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Inheritance of *p,p′*-DDE Phytoextraction Ability in Hybridized *Cucurbita pepo* Cultivars

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Cucurbita pepo ssp *pepo* (zucchini) has been shown to uniquely phytoextract percent level amounts of dichlorodiphenyldichloroethylene (DDE) and other organic contaminants from soil. Since *C. pepo* ssp *ovifera* (squash) does not have this ability, a three-year field trial was conducted to follow the inheritance pattern of DDE accumulation for cross pollinated *C. pepo* cultivars. Parental zucchini and squash cultivars (3 each) had stem-to-soil bioconcentration factors (BCF, contaminant ratio of stem to soil) of 16 and 1.7, respectively, and phytoextracted 1.8 and 0.18% of the DDE from soil. The 18 possible first filial (F1) hybrids of zucchini and squash accumulated significantly different DDE levels than the respective parents. The zucchini F1 hybrid (zucchini pollinated with squash) stem BCFs and percent phytoextraction values were 10 and 0.96, respectively, or 36% and 47% *less* than the parental zucchini. The squash F1 hybrid (squash pollinated with zucchini) stem BCFs and percent phytoextraction values were 8.3 and 0.68, respectively, or 490% and 370% greater than the parental squash. When backcrossed (BC) with the original parent, the nine zucchini F1 BC cultivars did not regain the capability to take up DDE; stem BCFs and percent phytoextraction values were equivalent to those of the F1 generation. However, the nine squash F1 BC cultivars lost much of the DDE uptake capability of the F1 generation; stem BCFs and percent phytoextraction values were intermediate but closer to those of the parental squash. The inheritance patterns suggest single locus control for persistent organic pollutant (POP) uptake ability in *C. pepo* ssp *pepo*.

Introduction

Chlorinated hydrocarbons such as dichlorodiphenyltrichloroethane (DDT), chlordane, and polychlorinated biphenyls (PCBs) belong to a class of chemicals known as persistent organic pollutants (POPs) (*1, 2*). Persistent organic pollutants are of significant environmental concern due to molecular recalcitrance, toxicity, and global distribution. The half-lives of DDT and other POPs in soil are measured in years (*3*) and disappearance curves frequently plateau, resulting in a highly resistant residual fraction of contaminant (*4*). These chemicals are also highly hydrophobic; with log K_{ow} (octanol−water partition coefficients) values exceeding 6.0. Consequently, POPs bind strongly to soil organic matter and have been shown to become progressively sequestered with time (*4*). Due to contaminant hydrophobicity, POPs readily bioaccumulate in the lipids of exposed organisms (*5*). Subsequent biomagnification within food chains and significant negative impact on top trophic level organisms may then occur (*6, 7*). Most POPs are semivolatile and are readily transported globally via atmospheric processes (*8*). Organochlorines such as PCBs and DDT are routinely detected in Arctic and Antarctic biota (*9*−*11*).

Phytoremediation is the in situ use of vegetation to remove contaminants from soils, and several possible mechanisms exist for organic pollutants (*12*−*14*). Organic molecules such as chlorinated solvents (trichloroethelyene [TCE]), certain pesticides (atrazine), low molecular weight polycyclic aromatic hydrocarbons (PAHs), and lesser chlorinated PCBs may be degraded by root-exuded enzymes or by the exudates-stimulated microbial community in the rhizosphere (*13, 14*). Chemicals with high to moderate water solubility (octanol−water partition coefficient [Log K_{ow}] values −2.0 to 3.5) may enter the root with the transpiration stream and be subject to physiological processes such as degradation and evapotranspiration (*13, 15*). Hydrophobic, degradation-resistant contaminants such as POPs are not thought to be amenable to phytoremediation. However, *Cucurbita pepo* ssp *pepo* (zucchini, pumpkin) has demonstrated a unique ability to extract and translocate highly weathered hydrophobic pollutants from soil. Hülster et al. (*16*) first reported soil-to-plant uptake of dioxin into *Cucurbita* species; subsequent work has shown that *Cucurbita pepo* ssp *pepo* accumulates significant amounts of weathered chlordane, DDT/DDE/DDD, and PCBs from soil (*17*−*20*). Although the magnitude of pollutant removal varies with chemical properties and plant phylogeny, the contaminants are largely accumulated as parent compound (*19*). Persistent organic pollutant concentrations in *C. pepo* ssp *pepo* stems and roots may be 5−30 times greater than present in the soil, extracting up to 5% of the contaminant burden in a three month period (*21*).

Previous work has shown that the accumulation of weathered POPs also varies significantly at the subspecies level; *C. pepo* ssp *pepo* (zucchini) accumulates up to an order of magnitude more contaminant than does *C. pepo* ssp *ovifera* (squash) (*17*). In an effort to elucidate the underlying mechanisms responsible for contaminant uptake by *C. pepo* ssp *pepo* (zucchini), this subspecies level difference was exploited through traditional breeding and hybridization techniques. In a multiyear field study, three zucchini and three squash cultivars were grown in a DDE contaminated soil and all possible (*18*) first filial (F1) hybrids and F1 backcrosses (*18*) were created. Inheritance of the DDE-accumulating capability through the parental (P), F1, and F1 Backcross (BC) cultivars was analyzed to characterize the multigenerational expression pattern of the phytoextraction capability.

Experimental Section

Experimental Field Site. In three consecutive years, experimental plots were established at the Connecticut Agricultural Experiment Station's Lockwood Farm (Hamden, CT) in areas known to be contaminated with 60−500 ng/g weathered *p,p′*-DDE (*17*). The DDE is from historical DDT applications and the residues have been present for several decades. The soil is a fine sandy loam (56% sand, 36% silt, 8% clay) with 1.4% organic carbon and a pH of 6.7. The site was covered with 1000 m^2 of black polyethylene plastic sheeting, and 30-cm^2 squares were cut at 3.0-m^2 intervals. Each exposed area of soil served as a single replicate mound of vegetation (four plants per replicate). Individual parental and hybrid cultivars were grown in triplicate mounds according to a randomized block design. The parental generation consisted of 18 separate mounds; the hybrid generations consisted of 54 mounds.

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Parental and Hybrid Cultivars. Three cultivars of *Cucurbita pepo* ssp *pepo* ("Raven," "Goldrush," and "Costata Romanesco") and three cultivars of *Cucurbita pepo* ssp *ovifera* ("Zephyr," "Patty Pan," and "Yellow Crookneck") were purchased from Johnny's Selected Seeds (Albion, ME). These seeds served as the parental (P) generation and were germinated in the laboratory with germination paper. The seedlings were planted at the field site in June 2007; triplicate mounds of each cultivar were planted in a randomized block design (18 mounds total). All replicate plots were weeded as necessary during the first month of growth; after that, large plant size prohibited unwanted vegetation. On a daily basis, male and female flowers on the six parental cultivars were inspected in the afternoon; flowers that were likely to open the next morning were covered with paper bags fitted with a rubber band so as to exclude pollinators and preserve pollen. After the open flowers were exposed the following morning, stamens were removed from male flowers of predetermined cultivars and were used to pollinate the protected female flower of cultivars of the opposing subspecies. All 18 possible first filial (F1) hybrids were created; each cultivar pollinated with the three cultivars of the opposing subspecies. From this point forward, a hybrid zucchini describes a plant where a female zucchini flower was pollinated by a squash stamen. Similarly, a hybrid squash is a plant where a female squash flower was pollinated by a male zucchini. To acquire viable seeds from the F1 hybrids, fruit were left on the plant for several months. The seeds were harvested, washed, air-dried, and soaked in 10% sodium hypochlorite. After rinsing with reverse osmosis (RO) water, the seeds were air-dried and stored at room temperature (20−25 °C).

In June 2008, triplicate mounds of all 18 F1 hybrids were planted at Lockwood farm. The plants were cultivated as above. During the 2008 growing season, the F1 hybrids were backcrossed with the appropriate parent from the P generation. Manual pollination, seed harvest, and storage were conducted as described above. In June 2009, triplicate mounds of all 18 backcrossed F1 (F1 BC) hybrids were planted at Lockwood farm. During each season, the remainder of the fruit (those not used for hybridization and seed collection) was harvested at market-size throughout the summer. Destructive harvest of the plants in the P, F1, and F1 BC generations began in mid-August of each year.

Soil Extraction. The *p,p′*-DDE concentration in soil samples from each replicate mound for all cultivars and hybrids was determined. In each growing season and prior to planting, four soil cores (2.5 cm diameter, 6−10 cm depth) were collected from the corners of each individual replicate mound and were composited in whirlpak bags. The soils were air-dried, sieved to 2 mm, and 3-g portions were weighed into 20-mL vials with Teflon-lined caps. The vials were amended with 15 mL of hexanes and 1 μg of *o,p*-DDE (in 100 μL of 2,2,4-trimethylpentane) as an internal standard. The vials were capped and heated at 65 °C for 4 h. Particulates were removed from the solvent by passing 1 mL of the supernatant through a glass micro fiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) and the eluent was collected in a chromatography vial. Soil extracts were stored at −4 °C prior to analysis. The moisture content of soil from each replicate mound was determined by heating 3.0-g portions at 100 °C for 24 h.

Vegetation Extraction. At destructive harvest, plant stems were severed at ground level and a 1.0 m × 1.0 m × 0.25 m volume of soil containing the roots of individual mounds of vegetation was excavated. The wet mass of fruit, leaf, stem, and root tissues was determined. The vegetation was separated by cultivar and tissue type, and all samples were rinsed thoroughly with tap water. The vegetation was chopped finely and samples were transferred to whirlpak bags for storage at −4 °C. Samples were thawed and individual 25-g (wet weight) samples of plant material were mixed in an explosion-proof blender with 25 mL of 2-propanol (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) amended with 1 μg of *o,p*-DDE as an internal standard (in 100 μL of 2,2,4-trimethylpentane) (*17*). After blending on medium speed for 30 s, 50 mL of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) was added and the sample was blended for 5 min. The extracts were filtered through a glass-wool lined funnel and the eluent was collected in a 500-mL glass separatory funnel with Teflon stopcock. After draining for 15 min, the ether was subsequently rinsed three times at 20 min intervals with RO water and a saturated sodium sulfate solution. The petroleum ether was drained into an amber vial containing 10 g of anhydrous sodium sulfate. A 1-ml portion of the extract was removed for cleanup on 4-mL florisil cartridges (200 mg; Alltech, Deerfield, IL) that had been preconditioned with 5 mL of petroleum ether. The 1-mL vegetation extract was loaded onto the cartridge, which was then eluted with 6 mL of 6% diethyl ether in petroleum ether. The extract was collected in an 8-mL vial and the volume of each extract was reduced to 1 mL under nitrogen on a heating block at 35 °C. The samples were then transferred to chromatography vials for storage at −4 °C until analysis.

Chemical Analysis. Crystalline *p,p′*-DDE and *o,p′*-DDE were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD). A 1000 mg/L stock of *p,p′*-DDE in 2,2,4-trimethylpentane was created and diluted to calibration standards of 10, 25, 50, 100, 250, and 500 ng/mL. One hundred ng/mL *o,p*-DDE in 2,2,4-trimethylpentane was added to each calibration level as an internal standard. The concentration of *p,p′*-DDE in the soil and vegetation samples was determined on an Agilent (Avondale, PA) 6890 gas chromatograph (GC) with a ^{63}Ni microelectron capture detector (ECD) by internal standard calibration. An Equity-5 (Supelco, Bellefonte, PA, 28089-U) GC column (30 m × 0.25 mm ×0.25 μm) was used and the oven program was as follows: 75 °C initial temperature ramped at 20 °C/min to 217 °C, then ramped at 0.25 °C/min to 219 °C, then ramped at 15 °C/min to 280 °C with a final hold time of 2.0 min. The injection port was maintained at 250 °C and a 2-μL splitless injection was used. The carrier gas over the column was He. The ECD was maintained at 300 °C and the makeup gas to the detector was 5% CH_4 in Ar at 60 mL/min.

Statistical Analysis. Four replicates of soil from each replicate mound of vegetation were extracted. Triplicate mounds of each cultivar or hybrid were grown. For each individual replicate mound of vegetation, individual leaf and fruit tissues were extracted once; for roots and stems, the tissues were subsampled and extracted in duplicate. Recovery values of spiked internal standard were 98% (±7.5). All values of DDE contamination were expressed on a dry weight basis. The following indices were determined: tissue (root, stem, leaf, or fruit) bioconcentration factors (BCFs), translocation factor (TF), biomass, and percentage of DDE phytoextracted. Bioconcentration factors are the dry weight ratios of DDE concentration in the plant tissue to that in the soil, and the TF is the stem BCF divided by the root BCF. To determine percent phytoextraction, the absolute DDE mass in the plant is obtained by multiplying concentration by dry biomass. The soil DDE mass is determined similarly, with the volume of soil impacted by the root being estimated at 1.0 m by 1.0 m by 0.25 m, yielding an estimated of 289 kg (field moisture) per replicate mound of vegetation. The DDE mass in the plant is then expressed as a percentage of that in the soil compartment. An analysis of variance (ANOVA) followed by a Dunns or Student−Newman−Keuls Multiple Comparison Test was used to determine the statistical significance of differences among true replicates between these parameters across P, F1, and F1 BC generations and cultivars.

TABLE 1. Biomass and DDE Accumulation Values for Six Parental Generation (P) Cultivars

plant type	root mass (g)	stem mass (g)	leaf mass (g)	fruit mass (g)	% phytoextracted	root BCF	stem BCF	leaf BCF	fruit BCF
				zucchini					
Goldrush	134	4580	2330	16000	1.57	16.8	11.1	0.228	0.249
Costata	87.1	4710	1390	13600	1.85	23.0	11.3	0.163	0.417
Raven	85.3	2010	925	9870	1.66	19.9	23.7	0.205	0.511
average	112 A[a]	3750 A	1660 A	13400 A	1.82 A	19.1 A	15.7 A	0.206 A	0.373 A
				squash					
Zephyr	74.3	3640	2070	20400	0.164	6.65	1.63	0.140	0.041
Crook	66.5	1950	1070	4230	0.207	5.11	2.23	0.240	0.506
Patty	66.8	2540	1240	11900	0.112	3.54	0.949	0.150	0.057
average	73 A	2520 A	1350 A	10400 A	0.183 B	4.95 B	1.71 B	0.185 A	0.253 A

[a] Within a column (across subspecies), average values followed by different letters are significantly different (ANOVA with multiple comparison test).

Results and Discussion

Soil DDE Concentration. The average soil DDE content for the P, F1, and F1 BC growing seasons was 211 ng/g (±107; 84−490 ng/g) (dry weight), 186 ng/g (±80.8; 74−450 ng/g), and 182 ng/g (±105; 50−430 ng/g), respectively. However, for a given mound, variability among the replicate soil extractions was consistently less than 10%. These are indicative of historical contamination with DDT (*17, 22*) and are in agreement with previous findings indicating that DDT dechlorination to DDE can be extensive in temperate soils (*23, 24*).

Parental Generation. The average total wet biomass of the zucchini and squash cultivars was 18 900 g and 14 300 g, respectively (not significantly different). In addition, the masses of individual tissue compartments were not significantly different from each other (Table 1). The tissues of all 6 cultivars contained measurable DDE. The highest concentrations were typically detected in the roots, followed by decreasing amounts in the stems, leaves, and fruit. As expected, the two different subspecies of *C. pepo* contained significantly different amounts of DDE. The highest root and stem DDE concentrations in zucchini (*C. pepo* ssp *pepo* cv Goldrush) were 6740 and 5340 ng/g, respectively; the highest root and stem contaminant levels in squash (*C. pepo* ssp *ovifera* cv Zephyr) were 2624 and 444 ng/g, respectively. For both subspecies, the contaminant concentrations in the leaves and fruit did not differ significantly and were typically 1−3 orders of magnitude lower than the roots and stems.

Bioconcentration factors or BCFs are dry weight ratios of DDE tissue content to that in the soil and permit direct comparison of contaminant content in plants grown in soils with different contaminant levels. The data for individual cultivars are shown in Table 1. The root and stem BCFs of zucchini were significantly greater that those of squash. The average zucchini root and stem BCFs were 19.1 and 15.7, respectively; the squash root and stem BCFs were 4.95 and 1.71, respectively. The translocation factor (TF), defined as the stem BCF/root BCF, also differed significantly among the two subspecies; the average zucchini and squash TF values were 0.83 and 0.34, respectively (significantly different, $p < 0.05$). The leaf and fruit BCFs ranged from 0.19 to 0.37 and did not differ among the subspecies. Similarly, the total amount of contaminant phytoextracted by each parental subspecies differed significantly ($p < 0.05$). Zucchini removed an average 1.8% of the DDE with a maximal phytoextraction of 3.7% (*C. pepo* ssp *pepo* cv Costata Romanesco). Squash plants removed an average 0.18% of the DDE with a maximal phytoextraction of 0.34% (*C. pepo* ssp *ovifera* cv Yellow Crookneck). These findings agree with our previous work and highlight the unique ability of *C. pepo* ssp *pepo* to accumulate weathered POPs from soil (*17, 20*).

First Filial Generation (F1). The uptake and translocation of weathered DDE was investigated in all 18 possible F1 hybrids created from the original 6 parental cultivars. The average total wet biomass of the zucchini and squash hybrids was 15 800 g and 11 300 g, respectively. These masses are significantly different ($p < 0.05$) from each other but neither is different from the respective parents. In addition, the individual root, leaf, and fruit masses of the squash F1 hybrids are significantly less than those of the zucchini F1 hybrids (SI Table 1). Similar to the parental generation, the highest DDE concentrations were found in the roots of both types of hybrids, with decreasing amounts in the aerial tissues. The highest root and stem DDE concentrations in a zucchini hybrid (Goldrush × [yellow crook]) were 7010 and 5600 ng/g, respectively; the highest root and stem contaminant levels in a squash hybrid (Zephyr × [raven]) were 5820 and 2640 ng/g, respectively. Again, similar to the P generation, DDE content in the leaves and fruit were typically less than 100 ng/g.

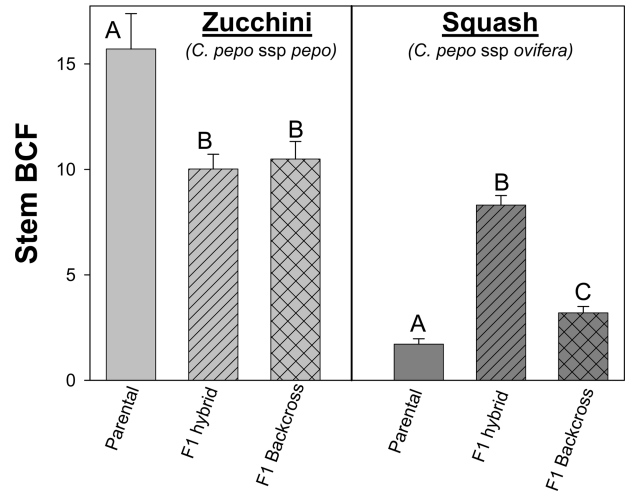

FIGURE 1. Stem-to-soil DDE bioconcentration factors (BCFs) for parental (P), first filial (F1) zucchini/squash hybrids, and F1 backcrosses of zucchini and squash. Within a subspecies, bars (standard error) with different letters are significantly different (one-way ANOVA followed by Student−Newman−Keuls multiple comparison test). For parental generation, $n = 6$. For F1 generations, $n = 18$.

Unlike the P generation, the average root and stem BCFs of zucchini hybrids and squash hybrids were not significantly different. The average zucchini hybrid root and stem BCFs were 16.4 and 10.0, respectively; the squash hybrid root and stem BCFs were 15.5 and 8.31, respectively (Figures 1 and 2). The root BCFs for zucchini parents and F1 hybrids are not significantly different; however, the stem BCFs for the F1 generation are *reduced* by 36% relative to the parental zucchini (significant at $p < 0.05$). Conversely, the squash F1

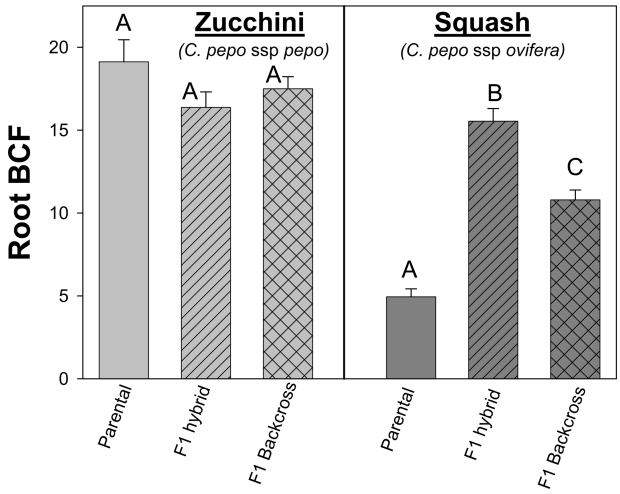

FIGURE 2. Root-to-soil DDE bioconcentration factors (BCFs) for parental (P), first filial (F1) zucchini/squash hybrids, and F1 backcrosses of zucchini and squash. Within a subspecies, bars (standard error) with different letters are significantly different (one-way ANOVA followed by Student−Newman−Keuls multiple comparison test). For parental generation, $n = 6$. For F1 generations, $n = 18$.

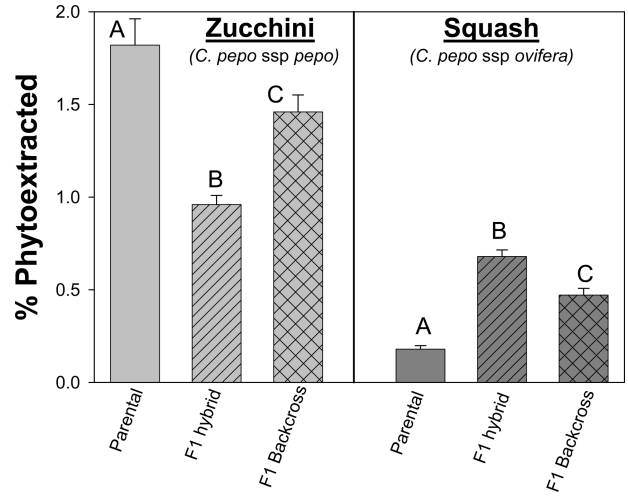

FIGURE 3. Percent of DDE phytoextracted by parental (P), first filial (F1) zucchini/squash hybrids, and F1 backcrosses of zucchini and squash. Within a subspecies, bars (standard error) with different letters are significantly different (one-way ANOVA followed by Student−Newman−Keuls multiple comparison test). For parental generation, $n = 6$. For F1 generations, $n = 18$.

hybrid stem and root BCFs were significantly *greater* than those of the squash parental generation. The TF values for the zucchini and squash hybrids were 0.65 and 0.58, respectively; these factors were not significantly different from each other or from the parental zucchini but all are significantly greater than the parental squash ($p < 0.05$). The leaf BCFs for the hybrid zucchini and squash were 0.16 and 0.17, respectively; these values were not significantly different from each other or from the P generation. Conversely, the fruit BCFs of the zucchini and squash hybrids were 0.57 and 0.49, respectively; these factors were not significantly different from each other or from the parental zucchini but all are significantly greater than the parental squash ($p < 0.05$). The zucchini and squash hybrids phytoextracted 0.96 and 0.68% of the weathered DDE. These DDE phytoextraction values are not only significantly different from each other but both values are also significantly different from the respective parents ($p < 0.05$)(Figure 3). Data for the individual 18 F1 hybrid cultivars can be found in SI Table 1.

First Filial Backcrosses (F1 BC). The DDE accumulation of all 18 possible F1 backcrosses (BC) created from the F1 hybrids and parents was determined. The average total wet biomass of the zucchini and squash F1 BC cultivars was 17 100 g and 15 500 g, respectively. These masses are not significantly different from each other, the respective F1 hybrids, or parents. However, some statistically significant differences exist in the masses of individual tissues. For example, the leaf and stem mass of the zucchini hybrid BC were significantly greater than the corresponding F1 hybrids ($p < 0.05$). Also, the stem and root mass of the squash F1 BC was significantly greater than both the squash parental and F1 generations ($p < 0.05$). Similar to the parental and hybrid generations, contaminant concentrations were highest in the roots and decreased throughout the shoots. The highest root and stem DDE concentrations in a zucchini F1 BC were 6300 ng/g (Goldrush × [patty] BC) and 3700 ng/g (Raven × [crook] BC), respectively; the highest root and stem DDE concentrations in a squash F1 BC were 5200 ng/g (Goldrush × [patty] BC) and 1500 ng/g (Raven × [crook] BC), respectively. Again, similar to the P and F1 hybrid generations, DDE content in the leaves and fruit were typically less than 100 ng/g.

Similar to the P generation but unlike the F1 hybrids, the average root and stem BCFs of zucchini and squash hybrid BC were significantly different. The average zucchini hybrid BC root and stem BCFs were 17.5 and 10.5, respectively; the squash hybrid BC root and stem BCFs were 10.8 and 3.19, respectively (significantly different by tissue across subspecies at $p < 0.05$). Within the three generations of zucchini, the root BCFs are equivalent but for stem BCFs, the hybrids and BC hybrids are statistically equivalent but significantly *less* than the value for the parental generation (Figures 1 and 2). Conversely, for the three squash generations, the root and stem BCFs were all statistically different from each other. The parental generation is lowest, with the F1 hybrid levels being greatest and the backcrosses having intermediate value (Figures 1 and 2).

The TF values for the zucchini and squash hybrid BC were 0.59 and 0.30, respectively; these values were significantly different from each other ($p < 0.05$). Across all three zucchini generations, the patterns of statistically significant differences in TF match those of the stem BCFs (P > F1= F1 BC) but for the squash, the hybrids are significantly greater than the parents or backcrosses (F1 > P = F1 BC). The leaf BCFs for the hybrid BC zucchini and squash were 0.40 and 0.19; these values are not significantly different from each other or from the other generations. Conversely, the fruit BCFs of the zucchini and squash hybrid BC were 0.55 and 0.18, respectively; these values are significantly different from each other. For the zucchini, the fruit BCFs are statistically equivalent across all three generations. For the squash, the F1 hybrid fruit BCFs are significantly greater than either P or F1 BC generations (F1 > P = F1 BC). The zucchini and squash hybrid backcrosses phytoextracted 1.5 and 0.47% of the weathered DDE. These DDE phytoextraction values are not only significantly different from each other but are also significantly different from the respective parental and F1 hybrid generations ($p < 0.05$)(Figure 3). Data for all 18 F1 hybrid BC cultivars can be found in SI Table 2.

Inheritance Patterns. The inheritance patterns of DDE phytoextraction capability of *C. pepo* parental, F1 hybrid, and F1 backcrossed hybrid zucchini and squash cultivars were evaluated. The stem BCFs are typically the most commonly used parameter for differentiating the POP uptake abilities of *C. pepo* cultivars and other plants (*17, 20, 25, 26*). However, the inheritance pattern for the squash is the same across the three generations when measured by stem BCFs, root BCFs, and total % phytoextraction (Figure 1−3). This pattern of high uptake ability with the F1 hybrid and

intermediate ability for the backcrossed squash cultivars clearly suggests single locus control with a possible gene dosage effect (*27*). The data for zucchini are mixed; the root BCFs are statistically equivalent across generations and the stem BCFs for the F1 hybrids and backcrosses are significantly less than the parents. This in part may be due to the large variability in the data. However, the percent phytoextraction values across the three zucchini generations are consistent with the squash data (Figure 3); the F1 backcrossed cultivars are intermediate relative to the parental and F1 generations and suggest molecular control at a single or dominant locus.

Not surprisingly, little is known about the molecular basis of the phytoextraction abilities of *C. pepo* ssp *pepo*. In a recent hydroponic study (*26*), PCR select Suppression Subtraction Hybridization followed by semiquantitative RT-PCR was used to identify differentially expressed genes in DDE-exposed zucchini relative to DDE-exposed squash or nonexposed zucchini. In a cDNA library created across subspecies (zucchini relative to squash), 34 of 40 shoot cDNA sequences had partial homology to phloem filament protein 1 (PP1). Given the unique nature of the root-to-shoot translocation of POPs in *C. pepo* ssp *pepo*, this finding is of great interest and is the topic of ongoing investigation. In the root tissue, two of 6 cDNAs were homologous to cytochrome P450 like proteins, and another matched a putative senescence associated protein. A second library was created from DDE-exposed and nonexposed zucchini. Of 22 differentially expressed genes, 14 cDNAs had homology with genes involved in abiotic stresses, signaling, photosynthesis and lipid metabolism. The remainder encoded novel proteins whose role in DDE accumulation and metabolism is unknown (*26*).

Soils contaminated with persistent organic pollutants such as DDT and metabolites is ubiquitous but remedial options are limited and expensive. *Cucurbita pepo* ssp *pepo* has a unique ability to phytoextract percent level amounts of weathered POPs from soil and accumulate much of that chemical in aerial tissues. Current research on clarifying cellular mechanisms and governing molecular controls for POP phytoextraction ability will enable remedial optimization in the field as well as potential gene transfer to perennial noncrop species.

Acknowledgments

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Supporting Information Available

This material is available free of charge via the Internet at http://pubs.acs.org.

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