Uptake and Translocation of Hydrophobic Organic Chemicals in Several Plant Species

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Contents

論文要旨

Hexachlorocyclohexanes（HCH 類）やシクロジエン系化合物といった有機塩素系殺虫剤は、1950 - 60 年代にかけて広く日本の農耕地に施用された。これらの有機塩素系殺虫剤は 1970 年代初頭に使用禁止となったが、現在も土壌中に残留しており、近年、ディルドリンやヘプタクロル類といったシクロジエン系化合物によるウリ科果実の汚染が問題となっている。これらの有機化学物質は、疎水性を表す指標である *n*-オクタノール－水分配係数 （log K_{OW}）が 3 以上と疎水性の高い物質である。一般に、高疎水性の有機化学物質は根に濃縮され茎葉部へは移行しないとされているが、ウリ科植物では疎水性有機化学物質が茎葉部へ移行しやすいことが知られている。これまでの研究で、HCH 類、シクロジエン系化合物、DDT 類を添加した土壌でウリ科を含む 5 科 8 種の植物を栽培し、これらの化合物の取込み性を比較したところ、シクロジエン系化合物と dichlorodiphenyltrichloroethanes（DDT 類）の茎葉部濃度はウリ科植物でのみ高かったが、HCH 類では植物種間差が不明瞭であった。また、根部濃度は全ての化合物群においてウリ科植物が高かった。これらの結果は疎水性有機化学物質の取込みや移行に植物種間差があることを示している。しかし、これらの化合物が高い疎水性を持つために土壌に強く吸着され、植物が取込めるような可給性の疎水性有機化学物質の濃度は低い。したがって、土耕試験ではこれらの高疎水性有機化学物質の取込みや移行における植物種間差を詳細に比較することは困難である。本研究では、疎水性の度合いの異なる β-HCH（log K_{OW} 3.8）、ディルドリン（log K_{OW} 5.2）を 5 科 5 種の植物に水耕条件下で取込ませ、植物による疎水性有機化学物質の根への取込みや茎葉部への移行における種特異性と移行メカニズムを明らかにすることを目的とした。

1. 水耕条件下での各種植物による β-HCH およびディルドリンの取込みの経時変化

供試植物はオオムギ（イネ科）、ダイズ（マメ科）、トマト（ナス科）、ブロッコリ（アブラナ科）、ズッキーニ（ウリ科）とした。β-HCH およびディルドリンを添加していない培養液で前培養した植物を用い、10 ppb の β-HCH およびディルドリンを含む試験水（0.5 mM $CaCl_2$、2 mM MES、pH 5.8）を用いた取込み実験を 25℃、湿度 60%、明条件で行った。処理開始から 1、2、4、8、24 時間後に茎葉部と根部に分けてサンプリングし、試験水、茎葉部、根部の β-HCH、ディルドリン濃度および蒸散量を測定した。

有機化学物質の培地から根への取込み性は、根部濃度を培地濃度で除した Root Concentration Factor（RCF）によって評価される。RCF は全ての植物においてディルドリンが β-HCH よりも 10 倍高く、RCF は log K_{OW} に依存して増加するというこれまでの知見と一致した。また、全ての供試植物において β-HCH とディルドリンの RCF は 1 時間で急激に増加後、漸増したことから、根への取込みは吸着によると考えられた。取込みの過程における植物種間差について、β-HCH では、ブロッコリの RCF が他の植物より高かったものの、その他の植物では差異は認められなかった。ブロッコリにおける β-HCH の RCF が高かった要因として、根の表面積などの違いによるものと推察された。ディルドリンでは RCF に植物種間差は認められなかった。

茎葉部への移行性は、導管液中濃度を培地濃度で除した Transpiration Stream Concentration Factor（TSCF）によって評価される。しかし、導管液中濃度を直接測定することが困難であることから、茎葉部含量 / 蒸散量を導管液中濃度として準用することも認められている。ここでは、TSCF を（茎葉部中含量/蒸散量）/ 培地濃度で算出した。β-HCH の場合、ズッキーニが最も早い 2 時間、次いでトマトとブロッコリが 8 時間、最後にオオムギとダイズは 24 時間で茎葉部から検出されており、TSCF も概ね同様の傾向が認められ、24 時間処理時の TSCF はブロッコリ＞ズッキーニ・トマト＞オオムギ・ダイズとなった。RCF は全ての植物で処理開始から 1 時間までで速やかに増加しており、茎葉部から β-HCH が検出されるまでの植物種間での時間差は根から茎葉部への移行過程で生じたものと考えられた。一方、ディルドリンの TSCF はズッキーニでのみ顕著に高く、他の植物では移行

はほとんど認められなかった。

2. 蒸散量制限下でのズッキーニにおける β-HCH およびディルドリンの取込み

ここまでの結果より、ズッキーニでは β-HCH およびディルドリンの茎葉部への移行性が高いことが明らかとなった。また、これらの疎水性有機化学物質は導管を経路として蒸散流に乗り輸送される可能性が考えられる。そこで、対照（25℃、湿度 60%、明条件）に対し、加湿（湿度 80%）、ABA 添加（試験水に 100 μM アブシシン酸添加）、暗所（24h 暗処理）、加熱（root）（根を 5 分間 70℃で前処理）、加熱（whole）（植物体を 5 分間 70℃で前処理）の各種処理により蒸散量を制限した条件下で、取込みおよび移行性を比較した。

蒸散量は対照区と比較して、加湿で 50%、ABA 添加で 70%、暗所で 41%、加熱（root）で 24%、加熱（whole）で 100%減少した。β-HCH の RCF は対照区と比較して、加湿、ABA 添加および暗所では影響を受けなかったが、根あるいは植物体全身の加熱により増加した。ディルドリンの RCF は処理間に有意差は認められなかった。つまり、β-HCH とディルドリンの根への取込みは蒸散量の制限による影響を受けない。したがって、β-HCH やディルドリンは、主にマスフローや生理的な要因の関与しない物理化学的な吸着によって取込まれていることが示唆された。

茎葉部への移行について、まず、それぞれの茎葉部濃度は β-HCH では対照 ＞ 暗所、加熱（root）＞ 加湿、ABA 添加となり、ディルドリンでは対照 ＞ 加湿 ＞ 暗所、ABA 添加 ＞ 加熱（root）となった。なお、植物体全身の加熱処理では β-HCH とディルドリン共に定量下限以下であったことから、脂質組織を介した拡散による茎葉部への移行は考えにくい。ここで、TSCF を用いて茎葉部への移行性を比較すると、β-HCH では、暗所と根の加熱における TSCF と加湿と ABA 添加の TSCF との間に有意差は認められなかった。このことから、β-HCH の茎葉部への移行は主に蒸散量に依存した移行であることが示唆された。一方、ディルドリンの場合、TSCF は蒸散のみを制限した加湿や ABA 添加処理による影響は受けなかったのに対し、暗所と根の加熱処理によって低下した。過去の研究結果から、ウリ科植物では、ディルドリンの根から茎葉部への移行にディルドリン親和性のタンパク質様物質が関与することが示唆されている。本研究において、暗所や根の加熱処理によってディルドリンの茎葉部への移行が阻害されたことから、ズッキーニにおけるディルドリンの茎葉部への移行には親和性タンパク質のような蒸散以外の生理的・分子的な要因が関与している可能性が示された。

以上の研究により、ズッキーニは他の植物と比較して β-HCH やディルドリンを根から茎葉部へ移行しやすいが、それぞれの移行メカニズムは異なることが明らかとなった。β-HCH は log K_{OW} が 3.8 とディルドリン（log K_{OW} 5.2）に比べて親水性である。そのため、非ウリ科植物でも蒸散流によって β-HCH を茎葉部へ移行することができると推察された。しかしながら、ズッキーニは β-HCH の茎葉部への移行速度が速く、根表面から導管までの移動が何らかの要因によって他の植物よりもスムーズであると推察された。その要因としてはディルドリン同様の親和性タンパク質の関与あるいは根の形態学的な差異等が考えられた。一方、ディルドリンは根により強く吸着するため、根の表面から茎葉部までの移行にはディルドリン親和性タンパク質のような蒸散以外の生理的・分子的な要因の関与が不可欠である。ウリ科植物には疎水性有機化学物質親和性のタンパク質の存在が過去の研究において確認されているため、ズッキーニではディルドリンを特異的に茎葉部へ移行すると考えられた。今後、ズッキーニにおける疎水性有機化合物の根から茎葉部までの移動について組織・細胞レベルでの詳細な解析が必要である。

Keywords: β-HCH, dieldrin, *Cucurbita pepo*, root concentration factor, transpiration stream concentration factor

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INTRODUCTION

Persistent organic pollutants are toxic chemicals that stay in the environment for a long time, bioaccumulate through the food web, and adversely affect human health and the environment. Hexachlorocyclohexanes (HCHs) and chlorinated cyclodienes (aldrin, dieldrin, endrin, and heptachlor) were widely used on Japanese farmland from the 1950s to the 1960s. Although they were prohibited in the early 1970s, they remained detectable in the soil even after 40 years [1]. In recent years, dieldrin has been detected in cucurbit fruits in excess of maximum residue limits in Japan [2].

HCHs and chlorinated cyclodienes have relatively high hydrophobicity: the log K_{OW} (log *n*-octanol–water partition coefficient) values of these chemicals are more than 3. In general, hydrophobic organic chemicals (HOCs) such as HCHs and chlorinated cyclodienes are concentrated in roots and are little translocated to shoots [3, 4]. However, cucurbits are known to take up and translocate HOCs such as dieldrin and endrin [5], dichlorodiphenyldichloroethylene (DDE) [6, 7], polychlorinated dibenzodioxins/furans [8], and polychlorinated biphenyls (PCBs) [9] into above ground tissue. As for the translocation mechanisms of HOCs in cucurbits, it has been suggested that root produces protein-like materials in xylem sap that play a crucial role in the translocation of HOCs [10].

In my previous study [11], I investigated the uptake of HCHs, chlorinated cyclodienes, and dichlorodiphenyltrichloroethanes (DDTs) by non-cucurbits and cucurbits in a soil culture experiment. Shoot concentrations of chlorinated cyclodienes and DDTs were higher in cucurbits, but HCHs did not show clear differences. Root concentrations of HOCs tended to be higher in cucurbits. These data indicate differences among plant species in the uptake and translocation of

HOCs. However, as HOCs are sorbed strongly to soil because of their high hydrophobicity, and low concentrations of bioavailable HOCs, it was difficult to compare uptake and translocation of HOCs among species in detail in soil culture.

In this study, to overcome this problem I performed an uptake experiment with β-1,2,3,4,5,6-hexachlorocyclohexane (β-HCH) and 1,2,3,4,10,10-Hexachloro-6,7-epoxy-,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene (dieldrin) in water culture using 5 species belonging to different families. Plants were grown in a hydroponic medium containing β-HCH and dieldrin, and the time-course of the uptake from the medium to roots and translocation to the shoots was observed. To measure the uptake of organic chemicals, I calculated the root concentration factor (RCF) as the ratio of HOC concentrations in roots to those in the medium [3, 12, 13]. To measure translocation, I used the transpiration stream concentration factor (TSCF) [3, 4, 12, 13]. Although the TSCF is defined as the ratio of the concentration in the xylem sap to that in the medium [3], it is difficult to measure the concentration in the xylem sap directly. So I estimated it indirectly by dividing the amount of HOCs in the shoot by the volume of water transpired [14, 15]. Because it appeared that the HOCs were translocated to the shoot in the transpiration stream, I also investigated the influence of limiting transpiration by physical and chemical treatments on β-HCH and dieldrin translocation to discuss the mechanisms.

MATERIALS AND METHODS

Preparation of test medium

I used β-HCH and dieldrin in the plant uptake experiments. The log K_{OW} of β-HCH and dieldrin is 3.8 and 5.2, respectively [16]. The test medium was prepared with reference to OECD test guidelines for the preparation of poorly water-soluble substances [17]. β-HCH and dieldrin (Wako Pure Chemicals, Osaka, Japan) were dissolved in acetone, and a 0.01 g L^{-1} stock solution was prepared. A 1-mL aliquot of the stock solution was mixed into 1 L of a solution containing 0.5 mM $CaCl_2$ and 2 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.8). This solution was then ultrasonicated for 30 min. The measured final concentrations in test medium were 8.91 ± 0.21 μg L^{-1} β-HCH and 7.45 ± 0.09 μg L^{-1} dieldrin.

Time-course of uptake of β-HCH and dieldrin by plants in hydroponic medium

The schema of time-course of uptake experiment was shown in Figure 1. Seeds of *Hordeum vulgare* L. 'Hayadori-2', *Glycine max* Merrill 'Tachinagaha', *Solanum lycopersicum* Mill. 'Magnet', *Brassica oleracea* var. *italica* Plenck 'Stick Señor', and *Cucurbita pepo* L. 'Black Tosca' were sown in a nursery bed filled with granular perlite and germinated in a growth chamber (Koito Kogyo, Tokyo, Japan) at 25 °C under a 16:8-h light: dark cycle for 7 days. The seedlings were transplanted into a hydroponic apparatus (Home Hyponica 501; Kyouwa Co., Osaka, Japan) and grown for several days with aeration without β-HCH and dieldrin to achieve approximately the same fresh weight of roots (*H. vulgare*, 12 days; *G. max*, 6 days; *S. lycopersicum*, 12 days; *B. oleracea*, 16 days; *C. pepo*, 4 days). The leaf stage of each species at the transplanting was as follows: *H. vulgare*, 3rd; *G. max*, 3rd; *S. lycopersicum*, 4th; *B. oleracea*,

4th; *C. pepo*, 3rd. The apparatus held 9 L of medium containing (mg L^{-1}) N, 130; P, 26; K, 168; Ca, 82; Mg, 18; Mn, 0.6; B, 0.3; Fe, 1.4; Cu, 0.02; Zn, 0.05; Mo, 0.02. The pH was adjusted daily to between 5.8 and 6.2 with 6 N H_2SO_4 or 6 N KOH. The medium was renewed every 7 days.

The uptake experiment was begun 2h after a light period was started. One plant of each species was transferred to a stainless steel vessel (95 mm height × 70 mm inner diameter) with 300 mL of test medium. The experiment was run in a growth chamber (Nippon Medical & Chemical Instruments Co., Osaka, Japan) at 25 °C, 60% relative humidity (RH), under light. The test medium was not aerated so as to avoid the volatilization of the β-HCH and dieldrin during the treatment. After 1, 2, 4, 8, and 24 h, the shoots and roots were harvested separately. The roots were rinsed in 100 mL of Milli-Q water. Transpiration was calculated from the volume loss of the medium. The test medium evaporation from the test vessel directly was negligible because the volume of the medium in the vessel without the plant did not decrease after 24 h. Each sample was weighed to obtain the fresh weight. The experiment was conducted in quadruplicate.

Uptake of β-HCH and dieldrin by C. pepo *under limited transpiration*

The schema of uptake experiment by *C. pepo* under limited transpiration was shown in Figure 2. Seedlings of *C. pepo* were raised as above and grown for 7 days in the hydroponics apparatus. The uptake experiment was begun 2h after a light period was started. One plant was transferred to a stainless steel vessel (140 mm height × 82 mm inner diameter) with 600 mL of the test medium. The experiment was run in a growth chamber as above without aeration for 24 h. The control treatment used 60% RH (Non-treated). To change the transpiration rate, 5 treatments were applied: 80% RH (“High Humidity”) [18], 100 μM abscisic acid (“+ABA”; Wako Pure

Chemicals) in the medium [19]; darkness (“Dark”); heating the roots in water at 70 °C for 5 min before the experiment (“Heated (root)”); and heating the whole plant in water at 70 °C for 5 min before the experiment (“Heated (whole)”). As for Heated (root) plants, the roots wilted, but the shoots did not change apparently. As for Heated (whole) plants, both of the roots and shoots wilted.

After 24 h, the shoots and roots were harvested separately, and transpiration was calculated as above. The roots were rinsed in 200 mL of Milli-Q water. Each sample was weighed to obtain the fresh weight. The experiment was conducted in quadruplicate.

Analysis of β-HCH and dieldrin concentrations in test medium and plants

Test medium (5 mL) was spiked with 50 ng each of D_6-γ-HCH and $^{13}C_{12}$-dieldrin (Cambridge Isotope Laboratories, Andover, MA, USA) as internal standards. The medium was extracted twice with 2 mL of *n*-hexane with shaking for 1 min. The extract was passed through Na_2SO_4 for dehydration. The sample was syringe-spiked with 50 ng each of $^{13}C_{12}$-2,4,4′-trichlorobiphenyl and $^{13}C_{12}$-2,2′,4,4′,6,6′-hexachlorobiphenyl (Wellington Laboratories, Guelph, ON, Canada) and then concentrated to 50 µL under a gentle stream of nitrogen gas.

Each shoot and root sample was chopped finely and then homogenized in 150 mL of acetone for 3 min on a Polytron PT3100 homogenizer (Kinematica AG, Lucerne, Switzerland). The extract was passed through a 0.8-µm glass fiber filter and concentrated to 50 mL in a rotary evaporator at 40 °C. A 25-mL aliquot of the extract was spiked with the same internal standards as above and then concentrated to between 5 and 10 mL in a rotary evaporator at 40 °C. The concentrated extract was adsorbed with a diatomite column (InterSep K-solute; GL Science, Tokyo, Japan) for 20 min, eluted with 100 mL of *n*-hexane, and the eluate was concentrated to

between 1 and 2 mL in a rotary evaporator at 40 °C. The concentrated extract was purified through a graphite column and a primary/secondary amine column (ENVI-Carb-II/PSA column; Supelco, Bellefonte, PA, USA). The sample was syringe-spiked as above and then concentrated to 50 µL under a gentle stream of nitrogen gas.

β-HCH and dieldrin in the purified samples were measured by a gas chromatograph – mass spectrometer (GC-MS; HP6890-5973N; Agilent Technologies, Santa Clara, CA, USA) equipped with an ENV-8MS capillary column (30 m × 0.25 nm i.d. × 0.25 µm film thickness; Kanto Chemical, Tokyo, Japan). The limit of quantitation (LOQ) was calculated according to JIS K0312 [20]. The LOQs for β-HCH were 1.36 ng g^{-1} in shoot extracts, 3.04 ng g^{-1} in root extracts, and 0.61 µg L^{-1} in media; and those for dieldrin were 0.78 ng g^{-1} in shoot extracts, 1.74 ng g^{-1} in root extracts, and 0.35 µg L^{-1} in media.

Statistical analyses

Statistical analyses were performed in SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was followed by Tukey's multiple comparison test using a pairwise comparison matrix to determine which samples differed significantly.

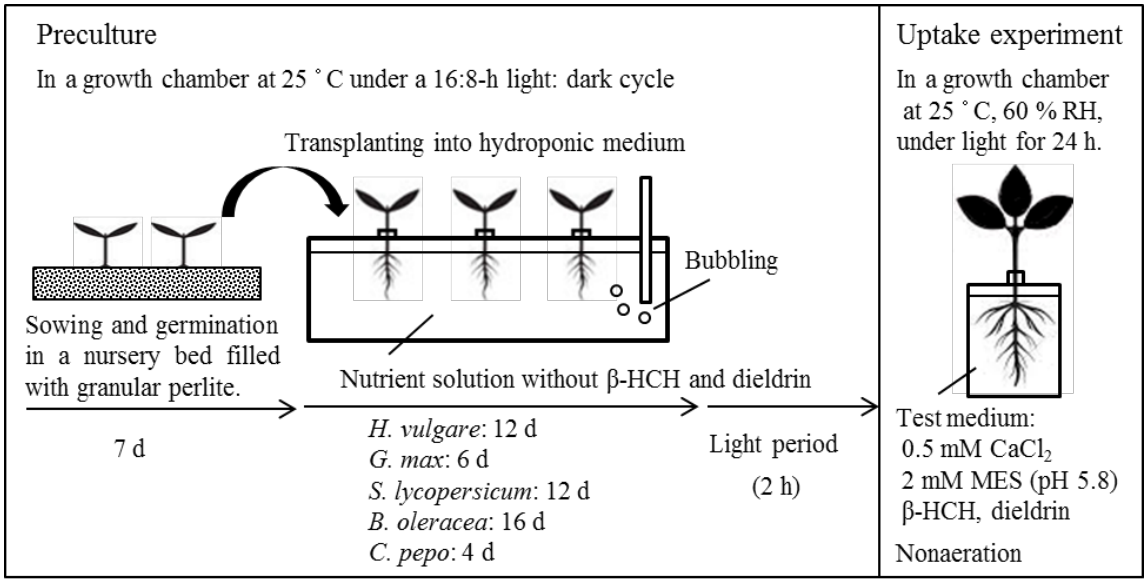

Figure 1 A schema of time-course of uptake experiment by several plants in hydroponic medium.

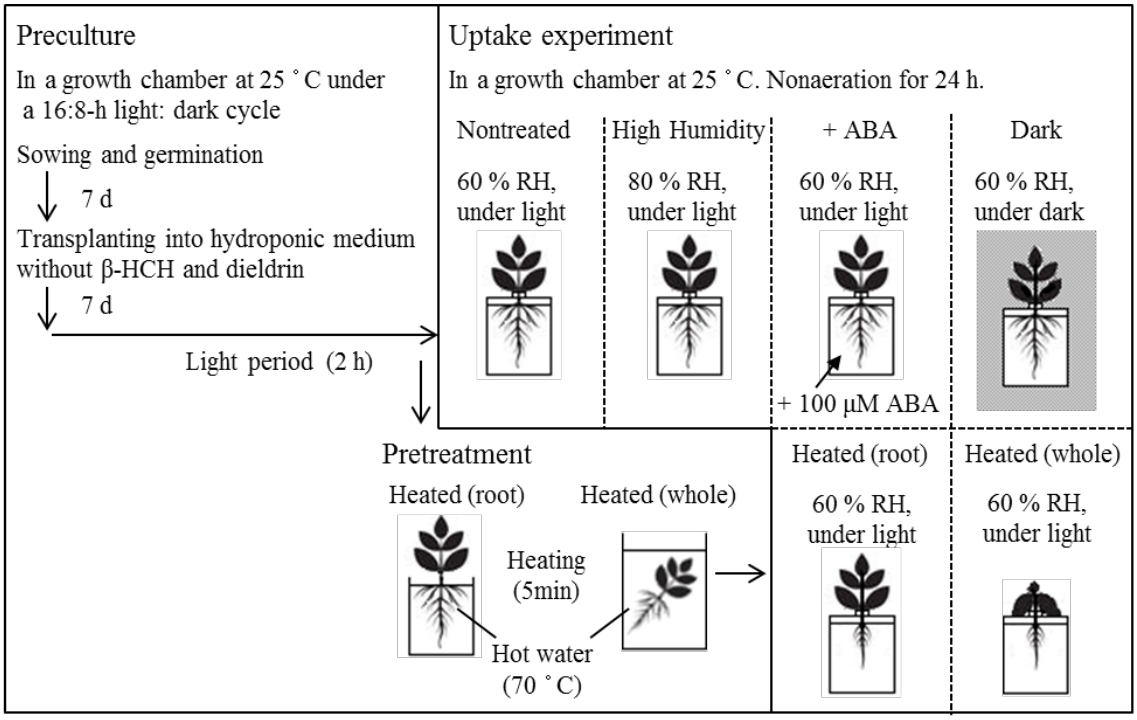

Figure 2 A schema of uptake experiment by *C. pepo* under limited transpiration.

RESULTS

Plant growth

The shoot fresh weights of *H. vulgare*, *G. max*, *S. lycopersicum*, and *C. pepo* did not differ significantly among treatment times, but that of *B. oleracea* was greater at 24 h than at the earlier times (Table 1). The root fresh weights of all species did not differ significantly among treatment times. The shoot fresh weights at 24 h decreased in the order of *B. oleracea* = *S. lycopersicum* > *C. pepo* = *G. max* > *H. vulgare*. The root fresh weights at 24 h of all species were approximately the same. Transpiration increased linearly with time in all species (Figure 3). Throughout the experiment, *S. lycopersicum* and *B. oleracea* had significantly higher transpiration than *H. vulgare*, *G. max*, and *C. pepo*. The transpiration volume of *C. pepo* at 24 h in this experiment without aeration (35.7 ± 0.7 mL) was not significantly different from those with aeration (35.9 ± 0.7 mL) and without β-HCH and dieldrin (*t*-test, $P = 0.85$). Therefore, in this experimental system, neither the presence of HOCs nor the absence of aeration influenced plant growth.

Concentrations of β-HCH and dieldrin in roots, shoots, and test medium

β-HCH and dieldrin were detected in the root extracts of all species at 1 h, and the concentrations increased gradually to 24 h (Table 2). Concentrations of β-HCH tended to be higher in *B. oleracea* root extracts than in the other species throughout the experiment, but those of dieldrin showed no marked differences among species.

On the other hand, β-HCH was not detected in the shoots of any species at 1 h, and dieldrin was detected only in the shoots of *C. pepo* at 1 h. The time at which β-HCH became detectable in the shoots differed among species: in *C. pepo* at 2 h, in *S. lycopersicum* and *B. oleracea* at 8 h,

and in *H. vulgare* and *G. max* at 24 h. At 24 h, the shoot concentrations of β-HCH increased in the order of *G. max* ≤ *H. vulgare* ≤ *S. lycopersicum* ≤ *C. pepo* < *B. oleracea*. This order agreed with the result in my previous soil culture experiment [11]. Dieldrin was not detected in the shoots of *H. vulgare*, *G. max*, or *B. oleracea* at any time. It was not detected in *S. lycopersicum* until 24 h. Only in *C. pepo* did the concentration of dieldrin increase with time. This result agreed with the high shoot concentrations of dieldrin in cucurbits in my previous soil culture experiment [11].

The medium concentrations in non-plant control did not decrease after 24 h treatment (β-HCH, 9.06 ± 0.30 μg L^{-1}; dieldrin, 7.15 ± 0.20 μg L^{-1}). The concentrations in the medium with the plants decreased with time, at the rates dependent on chemicals and species. At 24 h, concentrations of β-HCH differed by a factor of 1.4 times and those of dieldrin by 1.6 times among species. β-HCH concentrations decreased by 64% to 90%, and those of dieldrin by 25% to 39%. The final concentrations of β-HCH were about 3 times those of dieldrin.

Root concentration factors of β-HCH and dieldrin

As the concentrations of β-HCH and dieldrin in the medium differed among species during the experiment, I calculated the root concentration factor (RCF) as:

$$\text{RCF} = (\text{concentration in root}) / (\text{concentration in medium}) \quad (1)$$

I used the concentration in the root extracts and the medium at the end of each treatment time to calculate RCF.

The β-HCH RCF values of all species increased sharply within 1 h and continued to increase gradually (Figure 4A). That of *B. oleracea* was higher than those of the other species throughout the experiment. The dieldrin RCF values of all species also increased sharply within 1 h and

continued to increase gradually, but there were no significant differences among species after 8 h (Figure 4B).

Transpiration stream concentration factors of β-HCH and dieldrin

Although the transpiration stream concentration factor (TSCF) is defined as the ratio of the concentration in the xylem sap to that in the medium [3], it is difficult to measure the concentration in the xylem sap directly. So I estimated it indirectly by dividing the amount of HOCs in the shoot by the volume of water transpired [14, 15]:

$$\text{TSCF} = (\text{amount in shoots} / \text{transpiration volume}) / (\text{concentration in medium}) \quad (2)$$

I used the concentration in the medium at the end of each treatment time to calculate TSCF.

The β-HCH TSCF values showed large differences among species (Figure 5A). At 24 h, they increased in the order of *H. vulgare* = *G. max* < *S. lycopersicum* = *C. pepo* < *B. oleracea*. In addition, the time at which values began to increase followed the same pattern as the time at which β-HCH became detectable in shoots. The β-HCH TSCF value in *C. pepo* rose faster than in the other species, but that at 24 h was less than the value in *B. oleracea*. The dieldrin TSCF value was remarkably high only in *C. pepo*, in which it rose rapidly (Figure 5B). In contrast, it remained negligible in the other 4 species.

Influences of transpiration inhibition on β-HCH and dieldrin uptake and translocation

I restricted the transpiration volume of *C. pepo* by various treatments and investigated the effect on β-HCH and dieldrin concentrations in the plants. Relative to the Non-treated (but exposed), the root fresh weights were unchanged by High Humidity, +ABA, and Dark treatments, but were

decreased by Heated (root) and Heated (whole) treatments (Table 3). The shoot fresh weights were unchanged by most treatments but were decreased by the Heated (whole) treatment. The transpiration volumes were decreased by all treatments: by 24% by Heated (root), by 41% by Dark, by 50% by High Humidity, by 70% by +ABA, and by 100% by Heated (whole).

Relative to the Non-treated, the root concentrations of β-HCH and dieldrin were increased by Heated (root) and Heated (whole) treatments, but were unchanged by High Humidity, +ABA, and Dark treatments (Table 4). The shoot concentrations of β-HCH decreased in the order of Non-treated > Dark = Heated (root) > High Humidity = +ABA >> Heated (whole). Those of dieldrin decreased in the order of Non-treated > High Humidity > Dark = +ABA > Heated (root) >> Heated (whole). The order of the shoot β-HCH concentrations approximated that of the transpiration volumes, but that of the shoot dieldrin concentrations did not. The concentrations of β-HCH in the medium were not significantly different among treatments and Non-treated, but those of dieldrin were higher in Heated (root) and Heated (whole) treatments.

Relative to the Non-treated, β-HCH RCF values in High Humidity, +ABA, and Dark treatments were not significantly different, but those in Heated (root) and Heated (whole) were about 50% higher (Figure 6A). Dieldrin RCF values were not significantly different among the treatments, although that in High Humidity was about 40% higher than the Non-treated.

Relative to the Non-treated, β-HCH TSCF values were decreased by all treatments, but there were no significant differences among treatments (Figure 6B). Dieldrin TSCF values were not significantly different in High Humidity and +ABA treatments, but they were significantly decreased in Dark treatment and remarkably decreased in Heated (root) treatment. TSCF values in Heated (whole) treatment could not be calculated because transpiration was 0.

Table 1 Shoot and root fresh weights after each treatment time.

Plants	Treatment time				
	1 h	2 h	4 h	8 h	24 h
Fresh weight of shoot (g)					
H. vulgare	1.40±0.19^{a}	1.31±0.06^{a}	1.39±0.01^{a}	1.29±0.17^{a}	1.51±0.07^{a}
G. max	2.92±0.19^{b}	2.51±0.14^{ab}	2.67±0.13^{b}	3.04±0.22^{b}	3.71±0.58^{b}
S. lycopersicum	5.40±0.21^{d}	5.79±0.29^{d}	5.47±0.16^{d}	6.19±0.38^{c}	6.90±0.44^{c}
B. oleracea	4.44±0.18^{cd}	4.85±0.66^{cd}	4.57±0.26^{cd}	5.08±0.20^{c}	7.22±0.19^{c}
C. pepo	4.25±0.18^{c}	3.62±0.12^{bc}	3.68±0.26^{bc}	3.72±0.16^{b}	4.70±0.14^{b}
Fresh weight of root (g)					
H. vulgare	1.70±0.22^{a}	1.68±0.12^{ab}	1.53±0.02^{ab}	1.65±0.18^{a}	1.62±0.11^{a}
G. max	1.49±0.10^{a}	1.45±0.07^{a}	1.42±0.11^{a}	1.58±0.06^{a}	1.99±0.25^{a}
S. lycopersicum	1.84±0.05^{a}	2.16±0.08^{b}	1.94±0.09^{ab}	2.16±0.19^{a}	2.24±0.12^{a}
B. oleracea	1.61±0.12^{a}	1.75±0.17^{ab}	1.65±0.09^{ab}	2.04±0.13^{a}	2.06±0.05^{a}
C. pepo	2.09±0.09^{a}	1.92±0.04^{ab}	1.97±0.13^{b}	1.93±0.12^{a}	2.09±0.17^{a}

Means ± SEM (n = 4).

Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a column, means followed by the same letter are not significantly different.

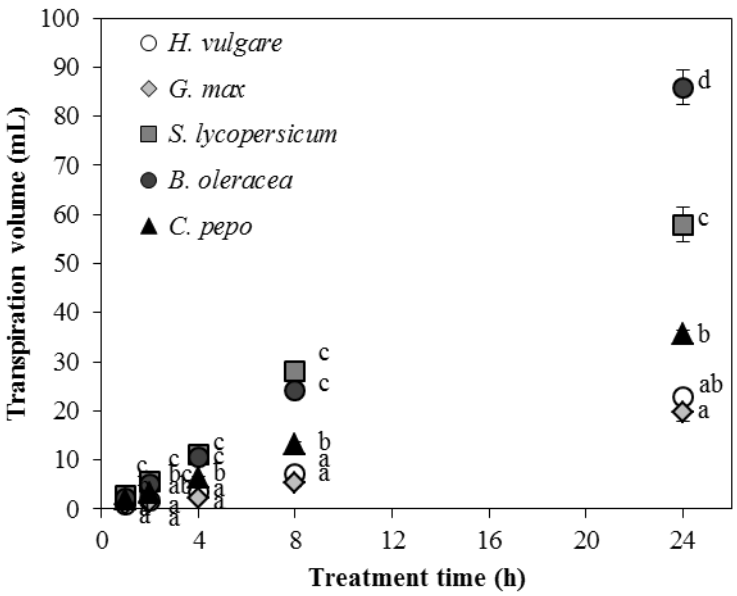

Figure 3 Transpiration volumes. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.01$). Within a treatment time, means followed by the same letter are not significantly different.

Table 2 Concentrations of β-HCH and dieldrin in roots, shoots, and test medium after each treatment time.

Plants	Treatment time				
	1 h	2 h	4 h	8 h	24 h
Concentration of β-HCH in root (ng g^{-1})					
H. vulgare	53.07±3.91^{a}	81.69±11.71^{a}	78.31±6.10^{a}	92.67±4.18^{a}	104.29±4.06^{a}
G. max	61.33±6.14^{a}	95.99±18.02^{a}	63.57±8.05^{a}	115.31±4.73^{ab}	110.88±6.17^{a}
S. lycopersicum	110.57±5.88^{b}	99.10±6.72^{a}	121.50±5.88^{b}	129.93±7.73^{ab}	131.84±10.87^{a}
B. oleracea	126.12±2.61^{b}	130.41±4.75^{a}	136.01±7.66^{b}	154.95±12.06^{b}	192.60±5.20^{b}
C. pepo	74.76±1.95^{a}	92.65±5.55^{a}	80.47±4.28^{a}	96.24±6.31^{a}	144.98±9.00^{a}
Concentration of dieldrin in root (ng g^{-1})					
H. vulgare	419.64±43.38^{ab}	454.49±24.50^{ab}	541.66±8.84^{ab}	691.73±54.94^{a}	856.30±11.46^{b}
G. max	280.96±16.68^{a}	369.85±21.23^{a}	452.78±35.17^{ab}	673.09±22.06^{a}	704.44±58.37^{ab}
S. lycopersicum	471.01±10.50^{b}	449.57±25.57^{ab}	576.49±22.57^{bc}	665.96±35.25^{a}	732.55±26.10^{ab}
B. oleracea	525.47±37.89^{b}	573.78±15.99^{b}	691.03±39.12^{c}	675.28±37.07^{a}	822.84±15.40^{b}
C. pepo	306.47±11.36^{a}	380.87±56.27^{a}	395.71±13.51^{a}	612.98±32.61^{a}	545.88±40.73^{a}
Concentration of β-HCH in shoot (ng g^{-1})					
H. vulgare	< 1.36^{a*}	< 1.36^{a}	< 1.36^{a}	< 1.36^{a}	29.52±2.67^{ab}
G. max	< 1.36^{a}	< 1.36^{a}	< 1.36^{a}	< 1.36^{a}	13.53±4.59^{a}
S. lycopersicum	< 1.36^{a}	< 1.36^{a}	< 1.36^{a}	18.89±0.79^{c}	40.44±0.36^{bc}
B. oleracea	< 1.36^{a}	< 1.36^{a}	< 1.36^{a}	17.24±1.27^{bc}	81.83±5.34^{d}
C. pepo	< 1.36^{a}	1.81±0.12^{b}	7.27±0.25^{b}	13.68±0.50^{b}	52.41±2.09^{c}
Concentration of dieldrin in shoot (ng g^{-1})					
H. vulgare	< 0.78^{a*}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}
G. max	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}
S. lycopersicum	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	1.52±0.13^{a}
B. oleracea	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}	< 0.78^{a}
C. pepo	2.26±0.18^{b}	7.61±0.87^{b}	19.55±0.85^{b}	46.44±1.10^{b}	49.06±2.47^{b}
Concentration of β-HCH in test medium (μg L^{-1})					
H. vulgare	7.36±0.18^{ab}	7.04±0.20^{a}	7.13±0.13^{a}	7.15±0.05^{a}	7.10±0.30^{ab}
G. max	8.88±0.37^{c}	7.86±0.48^{a}	9.12±0.10^{b}	8.41±0.35^{b}	7.68±0.16^{b}
S. lycopersicum	8.69±0.10^{bc}	7.47±0.08^{a}	8.38±0.08^{b}	8.53±0.24^{b}	7.55±0.18^{b}
B. oleracea	6.84±0.30^{a}	6.96±0.15^{a}	6.38±0.17^{a}	6.59±0.17^{a}	5.72±0.33^{a}
C. pepo	6.63±0.26^{a}	7.17±0.33^{a}	7.02±0.26^{a}	8.65±0.16^{b}	7.98±0.40^{b}
Concentration of dieldrin in test medium (μg L^{-1})					
H. vulgare	5.32±0.27^{bc}	5.04±0.22^{a}	5.00±0.25^{cd}	4.37±0.21^{b}	2.93±0.22^{b}
G. max	6.31±0.18^{c}	5.00±0.39^{a}	5.36±0.06^{d}	4.03±0.30^{ab}	2.37±0.26^{ab}
S. lycopersicum	5.30±0.19^{bc}	4.35±0.08^{a}	4.19±0.07^{bc}	3.51±0.22^{ab}	2.49±0.10^{ab}
B. oleracea	4.75±0.32^{ab}	4.45±0.21^{a}	3.47±0.15^{ab}	3.36±0.08^{ab}	2.66±0.20^{ab}
C. pepo	3.74±0.11^{a}	4.04±0.24^{a}	2.99±0.14^{a}	2.97±0.08^{a}	1.86±0.06^{a}

Means ± SEM (n = 4).

*Under the limit of quantitation.

Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a column, means followed by the same letter are not significantly different.

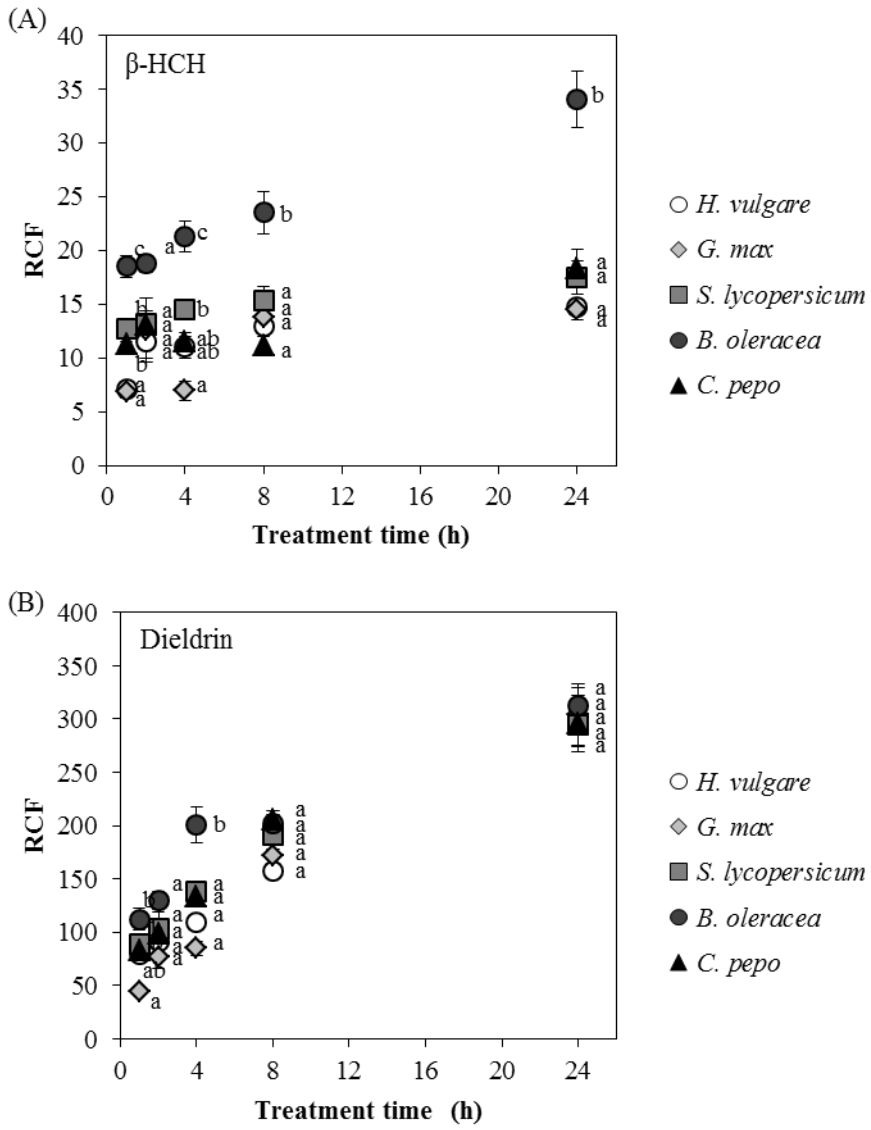

Figure 4 Root concentration factors (RCF) of (A) β-HCH and (B) dieldrin. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.01$). Within a treatment time, means followed by the same letter are not significantly different.

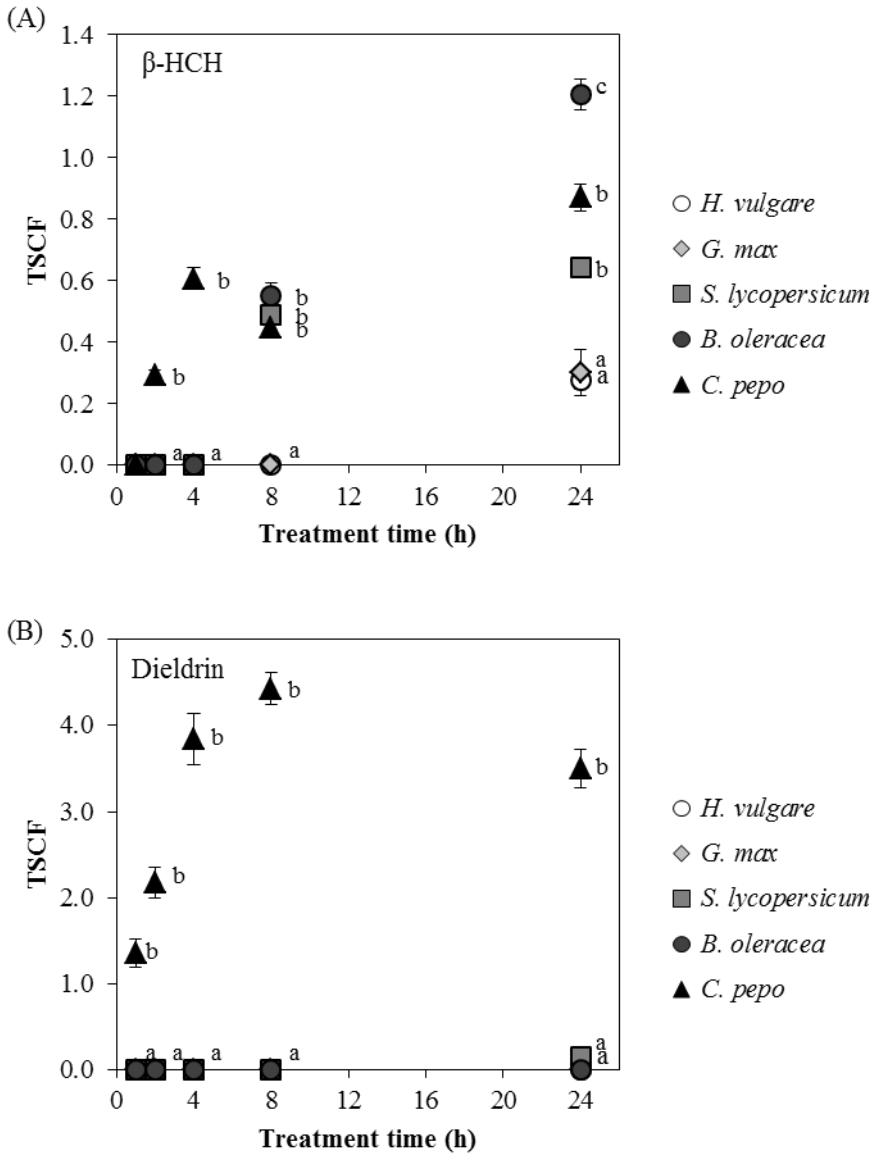

Figure 5 Transpiration stream concentration factors (TSCF) of (A) β-HCH and (B) dieldrin. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a treatment time, means followed by the same letter are not significantly different.

Table 3 Root and shoot fresh weights and transpiration volume in each transpiration-limiting treatment in *C. pepo*.

Treatment	Fresh weight (g)		Transpiration volume (mL)
	Root	Shoot	
Non-treated	5.06 ±0.20[a]	15.07 ±0.78[a]	137.0 ±7.5[a]
High Humidity	5.70 ±0.20[a]	16.17 ±0.65[a]	68.8 ±2.6[c]
+ABA	5.62 ±0.41[a]	14.07 ±0.71[a]	40.3 ±2.4[d]
Dark	4.45 ±0.23[ab]	13.65 ±0.64[a]	81.3 ±3.1[c]
Heated (root)	3.40 ±0.06[bc]	14.41 ±0.36[a]	103.8 ±4.1[b]
Heated (whole)	3.02 ±0.19[c]	10.06 ±0.72[b]	0[e]

Means ± SEM (n = 4).

Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a column, means followed by the same letter are not significantly different.

Table 4 Concentrations of β-HCH and dieldrin in roots, shoots, and test medium in each transpiration-limiting treatment in *C. pepo*.

Treatment	Root (ng g^{-1})		Shoot (ng g^{-1})		Test medium (μg L^{-1})	
	β-HCH	Dieldrin	β-HCH	Dieldrin	β-HCH	Dieldrin
Non-treated	130.84±1.90^{a}	788.75±23.78^{a}	53.72±0.97^{a}	54.56±1.04^{a}	9.15±0.22^{a}	2.05±0.15^{abc}
High Humidity	118.24±1.60^{a}	816.92±27.10^{a}	13.91±0.31^{c}	26.60±2.02^{b}	8.98±0.17^{a}	1.52±0.14^{a}
+ABA	124.90±1.72^{a}	803.83±27.67^{a}	9.30±0.34^{c}	17.50±0.17^{c}	8.48±0.15^{a}	1.83±0.12^{ab}
Dark	140.79±2.51^{ab}	870.40±37.73^{a}	27.36±0.74^{b}	17.84±1.76^{c}	9.32±0.04^{a}	2.30±0.06^{bcd}
Heated (root)	171.20±2.46^{bc}	1196.88±29.29^{b}	26.50±1.67^{b}	2.05±0.22^{d}	8.98±0.24^{a}	2.60±0.10^{cd}
Heated (whole)	184.85±10.46^{c}	1258.83±28.59^{b}	< 1.36*^{d}	< 0.78*^{d}	8.80±0.06^{a}	2.93±0.16^{d}

Means ± SEM (n = 4).

*Under the limit of quantitation.

The initial concentrations in test medium were 8.91 ± 0.21 μg L^{-1} β-HCH and 7.45 ± 0.09 μg L^{-1} dieldrin.

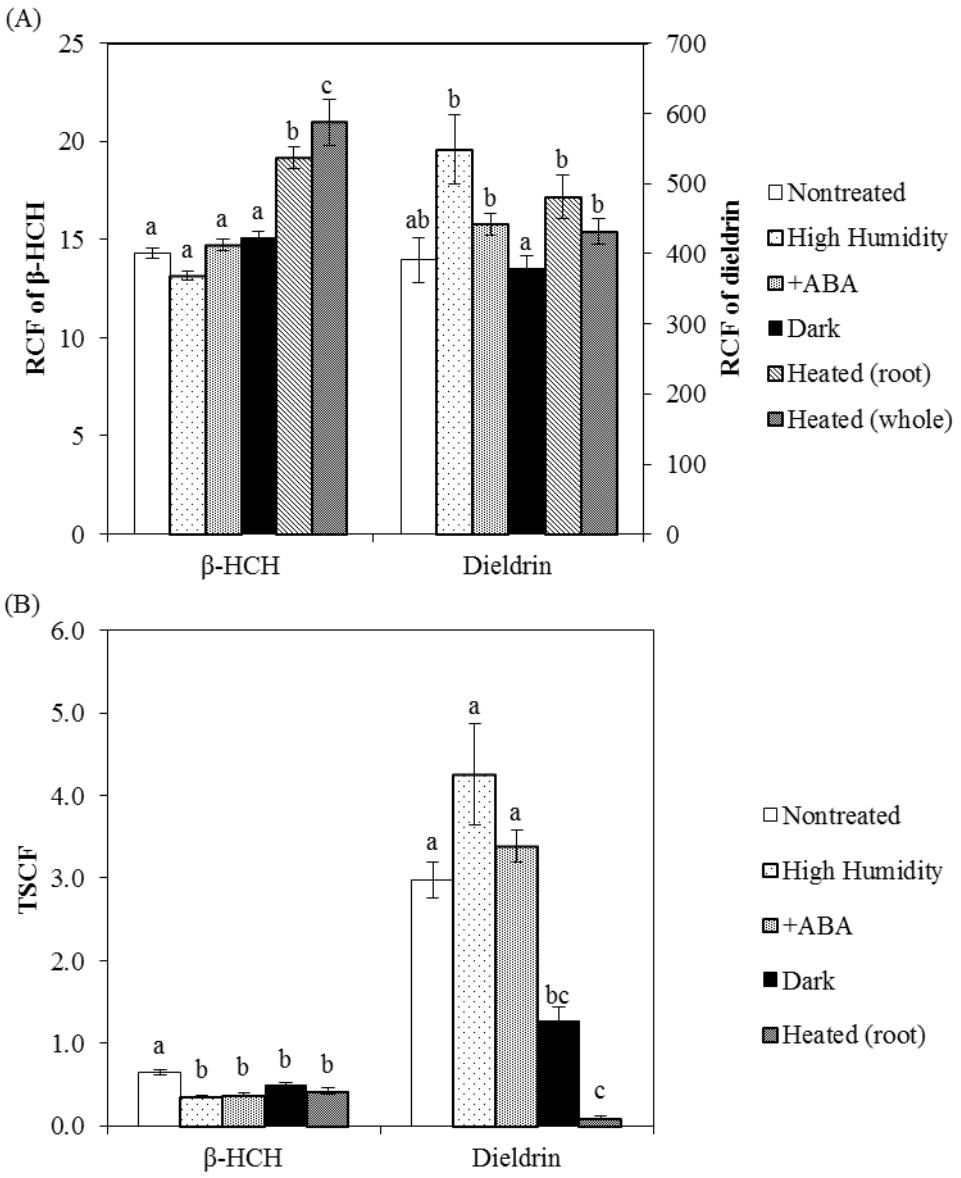

Figure 6 (A) Root concentration factors (RCF) and (B) transpiration stream concentration factors (TSCF) of β-HCH and dieldrin in each transpiration-limiting treatment. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.01$). Bars with the same letter are not significantly different. TSCF was not calculated in "Heated (whole)" treatment.

DISCUSSION

Mass balance of β-HCH and dieldrin in hydroponic culture

By calculating the rate of recovery of β-HCH and dieldrin as the sum of each in the shoots, roots, and medium divided by the initial amount supplied in the medium, I could account for any losses from the system by volatilization, metabolism by the plant, biodegradation by microorganisms, or supply from the atmosphere.

I calculated the recovery rate of β-HCH and dieldrin in each plant and at each time (Table 5). The recovery of each varied around 100% in all species during the experiment. Thus, I considered that losses were negligible. In addition, β-HCH and dieldrin were not detected in *C. pepo* plant grown in the medium without β-HCH and dieldrin in the growth chamber (data not shown). These results confirm the appropriateness of evaluating uptake and translocation by comparing quantities in the roots and shoots.

Differential uptake of β-HCH and dieldrin from medium to roots among species

RCF is often used to describe the uptake of organic chemicals from media into roots [3, 12, 13]. RCF values of dieldrin were approximately 10 times those of β-HCH (Figure 4). This tendency agreed with previous reports that RCF increased with log K_{OW} [3, 21]. Briggs *et al*. (1982) [3] modeled RCF of *H. vulgare* as:

$$\log (RCF - 0.82) = 0.77 \log K_{OW} - 1.52 \quad (3)$$

I calculated RCF by using log K_{OW} values of 3.8 for β-HCH and 5.2 for dieldrin [16], obtaining RCF values of 26.3 for β-HCH and 305.6 for dieldrin. The RCF values of each species after 24 h in my experiment were close to these calculated values.

Highly hydrophobic chemicals such as HOCs are taken up by roots mainly by sorption [22, 23]. And it was suggested that the sorption was occurred by the interaction between organic chemicals and a root surface [3]. The results in this study support uptake by sorption because the RCF values of β-HCH and dieldrin were increased immediately within 1 h and continued to increase gradually with time in all species (Figure 4). In general, hydrophobic (high log K_{OW}) chemicals are strongly sorbed to roots [3, 21], and therefore the difference in root concentrations between the HOCs (β-HCH < dieldrin) could be explained by the difference in hydrophobicity (log K_{OW}) between them.

The RCF values of β-HCH and dieldrin did not so differ among species, except the β-HCH RCF value of *B. oleracea* was double those of the other 4 species. The reason of the high RCF of β-HCH in *B. oleracea* was not clear, but I speculate that such a difference might be caused by differences in the roots' specific surface area or lipid contents or in the composition of lipids that act as sorbents at the root surface.

Differential translocation of β-HCH and dieldrin from roots to shoots among species

TSCF is widely used to describe the translocation of xenobiotic organic chemicals from roots to shoots [3, 4, 12, 13]. I considered the numerator of the TSCF equation, which was the amount of HOCs in the shoots divided by the transpiration volume (Eq. 2), as the mean concentration of HOCs in the xylem sap during treatment [24].

The rate of translocation from roots to shoots over time described by TSCF was clearly different among species. It is known that *C. pepo* has superior ability to accumulate HOCs in the aerial parts [5, 6]. β-HCH was detected earliest in *C. pepo* (2 h), then in *S. lycopersicum* and *B. oleracea* (8 h), and last in *H. vulgare* and *G. max* (24 h) (Table 2), and TSCF showed the same

tendency (Figure 5A). As the β-HCH RCF values of all species increased immediately within 1 h, the time lags in the detection of β-HCH in the shoots were due to the process of translocation from roots to shoots. The order in which β-HCH reached to the shoots was nearly the same that of the TSCF at 24 h: that is, *B. oleracea* > *C. pepo* = *S. lycopersicum* > *H. vulgare* = *G. max*.

The TSCF of dieldrin was high in *C. pepo* (Figure 5B). It became clear that this ranking of *C. pepo* was due not to the process of uptake by the roots but to the process of translocation to the shoots. In contrast, the TSCFs of *H. vulgare*, *G. max*, *B. oleracea*, and *S. lycopersicum* were lower. Although non-cucurbits are nearly equal to *C. pepo* in their ability to take up dieldrin by their roots, they have much less ability to translocate it to their shoots.

Superiority of β-HCH and dieldrin translocation ability in C. pepo

In the uptake and transportation of HOCs in the medium to the aboveground parts of plants, the transpiration stream seems to function as a driving force [23, 25]. *Cucurbita pepo* was not superior to the other species in uptake by the roots as estimated by RCF, but it was superior in translocation to the shoots as estimated by TSCF. Thus, I investigated the effect of the inhibition of transpiration on RCF and TSCF in *C. pepo*.

The high humidity, addition of ABA, and dark treatments had no significant effect on the RCF of β-HCH. No treatment had a significant effect on the RCF of dieldrin. Thus, the inhibition of transpiration had little or no effect on the RCF of either HOC. The uptake of β-HCH and dieldrin by the roots was not due to mass flow. In addition, RCF was not decreased by the heating and dark treatments. These results suggest that the uptake of β-HCH and dieldrin by roots is unrelated to root physiological functions, and support sorption as the main contributor to the uptake of β-HCH and dieldrin in plants.

I calculated TSCF to consider the effects of each treatment on translocation to the shoots (Figure 6B). The TSCF for those of the heated whole plants could not be calculated because transpiration was 0. However, as β-HCH and dieldrin were not detected in the shoots in this treatment, translocation to the shoots by diffusion through lipid tissues was unlikely.

Since the TSCF of β-HCH was not further decreased by the dark and heated roots treatments than by the high humidity and addition of ABA treatments (Figure 6B), the translocation of β-HCH might not rely on transport proteins. To reach the xylem vessels, HOCs adsorbed on the root surface need to pass through the root epidermis, cortex, endodermis, pericycle, and stele via the apoplastic and symplastic pathways. Because *C. pepo* translocated β-HCH to the shoots faster than the other species did, it might have a way to transport HOCs smoothly in the root tissues.

Though TSCF of dieldrin was not significantly different in high humidity and addition of ABA treatments that limited only transpiration, it was decreased by the dark and heated roots treatments. Murano *et al*. (2010) [10] previously suggested that transport proteins play an important role in the translocation of dieldrin from roots to shoots in cucurbits. Major latex-like proteins in *C. pepo* are involved in the translocation of dioxins, which also have high hydrophobicity [26]. Therefore, I infer that the plant's ability to translocate dieldrin was lost owing to the denaturation of transport proteins in the root by heating. If the transport proteins are influenced by photosynthesis, the decrease in TSCF by the dark treatment was likely due to repression of the production and/or translocation of transport proteins. This result supports the hypothesis that transport proteins produced in the roots contribute to the translocation of dieldrin from the roots to the shoots in cucurbits.

In summary, *C. pepo* is better able to translocate β-HCH and dieldrin from the roots to the shoots than the other species. However, the mechanisms of transport seem to differ between

HOCs. β-HCH, which is more hydrophilic (log K_{OW} 3.8) than dieldrin (log K_{OW} 5.2) [16], may be more readily transported in the transpiration stream, even in non-cucurbits. However, translocation ability differs among species, and *C. pepo* seems to transport β-HCH more smoothly from the roots to the xylem than the other species, although I don't yet know how it does. It will be important to directly observe the translocation of HOCs through the root tissues to the xylem in detail. On the other hand, because dieldrin is more strongly sorbed to the root, translocation from the root surface to the xylem appears to require transport proteins. Hence, I consider that cucurbits can synthesize transport proteins that can translocate dieldrin from the roots to the shoots.

Table 5 Mass balances of β-HCH and dieldrin.

Plant	Treatment time	β-HCH				Dieldrin			
		Contents (μg)			Recovery (%)*	Contents (μg)			Recovery (%)
		in root	in shoot	in test medium		in root	in shoot	in test medium	
H. vulgare	1 h	0.09±0.01	< 0.003 **	2.20±0.05	97.0±2.5	0.69±0.07	< 0.002 **	1.59±0.08	88.8±4.9
	2 h	0.14±0.02	< 0.003	2.10±0.06	88.9±3.1	0.76±0.01	< 0.002	1.50±0.06	101.8±3.3
	4 h	0.12±0.01	< 0.003	2.11±0.04	91.0±1.4	0.83±0.02	< 0.002	1.48±0.08	100.2±2.6
	8 h	0.15±0.02	< 0.003	2.10±0.01	92.9±0.9	1.13±0.22	< 0.002	1.28±0.06	104.4±5.7
	24 h	0.17±0.01	0.04±0.00	1.97±0.08	89.9±3.9	1.38±0.09	< 0.002	0.81±0.06	95.6±0.9
G. max	1 h	0.09±0.01	< 0.003	2.66±0.11	95.5±3.8	0.42±0.02	< 0.002	1.89±0.05	97.2±1.7
	2 h	0.14±0.04	< 0.003	2.39±0.17	88.9±4.5	0.53±0.04	< 0.002	1.49±0.12	88.4±3.9
	4 h	0.08±0.00	< 0.003	2.71±0.03	95.2±1.1	0.64±0.04	< 0.002	1.60±0.02	90.1±1.5
	8 h	0.18±0.01	< 0.003	2.48±0.10	102.3±3.6	1.07±0.07	< 0.002	1.19±0.09	101.3±1.6
	24 h	0.22±0.03	0.04±0.01	2.15±0.05	84.1±1.0	1.37±0.10	< 0.002	0.67±0.08	87.5±1.2
S. lycopersicum	1 h	0.20±0.01	< 0.003	2.59±0.03	98.9±1.3	0.87±0.02	< 0.002	1.58±0.06	92.4±1.5
	2 h	0.21±0.01	< 0.003	2.20±0.02	97.7±1.3	0.96±0.02	< 0.002	1.28±0.02	101.4±1.2
	4 h	0.23±0.00	< 0.003	2.42±0.02	100.5±0.9	1.11±0.04	< 0.002	1.21±0.02	94.0±1.1
	8 h	0.28±0.03	0.12±0.01	2.32±0.07	103.8±1.9	1.42±0.08	< 0.002	0.96±0.07	102.8±0.9
	24 h	0.29±0.02	0.28±0.02	1.83±0.03	97.1±1.5	1.63±0.03	0.01±0.00	0.60±0.03	102.6±1.1
B. oleracea	1 h	0.20±0.01	< 0.003	2.04±0.09	95.9±3.4	0.84±0.04	< 0.002	1.42±0.10	96.1±2.6
	2 h	0.23±0.03	< 0.003	2.05±0.04	97.2±2.7	1.00±0.08	< 0.002	1.31±0.06	101.6±2.5
	4 h	0.22±0.01	< 0.003	1.85±0.05	92.5±2.1	1.13±0.04	< 0.002	1.00±0.04	97.3±1.8
	8 h	0.31±0.01	0.09±0.00	1.82±0.04	94.9±1.9	1.36±0.03	< 0.002	0.93±0.02	95.6±1.0
	24 h	0.40±0.02	0.59±0.04	1.23±0.09	104.9±4.6	1.70±0.03	< 0.002	0.57±0.05	115.6±3.5
C. pepo	1 h	0.16±0.01	< 0.003	1.98±0.08	94.2±3.5	0.64±0.02	0.01±0.00	1.12±0.03	89.2±1.1
	2 h	0.18±0.01	0.01±0.00	2.13±0.10	102.6±4.0	0.72±0.09	0.03±0.00	1.20±0.07	109.5±3.0
	4 h	0.16±0.01	0.03±0.00	2.06±0.08	106.9±3.8	0.77±0.04	0.07±0.01	0.88±0.04	95.7±0.8
	8 h	0.18±0.00	0.05±0.00	2.48±0.05	98.6±1.7	1.17±0.03	0.17±0.00	0.85±0.02	93.0±1.3
	24 h	0.30±0.02	0.25±0.01	2.11±0.10	119.0±5.1	1.12±0.03	0.23±0.01	0.49±0.02	111.4±2.3

Means ± SEM (n = 4).

*Recovery rate was calculated by dividing the total amount of HOCs by the amount supplied in the test medium.

**Under the limit of quantitation.

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References

1. Seike N, Eun H, Otani T. 2007. Temporal change in organochlorine pesticide contamination in Japanese paddy soils. *Organohalogen Compounds* 69: 28–31.
2. Hashimoto Y. 2005. Dieldrin residue in the soil and cucumber from agricultural field in Tokyo. *J Pestic Sci* 30: 397–402.
3. Briggs GG, Bromilow RH, Evans AA. 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic Sci* 13:495–504.
4. Dettenmaier EM, Doucette WJ, Bugbee B. 2009. Chemical hydrophobicity and uptake by plant roots. *Environ Sci Technol* 43: 324–329.
5. Otani T, Seike N, Sakata Y. 2007. Differential uptake of dieldrin and endrin from soil by several plant families and Cucurbita genera. *Soil Sci Plant Nutr* 53: 86–94.
6. White JC. 2001. Plant-facilitated mobilization and translocation of weathered 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p′*-DDE) from an agricultural soil. *Environ Toxicol Chem* 20: 2047–2052.
7. Gent MP, White JC, Parrish ZD, Isleyen M, Eitzer BD, Mattina MI. 2007. Uptake and translocation of *p,p′*-dichlorophenyl-1,1-dichloroethylene supplied in hydroponics solution to *Cucurbita*. *Environ Toxicol Chem* 26: 2467–2475.
8. Hülster A, Müller JF, Maeschner H. 1994. Soil–plant transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (Cucurbitaceae). *Environ Sci Technol* 28: 1110–1115.
9. Whitfield Åslund ML, Zeeb BA, Rutter A, Reimer KJ. 2007. *In situ* phytoextraction of polychlorinated biphenyl-(PCB) contaminated soil. *Sci Total Environ* 374: 1–12.

10. Murano H, Otani T, Seike N. 2010. Dieldrin-dissolving abilities of the xylem saps of several plant families, particularly *Cucurbita pepo* L. *Environ Toxicol Chem* 29: 2269–2277.
11. Namiki S, Otani T, Seike N. 2013. Fate and plant uptake of persistent organic pollutants in soil. *Soil Sci Plant Nutr* 59: 669–679.
12. Shone MGT, Wood AV. 1974. A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley. *J Exp Bot* 25: 390–400.
13. Trapp S, Pussemier L. 1991. Model-calculations and measurements of uptake and translocation of carbamates by bean-plants. *Chemosphere* 22: 327–339.
14. Shone MGT, Bartlett OB, Wood AV. 1974. A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley. II. Relationship between uptake by roots and translocation to shoots. *J Exp Bot* 25: 401–409.
15. Trapp S. 2000. Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. *Pest Manag Sci* 56: 767–778.
16. Mackay D, Shiu WY, Ma KC. 1997. Insecticides. In *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals Vol. V: Pesticide Chemicals*, Lewis Publishers, New York, pp 334–648.
17. OECD. 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. Paris.
18. Rawson HM, Begg JE. 1977. The effect of atomospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta* 134: 5–10.
19. Jones RJ, Mansfield TA. 1970. Suppression of stomatal opening in leaves treated with abscisic acid. *J Exp Bot* 21: 714–719.

20. Japanese Standards Association. 1999. Method for determination of tetra- through octa-chlorodibenzo-*p*-dioxins, tetra-through octa-chlorodibenzofurans, and coplanar polychlorinated biphenyls in industrial water and waste water. K0312. Tokyo.
21. Burken JG, Schnoor JL. 1998. Predictive relationship for uptake of organic contaminants by hybrid poplar trees. *Environ Sci Technol* 32: 3379–3385.
22. Gao Y, Collins CD. 2009. Uptake pathways of polycyclic aromatic hydrocarbons in white clover. *Environ Sci Technol* 43: 6190−6195.
23. Collins C, Fryer M, Grosso A. 2006. Plant uptake of non-ionic organic chemicals. *Environ Sci Technol* 40: 45–52.
24. Murano H, Otani T, Seike N, Sakai M. 2010. Dieldrin uptake and translocation in plants growing in hydroponic medium. *Environ Toxicol Chem* 29: 142–148.
25. Hsu FC, Marxmiller RL, Yang AY. 1990. Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. *Plant Physiol* 93: 1573–1578.
26. Inui H, Sawada M, Goto J, Yamazaki K, Kodama N, Tsuruta H, Eun H. 2013. A major latex-like protein is a key factor in crop contamination by persistent organic pollutants. *Plant Physiol* 161: 2128–2135.