF is calculated when it reaches equilibrium. Where the shapes of TSCF curves for soybean and squash "zephyr" were almost identical, zucchini "gold rush" made significant increase on TSCF after 100 and 150 minutes of sampling. The results illustrated in the Figure 23 indicate that there is some significant physical characteristics difference in zucchini "gold rush" that accelerates the root to shoot transfer of the hydrophobic compounds when compared with other species. The difference may be found in the composition of xylem sap or in the composition of the root tissue.

Root lipid analysis

The physical characteristics of plant roots are not commonly reported, even though the composition of the root tissue might be just as important as the composition of the xylem saps. The carbon contents of the root tissue were analyzed first, to determine potential for sorption property differences between plant species. As it was mentioned in the method section, the carbon contents of the plants are usually reported between 30 to 40 % of the whole plants. The TOC measured in the experiments shown in the Table 3 falls into the expected range.

Secondly, the lipid contents of the root tissue were analyzed. The lipid content is believed to be one of the key factors that could affect the uptake of hydrophobic organic compounds because hydrophobic compounds tend to adsorb on lipids. Some models are developed using the lipid content as one of the main factors, however, relatively few values for lipid contents have been reported. Table 3 illustrates high root lipid content found in zucchini "gold rush," almost twice as much as other two plant species.

The results indicate that sorption of hydrophobic organics to the roots of zucchini "gold rush" should be greater than the other two plant species [5, 21-26]. Assuming sorption is proportional to the root lipid content, zucchini "gold rush" should sorb twice the amount of hydrophobic compounds than the other two plant species. This root tissue analysis shows the high potential of the zucchini to have the effect the adsorption on the root surface.

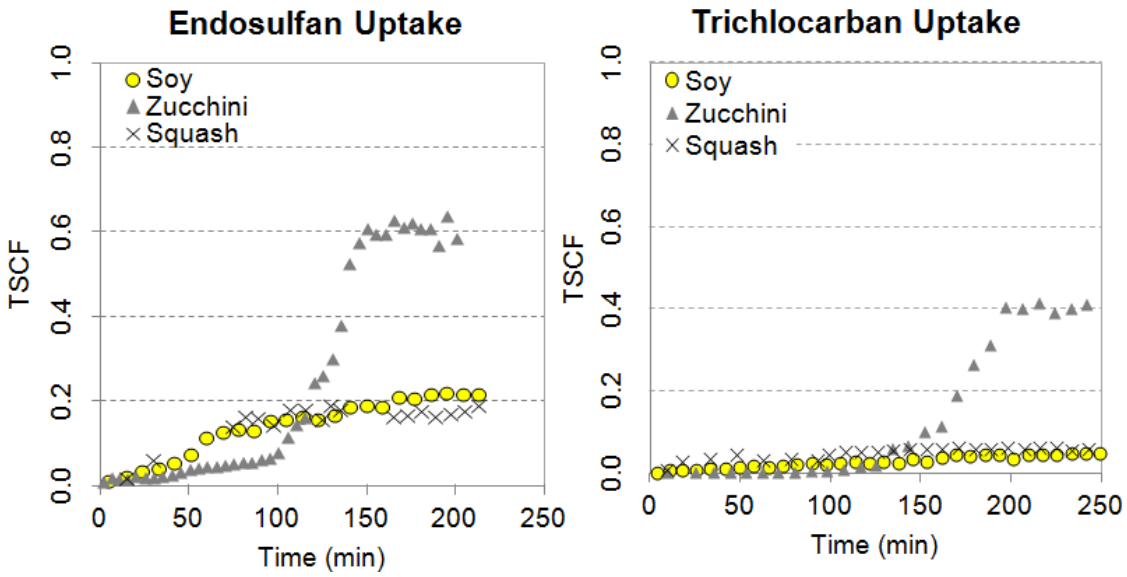

Fig. 23. Example comparison of the three species on hydrophobic compounds (Left) Uptake of endosulfan (Right) Uptake of triclocarban (Data points are from three individual experiment)

Table 3. Root tissue analysis of the three plant species

	Total Organic Carbon Conc. (% dry)	95 % CI(±)	Lipid Content (% Lipid wet)	95 % CI(±)
Soybean	**35.52**	0.21	**0.047**	0.0008
Zephyr	**36.43**	0.14	**0.062**	0.0075
Gold Rush	**38.56**	0.24	**0.127**	0.0019

Xylem sap composition

The xylem sap compositions of the three species were also examined. The xylem sap was produced using the pressure chamber technique without any spiking compound in the nutrient solution. Generally, the solubility of hydrophobic organic compounds increases when there is more dissolved polar organic matter in the solution, so, total carbon concentration was analyzed on the xylem sap (Table 4). The measured total carbon contents in xylem sap were statistically identical for all of the species used, indicating that the carbon content is not the significant difference among the plant species.

Secondly, the protein content of the xylem saps was analyzed. The protein contents are be commonly reported in plants xylem sap; however, the protein contents in xylem sap could result in higher solubility and/or facilitated transport of the hydrophobic compounds.

The protein concentrations measured for soybean and squash “zephyr” are almost identical but only half of the concentration found in zucchini “gold rush.” This difference in protein content may be associated with the higher root to shoot transfer observed for zucchini “gold rush” through a solubility enhancement or facilitated transport through the root membranes. While beyond the scope of this study, additional characterization of the

xylem sap and the associated proteins would be necessary to better understand the actual mechanism.

Buhtz et al. [65] studied the composition of xylem sap protein and found that there are glycine rich proteins (GRP) found only in the *cucurbitae* family. Even though information on direct functional evidence is missing, some think that GRPs could be involved in stabilization of differentiated water transporting elements above ground [80,81]. It may be possible that while GRP travels from roots to shoots, it takes the hydrophobic compounds along with it.

Solubility in xylem sap

Finally, the solubility of two compounds, caffeine and triclocarban, were analyzed using xylem sap from two plant species, soybean and zucchini "gold rush." The xylem sap from the squash "zephyr" plant was not used because its measured TSCF values are statistically identical to that of soybeans and the analysis results suggest that the soybean and squash "zephyr" have similar characteristics. The solubility of caffeine in the soybean and zucchini "gold rush" xylem saps was 21.8 ±1.38 g/L and 21.3 ±1.62 g/L, respectively. The solubility in deionized water was determined as 21.3 ±0.66 g/L and reported aqueous solubility is 21.6 g/L [82] (Fig. 24). The analysis of variance and Tukey's HSD confirms that the differences among the two xylem saps and deionized water are insignificant (see Appendix C-2 for details).

The solubility of triclocarban was measured with the mean of 20.6 ± 0.40 mg/L and 10.6 ± 0.16 mg/L for the zucchini "gold rush" and soybeans, respectively, This is compared to a mean solubility in deionized water of 11.2 ± 0.39 mg/L. The solubility of triclocarban in the literature is 11 mg/L [79] (Fig. 25). It took 120 hours before the

solubility reached equilibrium with zucchini "gold rush" xylem sap compared to 48 hours for the soybean xylem sap. The reason for the difference in kinetics is uncertain.

In summary, zucchini "gold rush" roots have twofold higher lipid content than soybean and squash "zephyr" and a higher root concentration factor. The concentration of protein in zucchini "gold rush" xylem sap is also two times higher than in soybean and squash "zephyr." The higher protein concentration may be associated with higher root to shoot transport of hydrophobic organics observed in zucchini "gold rush." The solubility of the hydrophobic triclocarban (log K_{ow} = 4.9) in zucchini "gold rush" xylem sap was twice that measured deionized water and the xylem sap of soybean. Overall, the observed physiological differences between zucchini "gold rush" and the other plants suggests that the composition of the xylem sap may play an important role in understanding the root to shoot transfer of hydrophobic compounds.

Table 4. Xylem sap analysis of the three plant species

	Total Carbon		Protein Content	
	Conc. (mg/L)	95 % CI(±)	Conc. (mg/L)	95 % CI(±)
Soybean	**355**	37	**116**	30
Zephyr	**380**	41	**140**	24
Gold Rush	**370**	31	**250**	41

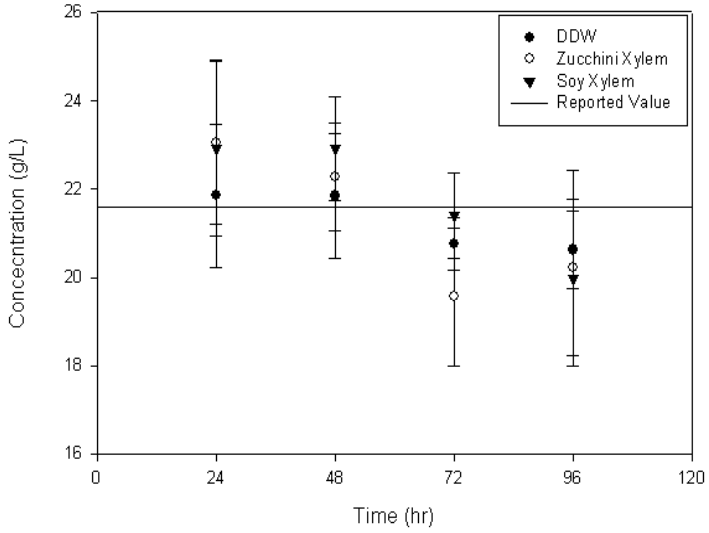

Fig. 24. Caffeine solubility analysis (Error bar represents 95 % confidence interval)

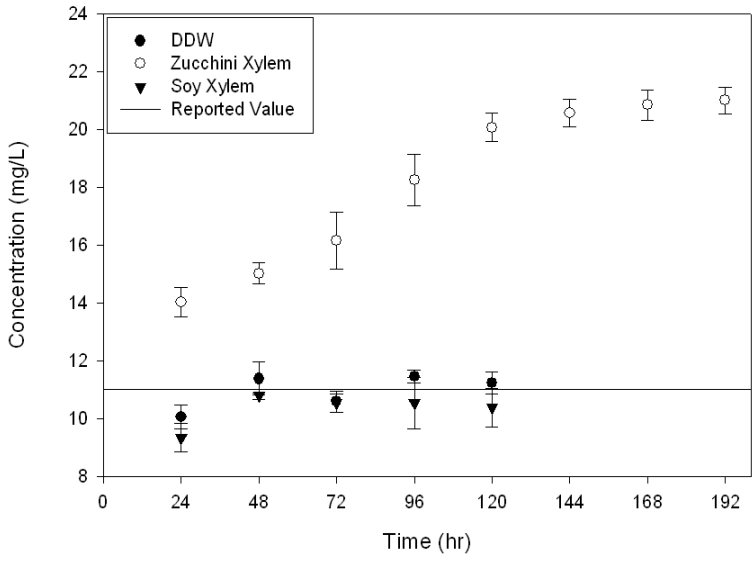

Fig. 25. Triclocarban solubility analysis (Error bars represent 95 % confidence interval)

CHAPTER 5

SUMMARY AND CONCLUSIONS

Soybean, squash "zephyr," and zucchini "gold rush" plants were evaluated for their potential to uptake and transport xenobiotic organic contaminants from roots to shoots using the pressure chamber technique. Values of TSCF were measured for caffeine, endosulfan, and triclocarban. For caffeine, the measured TSCF values were statistically identical for all of the three species. For zucchini "gold rush," however, the TSCF values for endosulfan and triclocarban were threefold and almost tenfold higher than for soybean and squash "zephyr." This shows that the unique uptake ability of zucchini "gold rush" is especially significant for hydrophobic contaminants.

Based on the differences in the root to shoot transport measured in this study, the physiological differences in the root tissue and in the xylem sap for the three plant species used in this study were examined. The root tissue analysis showed that zucchini "gold rush" roots have twice as much lipid as soybean and squash "zephyr" suggesting higher root concentration factors for zucchini "gold rush." The xylem sap analysis found twice as much protein in zucchini "gold rush" xylem sap suggesting the potential for enhanced solubility and/or facilitated transport of the contaminants within zucchini "gold rush." The higher solubility of triclocarban in the xylem saps showed the potential for enhanced solubility for more hydrophobic compounds.

The results from this laboratory study indicate that the uptake of hydrophobic contaminants in zucchini "gold rush" is significant compared to other food crop species due to its high lipid content in the root tissue, enhanced solubility within the xylem sap, and possibly enhanced facilitated transport from the root surface to xylem vessels

because of the high protein content in the xylem sap. However more complete characterization of the xylem sap is needed to understand the mechanism associated with the zucchini's unique ability to transport hydrophobic compounds from root to shoots. Additional data for other hydrophobic compounds and physiological data for xylem saps are needed to refine and validate plant root uptake models.

Results presented in this thesis confirmed that the root uptake of hydrophobic compounds by zucchini "gold rush" is significantly higher than soybean and squash "zephyr." The mechanism is not understood; however, the higher root tissue lipid content and xylem sap protein levels found in zucchini "gold rush" may be related to the higher root to shoot transfer. Further characterization of the xylem sap, including amino acid analysis, should be conducted.

As previously mentioned, most existing plant root uptake models are appropriate only for the neutral compounds mainly due to the lack of data for charged compounds. Additional plant uptake data for ionizable organic compounds is needed to expand the applicability of such models. Pharmaceutical and personal care products (PPCPs) are a relatively recent environmental concern and would be an appropriate class of compounds to examine for root uptake since most are relatively low in hydrophobicity and often ionized in the environment.

To address this concern, preliminary TSCF values were obtained for five common pharmaceutical and personal care products (PPCPs): carbamazepine, tris (2chloroethyl) phosphate, fluoxetine, progesterone, and sulfamethoxazole. Detailed environmentally relavant parameters including a structure of each compound can be found in Appendix A-2. For zucchini "gold rush," TSCF values for carbamazepine tris (2chloroethyl)

phosphate, and fluoxetine were all significantly higher than the predicted values using the Dettenmaier's model [5] (Table 5).

Based on fluoxetine's characteristics as weak base indicated by its acid dissociation constant, the TSCF should be very low or not recognized within the plants because of high energy requirement [42]; however, the TSCF value measured in this experiment was 0.70 which is relatively high for corresponding log K_{ow} and pK_a values. Even though some models and data suggest that plants don't take up charged molecules, the lab data says it differently [22], reporting the TSCF value of ionized fenpropimorph as 0.51. As it was mentioned previously, it will be interesting to determine the root uptake of charged compounds including PPCPs.

Table 5. Summary of zucchini's pressure chamber experiment

	Log K_{ow}	pK_a	**TSCF** Zucchini	**Model Prediction**
Sulfamethoxazole	0.95	5.7	**<0.01***	**0.82**[A]
Tris (2chloroethyl) phosphate	1.44	Not Ionizable	**0.87***	**0.74**[A]
Carbamazepine	2.45	Not Ionizable	**0.77***	**0.51**[A]
Fluoxetine	3.82	9.53	**0.70***	**0.22**[A]
Progesterone	3.87	Not Ionizable	**<0.01***	**0.21**[A]

(*No Statistical analysis was reported due to single measurement of data points. A: Dettenmaier et al. (2009) [5])

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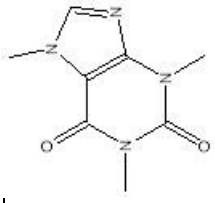
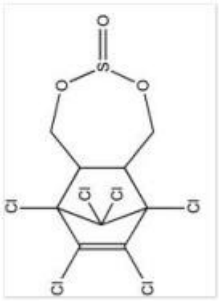
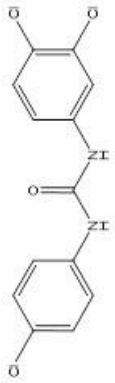
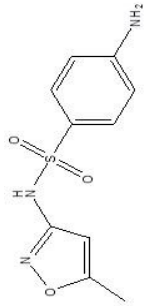
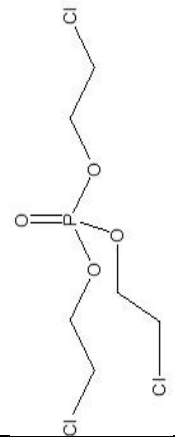
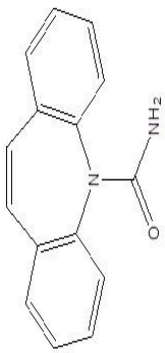
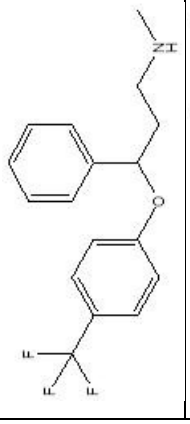
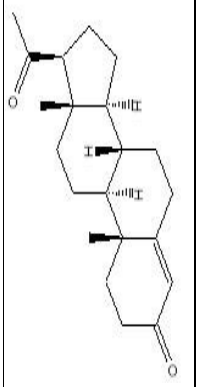
APPENDICES

Appendix A-1: Detailed Properties of Study Compounds

	14C/3H-labeled			
Compounds	$^{3}H_2O$	Caffeine	Endosulfan	Triclocarban
CAS Number	7732-18-5	58-08-2	115-29-7	101-20-2
Common Use	-	stimulant	insecticide	additive in antibacterial soaps
Solubility (mg/L) (25 C)	0	21600[A]	0.45 -0.51[B]	11[B]
Log Kow	-1.38[C]	-0.07[D]	3.83[C]	4.9[C]
SPARC calculated pKa (25C)	N/A	0.05	N/A	N/A
structure				

(N/A: Not Applicable, A: Yalkowsky & Dannenfelser 1992 [82] B: Syracuse Research Corporation 2004 [79] C: Hansch and Leo, 1995 [71] D: Hansch et al., 1989 [72])

Appendix A-2: Detailed Properties of PPCP Compounds

Compounds	Sulfa-methoxazole	tris (2chloroethyl) phosphate	carbamazepine	fluoxetine	progesterone
CAS Number	723-46-6	115-96-8	298-46-4	54910-89-3	57-83-0
Common Use	Antibiotic	Plasticizer additive	Anti-convulsant	Anti-depressant	Steroid Hormone
Solubility (mg/L) (25 C)	610 (37C)[A]	7000[B]	112[C]	38.35[D]	8.81[A]
Log K_{ow}	0.95 (pH5.5)[E]	1.44[F]	2.45[G]	3.82[H]	3.87[E]
SPARC calculated pKa (25C)	9.28	N/A	N/A	9.53	N/A
structure					

(N/A: Not Applicable, A: Yalkowsky & Dannenfelser 1992 [82] B: Muir 1984 [83] C: Ferrari et al. 2003 [84] D: EPI Suite wsK_{ow}win v1.67 estimate E: Hansch and Leo, 1995 [71]F: Chemicals Inspection and Testing Institute, 1992 [85]G: Dalpozzo et al., 1989 [86] H: Adlard et al., 1995 [87])

Appendix B: Dicot Nutrient Solution [73]

NUTRIENT SOLUTION FOR DICOTS (Soy, Lettuce, Tomato)

Current as of March 2005

		STARTER		VEGETATIVE GROWTH	
SALT	STOCK CONC.	mL per 100 L	FINAL CONC	mL per 100 L	FINAL CONC
$Ca(NO_3)_2$	1M	100	1 mM	200	2 mM
$K(NO_3)$	2 M	50	1 mM	150	3 mM
KH_2PO_4	0.5 M	100	0.5 mM	250	1.25 mM
$MgSO_4$	1 M	50	0.5 mM	150	1.5 mM
K_2SiO_3	0.1 M	100	0.1 mM	100	0.1 mM
K_2SO_4	0.5 M	0 (do not add)	0 mM	0 (do not add)	0 mM
$FeCl_3$	50 mM	10	5 μM	3	1.5 μM
EDDHA (red)	100 mM	40	40 μM	10	10 μM
$MnCl_2$	60 mM	10	6 μM	15	9 μM
$ZnCl_2$	20 mM	30	6 μM	20	4 μM
H_3BO_3	40 mM	100	40 μM	100	40 μM
$CuCl_2$	20 mM	20	4 μM	20	4 μM
Na_2MoO_4	1 mM	10	0.1 μM	10	0.1 μM

ALWAYS add acid or base as needed to adjust initial pH to 5.6

Appendix C-1: TSCF THSD Test by R

```
A='A'                (A=3H2O)
B='B'                (B=caffeine)
C='C'                (C=endofulfan)
D='D'                (D=triclocarban)
i='i'                (i=Soybean)
ii='ii'              (ii=Zucchini)
iii='iii'            (iii=Squash)
Run=c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,
32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54)
Comp=c(A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,A,B,B,B,B,B,B,B,B,C,C,C,C,
C,C,C,C,C,D,D,D,D,D,D,D,D,D)
Species=c(i,i,i,i,i,i,i,i,i,ii,ii,ii,ii,ii,ii,ii,ii,iii,iii,iii,iii,iii,iii,iii,iii,iii,i,i,i,ii,ii,ii,ii,ii,iii,iii,iii,i,i,i,ii,ii,ii,iii,iii,
iii)
Value=c(0.98,1.084,1.04,1.01,0.99,1.06,1.011,1.02,1.032,1.01,1.04,1.032,0.996,0.989,0.991,1
.03,0.97,1,1.064,1.051,0.979,1.091,1.032,1.003,0.999,1,1.031,0.84,0.76,0.75,0.83,0.81,0.8,0.8
6,0.81,0.82,0.226,0.21,0.208,0.631,0.608,0.611,0.201,0.193,0.189,0.039,0.051,0.041,0.43,0.3
9,0.38,0.071,0.054,0.06)
N.df=data.frame(Run,Comp, Species, Value)

N.aov<-aov(Value~(Comp+Species)^2,data=N.df)
N.aov
Call:
aov(formula=Value~Comp+Species)^2, data=N.df)

Terms:
Comp                Species    Comp:Spe Residuals
Sum of Squares     6.517583   0.166821    0.421127   0.03246
Deg. Of Freedom           3          2           6        42

Residual standard error:                    0.02780031
Estimated effects may be unbalanced

N.THSD<-TukeyHSD(N.aov)
N.THSD
Tukey multiple comparisons of means
95% family-wise confidence level

Fit:aov(formula=Value~(Comp+Species)^2, data=N.df)

$Comp
                    diff         lwr          upr          p adj
B-A            -0.21093   -0.23955    -0.182303              0
C-A            -0.67793   -0.70655    -0.649303              0
D-A            -0.85137   -0.87999   -0.8227474              0
C-B              -0.467   -0.50206   -0.4319442              0
D-B            -0.64044    -0.6755   -0.6053886              0
D-C            -0.17344    -0.2085   -0.1383886              0
```

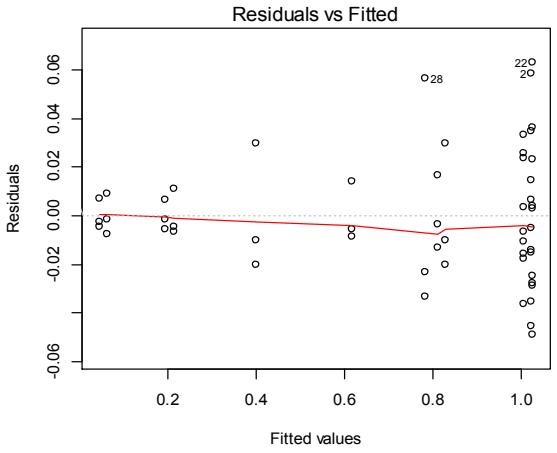
$Species

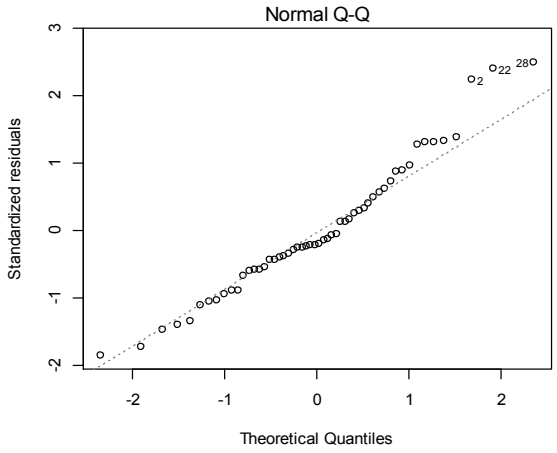
	diff	lwr	upr	p adj
ii-i	0.122	0.099486	0.14451356	0
iii-i	0.008667	-0.01385	0.03118023	0.621326
iii-ii	-0.11333	-0.13585	-0.09081977	0

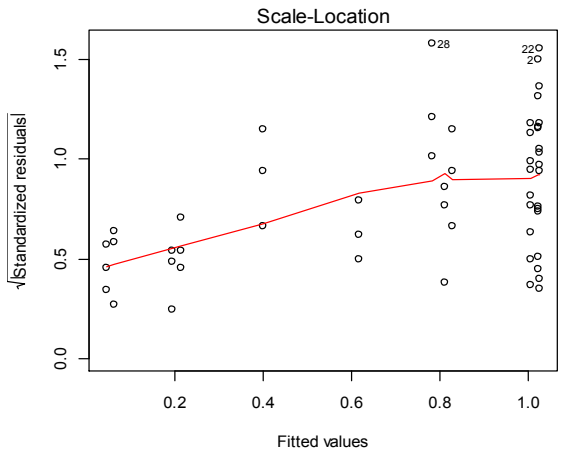
$`Comp:Species`

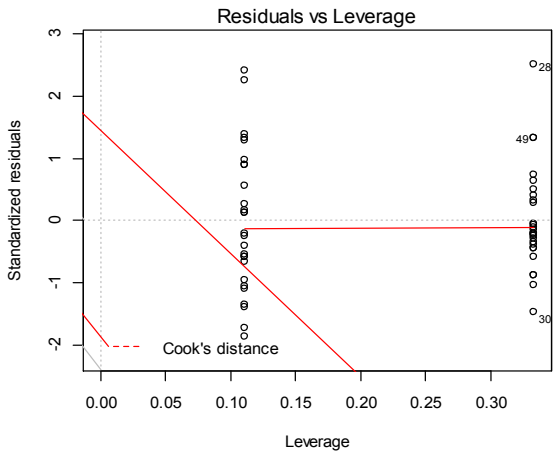
	diff	lwr	upr	p adj
B:i-A:i	-0.24189	-0.30598	-0.17780177	0
C:i-A:i	-0.81056	-0.87464	-0.74646844	0
D:i-A:i	-0.98156	-1.04564	-0.91746844	0
A:ii-A:i	-0.01878	-0.06409	0.02653866	0.949829
B:ii-A:i	-0.21189	-0.27598	-0.14780177	0
C:ii-A:i	-0.40856	-0.47264	-0.34446844	0
D:ii-A:i	-0.62522	-0.68931	-0.56113511	0
A:iii-A:i	0.002556	-0.04276	0.04787199	1
B:iii-A:i	-0.19522	-0.25931	-0.13113511	0
C:iii-A:i	-0.83089	-0.89498	-0.76680177	0
D:iii-A:i	-0.96356	-1.02764	-0.89946844	0
C:i-B:i	-0.56867	-0.64716	-0.4901763	0
D:i-B:i	-0.73967	-0.81816	-0.6611763	0
A:ii-B:i	0.223111	0.159024	0.28719823	0
B:ii-B:i	0.03	-0.04849	0.10849037	0.971368
C:ii-B:i	-0.16667	-0.24516	-0.0881763	3E-07
D:ii-B:i	-0.38333	-0.46182	-0.30484297	0
A:iii-B:i	0.244444	0.180357	0.30853156	0
B:iii-B:i	0.046667	-0.03182	0.12515703	0.654573
C:iii-B:i	-0.589	-0.66749	-0.51050963	0
D:iii-B:i	-0.72167	-0.80016	-0.6431763	0
D:i-C:i	-0.171	-0.24949	-0.09250963	2E-07
A:ii-C:i	0.791778	0.727691	0.85586489	0
B:ii-C:i	0.598667	0.520176	0.67715703	0
C:ii-C:i	0.402	0.32351	0.48049037	0
D:ii-C:i	0.185333	0.106843	0.2638237	0
A:iii-C:i	0.813111	0.749024	0.87719823	0
B:iii-C:i	0.615333	0.536843	0.6938237	0
C:iii-C:i	-0.02033	-0.09882	0.05815703	0.998823
D:iii-C:i	-0.153	-0.23149	-0.07450963	2.1E-06
A:ii-D:i	0.962778	0.898691	1.02686489	0
B:ii-D:i	0.769667	0.691176	0.84815703	0
C:ii-D:i	0.573	0.49451	0.65149037	0
D:ii-D:i	0.356333	0.277843	0.4348237	0
A:iii-D:i	0.984111	0.920024	1.04819823	0
B:iii-D:i	0.786333	0.707843	0.8648237	0
C:iii-D:i	0.150667	0.072176	0.22915703	0.000003
D:iii-D:i	0.018	-0.06049	0.09649037	0.999619
B:ii-A:ii	-0.19311	-0.2572	-0.129024	0
C:ii-A:ii	-0.38978	-0.45386	-0.32569066	0

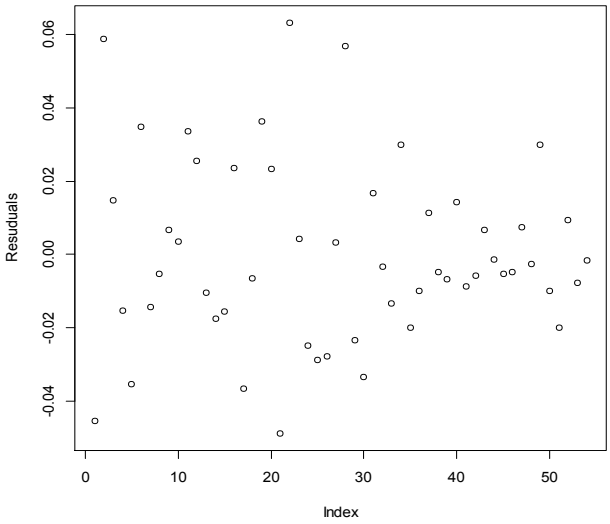
A:iii-A:ii	0.021333	-0.02398	0.06664977	0.888801
B:iii-A:ii	-0.17644	-0.24053	-0.11235733	0
C:iii-A:ii	-0.81211	-0.8762	-0.748024	0
D:iii-A:ii	-0.94478	-1.00886	-0.88069066	0
C:ii-B:ii	-0.19667	-0.27516	-0.1181763	0
D:ii-B:ii	-0.41333	-0.49182	-0.33484297	0
A:iii-B:ii	0.214444	0.150357	0.27853156	0
B:iii-B:ii	0.016667	-0.06182	0.09515703	0.999817
C:iii-B:ii	-0.619	-0.69749	-0.54050963	0
D:iii-B:ii	-0.75167	-0.83016	-0.6731763	0
D:ii-C:ii	-0.21667	-0.29516	-0.1381763	0
A:iii-C:ii	0.411111	0.347024	0.47519823	0
B:iii-C:ii	0.213333	0.134843	0.2918237	0
C:iii-C:ii	-0.42233	-0.50082	-0.34384297	0
D:iii-C:ii	-0.555	-0.63349	-0.47650963	0
A:iii-D:ii	0.627778	0.563691	0.69186489	0
B:iii-D:ii	0.43	0.35151	0.50849037	0
C:iii-D:ii	-0.20567	-0.28416	-0.1271763	0
D:iii-D:ii	-0.33833	-0.41682	-0.25984297	0
B:iii-A:iii	-0.19778	-0.26186	-0.13369066	0
C:iii-A:iii	-0.83344	-0.89753	-0.76935733	0
D:iii-A:iii	-0.96611	-1.0302	-0.902024	0
C:iii-B:iii	-0.63567	-0.71416	-0.5571763	0
D:iii-B:iii	-0.76833	-0.84682	-0.68984297	0
D:iii-C:iii	-0.13267	-0.21116	-0.0541763	3.94E-05


Residuals vs Fitted
Residuals
Fitted values
28
22
2


Normal Q-Q
Standardized residuals
Theoretical Quantiles
2
22
28

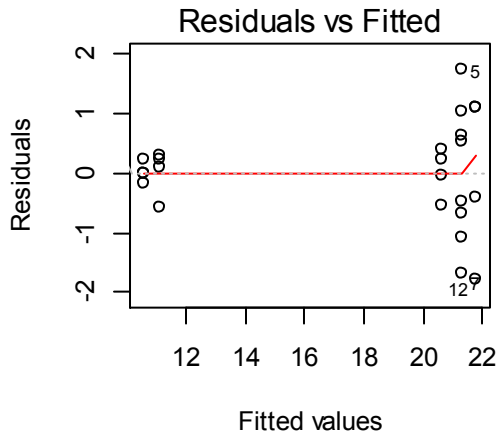

Scale-Location
√|Standardized residuals|
Fitted values
28
22
2

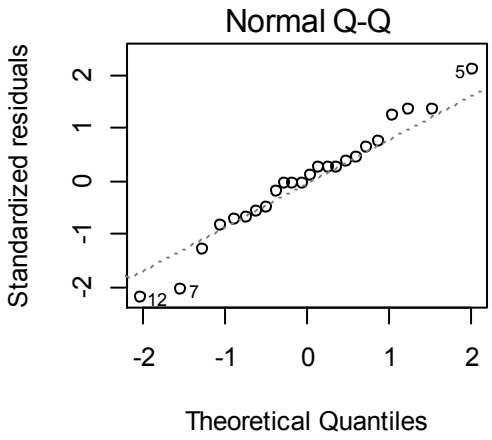

Residuals vs Leverage
Standardized residuals
Leverage
Cook's distance
28
49
30

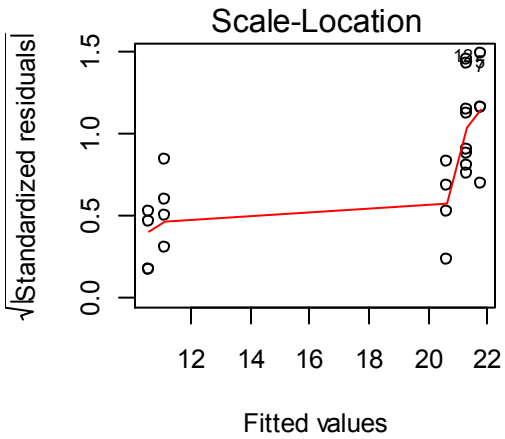

Resuduals
Index

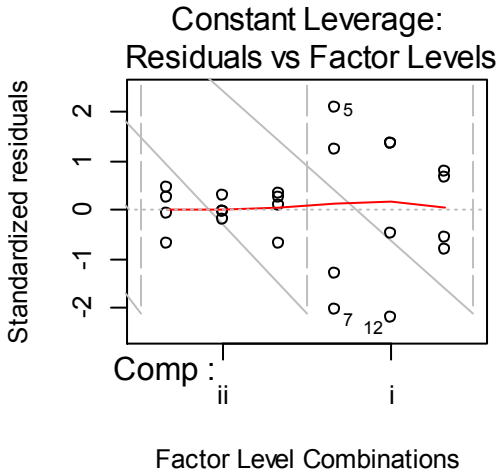
Appendix C-2: Solubility THSD Test by R

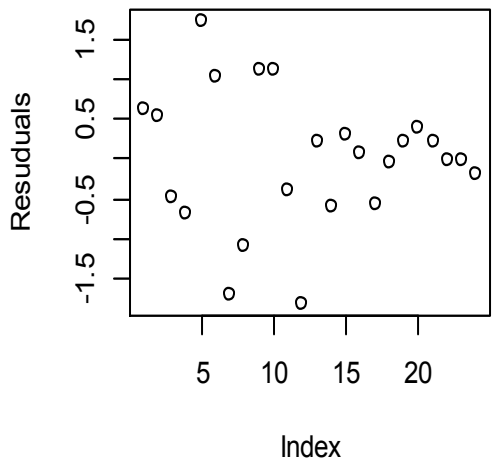
```
> A='A'                    (A=DDW)
> B='B'                    (B=zucchini)
> C='C'                    (C=soybean)
> i='i'                    (i=caffeine)
> ii='ii'                  (ii=triclocarban)
> Run=c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24)
> Comp=c(i,i,i,i,i,i,i,i,i,i,i,ii,ii,ii,ii,ii,ii,ii,ii,ii,ii,ii)
> Species=c(A,A,A,A,B,B,B,B,C,C,C,C,A,A,A,A,B,B,B,B,C,C,C,C)
>Value=c(21.9,21.8,20.8,20.6,23.0,22.3,19.6,20.2,22.9,22.9,21.4,20,11.38,10.5
9,11.46,11.25,20.07,20.58,20.85,21.01,10.79,10.54,10.54,10.39)
> S.df=data.frame(Run,Comp,Species, Value)
> S.aov<-aov(Value~(Comp+Species)^2,data=S.df)
> S.THSD<-TukeyHSD(S.aov)
> S.THSD
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Value ~ (Comp + Species)^2, data = S.df)
$Comp
          diff       lwr       upr     p adj
ii-i -7.329167 -8.14329 -6.51505         0

$Species
          diff       lwr       upr     p adj
B-A    4.72875  3.517504  5.939996         0
C-A      -0.04  -1.25125  1.171246  0.996092
C-B    -4.76875     -5.98   -3.5575         0

$`Comp:Species`
              diff       lwr       upr     p adj
ii:A-i:A -1.01E+01   -12.238  -7.97197         0
i:B-i:A   3.55E-15  -2.13303  2.133034         1
ii:B-i:A -6.48E-01  -2.78053  1.485534  0.923236
i:C-i:A   5.25E-01  -1.60803  2.658034  0.967098
ii:C-i:A -1.07E+01   -12.843  -8.57697         0
i:B-ii:A  1.01E+01  7.971966  12.23803         0
ii:B-ii:A 9.46E+00  7.324466  11.59053         0
i:C-ii:A  1.06E+01  8.496966  12.76303         0
ii:C-ii:A -6.05E-01  -2.73803  1.528034  0.941159
ii:B-i:B  -6.48E-01  -2.78053  1.485534  0.923236
i:C-i:B   5.25E-01  -1.60803  2.658034  0.967098
ii:C-i:B -1.07E+01   -12.843  -8.57697         0
i:C-ii:B  1.17E+00  -0.96053  3.305534  0.520891
ii:C-ii:B -1.01E+01  -12.1955  -7.92947         0
ii:C-i:C -1.12E+01   -13.368  -9.10197         0
```


Residuals vs Fitted
Residuals
Fitted values
5
12
7


Normal Q-Q
Standardized residuals
Theoretical Quantiles
5
12
7


Scale-Location
√|Standardized residuals|
Fitted values


Constant Leverage:
Residuals vs Factor Levels
Standardized residuals
Comp :
ii
i
Factor Level Combinations
5
7
12


Resuduals
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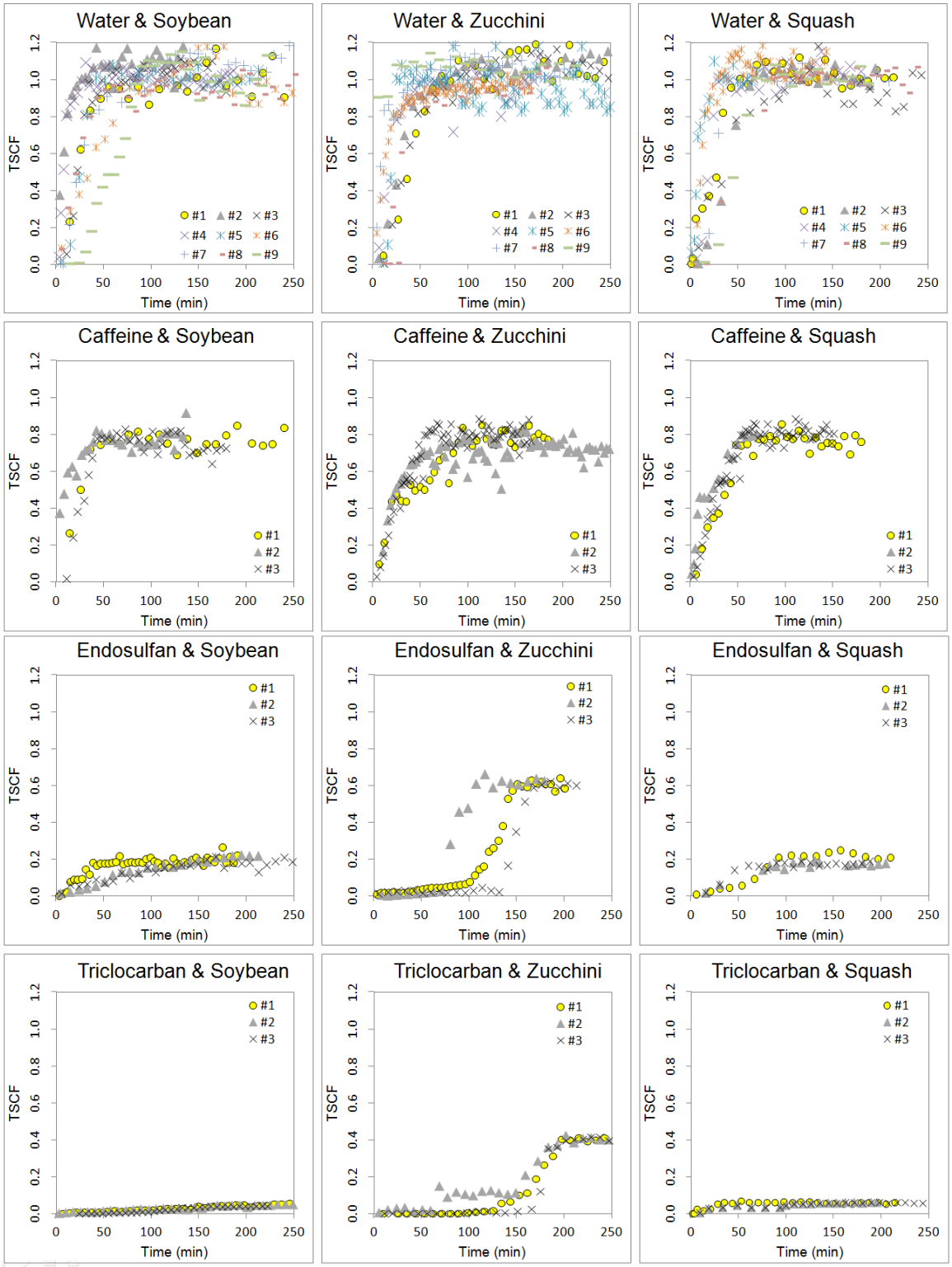
Appendix D: TSCF vs Time plots for each combination
(Replicates represent the order of sample measurements taken from three individual plants.)



Appendix E: Non linear regression determination for the new fit

F.nls
Nonlinear regression model
 model: TSCF ~ (alpha/(alpha + gamma^LogK))
  data: F.df
 alpha gamma
12.126 1.768
 residual sum-of-squares: 0.06805

Number of iterations to convergence: 5
Achieved convergence tolerance: 4.103e-06
> summary(F.nls)

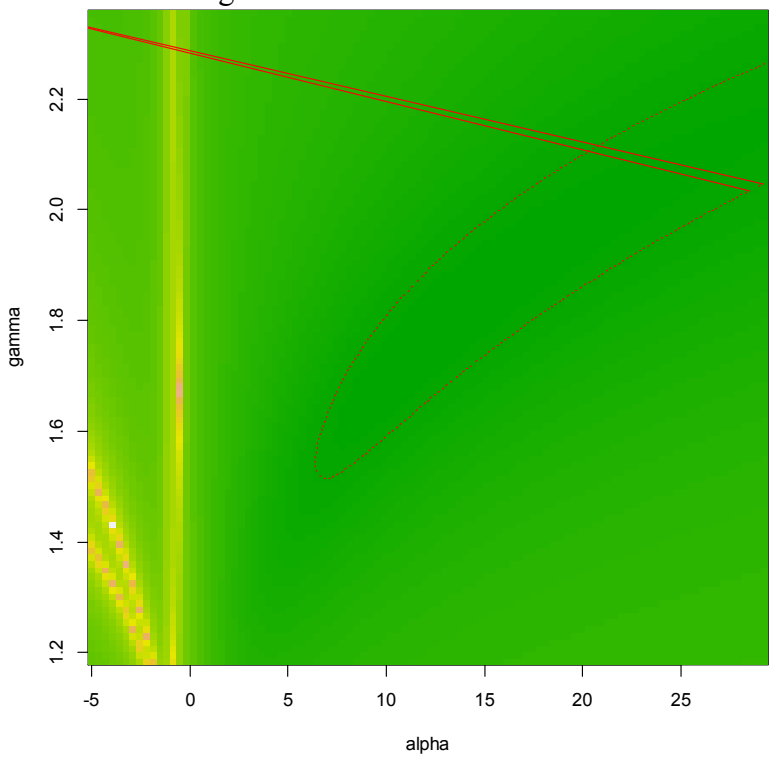
Formula: TSCF ~ (alpha/(alpha + gamma^LogK))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
alpha 12.1257   4.0358   3.005   0.0084 **
gamma   1.7685   0.1382 12.800 8.04e-10 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.06522 on 16 degrees of freedom

Number of iterations to convergence: 5
Achieved convergence tolerance: 4.103e-06



Since alpha is estimated 12.126 with an error of 4.36, the value of alpha was fixed to 11 as Dettenmaier's model.

```
 F2.nls=nls(m2,data=F.df,start=list(gamma=2.6))
 F2.nls
Nonlinear regression model
  model:  TSCF ~ (11/(11 + gamma^LogK))
   data:  F.df
gamma
1.731
 residual sum-of-squares: 0.06846

Number of iterations to convergence: 4
Achieved convergence tolerance: 6.144e-06
> summary(F2.nls)

Formula: TSCF ~ (11/(11 + gamma^LogK))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
gamma  1.73109    0.04159   41.62   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.06346 on 17 degrees of freedom

Number of iterations to convergence: 4
Achieved convergence tolerance: 6.144e-06
```