Accumulation of Weathered *p,p'*-DDTs in Grafted Watermelon

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ABSTRACT: The grafting of melon plants onto cucurbit rootstocks is a common commercial practice in many parts of the world. However, certain cucurbits have been shown to accumulate large quantities of weathered persistent organic pollutants from the soil, and the potential contamination of grafted produce has not been thoroughly evaluated. Large pot and field experiments were conducted to assess the effect of grafting on accumulation of weathered DDX (the sum of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE) from soils. Intact squash (*Cucurbita maxima × moschata*) and watermelon (*Citrullus lanatus*), their homografts, and compatible heterografts were grown in pots containing soil with weathered DDX at 1480−1760 ng/g soil or under field conditions in soil at 150−300 ng/g DDX. Movement of DDX through the soil−plant system was investigated by determining contaminant levels in the bulk soil and in the xylem sap, roots, stems, leaves, and fruit of the grafted and nongrafted plants. In all plants, the highest DDX concentrations were detected in the roots, followed by decreasing amounts in the stems, leaves, and fruit. Dry weight concentrations of DDX in the roots ranged from 7900 ng/g (intact watermelon) to 30100 ng/g (heterografted watermelon) in the pot study and from 650 ng/g (intact watermelon) to 2430 ng/g (homografted squash) in the field experiment. Grafting watermelon onto squash rootstock significantly increased contaminant uptake into the melon shoot system. In the pot and field studies, the highest stem DDX content was measured in heterografted watermelon at 1220 and 244 ng/g, respectively; these values are 140 and 19 times greater than contaminant concentrations in the intact watermelon, respectively. The xylem sap DDX concentrations of pot-grown plants were greatest in the heterografted watermelon (6.10 μg/L). The DDX contents of the leaves and fruit of watermelon heterografts were 3−12 and 0.53−8.25 ng/g, respectively, indicating that although the heterografted watermelon accumulated greater pollutant levels, the resulting contamination is not likely a food safety concern.

KEYWORDS: grafted watermelon, homografted, heterografted, xylem sap, *p,p'*-DDE, *p,p'*-DDT

■ INTRODUCTION

Grafted cucurbits are commonly produced in countries such as China, Korea, Spain, Italy, and Turkey.[1−3] Turkey is the second largest watermelon producer in the world,[2] with production of approximately 4 million tons in 2009.[4] The use of grafted watermelon seedlings has increased significantly,[2] equivalent to >95% of the Turkish watermelon market in 2009. Watermelons (*Citrullus lanatus*) are grafted onto *Cucurbita* spp. rootstocks to achieve better control of soilborne diseases and to increase tolerance to viral infection.[5−7] Although there has been much research on the advantages of grafting for fruit yield[8] and fruit quality,[9−13] little is known about the impact of grafting on the accumulation of soilborne organochlorine pesticides such as 2,2-bis(chlorophenyl)-1,1,1-trichloroethane (DDT) and metabolites.

During the 1960−1970s, DDT was one of the most extensively used organochlorine pesticides throughout the world and was widely applied in Turkey until being banned in 1985.[14] In soil, *p,p'*-DDT residues can be biotically and abiotically converted to one of two metabolites: 2,2-bis(chlorophenyl)-1,1-dichloroethane (*p,p'*-DDD) and 2,2-bis(chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE).[15,16] *p,p'*-DDT and the metabolites *p,p'*-DDD and *p,p'*-DDE are persistent organic pollutants (POPs) and have been targeted for global elimination under the Stockholm Convention. As a group, POPs represent a class of highly toxic organic pollutants, including compounds with known or suspected mutagenic,

estrogenic, and carcinogenic effects.[17,18] POPs are highly hydrophobic, with log K_{ow} (octanol−water partition coefficients) values of >5.0 and, thus, bind strongly to soil organic matter. Although the bioavailability of POPs declines sharply over time,[19] contaminants such as *p,p'*-DDE are known to bioaccumulate in the lipids of exposed organisms, resulting in a potential biomagnification within food chains.[20,21] The half-lives of POPs such as DDT and metabolites (DDX) in soil are frequently measured in years,[22] and conventional treatment technologies are often cost prohibitive or ineffective. Hulster et al.[23] first reported that *Cucurbita* species accumulate dioxin and furan in their plant tissues. Recent studies have shown that *Cucurbita* species have a unique potential to accumulate weathered *p,p'*-DDE, polychlorinated biphenyls (PCBs), and chlordane from contaminated soil, but that other squash and melon species, including watermelon, do not have this ability.[24−30] *Cucurbita pepo* has repeatedly been shown to extract high levels of *p,p'*-DDE and other POPs from soil, with the highest contaminant concentrations in the roots and stems, with significantly lower levels in the leaves and fruit.[32] The amount of contaminant accumulated in the vegetation depends on both plant phylogeny and the physical/chemical character-

istics of the pollutant.[26] White[31] showed varied *p,p'*-DDE uptake potentials among squash and pumpkin (*Cucurbita*) but demonstrated minimal contaminant accumulation by cucumber and melon (*Cucumis*). Notably, the mechanism of POP uptake by *Cucurbita* species remains unknown. In addition, the role of *Cucurbita* rootstock on the uptake of weathered POPs into grafted watermelon tissue, including fruit, is unknown and could be an important food safety concern as an uncharacterized pathway of human exposure to these pollutants.[32,33] This research investigates the accumulation of weathered DDX in the roots, shoots, leaves, fruit, and xylem sap of intact, homografted, and heterografted squash interspecific hybrid (*Cucurbita maxima* × *Cucurbita moschata*) and watermelon (*Citrullus lanatus* cv. Crimson Tide) plants grown in contaminated soil under greenhouse and field conditions.

■ MATERIALS AND METHODS

Soil. The soil used is from a 100 m^2 area in northern Karasu of Sakarya Providence, Turkey, that is known to be contaminated with 52−2925 ng/g weathered *p,p'*-DDX[34] from historical use. For the field experiment, 30 plants (6 replicates per treatment) were each planted in a 30 cm^2 mound at 2 m intervals. Soil samples were collected from each mound before planting in 2010. The soil is a loam with 3.95% organic carbon. For the pot experiments, 600 kg of soil was collected and was passed through a 2 mm sieve, and each of 30 pots was filled with 14 kg of the DDX-contaminated soil. For vegetated controls, four pots per plant type were filled with 14 kg of sieved DDX-free loam soil containing 3.70% organic carbon; this soil was obtained from an area approximately 500 m east of the contaminated field. Six additional pots were amended with 14 kg of DDX contaminated soil but were left unplanted. Soil samples were collected from each pot in the greenhouse prior to planting in 2010. The soil samples were subsequently air-dried at room temperature for 7 days (to ensure a moisture content of <10%) and stored in 250 mL amber bottles tightly sealed with Teflon-lined screw caps until analysis.

Plants. A squash interspecific hybrid (*Cucurbita maxima* × *Cucurbita moschata*) was chosen as rootstock in this study because *Citrullus lanatus* (Crimson Tide; watermelon) is most commonly grafted onto this type of rootstock in Turkey. *Cucurbita maxima* × *Cucurbita moschata* (squash) and *Citrullus lanatus* (Crimson Tide; watermelon) intact plants were used, along with their homografts and heterografts. To acquire plants representative of the commercial market, grafted and nongrafted plants were purchased from a professional grafting company in Antalya, Turkey. Scions and rootstocks were grafted when cotyledons and first true leaves started to develop and then were subsequently planted in vermiculite. The scion was inserted onto the prepared rootstock and fixed tightly by a grafting clip. Grafted plants were kept in a dark room at 25 °C and 95% humidity for 3 days, and then the plants were kept in a greenhouse at 21−30 °C after their junctions had healed. At 21 days, robust and healthy grafted and nongrafted plants were used for transplanting into the treatment pots or mounds. The field and greenhouse experiments consisted of five and seven different treatments, respectively: (1) intact squash interspecific hybrid (*Cucurbita maxima* × *Cucurbita moschata*); (2) intact watermelon (*Citrullus lanatus*); (3) homografted squash interspecific hybrid (the scion and rootstock are squash interspecific hybrid); (4) homografted watermelon (the scion and rootstock are watermelon); (5) heterografted watermelon (rootstock is squash interspecific hybrid, and the scion is watermelon); (6) vegetated controls (four replications of each cultivar were grown in the pots packed with DDX-free soil); (7) nonvegetated controls (six pots packed with DDX-contaminated soil were left unplanted). All pots (vegetated and controls) in the greenhouse and mounds in field were watered twice daily throughout the growing period. Cultivars were harvested at 65 and 68 days for field and greenhouse experiments, respectively. Both xylem sap and pore water were collected from replicate pots in the greenhouse. For all plants, root, shoot, leaf, and fruit tissues were weighed, washed, and homogenized with a blender by replicate and treatment type. The dry weight of a portion of each tissue sample was determined by heating at 105 °C for 24 h. All tissue samples were stored in a freezer at −4 °C until analysis.

Quantitation of DDX in Plants and Soil. A method published previously was used for extraction of plant tissues.[35] Briefly, six replicates each of root (1 g), stem (10 g), leaf (10 g), or fruit (10 g) tissue were weighed into 40 mL Teflon-lined screw-cap vials that were amended with 5 mL of 2-propanol, 10 mL of *n*-hexanes, and 506 ng of *α*-benzene hexachloride (*α*-BHC) as an internal standard. The vials were heated at 65 °C for 2.5 h. After a cooling period of 5 min, the extracts were decanted through a funnel lined with glass wool and collected in 500 mL glass separatory funnels. An additional 15 mL of 1:2 v/v 2-propanol/hexane was used to rinse the vials; the rinsate was then added to the separatory funnels. Extracts were amended with 100 mL of reverse osmosis (RO) water and 10 mL of saturated sodium sulfate; the extracts were then shaken rigorously for 5 s. After phase separation (∼2 min), this step was repeated with 50 mL of RO water and saturated sodium sulfate. Hexane extracts were collected in 40 mL amber Teflon screw-cap vials containing 5 g of granular anhydrous sodium sulfate.

Soil samples were extracted as five replicates per pot or three replicates per mound, using a slightly modified method published previously.[36] Briefly, a 3 g soil sample was weighed into a 40 mL Teflon-lined screw-cap vial and amended with 10 μL of an internal standard (IS) solution containing 506 ng of *α*-BHC in hexane. The vials were amended with 15 mL of hexane and heated at 65 °C for 5 h. After a cooling period of 10 min, hexane extracts were decanted into 40 mL amber Teflon screw-cap vials containing 5 g of granular anhydrous sodium sulfate. After 24 h, a portion of the soil or plant extracts was passed through a glass microfiber filter and stored in chromatography vials at −4 °C until analysis.

Xylem Sap and Pore Water Collection. For pot-grown plants, xylem sap was collected from vegetated pots at harvest using a previously published method that was slightly modified.[26,34] The pots were oversaturated with tap water, which was subsequently drained. After 2 h, the pots were placed at a 25° angle and the stem of the plant was cleaned of the soil particles and severed 2 cm above the graft point. The xylem sap was allowed to flow freely for 2 min, and then the severed stem was placed into a 40 mL amber glass vial and wrapped with parafilm. No water was added to the pot during the collection period. The sap volume was measured at the end of the 8 h collection period, and the xylem sap flow rate was calculated as milliliters of sap per hour. At the end of the xylem sap collection period, bulk soil pore water was collected from the pots. A 15 g soil sample from the pots was weighed into 50 mL polypropylene screw-cap tubes and centrifuged at 8000 rpm for 10 min. The supernatant was then filtered through a glass microfiber filter and collected in a chromatography vial. Xylem sap and pore water samples were stored at −4 °C until analysis.

Solid Phase Microextraction (SPME). The sap and pore water samples were subjected to a SPME method optimized by adjusting parameters that affect analyte absorption into and desorption from the fiber, including extraction time and temperature, fiber type, desorption time, and desorption temperature. A 65 μm PDMS-DVB fiber was used on xylem sap and pore water samples[26,34] (Supelco, USA). A 990 μL aliquot of either sap or pore water was transferred into a 2 mL autosampler vial containing 0.1412 ng/μL of *α*-BHC in 10 μL of methanol as an IS. The SPME method was also conducted on 10 μL of calibration standards with IS in methanol spiked into 990 μL of distilled water. The PDMS-DVB fiber was precleaned by thermal desorption and then was inserted through the septum on the vial's cap into the solution. The SPME apparatus and the vial were then placed on a thermal heating block (Heidolph, Germany) at 45 °C for 30 min. The analytes absorbed onto the fiber were desorbed at 300 °C for 5 min from the fiber directly into the injection port of the GC-ECD.

Instrument Conditions. The DDX content in the samples was determined on an Agilent 6890N gas chromatograph (GC) with a [63]Ni microelectron capture detector (μ-ECD). A HP-5MS (Supelco) column (30 m × 0.25 mm × 0.25 μm film) was used. The GC

Table 1. DDX Concentrations in Soil, Pore Water, and Xylem Sap

cultivar	DDX in soil[a] (ng/g)		pore water[b] (μg/L)	xylem sap[b] (μg/L)	BCF[c]
	field	pot			
intact plant of watermelon (nongrafted watermelon)	290 (B)[d]	1670 (A)	0.41 (A)	0.13 (A)	0.32
homografted watermelon (watermelon + watermelon)	300 (B)	1760 (A)	0.45 (A)	0.21 (A)	0.47
heterografted watermelon (squash + watermelon)	180 (AB)	1750 (A)	0.50 (A)	6.10 (B)	12.2
homografted squash (squash + squash)	150 (A)	1740 (A)	0.38 (A)	2.46 (C)	6.48
intact plant of squash (nongrafted squash)	220 (AB)	1480 (A)	0.42 (A)	3.00 (C)	7.13
nonvegetated controls (no plant)		1640 (A)	0.40 (A)		
vegetated control		ND[e]	ND	ND	

[a]Average concentration in ng/g soil on dry weight basis. DDX concentrations were calculated as the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE. [b]Average concentration in μg/L. Detection limit of the instrument for developed SPME methods is 0.012 μg/L. [c]BCF was calculated based on an average concentration of DDX in xylem sap over an average concentration of DDX in pore water. [d]Within a column, average values followed by different letters are significantly different (ANOVA with multiple comparison test). [e]ND, nondetectable.

oven was programmed as follows: initial temperature, 80 °C; held for 2 min; raised at 25 °C/min to 190 °C; raised at 5 °C/min to 280 °C; raised at 25 °C/min to 300 °C; held for 2 min. The total run time was 27.2 min. A 1 μL splitless injection was used, and the injection port and the electron capture detector were maintained at 300 °C. The carrier gas was N_2, and the makeup gas was N_2 at 60 mL/min. The retention times of α-BHC, p,p'-DDE, p,p'-DDD, and p,p'-DDT were 8.16, 13.03, 14.26, and 15.24 min, respectively.

RESULTS AND DISCUSSION

Soil DDX Concentration. Soil DDX concentrations were calculated as the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE and are given in Table 1. The DDX content in the pot soils ranged from 1480 to 1760 ng/g; these values are not significantly different (ANOVA with multiple-comparison tests). For the field experiment, the soils ranged from 150 to 300 ng/g (significantly different; see Table 1). For a pot or mound, variability among the replicate soil extractions was <8%. In the pot experiment, DDX was not detected in vegetated controls (detection limit < 0.1 ng/g). Internal standard recoveries were 94 ±7%.

Pot Study: Pore Water DDX Concentrations. The concentrations of DDX in the soil pore water are driven by partitioning between rhizosphere soil and water surrounding the root systems of the plants. Given the potentially different interactions between grafted and nongrafted root systems and the soil structure, soil pore water samples were collected. In all of the plants grown in vegetated control pots, pore water DDX concentrations were below the detection limit of the instrument (<0.012 μg/L). For nonvegetated control pots, average pore water DDX concentration was 0.40 μg/L. An average DDX concentration in pore water of vegetated pots ranged from 0.38 μg/L (homografted squash) to 0.50 μg/L (heterografted watermelon), but there were no statistically significant differences ($p > 0.05$) among the different plant types (Table 1). Because the soil DDX contents varied from 1480 to 1760 ng/g across cultivars, it is inappropriate to directly compare DDX contents in pore water without first normalization to the appropriate soil concentration. However, the normalized pore water data also showed no significant differences among the cultivars. Root exudates are known to affect soil structure, thereby influencing the subsequent release of weathered organic contaminants.[24] The exudation patterns of plant roots are known to vary significantly with genotype, nutritional status, life cycle stage, and a range of other factors. In this study, although no information on root exudation was sought, clearly these processes had little impact on the amount of contaminant present in the soil pore water. A previous grafting study using

grafted squash and cucumber in rhizotrons showed that pore water DDT concentrations ranged from 1.04 to 1.23 ng/mL among the different plant types but were not significantly different.[33]

Pot Study: Xylem Sap DDX Concentrations. Because pore water DDX concentrations are equivalent across the grafted and nongrafted plant types, differences in xylem sap contaminant concentrations may highlight unique accumulating abilities of the investigated plants. The DDX concentrations in xylem sap of vegetated control pots were below the detection limit (<0.012 μg/L), demonstrating that the potential cross-contamination and airborne deposition of POPs were negligible. As such, the differences in DDX concentration between grafted and nongrafted plant types depends exclusively on the ability of the vegetation to accumulate DDX from contaminated soil.

Xylem sap DDX concentrations are given in Table 1. The DDX concentrations increase in the following order: intact plant of watermelon (0.13 μg/L) < homografted watermelon (0.21 μg/L) < homografted squash (2.46 μg/L) < intact plant of squash (3.00 μg/L) < heterografted watermelon (6.10 μg/L). The results show that homografting (the scion and rootstock are from the same plant type) did not affect the accumulation of DDX in the xylem sap of the cultivars when compared to the respective intact plants (Table 1). For example, xylem sap concentrations of homografted and intact watermelon were 0.21 and 0.13 μg/L, respectively, and are not different ($p > 0.05$) from each other. However, both values are significantly different ($p < 0.05$) from the plants with homografted squash, intact squash, and heterografted watermelon. The highest xylem sap DDX concentrations were observed in heterografted watermelon (the scion is watermelon, and the rootstock is squash) at 6.10 μg/L. This value is significantly greater than those of all other plant types (Table 1). The concentrations of DDX in the xylem sap of intact and homografted watermelon plants were not significantly different than their respective pore water concentrations, highlighting the low contaminant accumulation historically observed with these plants. Conversely, for the plants having a squash rootstock, which includes heterogafted watermelon and homografted and intact squash, the xylem sap concentrations of DDX were 6–12 times higher than the respective pore water concentrations. Regardless of scion identity, the squash rootstock increased DDX concentrations in xylem sap relative to the pore water 12–47 times more than intact watermelon and homografted watermelon. Notably, the accumulation of DDX in xylem sap is also influenced by other unknown factors;
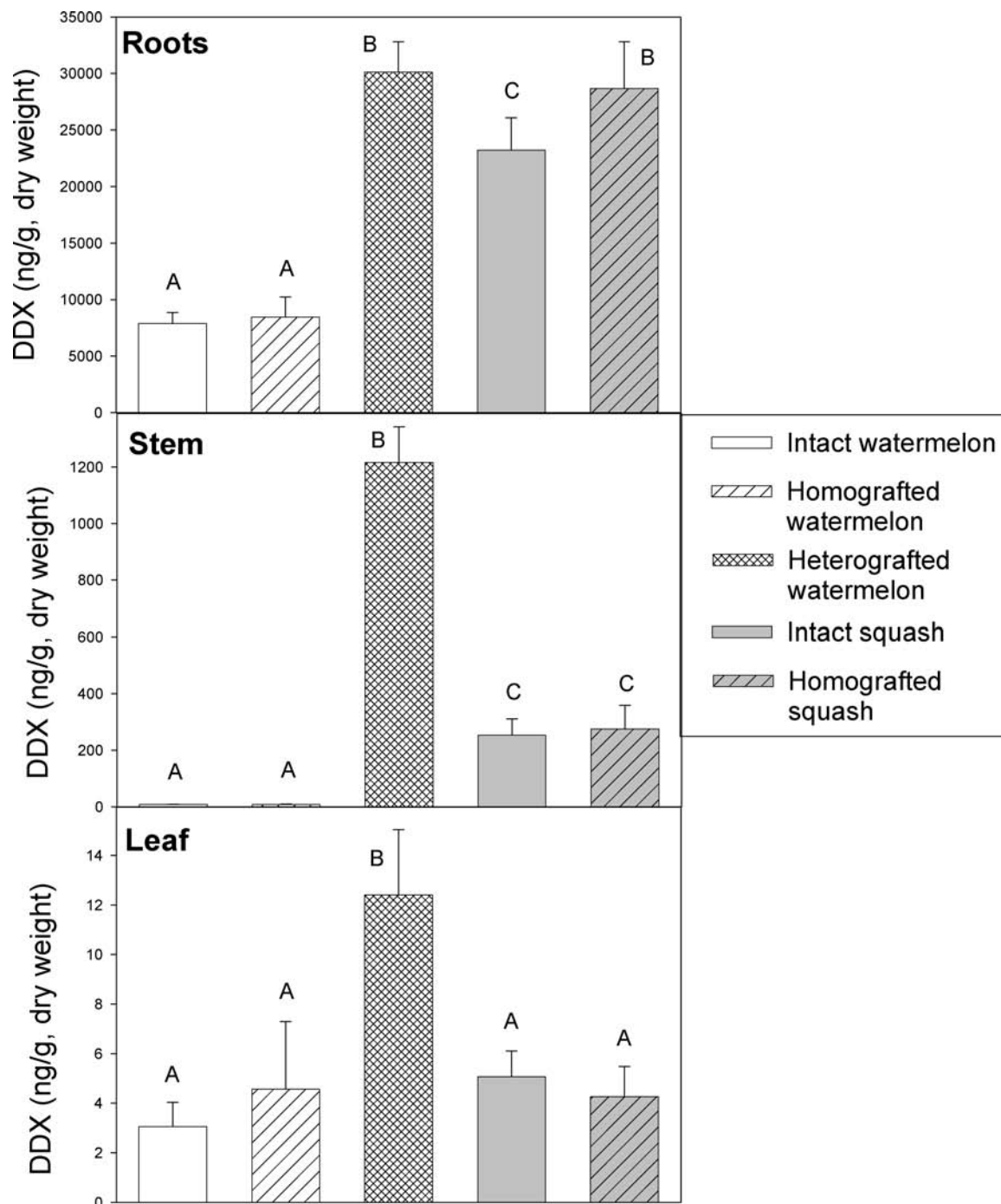
Figure 1. DDX accumulation in the roots, stems, and leaves of pot-grown plants. Error bars are the standard deviation of replicates. Within a tissue, different letters over the bars show significant differences (ANOVA with multiple-comparison tests). DDX concentrations were calculated as the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE on a dry weight basis.

thus, heterografted watermelons and intact/homografted squash plants have the same rootstocks but significantly different contaminant levels. Isleyen and Sevim,[34] who looked only at the xylem sap of grafted plants, reported sap p,p'-DDE concentrations of plants with zucchini rootstock up to 280 times greater than those of intact or homografted melon. Again highlighting the importance of plant phylogeny, Isleyen and Sevim[36] noted that although the xylem sap levels of melon grafted onto zucchini rootstock were significantly greater than those of the intact watermelon, the values were half that observed in intact or homografted zucchini sap.

Bioconcentration factors (BCFs) were calculated from DDX concentrations in xylem sap and pore water using eq 1:

$$\text{BCF} = \frac{C_{\text{xylem sap}}}{C_{\text{pore water}}} \tag{1}$$

Similar to xylem sap concentrations, BCFs (Table 1) are in the order intact plant of watermelon (0.32) < homografted watermelon (0.47) < homografted squash (6.48) < intact plant of squash (7.13) < heterografted watermelon (12.2).

Pot Study: DDX in Plant Tissues. Measurable levels of DDX, all expressed on a dry weight basis, were found in the tissues of all plant types. The highest concentrations were typically detected in the roots (Figure 1), followed by decreasing amounts in the stems, leaves, and fruit. DDX concentrations in the roots of the plants are divided into two categories; those with watermelon rootstock and those with squash rootstock. The average root DDX concentrations of intact and homografted watermelon were 7870 and 8450 ng/g, respectively. The average root contaminant levels in hetero-grafted watermelon, intact squash, and homografted squash plants were 30100, 23200, and 28700 ng/g, respectively. The contaminant concentrations of intact and homografted water-melon were not different from each other, but both values were significantly less (ANOVA with multiple comparison test; $p < 0.05$) than that of plants with the squash rootstock (squash, heterografted watermelon, and homografted squash). For heterografted watermelon and homografted squash plants, root DDX concentrations are different ($p < 0.05$) from the intact squash, but neither is different from each other. Similar to xylem sap concentrations, root DDX concentration in the heterografted watermelon was the highest among the cultivars.

The DDX concentrations in stems, leaves, and fruit were 1–3 orders of magnitude lower than that in the roots. Similar to xylem sap and root data, the highest stem DDX concentration was in the heterografted watermelon at 1220 ng/g (Figure 1). The average contaminant concentrations in the stems of intact and homografted squash plants were 253 and 275 ng/g, respectively. These values are not significantly different from each other, but notably, they values are 5 times less than that of heterografted watermelon (significantly different by an ANOVA with multiple-comparison test; $p < 0.05$). The lowest stem DDX concentration was 9 ng/g, detected in intact and homografted watermelon. The DDX concentrations in the leaves ranged from 3 to 12 ng/g, with levels in heterografted watermelon being significantly greater than in the other plant types (Figure 1). The fruit DDX concentrations of the different plant types are shown in Table 2 and increase in the following

Table 2. Fruit DDX Concentrations of Different Plant Types

	DDX[a] (ng/g)	
cultivar	pot	field
intact plant of watermelon	3.2 (A)[b]	1.0 (A)
homografted watermelon	5.7 (B)	0.53 (A)
heterografted watermelon	8.3 (C)	3.2 (B)
homografted squash	2.8 (A)	1.6 (A)
intact plant of squash	[c]	1.9 (A)

[a]Average concentration in ng/g fruit on dry weight basis. DDX concentrations were calculated as the sum of p,p′-DDT, p,p′-DDD, and p,p′-DDE. [b]Within a column, values followed by different letters are significantly different (ANOVA with multiple-comparison test). [c]No data.

order: homografted squash (2.8 ng/g) < intact plant of watermelon (3.2 ng/g) < homografted watermelon (5.7 ng/g) < heterografted watermelon (8.3 ng/g). No viable fruit could be harvested from the intact squash plants. Although the levels are quite low, the highest DDX concentration was detected in heterografted watermelon. Presumably, the highest DDX concentration detected in the aerial plant compartments in heterografted watermelon is a function of the increased contaminant burden in the root tissues of these plants, resulting in greater DDX amounts being loaded into the xylem sap for delivery to the shoot system.

BCFs are the dry weight ratios of DDX in a given tissue to that in the soil and permit direct comparison of data from plants grown in soils with different contaminant levels. BCF calculations for these data may not be critical as the DDX levels in the soils for the different treatments varied over a relatively narrow range (1480–1760 ng/g) and were not significantly different. Regardless, the BCFs for all plant types are shown in Table 3. The root BCFs of heterografted watermelon, intact squash, homografted squash, homografted watermelon, and intact watermelon were 17.1, 16.4, 16.6, 4.75, and 4.92, respectively. Similar to the non-normalized DDX concentration data, it is clear that plants with watermelon rootstocks contained significantly less contaminant ($p < 0.05$) than did those with squash rootstocks. The stem BCF values are approximately 2 orders of magnitude lower than the root data. The BCF of intact and homografted watermelon was 0.005. The BCF values for intact and homografted squash BCFs were 0.18 and 0.15, respectively. These values are significantly higher than that of watermelon but less than that of heterografted melon, which had a stem BCF of 0.69. The BCF values for leaves and fruit followed a pattern similar to that of the roots and stems; heterografted watermelon had the highest values.

Field Study: DDX in Plant Tissues. Similar to the pot study, DDX was found in all plant tissues, with the highest concentrations detected in the roots (Figure 2), followed by decreasing amounts in the stems, leaves, and fruits. The average root DDX concentrations of intact, homografted, and heterografted watermelon were 650, 1020, and 590 ng/g, respectively; the levels in intact squash and homografted squash plants were 2330 and 2430 ng/g, respectively. The contaminant concentrations of intact and homografted squash were not different from each other, but both values were significantly greater (ANOVA with multiple-comparison test; $p < 0.05$) than those of plants with the watermelon scion (intact, heterografted watermelon, and homografted watermelon). Although hetero-grafted watermelon has squash rootstock, the lowest root DDX concentration were detected in heterografted watermelon plants; the value is significantly less than ($p < 0.05$) those of intact and homografted squash plants but is not different from those plants with watermelon scion. This pattern of root DDX content differs from that in the pot study, where the heterografted watermelon had values more similar to the other plants with squash rootstock. The reasons for this difference are unknown but are not perceived as important due to the difficulty in differentiating surface adsorbed and truly accumulated POP residues in the root compartment. As such, contaminant levels in the various shoot tissues are generally regarded as more indicative of true contaminant accumulation potential.

The DDX concentrations in aerial tissues were up to 3 orders of magnitude lower than those in the roots. The highest stem DDX concentration was in heterografted watermelon at 244 ng/g; this value was not significantly different from that of intact squash plants (227 ng/g). The stems of homografted squash contained 140 ng/g, a value significantly less than those of heterografted watermelon and intact squash (ANOVA with multiple-comparison test; $p < 0.05$). The stem DDX contents in homografted and intact watermelon plants were 9 and 13 ng/g, respectively. The leaf DDX concentrations ranged from 4 to 176 ng/g, with the level in homografted squash being significantly greater than in the other plant types. DDX

Table 3. Root, Stem, Leaf, and Fruit DDX Bioconcentration Factors (BCF) for Pot- and Field-Grown Plants

		BCFs[a]			
		roots	shoots	leaves	fruit
intact plant of watermelon	pot	4.92 (A)[b]	0.005 (A)	0.002 (A)	0.002 (A)
	field	2.67 (a)	0.055 (a)	0.021 (a)	0.004 (a)
homografted watermelon	pot	4.75 (A)	0.005 (A)	0.003 (A)	0.003 (A)
	field	4.07 (a)	0.048 (a)	0.015 (a)	0.002 (a)
heterografted watermelon	pot	17.1 (B)	0.69 (B)	0.007 (B)	0.006 (B)
	field	3.07 (a)	2.10 (c)	0.029 (a)	0.018 (b)
homografted squash	pot	16.6 (B)	0.152 (C)	0.003 (A)	0.002 (A)
	field	16.95 (c)	2.35 (c)	1.30 (b)	0.010 (a)
intact plant of squash	pot	16.4 (B)	0.176 (C)	0.003 (A)	
	field	9.74 (b)	2.12 (c)	0.75 (c)	0.009 (a)

[a]BCFs are dry weight ratios of DDX tissue content to that in the soil. [b]Within a column, values followed by different letters are significantly different (ANOVA with multiple-comparison test).

concentration in leaves of intact squash plants was 113 ng/g; 4−5 ng/g was found in plants with watermelon scions. The fruit DDX concentrations followed a pattern similar to the pot study: homografted watermelon (0.53 ng/g) < intact plant of watermelon (1.02 ng/g) < intact plant of squash (1.57 ng/g) < homografted squash (1.92 ng/g) < heterografted watermelon (3.15 ng/g).

The tissue BCFs for all plant types are shown in Table 3. The root BCFs decrease in the following order: homografted squash (16.95) > intact plant of squash (9.74) > homografted watermelon (4.07) > heterografted watermelon (3.07) > intact plant of watermelon (2.67). Similar to root DDX concentration data, it is clearly seen that plants with watermelon scions contained significantly less contaminant ($p < 0.05$) than did those with squash scions. Although heterografted watermelon root BCF data were significantly less than in those plants with squash rootstocks, the stem and fruit BCF values were significantly greater than those of both homografted and intact watermelon plants.

The data from the pot and field studies clearly show the significance of rootstock in mediating the uptake of weathered organic contaminants from soil. Regardless of shoot type, the root BCF values for DDX of plants with squash rootstocks were 4 times higher than those with watermelon rootstocks. These differences were exacerbated in the stem compartment, where stem BCFs of plants with the squash rootstock were 30−138 times greater than those with watermelon rootstock. There is significant evidence in the literature demonstrating that select cucurbits have the unique ability to accumulate weathered POPs from soil.[23,26,31,36,37] In fact, several groups are actively pursuing implementation of zucchini and related cucurbits as a phytoremediation strategy for POP-contaminated soils.[23,26,31,36,37]

The mechanism by which certain cucurbita mediate POP accumulation remains unknown. White[30] hybridized DDE accumulating and nonaccumulating squash and zucchini. When the resulting F1 and F1 backcrossed hybrids were grown in DDE-contaminated soil under field conditions, the inheritance pattern of the POP uptake ability followed classical Mendelian genetics, thereby suggesting single-locus control. Berger et al.[38] exposed chlordane-accumulating zucchini to the contaminant under hydroponic conditions to assess the possible role of

aquaporins in contaminant uptake. Hydrogen peroxide deactivates aquaporins, and the effect is reversible. Upon exposure to hydrogen peroxide, the uptake of chlordane by zucchini decreased significantly (12−60%) but upon peroxide removal, complete uptake ability was recovered. Separately, Chikkara et al.[39] exposed DDE-accumulating and -non-accumulating cucurbits to the contaminant under hydroponic conditions and then used subtractive hybridization techniques to evaluate differentially expressed genes. The authors observed that upon DDE exposure, contaminant-accumulating squash exhibited significant up-regulation of a gene with high sequence homology to a phloem loading protein (PP1) but that expression of the identical gene in the nonaccumulating squash was unaffected. In fact, upon DDE exposure, over two dozen genes were up-regulated in the contaminant-accumulating plants, including several with unknown function. Clearly, elucidation of the precise mechanism by which these plants extract and translocate weathered POPs has significance for both remediation and food safety concerns and, as such, remains a topic of ongoing study.

Although the increase in DDX accumulation by watermelon grafted onto *Cucurbita* rootstock was significant, the levels observed in the edible fruit were low. For example, the tolerance levels for organochlorine pesticides in produce in the United States (Connecticut) and Turkey are 100 and 50 ng/g, respectively; the levels observed in the grafted fruit are 6−12 times below those limits. However, White[40] observed that the DDE concentration in the peel of the zucchini fruit was twice as high as the whole fruit value and nearly 6 times higher than the concentration in the flesh alone. Clearly, the fate and disposition of contaminant within the fruit tissue are dynamic processes, likely dependent on the rate of fruit formation, overall size, and ratio of high-lipid components (peel, seeds) to high-water components (flesh). The transport of POPs such as DDX into the fruit will also be affected by plant genotype. White et al.[30] showed that although the average stem to soil BCF for DDE-accumulating zucchini was 5.4 (10 times greater than that of nonaccumulating squash), the range in BCF values among the zucchini was from 2 to 9. The reason for this variability in contaminant uptake potential remains unknown, as do the impacts of soil characteristics, co-contaminants, and other climatic effects on this phenomenon. This lack of
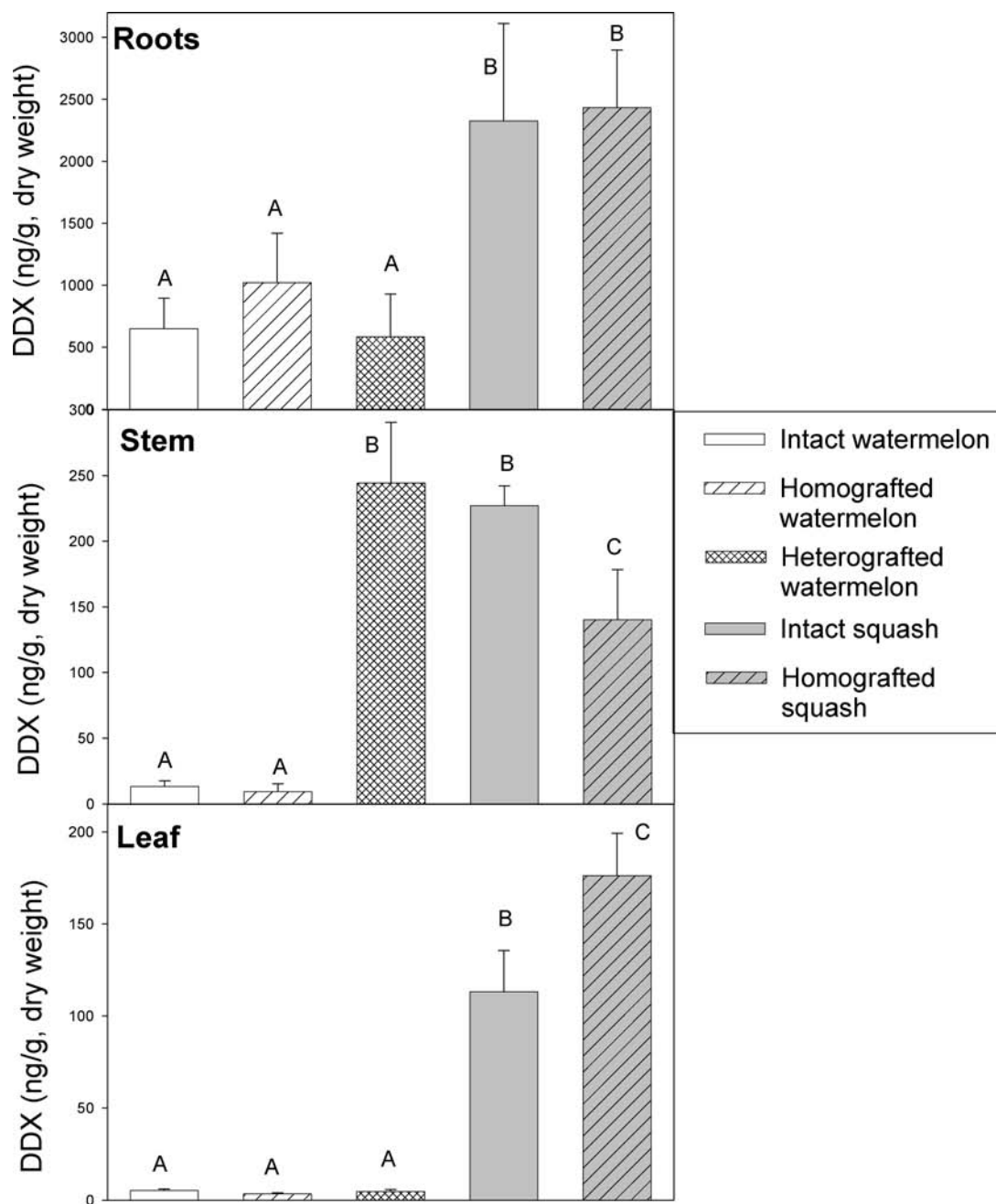
Figure 2. DDX accumulation in the roots, stems, and leaves of field-grown plants. Error bars are the standard deviation of replicates. Within a tissue, different letters over the bars show significant differences (ANOVA with multiple-comparison tests). DDX concentrations were calculated as the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE on a dry weight basis.

knowledge may be of special concern in Turkey, where although DDX has been detected in various commodities (honey,[41] mussels,[42] and butter[43]) and other matrices (water,[44] sediment,[45] and adipose tissue[46]), very little is known about the extent of organochlorine contamination in agricultural areas. Consequently, although relatively low DDX concentrations were observed in the fruit from the current study, caution is clearly warranted when grafting melons and other food crops onto *Cucurbita* rootstocks.

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