

Comparative effects of rootstock and scion on dieldrin and endrin uptake by grafted cucumber (*Cucumis sativus*)

Takashi OTANI* and Nobuyasu SEIKE

Organochemicals Division, National Institute for Agro-Environmental Sciences (NIAES),
3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan

(Received December 5, 2005; Accepted February 27, 2006)

We examined the effect of grafting on dieldrin and endrin uptake from soil into cucumber plants. We compared the concentrations of dieldrin and endrin in aerial tissues of 10 varieties of *Cucurbita* spp. and 23 varieties of cucumber (*Cucumis sativus* L.) grown in a drin-contaminated soil. We chose four varieties each of *Cucurbita* spp. and cucumber, made up of 16 combinations of grafted plants by combining the *Cucurbita* spp. as rootstocks and the cucumber as scions, and then compared the dieldrin and endrin concentrations in the aerial tissues of the grafted plants. The results of our grafting trials indicated that dieldrin and endrin concentrations in the grafted plants were influenced mainly by rootstock varieties. © Pesticide Science Society of Japan

Keywords: dieldrin, endrin, cucumber, *Cucumis sativus* L., *Cucurbita*, grafting.

Introduction

Drins (aldrin, dieldrin, and endrin) categorized as a group of persistent organic pollutants (POPs) were used extensively in Japan from 1958. They were not manufactured in Japan, but 3300 tons of aldrin, 683 tons of dieldrin, and 1500 tons of endrin were imported from 1958 to 1972.¹⁾ Although the amounts of drins used in agriculture are uncertain, large amounts appear to have been used on cultivated land in Japan. Because of the extreme persistence of drins in the environment²⁾ and that accumulation in agricultural products poses a potential threat to human health,³⁾ the Japanese Government banned drin use for food crops in 1971. After 1971, drins were used as wood preservatives for pest control, but in 1975, the domestic registration of drins as pesticides was canceled. Organochlorine pesticides such as DDT, chlordane, mirex, heptachlor, hexachlorobenzene, toxaphene, and the drins categorized as POPs were banned for use on crops in most developed countries by 1990³⁾; however, because of their long persistence, these compounds are still found in soils.⁴⁻⁷⁾ The half-lives of dieldrin and endrin in soils are relatively long (5–12 years).⁸⁾ Therefore, the risk of drin residues being present in crops cultivated in polluted soils might continue for years or decades.

Although drins have not been used in Japan on cultivated land for the past 30 years, dieldrin and endrin at residual concentrations exceeding the limit set by the Food Sanitation Law of Japan (dieldrin: <0.02 ppm, endrin: <0.005 ppm, fresh weight basis) have been detected in cucumbers produced in some agricultural areas.^{7,9)} In Japan, perhaps because of the high cucumber intake (mean, 11.2 g/day per person),¹⁰⁾ the residue limits in cucumbers are lower than those set by the Codex Alimentarius Commission (aldrin and dieldrin: <0.1 ppm, endrin: <0.05 ppm).¹¹⁾ Therefore, immediate measures need to be taken against the drin problem in cucumbers. One of the strategies for reducing the risks of hazardous chemicals in food is to use varieties that accumulate less of the chemicals. About 80% of cucumber producers in Japan have adopted grafted cultivation, and usually *Cucurbita* spp. is used as a rootstock.¹²⁾ Therefore, it is important to know which is the main contributor to the uptake of drins from the soil: the rootstock or the scion. Our objectives were to determine varietal differences in the uptake of dieldrin and endrin among cucumber varieties used as scions and *Cucurbita* spp. as rootstocks, and to assess the comparative effect of scion vs. rootstock on the uptake of weathered drins from agricultural soil.

Materials and Methods

1. Soil

The test soil was collected from a field that had been cultivated with cucumbers for over 10 years. Precise application records were not available, but this area of the farm had re-

* To whom correspondence should be addressed.
E-mail: otanit@niaes.affrc.go.jp
© Pesticide Science Society of Japan

ceived a regular application of drins for insect control from the late 1950s to the early 1970s. The soil was Acrudoxic Hapludand [classified according to Soil Taxonomy criteria¹³⁾], with a pH (H₂O) of 5.6, carbon content of 9.86%, nitrogen content of 1.86%, and loamy texture. The soil was air-dried, passed through a 2-mm sieve, and used for greenhouse trials and soil analysis.

2. Greenhouse trial 1

This trial was conducted from April 5 to May 14, 2004. Ten varieties of *Cucurbita* spp. (Shintosa-1gou, Kirameki, Kurodane, Yuyuikki-black, Yuyuikki-white, Hikaripower, Hikaripower-gold, New-super-unryu, Batorah, Sokodikara), and 23 varieties of cucumber (*Cucumis sativus* L.) (Natsusuzumi, Natsubayashi, Kinsei, Shin-hokusei, Nannkyoku-2gou, Yoshinari, Sachinari, Highgreen-21, Highgreen-22, Greenrakkusu, Tokiwa-21, Sharp-1, Sharp-301, Ankohru-8, Excellent-fushinari, Naoyoshi, Taisho, Taisho-2, Hokkikou-JP, V-road, Climber-1gou, Pilot, Ocean) were included in the first experiment. Seeds of the test plants were germinated on perlite culture. Fourteen days after sowing, the seedlings were transferred to polyethylene pots (volume 400 ml) filled with 270 g of the soil and then grown in a glasshouse at 25°C under natural light conditions. Each pot was set in a plastic tray for bottom-up irrigation and mulched with a sheet of silver plastic film to avoid or minimize drin contamination of the shoots by soil adhesion or outgassing. The soil in each pot was fertilized with a chemical fertilizer [0.5 g N as (NH₄)₂SO₄, 0.22 g P as Ca(H₂PO₄)₂·H₂O and 0.42 g K as KCl] and irrigated. New positions were assigned to the pots every day to achieve uniform conditions for plant growth. Three varieties of *Cucurbita* spp. (Shintosa-1gou, Kurodane, and Yuyuikki-black) and three varieties of cucumber (Natsusuzumi, Highgreen-22, Sharp-1) were also grown in the same manner except in soil in which drins were not detectable (drin-free soil). Twenty-five days after transferring the tested plants to the pots, the aerial tissues of the plants were harvested. All the treatments were replicated three times. Plant samples were chopped finely, mixed, and divided into two subsamples. One was dried at 70°C for measurement of the moisture content, and the other was frozen and stored at –20°C until extraction for dieldrin and endrin content analysis.

3. Greenhouse trial 2

This trial was conducted from June 15 to July 30, 2004. Four varieties of *Cucurbita* spp. (Shintosa-1gou, Hikaripower-gold, Kirameki, Yuyuikki-black) as rootstocks and four varieties of cucumber (Sharp-1, Yoshinari, Sharp-301, Natsubayashi) as scions were chosen for this grafting trial. Seeds of the test plants were germinated on perlite culture. Nine days (cucumber) and 7 days (*Cucurbita* spp.) after sowing, 16 grafted plants were created from the combination of four varieties of *Cucurbita* spp. as rootstocks and four varieties of cucumber

as scions, and grown in perlite culture. Eighteen days after grafting, the grafted plants were transferred to pots with the soil, and then grown in the same manner as in trial 1. Four combination plants (Sharp-1/Shintosa-1gou, Yoshinari/Hikaripower-gold, Sharp-301/Kirameki, Natsubayashi/Yuyuikki-black) were also grown in drin-free soil. Eighteen days after transferring the grafted plants to the pots, the aerial tissues (scion parts) were harvested. All the treatments were replicated four times. Plant samples were prepared for the following analysis in the same manner as in trial 1.

4. Sample extraction and cleanup

Approximately 10 g of the soil sample was weighed, spiked with 100 ng of ¹³C₁₂-labeled dieldrin and ¹³C₁₂-labeled endrin as internal standards, Soxhlet extracted with 300 ml of acetone for 16 hr, and then concentrated in a rotary evaporator to 5 ml. Approximately 30 g of a plant sample was weighed and spiked with 100 ng of ¹³C₁₂-labeled dieldrin and ¹³C₁₂-labeled endrin (Cambridge Isotope Laboratories) as internal standards. The plant sample was homogenized for 5 min in a Polytron® PT3100 (Kinematica AG) with 200 ml of acetone. The solution was passed through a glass fiber filter and concentrated in a rotary evaporator. The concentrated solution was poured into a 100-ml volumetric flask and diluted to a marked line with acetone. Thirty-milliliter portions were taken from the above 100 ml solutions, and concentrated to 5 ml in a rotary evaporator.

The extracts of the soil or plant samples were transferred with 25 ml *n*-hexane into a separatory funnel and then washed twice with 100 ml water pre-cleaned with *n*-hexane, and excess water removed over Na₂SO₄. The extract was concentrated using a rotary evaporator to 1 ml, and other interferences were removed by passing the extract through a column packed with 5 g of Florisil (Mega Bond Elute FL, Varian). The interferences were separated by elution with 30 ml of *n*-hexane, and then dieldrin and endrin were eluted with 80 ml of a mixture of dichloromethane and *n*-hexane (1 : 3 v/v). The latter fractions were concentrated in a rotary evaporator to 1 ml and applied to 500 mg of graphite carbon column (Envi-Carb, Supelco), then eluted with 10 ml of *n*-hexane. The eluate was concentrated in a rotary evaporator to 1 ml. Five nanograms of ¹³C₁₂-labeled 2,2',4,4',5,5'-HxCB (Wellington Laboratories) in decane were added as a syringe spike, and the eluate was further concentrated to 50 µl under a gentle stream of nitrogen.

5. Quantification

The cleaned-up samples were analyzed on a gas chromatographic mass spectrometer (GC/MS) (HP6890-5973N, Agilent Technologies) equipped with ENV-8MS (30 m×0.25 mm i.d.×0.25 µm film thickness, Kanto Kagaku). The oven temperature was programmed to 120°C and held for 1 min; then increased by 10°C/min to 180°C and held for 1 min; then increased by 5°C/min to 300°C and held for 10 min. The injec-

tion port was at 250°C. The carrier gas was ultrahigh-purity helium (1.0 ml/min). The MS was operated in electron impact (EI) and selective ion monitoring (SIM) mode, using the following ions: dieldrin (M^+ ; m/z 380, 382), $^{13}C_{12}$ -labeled dieldrin (M^+ ; m/z 392, 394), endrin ($[M-Cl]^+$; m/z 345, 347), $^{13}C_{12}$ -labeled endrin (357, 359), and $^{13}C_{12}$ -labeled 2,2',4,4',-5,5'-HxCB (M^+ ; m/z 372, 374). The dieldrin and endrin concentrations in soil and plants were expressed as nanograms per gram on the dry weight basis of the particular matrix. The limits of detection (LODs) for dieldrin and endrin analyses were calculated according to the method of JIS K 0312.¹⁴⁾ The LODs for dieldrin and endrin in soils were 0.4 ng/g and 0.3 ng/g, respectively. The LODs for dieldrin and endrin in plants ranged from 4.5 to 10.1 ng/g and 3.9 to 8.8 ng/g, respectively.

6. Quality control

Analysis of a procedural blank (no sample) and reference material (soil sample), and checking the recovery rates of the internal standards were performed for laboratory quality control. The results obtained with the procedural blanks indicated that the samples were not contaminated due to processing in the laboratory (extraction, purification and measurement with GC/MS). The reference material (JSAC 0441) obtained from The Japan Society for Analytical Chemistry was analyzed three times. The result was 74.3 ± 3.5 ng/g [coefficient of variance (CV), 4.74%], within the range of the certificated value (76 ± 14 ng/g). Recovery rates of $^{13}C_{12}$ -labeled dieldrin and $^{13}C_{12}$ -labeled endrin ranged from 50% to 120%. If the values obtained were beyond the range, the sample was reanalyzed by repeating the processes used after extraction.

7. Statistical analysis

All statistical analyses were performed using SPSS 12.0J for Windows® (SPSS Inc.). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine which samples differed, through a pair-wise comparison matrix. Two-way ANOVA was used to determine interaction effects between two factors.

Results and Discussion

1. Soil

Analysis of the tested soil showed that it contained 594 ng dieldrin/g and 58 ng endrin/g. Aldrin was also analyzed, but not detected (<0.1 ng/g). Therefore, we did not screen for aldrin in plant samples in this study. Dieldrin is formed from aldrin through oxidation in soil.¹⁵⁾ Although no precise records of drin application to this soil were available, our data corresponded well with previous reports in the literature⁶⁾: although aldrin was used in greater quantities than dieldrin, it was primarily the latter that was found in soil samples.

2. Varietal differences in dieldrin and endrin uptake

In all of the plants grown in drin-free soil, the concentrations

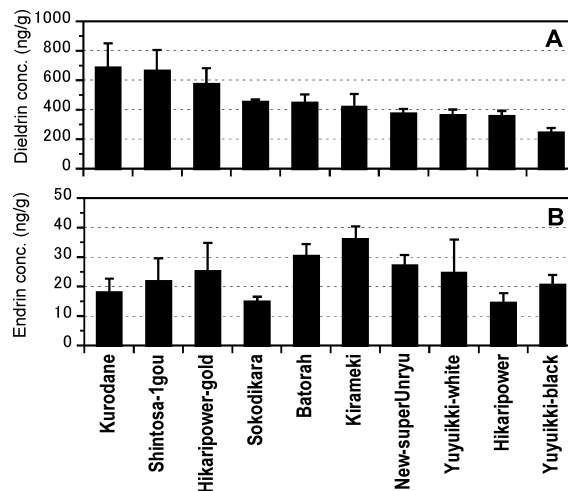


Fig. 1. Concentrations of dieldrin (A) and endrin (B) in the aerial tissues of *Cucurbita* spp. varieties. Error bars indicate standard deviation ($n=3$).

of dieldrin and endrin were below the detection limits in these trials. Therefore, the influence of airborne drins on aerial tissues was negligible, and the differences in dieldrin and endrin contents among the tested plants depended on the ability of the plants to take up dieldrin and endrin from the contaminated soil.

The concentrations of dieldrin and endrin in the aerial tissues of the test varieties of *Cucurbita* spp. and cucumber are shown in Figs. 1 and 2, respectively. In the 10 varieties of *Cucurbita* spp., concentrations of dieldrin varied from 249 to 689 ng/g, and of endrin from 15 to 36 ng/g. The varieties were not in the same ranking orders in terms of dieldrin and endrin concentrations. For example, 'Kirameki' had a moderate dieldrin concentration but had the highest endrin concentration.

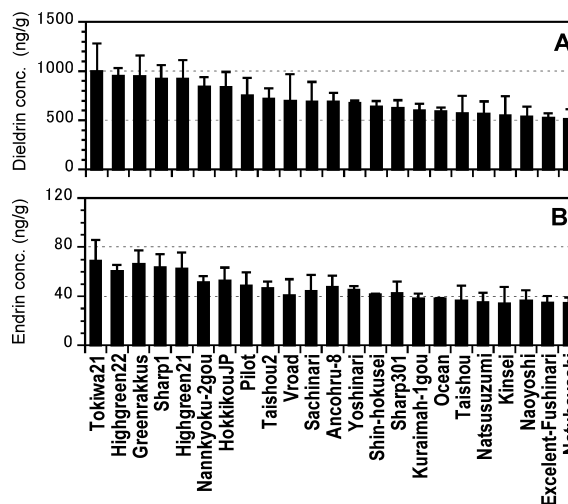


Fig. 2. Concentrations of dieldrin (A) and endrin (B) in the aerial tissues of cucumber varieties. Error bars indicate standard deviation ($n=3$).

On the other hand, ‘Kurodane’ had the highest dieldrin concentration but a relatively low endrin concentration. Therefore, the ratio of plant endrin/dieldrin (E/D) concentrations fluctuated (0.03 to 0.09) among the varieties. In the 23 cucumber varieties, dieldrin concentration varied from 514 to 998 ng/g, and that of endrin from 35 to 69 ng/g. The trend in the concentration pattern of dieldrin and endrin was well matched among the varieties, and the plant E/D ratios were nearly equal (0.06 to 0.07). The concentration of endrin in the soil was about 1/10th that of dieldrin, so the cucumber varieties tended to absorb dieldrin and endrin in accordance with the soil E/D ratio. On the other hand, the uptake efficiency of dieldrin and endrin seemed to differ among the varieties of *Cucurbita* spp. Mattina *et al.*¹⁶⁾ reported that the ratios of the chlordane-related components (*cis*-chlordane, *trans*-chlordane and *trans*-nonachlor) in zucchini were different from that in the soil in which the plants were grown. In our trials, we could only speculate that the differences in the plant E/D might have been related to the differential transport efficiencies of dieldrin and endrin from the soil to the aerial tissues among the varieties tested. However, it was noteworthy that the plant E/D among the tested varieties fluctuated much more in *Cucurbita* spp. than in cucumber. Moreover, the concentrations of dieldrin and endrin in the aerial tissues varied more widely in *Cucurbita* spp. (CV value among the 10 varieties: dieldrin, 30.7%; endrin, 29.1%) than in cucumber (CV value among the 23 varieties: dieldrin, 21.9%; endrin, 23.6%). All cucumber varieties tested belonged to a single species, *Cucumis sativus* L. In contrast, the varieties of *Cucurbita* spp. tested were obtained by crossings of different species (*C. maxima*, *C. ficifolia*, *C. moschata* and *C. pepo*), those are: ‘Kurodane’ (*C. ficifolia*), ‘Kirameki’ and ‘Sokodikara’ (*C. moschata*), Shintosa-1gou (a cross of *C. maxima* and *C. moschata*), ‘Yuyuikki-black’ and ‘Yuyuikki-white’ (*C. moschata* and *C. maxima* were used for crossing in the breeding process), and four other varieties (*C. moschata*, *C. maxima* and *C. pepo* were used for crossing in the breeding process). We could only speculate that the wider variation in the plant E/D or concentrations of dieldrin and endrin in *Cucurbita* spp. than in cucumber might have been due to the greater genetic diversity of the varieties of *Cucurbita* spp. tested.

Among the tested varieties, four varieties of *Cucurbita* spp. (as rootstocks) and four varieties of cucumber (as scions), with various dieldrin and endrin concentrations, were chosen for the grafting trial (Fig. 3). The dieldrin concentrations in the varieties of *Cucurbita* spp. decreased in the following order: ‘Shintosa-1gou’ ≥ ‘Hikaripower-gold’ ≥ ‘Kirameki’ ≥ ‘Yuyuikki-black’, and in the cucumber varieties decreased in the following order: ‘Sharp-1’ ≥ ‘Yoshinari’ ≥ ‘Sharp-301’ ≈ ‘Natsubayashi’. There were no significant differences in endrin concentration among the four varieties of *Cucurbita* spp., and the trend in endrin concentration among the four cucumber varieties was the same as for the dieldrin concentration.

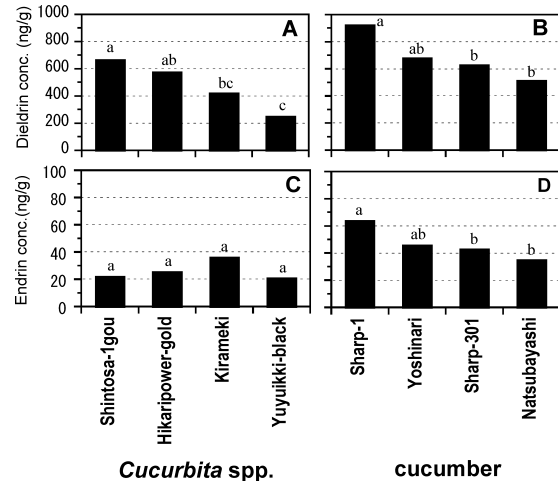


Fig. 3. Concentrations of dieldrin in the aerial tissues of four *Cucurbita* spp. (A) and four cucumber (B) varieties, and those of endrin in four *Cucurbita* spp. (C) and four cucumber (D) varieties, selected for the grafting trial. Columns with the same letter are not significantly different at $p < 0.05$ according to an ANOVA-protected Tukey's multiple range test.

3. Effect of grafting on dieldrin and endrin uptake

The concentrations of dieldrin and endrin in the aerial tissues of the grafted plants made up of various combinations of the four *Cucurbita* spp. rootstocks and the four cucumber scions are shown in Fig. 4. In general, we observed only small differences in dieldrin and endrin concentrations among grafted plants with the same rootstock but different scions, and much greater differences among grafted plants with the same scion but different rootstocks. We examined the effect of interaction between the two factors, rootstock variety (factor R) and scion (factor S), by two-way ANOVA. For dieldrin concentration in the grafted plants, the F value of factor $R \times$ factor $S (F_{R \times S}) =$

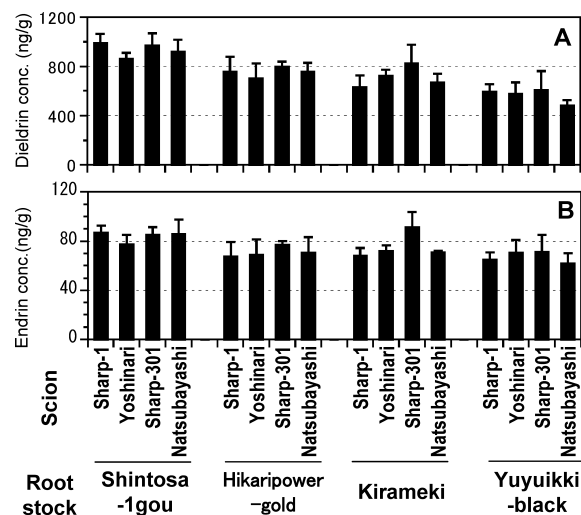


Fig. 4. Concentrations of dieldrin (A) and endrin (B) in the aerial tissues of grafted plants. Error bars indicate standard deviation ($n=4$).

$1.17 < F_{(9,48)}(0.05) = 2.08$. For endrin concentration in the grafted plants, the F value of factor $R \times$ factor $S (F_{R \times S}) = 0.52 < F_{(9,48)}(0.05) = 2.08$. So there was no interaction effect between rootstock (*Cucurbita* spp.) and scion (cucumber) in terms of the concentrations of dieldrin and endrin. This result indicated that the combination of rootstock and scion had little influence on the concentrations of dieldrin and endrin in the aerial tissues of the grafted plants.

As no interaction effect between rootstock and scion was recognized, we compared dieldrin and endrin concentrations in the grafted plants in terms of those in the same rootstock group (each group included four scion varieties) and those in the same scion group (each group included four rootstock varieties) (Fig. 5). Among rootstocks, the dieldrin concentration was highest in plants grafted on ‘Shintosa-1gou’, medium in those on ‘Hikaripower-gold’ and ‘Kirameki’, and lowest in those on ‘Yuyuikki-black’. The dieldrin concentration in plants grafted on ‘Shintosa-1gou’ rootstock was 1.6 times higher than in plants grafted on ‘Yuyuikki-black’. There were a few differences in the endrin concentration of grafted plants with four rootstocks, and in dieldrin and endrin concentrations of grafted plants with four scions, but they fell within a narrow range.

We then compared the trends in dieldrin and endrin concentrations of grafted plants with those of the self-rooted *Cucurbita* spp. and cucumber plants (Fig. 3). For dieldrin concentrations in self-rooted plants, the order among the *Cucurbita* spp. (‘Shintosa-1gou’ \geq ‘Hikaripower-gold’ $>$ ‘Kirameki’ \geq ‘Yuyuikki-black’) (Fig. 3A) matched well with that of plants grafted on the corresponding rootstocks (Fig. 5A). However, the order of self-rooted cucumber plants (‘Sharp-1’ \geq ‘Yoshinari’ \geq ‘Sharp-301’ \approx ‘Natsubayashi’) (Fig. 3B) did not reflect that of plants grafted with the corresponding scions, among which there were only small differences in dieldrin concentrations (Fig. 5B). For endrin concentration, no significant differences were recognized among self-rooted *Cucurbita* spp. (Fig. 3C) and only small differences were observed among plants grafted on different *Cucurbita* spp. rootstocks (Fig. 5C). Also, only small differences were observed among plants grafted with different cucumber varieties as scions (Fig. 5D), although the highest concentration of endrin in the self-rooted cucumber plants was twice the lowest concentration (Fig. 3D). As the trends in dieldrin and endrin concentrations of self-rooted varieties were reflected in those of plants grafted on the corresponding rootstocks, but not in those of plants grafted using the corresponding scions, this suggests that dieldrin and endrin uptake by grafted plants was controlled mainly by the rootstock, not the scion. Suda *et al.* reported that dieldrin and endrin concentrations in the fruits of grafted cucumber were influenced by rootstock plants.¹⁷⁾ It was also pointed out that rootstock plants controlled cadmium uptake in tobacco (*Nicotiana tabacum* L.) leaves.¹⁸⁾

We suggest that selecting low-uptake rootstocks is a promising way to decrease drin pollution in cucumbers. In the

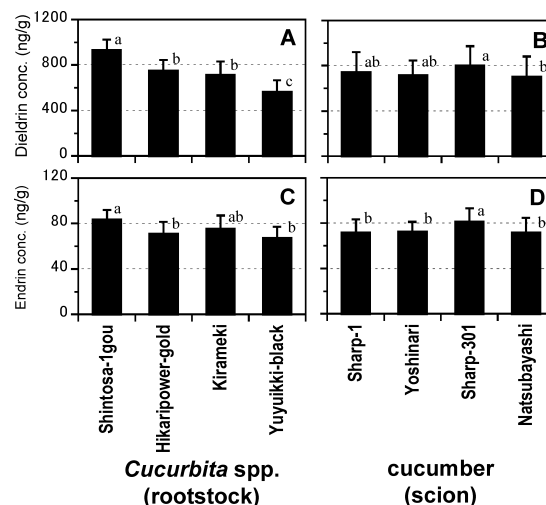


Fig. 5. Means of dieldrin (A and B) and endrin (C and D) concentrations in the aerial tissues of grafted plants using each of four *Cucurbita* spp. rootstocks (A and C) and four cucumber scions (B and D). Error bars indicate standard deviation ($n=16$). Columns with the same letter are not significantly different at $p < 0.05$ according to an ANOVA-protected Tukey’s multiple range test.

grafting trial, the highest dieldrin concentration (in the ‘Shintosa-1gou’ rootstock) was about 1.6 times the lowest (in ‘Yuyuikki-black’). Therefore, it is possible to reduce drin pollution by about half by using low-uptake rootstock varieties like ‘Yuyuikki-black’, without changing scion varieties. Moreover, in breeding approaches to decreasing drin concentrations in cucumber, it seems reasonable to aim for a low-drin-uptake *Cucurbita* spp. rootstock rather than a low-drin-uptake cucumber scion. In this study, the effect of rootstock on controlling drin uptake was recognized at an early growth stage of the plant’s aerial tissues. Further study is needed to confirm the effect of low-drin-uptake rootstock varieties in decreasing drin concentration on the cucumber fruit.

Acknowledgment

This work was supported in part by a Grant-in-aid (Hazardous Chemicals) from the Ministry of Agriculture, Forestry, and Fisheries of Japan (HC-05-2331-2).

References

- 1) ‘Noyaku Yoran,’ Japan Plant Protection Association, Tokyo, Japan, from 1958 to 1972 (in Japanese).
- 2) R. G. Nash and E. A. Woolson: *Science* **157**, 924–926 (1967).
- 3) J. L. Jorgenson: *Environ. Health Perspect.* **109**, 113–139 (2001).
- 4) M. J. I. Mattina, W. Iannucci-Berger, L. Dykas and J. Pardus: *Environ. Sci. Technol.* **33**, 2425–2431 (1999).
- 5) J. C. White: *Environ. Toxicol. Chem.* **20**, 2047–2052 (2001).
- 6) S. N. Meijer, C. J. Halsall, T. Harner, A. J. Peters, W. A. Ockenden, A. E. Johnston and K. C. Jones: *Environ. Sci. Technol.* **35**, 1989–1995 (2001).
- 7) Y. Hashimoto: *J. Pestic. Sci.* **30**, 397–402 (2005).
- 8) L. Ritter, K. R. Solomon, J. Forget, M. Stemeroff and C.

- O'Leary: "Assessment Report on Certain Persistent Organic Pollutants Prepared by the International Programme on Chemical Safety," United Nations Environment Programme (UNEP), Montreal, Canada, 1998.
- 9) H. Kondo, E. Amakawa, H. Sato, K. Yasuda, K. Onuki, M. Akiba and K. Kanaya: *Ann. Rep. Tokyo Metr. Inst. Public Health* **54**, 132–135 (2003) (in Japanese).
 - 10) "National Nutrition Survey in 2000," Tokyo, Japan, 2002.
 - 11) "Maximum Residue Limits for Pesticides," FAO/WHO, Rome, Italy, 2001.
 - 12) *Misc. Publ. Natl. Res. Inst. Veg., Ornam. Plants & Tea* **9**, 1–128 (2001).
 - 13) "Keys to Soil Taxonomy, 8th Ed.," Natural Resources Conservation Service, United States Department of Agriculture, Washington D.C., USA, 1999.
 - 14) "Method for Determination of tetra- through octa-chlorodibenzo-*p*-dioxins, tetra- through octa-chlorodibenzofurans, and coplanar polychlorinatedbiphenyls in industrial water and waste water (K0312)," JIS (Japan Industrial Standard), Tokyo, Japan, 1999.
 - 15) L. A. Barrie, D. Gregor, B. Hargrave, R. Lake, D. Muir, R. Shearer, B. Tracey and T. Bidleman: *Total Environ.* **122**, 1–74 (1992).
 - 16) M. J. I. Mattina, J. White, B. Eitzer and W. Iannucci-Berger: *Environ. Toxicol. Chem.* **21**, 281–288 (2002).
 - 17) T. Suda, N. Iwata and K. Yamada: *J. Pestic. Sci.* **1**, 59–63 (1976) (in Japanese).
 - 18) G. J. Wagner, T. G. Sutton and R. Yeagan: *Tob. Sci.* **32**, 88–91 (1988).