Differential uptake and translocation of β-HCH and dieldrin by several plant species from hydroponic medium

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Title: Differential uptake and translocation of β-HCH and dieldrin by several plant species from hydroponic medium

Abstract

To compare the uptake and translocation of hydrophobic organic chemicals (HOCs) by plant species, we performed uptake experiments with β-1,2,3,4,5,6-hexachlorocyclohexane (β-HCH) and 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1,4-*exo*-5,8-dimethanonaphthalene (dieldrin) using 5 species: *Hordeum vulgare*, *Glycine max*, *Solanum lycopersicum*, *Brassica oleracea*, and *Cucurbita pepo*. We evaluated uptake ability by root concentration factor (RCF) and translocation ability by transpiration stream concentration factor (TSCF). RCFs of β-HCH and dieldrin did not differ remarkably among species, except that that of β-HCH in *B. oleracea* was high. TSCFs of β-HCH and dieldrin were high in *C. pepo*, which was not superior in uptake as estimated by RCF. TSCF of dieldrin in *C. pepo* was decreased in darkness, and was markedly decreased by heating of roots. These results support the hypothesis that transport proteins produced in the root contribute to dieldrin translocation. On the other hand, TSCF of β-HCH was not decreased by these treatments. Therefore, translocation of β-HCH might not need the contribution of transport proteins. It is possible that *C. pepo* has a certain function to transport HOCs smoothly in root tissues.

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

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 >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 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 our previous study [11], we investigated the uptake of HCHs, chlorinated cyclodienes, and 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 concentrations of bioavailable HOCs are low, it was difficult to compare uptake and translocation of HOCs among species in detail in soil culture.

In this study, to overcome this problem, we 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-1,4,4a,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, we 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, we 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 we 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, we 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

We 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 L:D 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.

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 (Fig. 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 our 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 our previous soil culture experiment [5, 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, we calculated the root concentration factor (RCF) as:

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

We 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 (Fig. 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 (Fig. 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 we estimated it indirectly by dividing the amount of HOCs in the shoot by the volume of water transpired [14, 15]:

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

We 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 (Fig. 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 (Fig. 5B). In contrast, it remained negligible in the other 4 species.

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

We 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 (Fig. 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 (Fig. 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.

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, we could account for any losses from the system by volatilization, metabolism by the plant, biodegradation by microorganisms, or supply from the atmosphere.

We 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, we 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 (Fig. 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:

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

We calculated RCF by using log K_{OW} values of 3.8 for β-HCH and 5.2 for dieldrin [13], obtaining RCF values of 26.3 for β-HCH and 305.6 for dieldrin. The RCF values of each species after 24 h in our 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]. Our results 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 (Fig. 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 we 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]. We 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]. In our experiment, the TSCF of dieldrin was high in *C. pepo* (Fig. 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.

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 (Fig. 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*.

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, we investigated the effect of the inhibition of transpiration on RCF and TSCF in *C. pepo*.

The High Humidity, +ABA, and Dark treatments had no significant effect on the RCF of β-HCH (Fig. 6A). 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. If β-HCH and dieldrin in the medium were supplied to the roots by mass flow, the root uptake of both would depend on transpiration, so RCF should decrease in comparison with the Non-treated. Therefore, 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.

We calculated TSCF to consider the effects of each treatment on translocation to the shoots (Fig. 6B). The TSCF for Heated (whole) 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.

Though TSCF of dieldrin was not significantly different in High Humidity and +ABA treatments that limited only transpiration, it was decreased by the Dark and Heated (root) treatments. We previously suggested that transport proteins play an important role in the translocation of dieldrin from roots to shoots in cucurbits [10]. Major latex-like proteins in *C. pepo* are involved in the translocation of dioxins, which also have high hydrophobicity [26]. Therefore, we infer that the plant's ability to translocate dieldrin was lost owing to the denaturation of transport proteins in the root by heating. If 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, as the expression of the major latex-like protein *MLP151* in *Panax ginseng* was decreased by dark treatment [27]. 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.

On the other hand, since the TSCF of β-HCH was not further decreased by the Dark and Heated (root) treatments than by the High humidity and +ABA treatments (Fig. 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.

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. Because dieldrin is more strongly sorbed to the root, translocation from the root surface to the xylem appears to require transport proteins. Hence, we consider that cucurbits can synthesize transport proteins that can translocate dieldrin from the roots to the

shoots. On the other hand, β-HCH, which is more soluble in water (1.25 mg L^{-1}) than dieldrin (0.17 mg L^{-1} [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 we don't yet know how. It will be important to directly observe the translocation of HOCs through the root tissues to the xylem in detail.

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FIGURE LEGENDS

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

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

Fig. 3. Transpiration volume. 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.

Fig. 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.

Fig. 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.

Fig. 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.

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

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

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.

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

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*.

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.

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 5 Mass balances of β-HCH and dieldrin.

Means ± SEM (n = 4).

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

**Under the limit of quantitation.

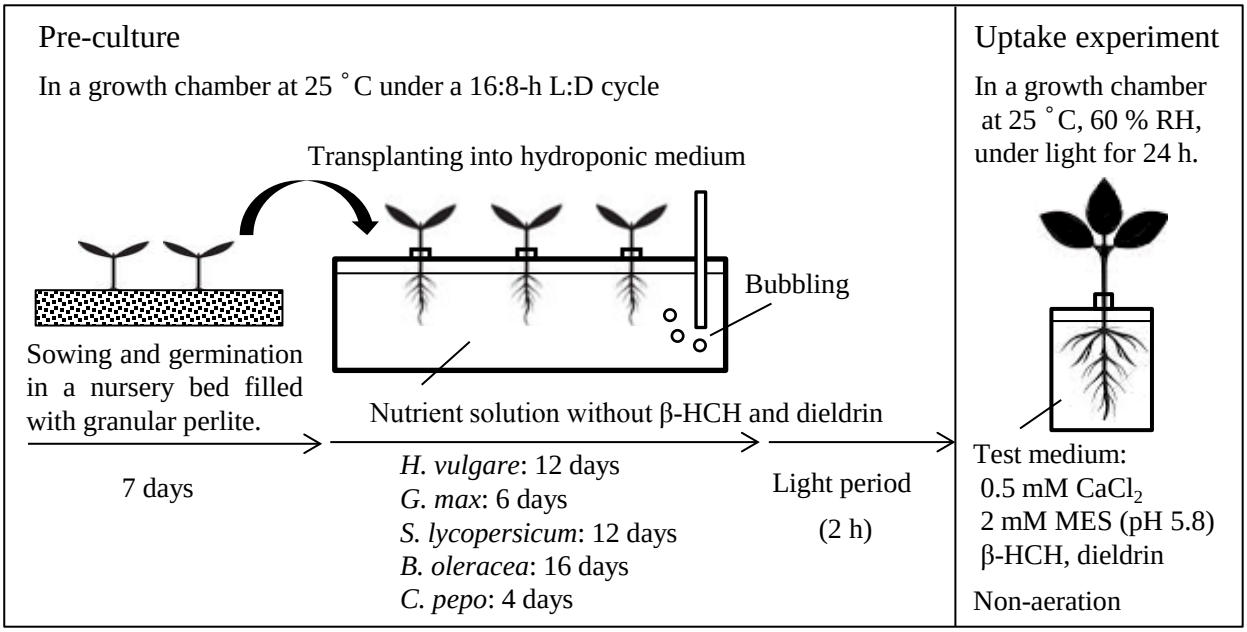

Fig. 1. A schema of time-course of uptake experiment by several plants in hydroponic medium.

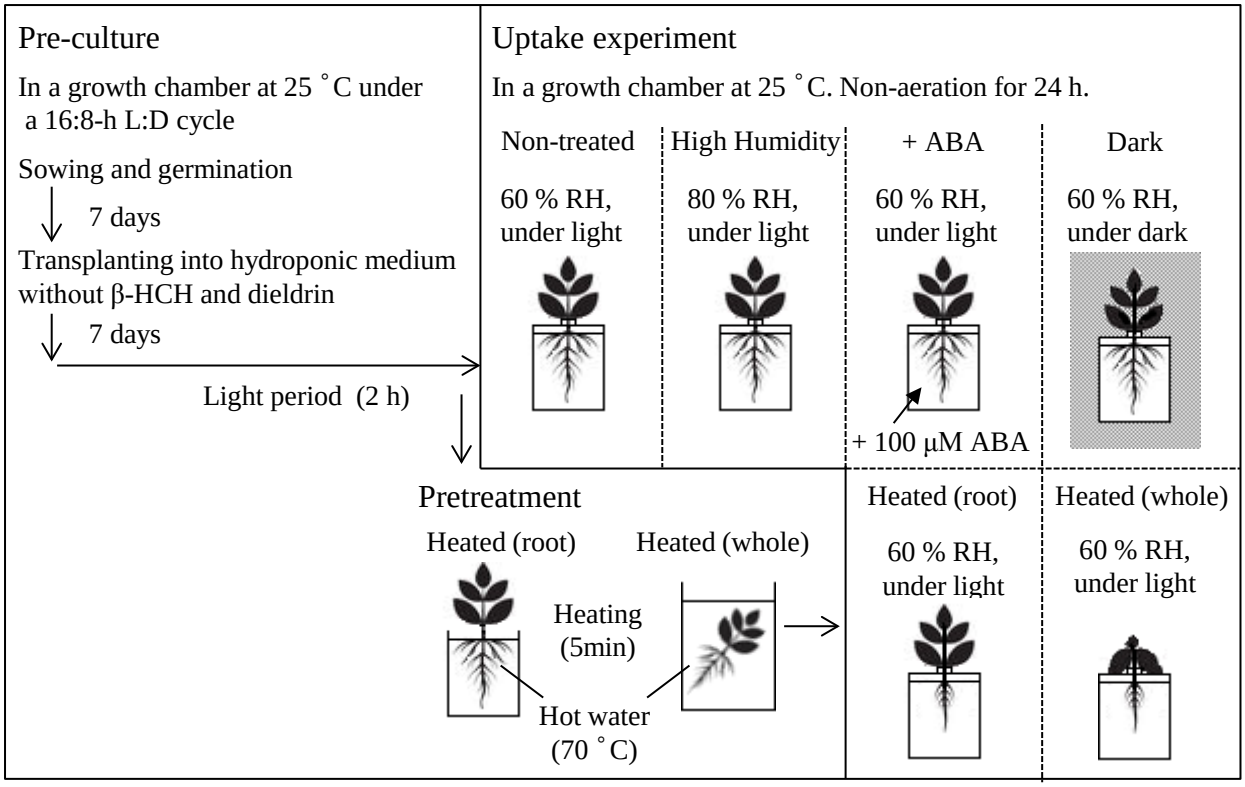

Fig. 2. A schema of uptake experiment by *C. pepo* under limited transpiration.

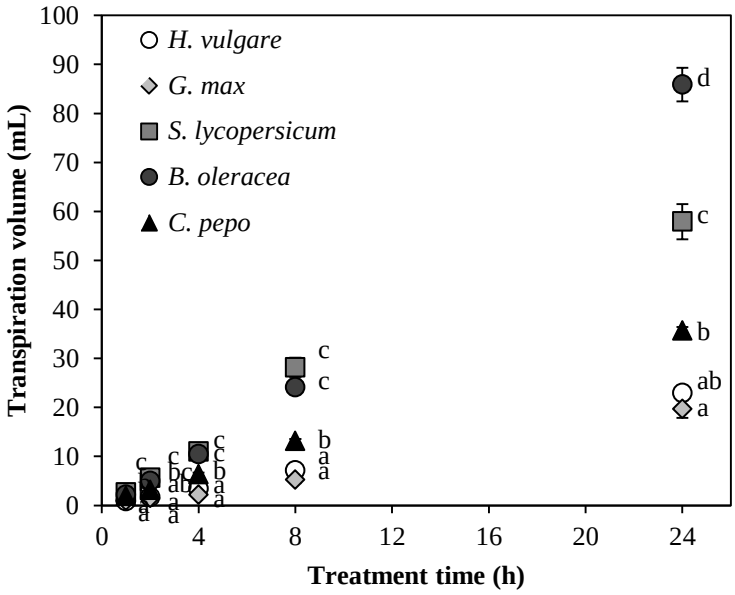

Fig. 3. Transpiration volume. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a treatment time, means followed by the same letter are not significantly different.

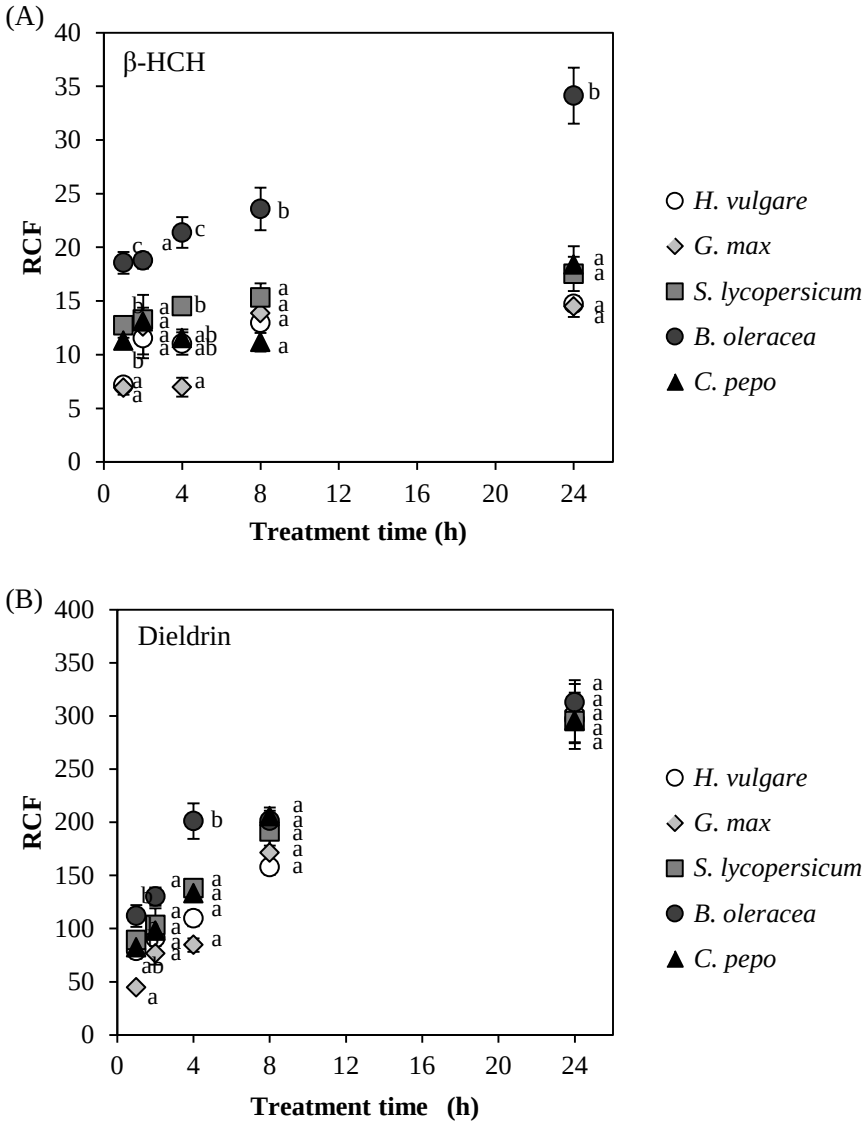

Fig. 4. Root concentration factors (RCF) of (A) β-HCH and (B) dieldrin. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a treatment time, means followed by the same letter are not significantly different.

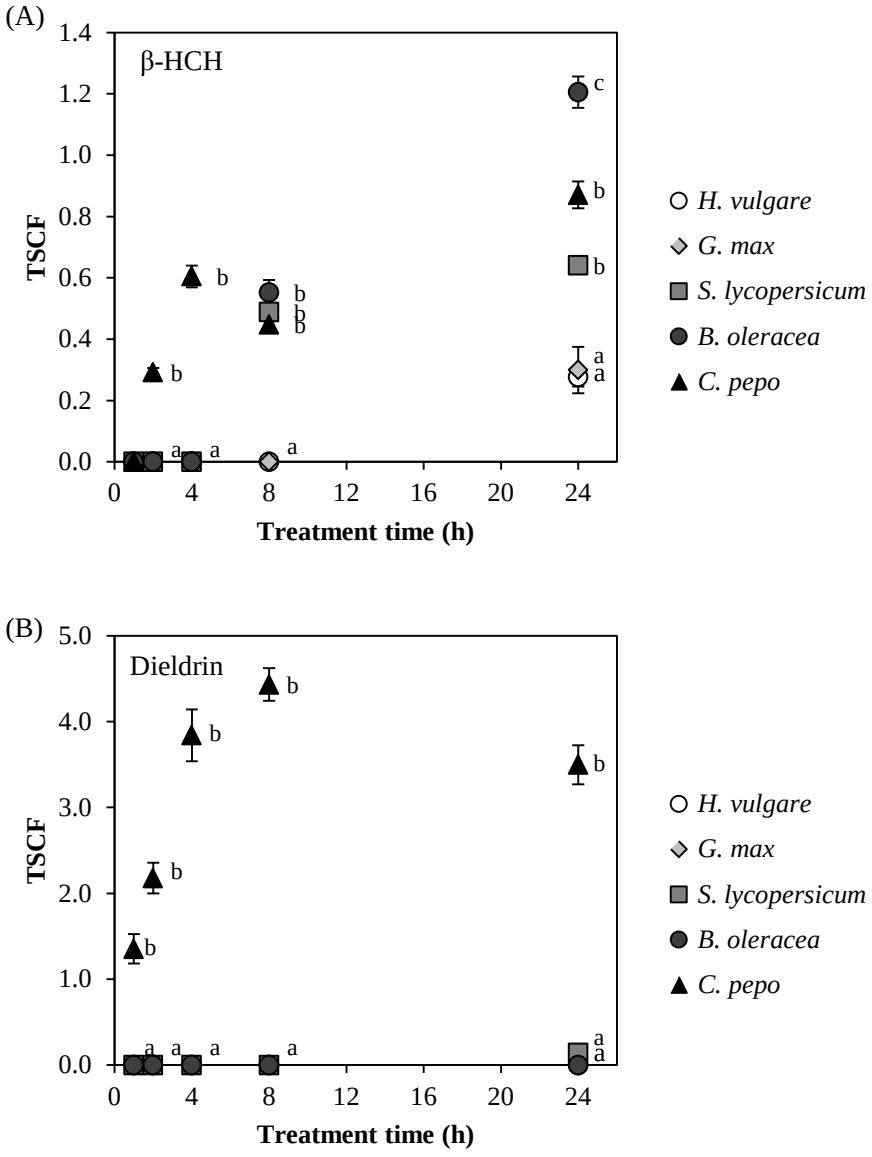

Fig. 5. Transpiration stream concentration factors (TSCF) of (A) β-HCH and (B) dieldrin. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.01). Within a treatment time, means followed by the same letter are not significantly different.

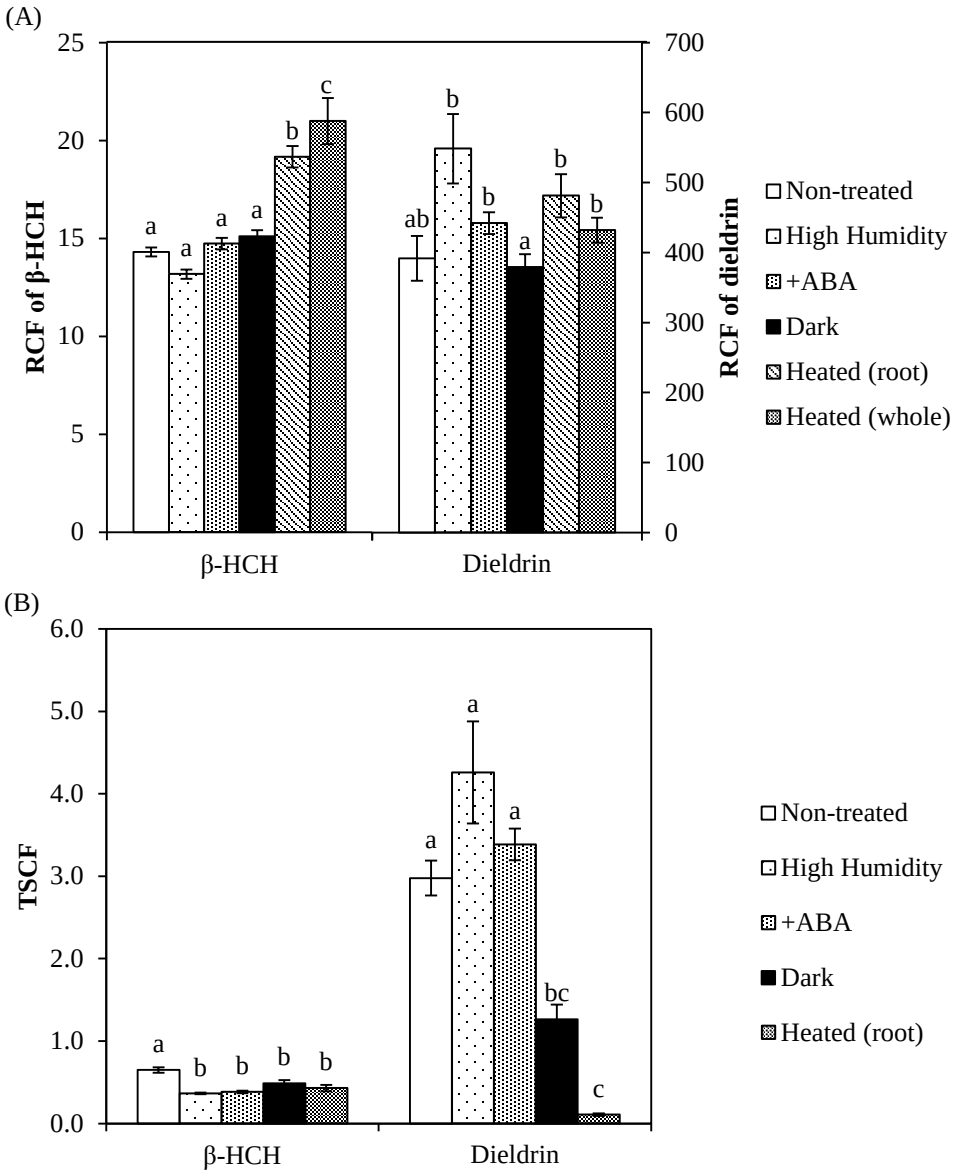

Fig. 6. (A) Root concentration factors (RCF) and (B) transpiration stream concentration factors (TSCF) of β-HCH and dieldrin in each transpiration-limiting treatment. Error bars indicate SEM (n = 4). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.01$). Bars with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

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.

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.

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 ($\mu g\ L^{-1}$)	
	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 concentration in test medium were 8.91 ± 0.21 μg L^{-1} β-HCH and 7.45 ± 0.09 μg L^{-1} dieldrin.

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 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 POPs by the amount supplied in the test medium.

**Under the limit of quantitation.