The influence of \textit{Brassica rapa} var. \textit{perviridis} growth conditions on the uptake and translocation of pesticides

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We cultivated \textit{Brassica rapa} var. \textit{perviridis} in soil mixed with four pesticides (fenobucarb, procymidone, flutolanil, and tolclofos-methyl) at different temperatures, day lengths, and soil water contents. We compared plants’ uptake and translocation abilities of the pesticides as affected by growth conditions. The root concentration factor (RCF) of pesticides tended to increase with rising temperature; however, the influence of temperature on the transpiration stream concentration factor (TSCF) differed for each pesticide. The RCFs and TSCFs of pesticides were high for short days. The soil water content had little or no effect on the uptake and translocation of pesticides. These results showed that it is necessary to consider growth conditions, especially the temperature and day length in plant uptake models for these pesticides. © Pesticide Science Society of Japan

\textbf{Keywords:} pesticides, temperature, day length, root concentration factor, transpiration stream concentration factor.

\section*{Introduction}

In Japan, agricultural chemicals used for primary crops and persisting in the soil were recently detected in succeeding crops at levels exceeding the maximum residue limits.¹ In such cases, production areas can be forced to take emergency measures, such as self-imposed halts on shipments or extensive inspection of soils and crops. It is necessary to predict the concentration of organic chemicals to prevent such problems of crop contamination.

Plant uptake of organic chemicals is generally explained by relationships with chemical properties and these are widely applied in plant uptake models. The root concentration factor (RCF) is an indication of root uptake ability and increases exponentially with increases in the logarithm of the \textit{n}-octanol–water partition coefficient ($K_{\text{OW}}$).² However, the relationship between log $K_{\text{OW}}$ and the transpiration stream concentration factor (TSCF), which indicates the translocation ability, often shows a bell-shaped curve with maximum values of approximately 1.8²³; in addition, different tested plants show different maximum values.⁴–⁶ It has also been reported that the relationship between TSCF and log $K_{\text{OW}}$ can be a negative sigmoid curve.⁷ Garvin \textit{et al.}⁸ reported that the TSCFs of endosulfan and triclocarban for \textit{Cucurbita pepo} L. ssp. \textit{pepo} were 3 and 10 times higher, respectively, than those for \textit{Glycine max} L. Thus, the TSCF relationships are different among plant species. However, few studies have focused on the influence of plant growth conditions on the RCF and TSCF.

Motoki \textit{et al.}⁹ investigated the relationship between the concentration of pesticides in \textit{Brassica rapa} L. var. \textit{perviridis} shoots and four typical Japanese soils. The concentrations of pesticides in shoots differed according to soil type, and they were positively correlated with water–extractable pesticide concentrations in the soil. Several studies have focused on relationships between plant concentrations and the behavior of organic chemicals in the soil.⁹,¹⁰ Despite plant growth conditions being important in affecting plant physiological reactions, the influence of these on pesticide uptake has not been investigated. In this study, we measured the concentrations of pesticides in shoots and roots of \textit{B. rapa} var. \textit{perviridis}, which has a relatively high pesticide uptake ability,¹¹ cultivated under conditions of different temperatures, day lengths, and soil water contents. Then, we compared the influence of each growth condition on the uptake and translocation ability of pesticides.

\section*{Materials and Methods}

\subsection*{1. Preparation of test soil}

We used four pesticides—fenobucarb, procymidone, flutolanil (these pesticides were obtained from Fujifilm Wako Pure Chemical, Osaka, Japan), and tolclofos-methyl (Kanto Chemical Co., Tokyo, Japan) with relatively high values of log $K_{\text{OW}}$ for plant uptake.
uptake experiments (Table 1). These pesticides were chosen because they are relatively difficult to degrade in soil and are not herbicides that affect plant growth. The pesticides were dissolved and mixed in acetone (Fujifilm Wako Pure Chemical) to 40 mg/L. Two liters of the mixture was then added to 400 g of Celite® powder (No. 545; Fujifilm Wako Pure Chemical), and the acetone was evaporated for 4 hr at room temperature in a draft chamber. Because chemicals were volatilized during the evaporation of acetone, the final contents of chemicals in 1 g of Celite® were 82.7 µg of fenobucarb, 81.0 µg of procymidone, 104.0 µg of flutolanil, and 102.9 µg of tolclofos-methyl. These pesticides were added to an uncontaminated Andosol of loam texture, pH [H₂O] 5.5, cation exchange capacity of 33.8 cmol(+)/kg, organic carbon of 52.1 g/kg, and water-holding capacity (WHC) of 747.1 mL/kg soil.

2. Plant culture in test soil
Plastic pots (600 mL) were filled with prepared soil (450 g of uncontaminated soil mixed with 5 g of prepared Celite®). The soil in each pot was fertilized with 5 g of chemical fertilizer (0.4 g N, 0.17 g P, and 0.34 g K) and 5 g of dolomite, and the soil moisture was adjusted with the cultivation conditions. We prepared the soil 1 day before transplanting the seedlings and placed the plastic pots in darkness in plant cultivation conditions. Seeds of B. rapa L. var. perviridis 'Yokattana' (Kaneko Seeds Co., Gunma, Japan) were sown in nursery soil and germinated in a growth chamber (Koito Kogyo, Tokyo, Japan) at 20°C in a 12:12-hr light:dark cycle. At 21 days, seedlings were transplanted into pots and raised for 21 days in a growth chamber (Nippon Medical & Chemical Instruments Co., Osaka, Japan). The control treatment was 20°C, a 12:12-hr light:dark cycle, and soil moisture of 60% WHC. Cultivation conditions were changed using six treatments (Table 2). We measured the weight of each pot and adjusted the soil moisture by supplying water according to the decrease, and we defined the total value of the decrease quantity for 21 days as the transpiration rate. At 21 days after transplanting, shoots and roots were harvested. The roots were washed under running tap water and sonicated in distilled water for 5 min to remove soil particles. Fresh weights of shoots and roots were measured for each sample and then cut finely, mixed, and divided into two subsamples. One subsample was dried at 70°C to measure the moisture content, and the other used to measure pesticide contents. These uptake experiments were conducted in quadruplicate.

3. Analysis of pesticide concentrations in plants
To extract pesticides, shoot and root samples were homogenized with 150 mL of acetone for 3 min. The extract was passed through a 0.8 µm glass fiber filter and concentrated to 100 mL in a rotary evaporator. To purify pesticides, a 50 mL aliquot of plant extract was spiked with 100 µL of acetone solution (2.5 µg/L) of internal standard (Diazinon-d₁₀; CDN Isotopes, Quebec, Canada). The extract was concentrated in a rotary evaporator. The concentrated extract was washed through a diatomite column (InterSep K-solute®; GL Science, Tokyo, Japan) with 120 mL of dichloromethane, and the eluate was concentrated to 0.5 mL in a rotary evaporator. The concentrated extract was dried under a nitrogen stream. The dried residue was dissolved in 2 mL of n-hexane–acetone (8:2) and then washed through a graphite column and a primary/secondary amine column (ENVI-Carb®/PSA column; Supelco, Bellefonte, PA, USA) by eluting with 10 mL of n-hexane–acetone (8:2). The eluate was concentrated to 0.2 mL in a rotary evaporator under a nitrogen stream. Pesticides in the purified samples were measured by gas chromatograph-mass spectrometry (GC-MS; HP6890–5973N; Agilent Technologies, Santa Clara, CA, USA) equipped with an ENV-8MS capillary column (φ0.25 mm × 30 m × 0.25 µm film thickness; Kanto Chemical, Tokyo, Japan).

4. Analysis of pesticide concentrations in the soil solution
To analyze the organic chemicals in the soil solution, we mixed 667 mg of prepared Celite® into 60 g of the uncontaminated soil in a stainless steel vessel (100 mL), adjusted the water content to 60% of the WHC with Milli-Q water, added the lid, and placed the stainless steel vessel in darkness at 15, 20, or 25°C. The water content was adjusted to 75% or 90% WHC with Milli-Q water, the lid was added, and the stainless steel vessel was placed in

| Table 1. Physicochemical properties of four pesticides |
|-----------------------------------------------|
| Chemicals     | Pesticide type | Substance group | Octanol–water partition coefficient (log KOW) |
|----------------|----------------|-----------------|-----------------------------------------------|
|                 |                |                 | a)  | b)  | c)  |
| Fenobucarb     | Insecticide    | Carbamate       | 2.67| 2.67| 2.78|
| Procymidone    | Fungicide      | Dicarboximide   | 3.14| 3.30| 3.3 |
| Flutolanil     | Fungicide      | Phenylbenzamide | 3.17| 3.77| 3.17|
| Tolclofos-methyl | Fungicide   | Chlorophenyl    | 4.56| 4.56| 4.56|

a) Values obtained from The Pesticide Manual (16th ed.). Values obtained from The 2011 Pesticide Handbook. Values obtained from the Footprint Pesticide Database of IUPAC.

| Table 2. Cultivation condition for plant uptake experiment |
|-----------------------------------------------|
| Treatment |
| Temperature | Day length | Soil water contents |
| 15°C | 15°C | 12 hr | 60% |
| 20°C | 20°C | 12 hr | 60% |
| 25°C | 25°C | 12 hr | 60% |
| Day length | |
| 8 hr | 20°C | 8 hr | 60% |
| 12 hr | 20°C | 12 hr | 60% |
| 16 hr | 20°C | 16 hr | 60% |
| Soil water contents | |
| 60% | 20°C | 12 hr | 60% |
| 75% | 20°C | 12 hr | 75% |
| 90% | 20°C | 12 hr | 90% |
darkness at 20°C. After 1, 3, 7, 14, and 22 days, the stainless steel vessel was placed on a receiver with a 0.8µm glass fiber filter and filtered by centrifugation at 15,800×g for 60 min to obtain the soil solution. To purify pesticides, a 10mL aliquot of soil solution was spiked with 100µL of acetone solution (2.5µg/L) of internal standard (Diazinon-d_{10}). The soil solution was purified as for the plant extract. Pesticides in the purified soil solution were measured as for plant extracts.

5. Statistical analyses
Statistical analyses were performed using SPSS 23 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 and Discussion

1. Plant growth
Shoot and root dry weights (DW) and transpiration rates of treatments are shown in Table 3. The data of 20°C, 12 hr, and 60% were those of the same sample. Shoot dry weights and transpiration rates increased with rising temperature. The transpiration rates at 20°C and 25°C were 1.9 and 2.5 times those at 15°C, respectively. Root dry weights did not significantly differ among temperature treatments. With longer photoperiods, shoot and root dry weights significantly.

Table 3. Shoot and root dry weights after 21 days of growth

| Treatment       | Dry weight (g-DW/pot) | Transpiration rate (L/21 days/pot) |
|-----------------|-----------------------|-----------------------------------|
|                 | Shoot                 | Root                              |
| Temperature     |                       |                                   |
| 15°C            | 4.71±0.09 a           | 1.10±0.07 a                       | 0.85±0.01 a |
| 20°C            | 5.49±0.21 b           | 1.17±0.13 a                       | 1.59±0.02 b |
| 25°C            | 6.73±0.06 c           | 0.98±0.06 a                       | 2.12±0.02 c |
| Day length      |                       |                                   |
| 8 hr            | 4.18±0.07 a           | 0.68±0.06 a                       | 1.16±0.04 a |
| 12 hr           | 5.49±0.21 b           | 1.17±0.13 a                       | 1.59±0.02 b |
| 16 hr           | 7.21±0.12 c           | 1.97±0.08 b                       | 1.66±0.03 b |
| Soil water contents |                   |                                   |
| 60%             | 5.49±0.21 a           | 1.17±0.13 a                       | 1.59±0.02 a |
| 75%             | 7.99±0.34 b           | 1.46±0.16 a                       | 2.95±0.10 b |
| 90%             | 8.85±0.25 b           | 1.55±0.06 a                       | 3.82±0.06 c |

Data were compared using 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 pesticides in shoot and root

| Treatment | Concentration (mg/kg-DW) |
|-----------|-------------------------|
|           | Fenobucarb | Procymidon | Flutolanil | Tolclofos-methyl |
| Shoot     |             |            |            |                  |
| Temperature |           |            |            |                  |
| 15°C       | 1.47±0.13 b | 2.08±0.15 a | 0.081±0.006 a | 0.007±0.0007 a |
| 20°C       | 0.73±0.08 a | 2.34±0.24 a | 0.059±0.010 a | 0.008±0.0007 a |
| 25°C       | 0.49±0.01 a | 2.89±0.13 a | 0.073±0.003 a | 0.006±0.0006 a |
| Day length |             |            |            |                  |
| 8 hr       | 1.40±0.08 b | 3.34±0.15 b | 0.145±0.007 b | 0.012±0.0009 b |
| 12 hr      | 0.73±0.08 a | 2.34±0.24 a | 0.059±0.010 a | 0.008±0.0007 ab|
| 16 hr      | 0.65±0.02 a | 2.21±0.05 a | 0.046±0.001 a | 0.007±0.0003 a |
| Soil water contents | |            |            |                  |
| 60%        | 0.73±0.08 a | 2.34±0.24 a | 0.059±0.010 a | 0.008±0.0007 a |
| 75%        | 0.58±0.13 a | 4.38±0.18 b | 0.135±0.019 b | 0.008±0.0003 a |
| 90%        | 0.65±0.08 a | 4.30±0.12 b | 0.119±0.009 ab| 0.012±0.0007 a |
| Root       |             |            |            |                  |
| Temperature |           |            |            |                  |
| 15°C       | 0.10±0.01 ab | 0.44±0.03 ab | 0.32±0.02 a  | 0.43±0.01 a  |
| 20°C       | 0.08±0.01 a  | 0.26±0.04 a  | 0.22±0.03 a  | 0.35±0.04 a  |
| 25°C       | 0.17±0.02 b  | 0.67±0.06 b  | 0.66±0.07 b  | 0.33±0.02 a  |
| Day length |             |            |            |                  |
| 8 hr       | 0.16±0.01 b  | 0.66±0.06 b  | 0.64±0.06 b  | 0.40±0.03 a  |
| 12 hr      | 0.08±0.01 a  | 0.26±0.04 a  | 0.22±0.03 a  | 0.35±0.04 a  |
| 16 hr      | 0.08±0.01 a  | 0.31±0.03 a  | 0.26±0.03 a  | 0.32±0.03 a  |
| Soil water contents | |            |            |                  |
| 60%        | 0.08±0.01 a  | 0.26±0.04 a  | 0.22±0.03 a  | 0.35±0.04 a  |
| 75%        | 0.18±0.01 b  | 0.53±0.02 a  | 0.47±0.03 b  | 0.40±0.03 a  |
| 90%        | 0.14±0.02 ab | 0.34±0.07 a  | 0.45±0.04 ab | 0.55±0.04 a  |

Data were compared using 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.
root dry weights and transpiration rates increased. Shoot dry weight for the 16 hr photoperiod increased by 1.7 times, root dry weight by 2.9 times, and transpiration rates by 1.4 times as compared with the 8 hr photoperiod. Shoot dry weights were higher for 75% and 90% WHC than for 60%; the transpiration rate increased 1.9 times for 75% WHC and 2.4 times for 90% as compared with 60%. Root dry weights did not significantly differ among soil water treatments.

2. Pesticide concentrations in shoots and roots
The pesticide concentrations in shoots and roots are shown in Table 4. The data of 20°C, 12 hr, and 60% were those of the same sample. The pesticides were not detected in shoots cultivated in non-contaminated soil (data not shown) and so pesticides in shoots were considered to be translocated from roots.

Table 5. Concentrations of pesticides in soil solution

| Chemicals          | Treatment | Concentration in soil solution (µg/L) | $C_t = C_0e^{-kt}$ | Mean Conc. (µg/L) |
|--------------------|-----------|--------------------------------------|---------------------|-------------------|
|                    |           | 1 day 3 day 7 day 14 day 22 day C_0  k   r²  |                     |                   |
| Temperature        |           |                                      |                     |                   |
| Fenobucarb         | 15°C      | 608.86 502.52 407.92 294.98 265.94 571.79 0.038966 0.925 365.28 |
|                    | 20°C      | 505.93 573.85 287.12 300.31 206.74 528.49 0.044152 0.805 318.07 |
|                    | 25°C      | 461.21 362.49 293.07 196.97 172.35 428.74 0.046052 0.931 252.46 |
| Procymidone        | 15°C      | 73.35 53.91 41.85 26.67 21.30 67.14 0.056911 0.945 34.89 |
|                    | 20°C      | 61.21 45.57 22.53 19.44 8.47 57.40 0.087200 0.938 21.06 |
|                    | 25°C      | 46.13 28.93 21.95 13.77 10.93 38.82 0.063728 0.909 18.65 |
| Flutolanil         | 15°C      | 161.22 131.40 98.13 78.75 46.37 159.46 0.055509 0.979 84.22 |
|                    | 20°C      | 121.29 112.17 54.76 43.86 17.29 131.44 0.090221 0.960 46.57 |
|                    | 25°C      | 124.74 68.47 55.60 34.49 27.27 98.86 0.064916 0.878 46.86 |
| Tolclofos-methyl   | 15°C      | 4.50 2.07 1.53 1.03 0.77 3.20 0.072191 0.831 1.39 |
|                    | 20°C      | 3.80 0.95 0.65 0.35 0.32 1.92 0.098014 0.710 0.62 |
|                    | 25°C      | 3.25 1.78 0.66 0.37 0.26 2.41 0.115006 0.873 0.64 |
| Soil water content | Fenobucarb| 60% 505.93 573.85 287.12 300.31 206.74 528.49 0.044152 0.805 318.07 |
|                    | 75%       | 713.21 697.72 413.18 187.31 178.97 743.90 0.074427 0.897 316.08 |
|                    | 90%       | 515.38 400.23 305.32 224.62 197.15 466.00 0.043921 0.907 281.21 |
| Procymidone        | 60%       | 61.21 45.57 22.53 19.44 8.47 57.40 0.087200 0.938 21.06 |
|                    | 75%       | 42.64 51.94 29.13 19.29 15.24 48.82 0.057064 0.907 25.33 |
|                    | 90%       | 33.78 43.61 24.19 15.61 14.91 47.82 0.062572 0.851 23.29 |
| Flutolanil         | 60%       | 121.29 112.17 54.76 43.86 17.29 131.44 0.090221 0.960 46.57 |
|                    | 75%       | 207.00 174.27 98.21 46.69 35.17 206.12 0.088473 0.949 74.52 |
|                    | 90%       | 203.04 101.45 62.37 39.25 37.04 142.13 0.073099 0.785 61.32 |
| Tolclofos-methyl   | 60%       | 3.80 0.95 0.65 0.35 0.32 1.92 0.098014 0.710 0.62 |
|                    | 75%       | 2.72 2.18 0.79 0.82 0.67 2.19 0.063432 0.708 1.06 |
|                    | 90%       | 3.42 2.37 0.97 0.62 0.71 2.60 0.075355 0.732 1.09 |
3. Effects of growth conditions on pesticide uptake by roots

According to Wauchope et al., temperature and soil water contents are factors that influence the fate of pesticides in the soil solution. Thus, we measured pesticide concentrations in the soil solution and calculated the mean concentrations during the experiment period using the following first-order equation:

$$C_t = C_0 e^{-kt}$$  \hspace{1cm} (1)

where $k$ is the rate constant, $C_t$ is the concentration after $t$ days, $C_0$ is the initial concentration, and $t$ is the number of days after the application of pesticide to the soil. We calculated the mean concentrations of pesticides in the soil and soil solution during the experiment period by integrating equation (1) over 1–22 days and dividing the integral by 21 (Table 5). Although we did not perform statistical analysis for these data, the concentrations of the four pesticides in soil solution tended to decrease with increasing temperature. Mean concentrations of fenobucarb and procymidone decreased in the order of $15^\circ C > 20^\circ C > 25^\circ C$. Those of flutolanil and tolclofos-methyl decreased in the order of $15^\circ C > 20^\circ C = 25^\circ C$. However, the relationships between soil water contents and pesticide concentrations were not clear: fenobucarb decreased in the order of $60\% \approx 75\% > 90\%$; flutolanil and tolclofos-methyl decreased in the order of $75\% \approx 90\% > 60\%$; and procymidone was not affected by the soil water content. Thus, the mean concentrations of pesticides resulted from differences in the temperature and soil water contents. Therefore, we calculated the RCF as the concentration in the root divided by the concentration in the soil solution to compare the uptake by plants.

The RCFs of fenobucarb, procymidone, and flutolanil were high for $25^\circ C$ in comparison to those of other temperatures (Fig. 1A). The soil sorption process of pesticides could be classified into first, a fast process sorbing the surface of the soil particle and, next, a slow process diffusing inside a soil particle. Although the fast process, which was controlled by sorption, was decreased by high temperatures, Ten Hulscher et al. assumed that a slow process controlled by diffusion was promoted by high temperatures. Our observed relationships between pesticide concentrations in the soil solution and temperature supported this hypothesis and indicated that soil sorption contributed to the decreases in pesticide concentrations at $25^\circ C$ in the soil solution (Table 5). Because plant roots can be considered as sorbents of pesticides, sorption to roots might be promoted at $25^\circ C$. In addition, the root weight and transpiration rate are regarded as the plant physiological factors that influence the uptake of organic chemicals. At $25^\circ C$, although the root dry weight did not differ from those at other temperatures, the transpiration rate that equaled the uptake amount of the water by the root was high. Therefore, the root uptake of these pesticides might have increased with the sorption of pesticides to roots, and the amount of water taken up may have been promoted at $25^\circ C$. The RCFs of tolclofos-methyl did not significantly differ with temperature treatment. The relatively high log $K_{ow}$ of tolclofos-methyl indicated that it was easily adsorbed by root surfaces as compared with the other tested pesticides; we surmised that it was hard to receive the influence by the change of plant physiological factor such as transpiration rate and metabolism by the temperature.
Based on the premise that concentrations in the soil solution were not affected by the day length, we used the concentrations at 20°C and 60% to calculate the RCF without measuring the concentrations in the soil solution for each day length. The RCFs of fenobucarb, procymidone, and flutolanil were high for the 8 hr (i.e., short day) photoperiod as compared with other photoperiods (Fig. 1B). The root dry weight and transpiration rate were low for the short day, indicating that these factors did not affect the uptake ability of pesticides. If metabolism were promoted by long days, the pesticides might be metabolized in roots. As a result, root concentrations might decrease under long days, and the RCFs would be low. Consequently, the RCFs of these pesticides might be relatively high with short days. The RCF of tolclofos-methyl was not changed by day length. It was reported that the RCFs of β-HCH and dieldrin were not changed by dark treatment. 21) Because tolclofos-methyl has similarly high hydrophobicity to β-HCH and dieldrin, it was thought that its uptake ability would not be affected by day length.

The RCFs of fenobucarb, procymidone, and flutolanil tended to increase with rising soil water content (Fig. 1C). These pesticides have a relatively low log $K_{OW}$ (Table 1) and are more easily dissolved in water than is tolclofos-methyl. In fact, fenobucarb concentrations in the soil solution were 10–100 times those of tolclofos-methyl (Table 5). Therefore, the amount of water taken up might make a larger contribution to the uptake of these pesticides than for tolclofos-methyl, and it was considered that the RCF increased under conditions of high soil water due to a high transpiration rate. However, the RCF of tolclofos-methyl was not affected by the soil water content (Fig. 1C). Thus, it is likely that plant physiological factors such as transpiration rate and metabolism did not influence the uptake ability for tolclofos-methyl.

4. Effects of growth conditions on the translocation of pesticides from root to shoot

Organic chemicals are translocated from root to shoot through the xylem by the transpiration stream. Thus, the TSCF is widely used to describe the translocation of organic chemicals to shoots.2–8,22) The TSCF is defined as the ratio of the concentration in the xylem sap to that in the medium. 2) It is difficult to directly measure the concentration in xylem sap, and so we estimated it indirectly by dividing the amount of pesticide in the shoot by the volume of water transpired23,24):

$$TSCF = \frac{\text{amount in shoot} + \text{transpiration volume}}{\text{concentration in soil solution}}$$ (2)

The TSCF of fenobucarb was high for the 15°C treatment, but the TSCF of procymidone increased with rising temperature (Fig. 2A). The TSCF of flutolanil did not significantly differ with temperature, indicating that temperature had little or no effect on its translocation to the shoot. The TSCF of tolclofos-methyl was high for 20°C and had no clear relationship with temperature. The above results suggest that the relationship between translocation ability and temperature varied according to the pesticide.

![Fig. 2. Transpiration stream concentration factors (TSCFs) of pesticides for (A) temperature, (B) day length, and (C) soil water contents. 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.](image-url)
We previously reported that the TSCF of dieldrin decreased with dark treatment for Cucurbita pepo and suggested that transport proteins playing an important role in dieldrin translocation were influenced by light.\textsuperscript{20,21} In addition, the TSCF of β-HCH (which has a lower log \(K_{OW}\) than does dieldrin) was not decreased by dark treatment, suggesting that the translocation of β-HCH might rely on the transpiration stream. The TSCFs of the four tested pesticides were higher for short days in contrast to dieldrin (Fig. 2B). Moreover, the TSCFs of the tested pesticides were affected by day length unlike β-HCH which was unaffected. Therefore, the translocation of these pesticides was not influenced by any transport proteins potentially inhibited by the dark condition. However, plant physiological factors, with the exception of transpiration rate, might play an important role in transporting these pesticides to shoots. Pesticide metabolism in the shoot could be a plant physiological factor that lowered the TSCF, as is also suggested for the RCF.

Although the TSCFs of tolclofos-methyl were lower for 75% and 90% WHC, the TSCFs of the other three pesticides remained constant at all soil water contents (Fig. 2C). Thus, the translocation of fenobucarb, procymidine, and flutolanil depended on the transpiration rate for soil water treatments. Because tolclofos-methyl is highly hydrophobic, it is difficult to translocate from root to shoot. There might be little tolclofos-methyl in the shoot for the transpiration rate. It is likely that the translocation of tolclofos-methyl basically depends on the transpiration rate.

5. General discussion
The RCF of pesticides tended to increase with rising temperature. However, the influence of temperature on the TSCF differed for each pesticide. The pesticide uptake increased with the rising transpiration rate due to high temperature; however, the relationship of translocation with temperature was not clear. The difference in translocation ability with temperature seemed to determine the pesticide concentrations in shoots. The RCFs of pesticides were high for short days, except for tolclofos-methyl, whose RCF did not change. In addition, TSCFs of pesticides were also high for short days. The day length might contribute to the metabolism of pesticides in plants, but any influence on uptake and translocation ability was not clarified. However, the pesticide concentrations in shoots were high for short days. Although there were significant differences in the values of RCFs and TSCFs of these pesticides, the soil water content had little or no effect on the uptake and translocation of pesticides. Thus, the shoot concentrations of pesticides caused by differences in soil water content could be explained by differences in transpiration rates and concentrations of pesticides in the soil solution.

In summary, the variations in pesticide concentrations depending on soil water contents could be estimated using the RCF and TSCF. However, it is necessary to consider that the RCF and TSCF were affected by temperature and day length when estimating their effect on pesticide concentrations in shoots. Because we do not yet know the plant physiological factors causing changes in the RCF and TSCF under different cultivation conditions, it is important to directly observe the behavior of pesticides in plant tissues.

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