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INHERITANCE PROFILE OF WEATHERED CHLORDANE AND *P,P′*-DDTS ACCUMULATION BY *CUCURBITA PEPO* HYBRIDS

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Cucurbita pepo ***ssp*** **pepo** ***(zucchini) accumulates significant levels of persistent organic pollutants in its roots, followed by unexpectedly high contaminant translocation to the stems. Most other plant species, including the closely related*** **C. pepo** ***ssp*** **ovifera** ***(squash), do not have this ability. To investigate the mechanism of contaminant accumulation, two cultivars each of parental zucchini and squash, as well as previously created first filial (F1) hybrids and F1 backcrosses (BC) of those parental cultivars, were grown under field conditions in a soil contaminated with weathered chlordane (2.29 μg/g) and DDX residues (0.30 μg/g; sum of DDT, DDE, DDD). The parental zucchini had stem-to-soil bioconcentration factors (BCF, contaminant ratio of stem to soil) for chlordane and DDX of 6.23 and 3.10; these values were 2.2 and 3.7 times greater than the squash, respectively. Chlordane and DDX translocation factors, the ratio of contaminant content in the stems to that in the root, were 2.1 and 3.2 times greater for zucchini than for squash. The parental zucchini and squash also differed significantly in chlordane component ratios (relative amounts of trans-nonachlor [TN], cis-chlordane [CC], trans-chlordane [TC]) and enantiomer fractions for the chiral CC and TC. Hybridization of the parental squash and zucchini resulted in significant differences in contaminant uptake. For both the three separate component ratios (CR) and two sets of enantiomer fraction (EF) values, subspecies specific differences in the parental generation became statistically equivalent in the F1 hybrid zucchini and squash. When backcrossed (BC) with the original parental plants, the zucchini and squash F1 BC cultivars reverted to the statistically distinct CR and EF patterns. This pattern of trait segregation upon hybridization suggests either single gene or single locus control for persistent organic pollutant (POP) uptake ability by*** **C. pepo** ***ssp*** **pepo.**

KEY WORDS: Chlordane, DDT, zucchini, squash, hybrids, Persistent Organic Pollutants (POPs), Bioconcentration Factors (BCFs)

INTRODUCTION

Persistent organic pollutants (POPs) such as chlordane and dichlorodiphenyl-trichloroethane (DDT) are of significant concern because of their persistence in

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environmental media, global distribution, and toxicity (Hagen and Walls 2005; Armitage and Gobas 2007; Nizzetto *et al.* 2010). Although the use of chlordane and DDT in the United States was discontinued 25–40 years ago, soils and sediments contaminated with low to moderate levels of these pollutants are ubiquitous (Mattina *et al.* 1999; Wong and Bidleman 2011). The persistence of these chemicals in the environment is in part due to synthetic molecular structure; with no natural analogs present, degradative pathways have not evolved. In addition, the high hydrophobicity of POPs results in strong binding to soil organic matter, with contaminant bioavailability often correlating negatively with residence time (Alexander 2000). Interestingly, many POPs are semivolatile and the long term outgassing of these chemicals from highly weathered soils to the atmosphere has resulted in both global distribution and contamination (Wania and Mackay 1996; Ross *et al.* 2008). Although POP residues retained in soils are highly sequestered, accumulation in the tissues of soil- and sediment-based biota is well documented (Ross *et al.* 2008), with biomagnification upon trophic transfer creating significant exposure and risk for top-level carnivores and omnivores. The many unique concerns of POPs are best demonstrated by the contamination of remote arctic and Antarctic wildlife (Ross *et al.* 2008; Miljeteig *et al.* 2009).

The POP properties of hydrophobicity and resistance to degradation greatly confound efforts to remediate contaminated soils. Short of natural attenuation or excavation, conventional remediation strategies such as sparging with air or water are ineffective (White 2008). Phytoremediation, a technology whereby vegetation facilitates the removal of contaminants from natural solids, has been shown to be highly effective for moderately soluble or degradable organic pollutants such as low molecular weight petroleum hydrocarbons, solvents and pesticides such as atrazine (Pilon-Smits 2005; Schweitzguébel *et al.* 2009). Given the known characteristics of POPs, phytoremediation is not predicted to be a viable treatment alternative. However, Hülster *et al.* (1994) first reported that certain *Cucurbita* species had an ability to accumulate dioxin into select aerial tissues after uptake into the roots. Subsequent work by others has shown that *Cucurbita pepo* ssp *pepo* (zucchini) can uniquely bioaccumulate high concentrations of weathered POPs, including chlordane, DDT/DDE/DDD, in root and more importantly, stem tissues (White 2001; White *et al.* 2003; Mattina *et al.* 2006; Whitfied Åslund *et al.* 2010). Zhang *et al.* (2009) and Murano *et al.* (2010a) have reported similar increased uptake of dioxin and dieldrin, respectively, by *C. pepo* relative to other plant species. The pollutants appear to be largely accumulated as unaltered parent compound, although the magnitude of contaminant accumulation varies significantly with both plant phylogeny and chemical characteristics. Contaminant levels in zucchini stems are frequently several times greater than present in the soil and have been shown to exceed other plants by 1–3 orders of magnitude. Single season removal or phytoextraction values often approach 1–5% in some soils (White 2009).

Importantly, the uptake of weathered POPs differs significantly at the subspecies level, with *C. pepo* ssp *pepo* (zucchini) accumulating up to an order of magnitude more contaminant than does *C. pepo* ssp *ovifera* (squash). In an effort to elucidate the mechanism of POP uptake, recent work has taken advantage of the ability to hybridize the different subspecies of *C. pepo* (White. 2010). In that study, the inheritance patterns of DDE uptake potential across parental (P), first filial (F1) and first filial backcross (F1 BC) generations suggested classical Mendelian segregation and potentially single locus control. Specifically, when DDE accumulating zucchini were crossed with non-accumulating squash, the resulting F1 hybrid had reduced contaminant uptake ability but upon backcrossing to the original zucchini parent, the F1 BC recovered the original extraction

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capacity. Similarly, the non-accumulating squash exhibited significant DDE uptake capacity when hybridized but lost the ability upon backcrossing to the parent. The goal of the current work was to determine the ability of parental and hybridized *C. pepo* cultivars to accumulate weathered POPs from soil co-contaminated with chlordane and DDTs. Because of the complex nature of chlordane residues, in addition to total contaminant uptake patterns, individual component ratios and chiral signatures were determined and provided a more intensive examination of chemical accumulation profile upon hybridization. As transport proteins are enantioselective, this detailed level of chemical analysis will ultimately facilitate determination of the mechanism of *C. pepo* ssp *pepo* accumulation of POPs and a potential novel phytoremediation approach for these contaminants in soil.

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MATERIALS AND METHODS

Field Site

An experimental plot was established at the Connecticut Agricultural Experiment Station's New Haven campus. The plot (approximately 12m × 21m) was treated with technical chlordane in the 1960s and was subsequently under turf until 1998, when the area was turned over for periodic experimentation. Chlordane residues ranging from 1–18 μg/g have been reported in this soil (6.5% organic matter) (Mattina *et al.* 1999; Eitzer *et al.* 2001). Anecdotal study of this site has also confirmed the presence of *p,p′*-DDT, as well as the metabolites *p,p′*-DDD and *p,p′*-DDE, at concentrations of 50–300 ng/g.

The site was covered with 250 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. Individual parental and hybrid cultivars were grown in triplicate mounds according to a randomized block design as described below.

Parental and Hybrid Cultivars

Two cultivars of *Cucurbita pepo* ssp *pepo* ("Raven," "Costata Romanesco") previously shown to accumulate significant amounts of weathered *p,p′*-DDE (White *et al.* 2003; White 2010) and two cultivars of *Cucurbita pepo* ssp *ovifera* ("Zephyr," "Patty Pan") previously shown to not accumulate weathered *p,p′*-DDE were purchased from Johnny's Selected Seeds (Albion, ME). These seeds served as the parental (P) generation. First filial (F1) hybrids and backcrossed F1 (F1 BC) hybrids of these four cultivars were created by manual cross-pollination as described in White (2010). Briefly, one day prior to opening, male and female flowers on the parental cultivars were covered with paper bags fitted with a rubber band so as to exclude pollinators and preserve pollen. The following morning, stamens from male flowers of pre-determined cultivars were used to pollinate the predetermined female flower of cultivars of the opposing sub-species. For this work, a hybrid zucchini describes a plant where a female zucchini flower was pollinated by squash pollen and a hybrid squash is a plant where a female squash flower was pollinated by a male zucchini. An F1 BC is a hybrid that is subsequently crossed with the original parent; e.g., a hybrid squash crossed with the original squash parent. The seeds from collected fruit were harvested, washed, air dried, and soaked in 10% sodium hypochlorite. After rinsing with reverse osmosis (R.O.) water, the seeds were air dried and stored at room temperature (20–25°C).

In June 2010, seeds were pre-germinated on germination paper and planted as 48 separate mounds of cucurbits with each mound consisting of four separate individuals. This included 12 replicate parental mounds (6 mounds each of zucchini [Raven, Costata], and 6 mounds of squash [Zephyr, Patty]; 18 replicate F1 mounds with three separate hybrid zucchini (Raven × Patty [3], Raven × Zephyr [3], and Costata × Zephyr[3]) and three hybrid squash (Patty × Raven[3], Zephyr × Raven[3], and Zephyr × Costata[3]); and 25 replicate F1 BC mounds. The plants were watered and weeded as necessary during the growing season. Destructive harvest of the mounds began in early September 2010. Because the goal of the study was to compare contaminant uptake between *C. pepo* ssp *pepo* and *C. pepo* ssp *ovifera*, the zucchini and squash cultivars were grouped and analyzed by subspecies (both parental and hybridized).

Soil Extraction

The chlordane and *p,p′*-DDTs (*p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE) concentration in soil samples from each replicate mound was determined. 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 (White 2009). 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 50 μL of a 15 μg/mL ^{13}C *p,p*'-DDE stock in toluene as an internal standard (IS)(50 ng/mL IS in sample). The vials were capped and heated on a hot block at 65°C for 4 h. One mL of the supernatant was passed through a glass micro-fiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) for particulate removal and was collected in a chromatography vial. Soil extracts were stored at −4°C prior to analysis. Because soil contamination levels are routinely expressed on a dry weight basis, the moisture content of soil from each replicate mound was determined by heating 3.0-g portions at 100°C for 24 h (White 2009).

Vegetation Extraction

At 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 vegetation was separated by cultivar and tissue type, and the mass of fruit, leaf, stem, and root tissues were determined. All tissue samples were rinsed thoroughly with tap water and finely chopped with a Hobart commercial food chopper. The samples were transferred to whirlpak bags for storage at -4°C. Individual plant tissues were extracted by a QuEChERs method based on that described by Anastassiades *et al.* (2003). Briefly, up to 15 g (wet weight) of sample was added to a 50 mL centrifuge tube containing 15 mL of acetonitrile and 100μL of ^{13}C *p,p′*- DDE at 2–5 μg/mL (13–33 ng/ml in solution; precise amount depended on tissue type) as an IS. The tubes were placed on a wrist-action shaker for 10 min. Six grams of magnesium sulfate and 1.5 g of sodium acetate were added to each tube, which were then centrifuged at 3000 rpm for 10 min. Fifteen mL plastic centrifuge tubes were amended with 1.5 g of magnesium sulfate and 500 mg of PSA. Two mL of toluene was added to wet the powders and 10 mL of sample extract were transferred to each tube. The tubes were shaken by hand for 30 s and centrifuged at 3000 rpm for 10 min. Six mL of each extract were concentrated to 1 mL under nitrogen. The samples were then transferred to chromatography vials for storage at –4°C until analysis.

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Chemical Analysis

All extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) on a Varian 3800 GC interfaced with a Varian 4000MS ion trap (Varian, Sugar Land, TX, USA). The injection port temperature was 230°C and the pressure settings for the injector port were as follows: initial pressure 13 psi, hold 0.1 min; ramped 40 psi/min to 25 psi, hold 3.4 min; 40 psi/min to 13 psi; 1.2 psi/min to 16.6 psi; 0.03 psi/min to 18.7 psi; 0.06 psi/min to 20.6 psi, hold 7.32 min. The column was a 30 m × 0.25 mm × 0.25 μm film β-cyclodextrin chiral column (Supelco, Bellefonte, PA, USA). The GC oven was programmed as follows: initial temperature 100°C, hold 4 min; ramped at 20°C/min to 160°C; ramped at 0.5°C/min to 195°C; ramped at 1.0°C/min to 228°C, hold 6 min. The mass spectrometer conditions were: emission current 60 μA; target total ion current 5,000 counts; maximum ionization time 25,000 μs; multiplier offset ±200V. The scan range was *m/z* 195 to 425. The major achiral component of technical chlordane, trans-nonachlor (TN), as well as the (+) and (−) enantiomers of chiral trans-chlordane (TC), and cis-chlordane (CC); were quantified. Because of the well-known but somewhat unpredictable breakdown of *p,p′*-DDT to *p,p′*-DDE/DDE in the GC inlet, total DDX was calculated as the sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE.

Statistical Analysis

Triplicate mounds of each plant type or hybrid were grown for a total of 48 *C. pepo* mounds. Triplicate samples of soil from each of the 48 mounds of vegetation were extracted. For each individual replicate mound of vegetation, individual leaf and fruit tissues were extracted once; root and stem tissues were extracted in duplicate. All values of contaminant concentration were expressed on a dry weight basis. The following indices were determined: tissue and whole plant biomass, tissue (root, stem, leaf, or fruit) bioconcentration factor (BCF), translocation factor (TF), component ratios (CR; chlordane only), and enantiomer fraction (EF; chlordane only). Bioconcentration factors are the dry weight ratios of contaminant concentration in the plant tissue to that in the soil, and the TF is the stem BCF divided by the root BCF. Component ratios were calculated for TN, TC, and CC as the amount of each component present as a fraction of the total chlordane (TN + TC + CC) measured. The enantiomer fraction (EF) was calculated for +/−TC and +/−CC for some of the chiral chlordane components using the following formula:

$$\mathrm{EF}_{+} = \frac{\mathrm{C}_{+}}{(\mathrm{C}_{+} + \mathrm{C}_{-})} \quad \text{(Harner et al. 2000)}$$

where C represents the concentration of each enantiomer. For analysis across subspecies, cultivars of a given subspecies were treated as replicates. An analysis of variance (ANOVA) followed by a Dunn's or Student Newman Keuls Multiple (SNK) Multiple Comparison Test (MCT) was used to determine the statistical significance of differences between plant types (zucchini and squash) within a given generation (parental, F1, or F1 BC). and across P, F1, and F1 BC generations for a given plant type (zucchini or squash). In all cases, a $p < 0.05$ was used as the level of statistical significance.

RESULTS AND DISCUSSION

Soil Contaminant Concentration

The average soil chlordane and DDX content (dry weight) was 2300 ng/g (±1110; 177–5730 ng/g) and 299 ng/g (±164; 14–1120 ng/g), respectively. This wide variation in contamination for both POPs was expected; however, for a given mound, variability among the triplicate soil extractions was less than 5% for chlordane and less than 10% for DDX. These levels of chlordane are in agreement with previous findings (Mattina *et al*. 1999, 2006) and the DDX concentrations are indicative of historical contamination with DDT (White 2001; White *et al.* 2007). For chlordane, the component ratios (CR) for TN, TC, and CC were 0.21 (±0.02), 0.32 (±0.02), and 0.46 (±0.02), respectively. The enantiomer fraction (EF) for TC was 0.43 (±0.04); the EF for CC was 0.57 (±0.02). In a 2006 study using this soil, the average total chlordane concentration was measured at 5670 ng/g, with CR values of 0.19, 0.38, and 0.43 for TN, TC, and CC, respectively (Mattina *et al.* 2006). In the 2006 study, the measured EFs were 0.47 for TC and 0.53 for CC. Although the CR and EF values in the current study are in general agreement with that of Mattina *et al.* (2006), the proportional increases in TN and CC do demonstrate continued weathering of the chlordane residues.

Parental Generation

The average total wet biomass of the zucchini and squash cultivars was 9,500 g (±2,490) and 13,512 g (±2100), respectively (significantly different; one way ANOVA with SNK MCT). The average wet masses of the individual tissue compartments are shown in Table 1; both the stem and leaf compartments of the squash were significantly larger than the corresponding zucchini tissues (one way ANOVA with SNK MCT). The reason for this difference in cultivar size is not known but the biomass of field-grown cucurbits can vary significantly; in this study, replicate variability in total biomass approached 15%. The roots, stems and fruit of all four cultivars contained measurable chlordane; DDX was detected only in the roots and stems of the plants. No measurable chlordane or DDX was found in the leaves of either plant type. For a given replicate mound, the highest chlordane and DDX concentrations were consistently detected in the roots, followed by significantly (one way ANOVA with SNK MCT) decreasing amounts in the stems and fruit. These findings agree with previous work where POP concentrations are frequently at or below the level of detection whereas stem and root values are often 10–100 times higher (White *et al.* 2003). Because of the variability in soil contaminant concentrations across the plot, all parental generation tissue contaminant levels are normalized to the respective soil chlordane or DDX content and expressed as bioconcentration factors (Table 2) (White *et al.* 2003). The root and fruit (chlordane only) bioconcentration factors are statistically equivalent across subspecies; this finding is not surprising as previous work has shown that the stem compartment is the most important tissue for evaluating the unique POP accumulating potential of zucchini (White *et al.* 2003; Mattina *et al.* 2006; White 2010). As expected, the two different subspecies of *C. pepo* contained significantly different amounts of the contaminants in the stem tissue. The zucchini stem chlordane and DDX BCFs were 2.2 and 3.7 times greater than that of the squash cultivars. Similarly, the translocation factors (TF), a measure of root to shoot contaminant transfer, were 2.1 and 3.2 times greater for the zucchini cultivars. These findings agree with our previous work and highlight the unique

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Table 1 Biomass and contaminant accumulation values for the parental and hybridized generations of cucurbits.

Parameter	Zucchini[a] (n)	Squash[b] (n)
Biomass[c]		
Root	52.6 A[d]a[e] (6)	60.8 Aa (5)
Stem	2250 Aa (6)	5150 Ba (5)
Leaf	604 Aa (6)	1250 Ba (5)
Fruit	6590 Aa (6)	7050 Aa (5)
	F1 Zucchini	F1 Squash
Biomass[c]		
Root	68.3 Aa (9)	47.1 Aa (8)
Stem	6160 Ab (9)	5440 Aa (8)
Leaf	1700 Ab (9)	1260 Aa (8)
Fruit	10190 Ab (9)	6590 Ba (8)
	F1 BC Zucchini	F1 BC Squash
Biomass[c]		
Root	43.6 Aa (9)	34.1Aa (9)
Stem	3510 Aa (9)	2470Aa (9)
Leaf	950 Aa (9)	681Aa (9)
Fruit	6180 Aa (9)	5470Aa (9)

[a]Values represent averages of Costata and Raven cultivars.
[b]Values represent averages of Zephyr and Patty cultivars.
[c]All biomass values are in grams (wet weight).
[d]Within a row (across subspecies), average values followed by different capital letters are significantly different (One way ANOVA with a Student Newman Keuls MCT; $p < 0.05$).
[e]Within a column and tissue type (zucchini or squash), average values followed by different lowercase letters are significantly different (One way ANOVA with a Student Newman Keuls MCT; $p < 0.05$).

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ability of *C. pepo* ssp *pepo* to accumulate weathered POPs from soil (White *et al.* 2003; Mattina *et al.* 2006; White 2010; Chhikara *et al.* 2010).

Further physiological differences between the two subspecies of *C. pepo* are evident upon analysis of the chlordane component ratios (CR) and enantiomer fractions (EF) in the different plant tissues (Table 3). It is noteworthy that for both zucchini and squash, all measured CR and EF were significantly different from those measured in the soil (one way ANOVA with SNK MCT). The roots of both plant types do not differ in CR or EF; this is in agreement with the root BCF values, which also show that the two *C. pepo* subspecies differ little in contaminant content in the roots. In terms of chlordane components, CC is disproportionately accumulated by both types of plant roots and both TN and TC are taken up to a significantly lesser extent. Upon translocation in both zucchini and squash, there is a trend toward decreased TN levels and increased CC amounts from roots to stems. These findings agree well with Mattina *et al.* (2004, 2006), where zucchini (cultivar "Black Beauty") showed similar component accumulation and transport patterns. However, after translocation of chlordane to the stem tissues, significant divergence in CR is evident between zucchini and squash. In the stem tissues of the two plant types, zucchini contains significantly greater amounts of TN and TC, as well as significantly less CC, than does squash. These findings are in partial agreement with Mattina *et al.* (2006), where squash aerial tissues (cultivar "Zephyr") contained lower relative amounts of TC than zucchini. However, in the 2006 study the squash stems contained greater relative amounts TN and

Table 2 Bioconcentration factors (BCFs) and translocation factors (TFs) for the parental and hybridized cucurbits. The number in parentheses is n.

Parameter	Zucchini[a]	Squash[b]	F1 Zucchini[c]	F1 Squash[d]	F1 BC Zucchini[e]	F1 BC Squash[e]
Chlordane						
Root	25.2[f] A[g]a[h] (11)	23.5 Aa (10)	41.6 Aa (18)	36.9 Aa (16)	30.7 Aa (18)	22.6 Aa (16)
Stem	6.23 Aa (12)	2.79 Ba (9)	9.64 Aa (17)	11.6 Ab (15)	7.45 Aa (17)	4.33 Aab (14)
Fruit	1.22 Aa (6)	0.94 Aa (5)	1.85 Aa (9)	2.47 Aa (8)	1.14 Aa (10)	1.52 Aa (9)
TF	0.248 Aa (12)	0.119 Ba (9)	0.179 Ab (17)	0.117 Aa (14)	0.243 Aab (17)	0.191 Aa (14)
DDX[e]						
Root	6.36 Aa (11)	5.45 Aa (10)	9.58 Aa (17)	5.87 Ba (15)	8.37 Aa (18)	5.28 Aa (16)
Stem	3.10 Aa (12)	0.83 Ba (9)	3.35 Aa (17)	2.33 Ab (16)	3.10 Aa (17)	1.30 Bab (14)
TF	0.488 Aa (12)	0.152 Ba (9)	0.323 Aa (17)	0.221 Aa (14)	0.370 Aa (17)	0.246 Aa (17)

[a]Values represent averages of Costata and Raven cultivars.
[b]Values represent averages of Zephyr and Patty cultivars.
[c]Hybrid zucchini- Raven × Patty, Raven × Zephyr, and Costata × Zephyr).
[d]Hybrid squash- Patty × Raven, Zephyr × Raven, and Zephyr × Costata).
[e]F1 backcross (BC) are the F1 hybrid zucchini and squash crossed with the original respective parent.
[f]BCF or bioconcentration factors are the dry weight ratio of contaminant in a tissue to that in the soil.
[g]Within a generaton (across subspecies), average values followed by different capital letters are significantly different (One way ANOVA with a Student Newman Keuls MCT; $p < 0.05$).
[h]Across generatons but within a plant type (zucchini or squash), average values followed by different lowercase letters are significantly different (One way ANOVA with Student Newman Keuls MCT; $p < 0.05$).
[i]TF or translocation factors are the replicate stem BCFs divided by the average root BCF for a given replicate.

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less CC, which disagrees with the current work. The reason for this discrepancy may be a function of growing conditions; the 2006 study was in 2.5 kg rhizotrons with relatively small plants that did not fruit while being grown for shorter periods of time whereas the current study was under field conditions with very large fruiting plants. Earlier work has shown that the accumulation of DDE can vary significantly with plant age, physiological stage, and cultivation conditions (Wang *et al.* 2004; White *et al.* 2006). Interestingly, in the fruit, the observed component ratio pattern reverses relative to the stems; zucchini fruit contain significantly less TN and more CC than does the squash.

For EFs, there is a significant shift for TC upon entry into the plants; in the soil, -TC is the dominant enantiomer but in plant roots, +TC is most abundant. For zucchini, the +TC enantiomer remains dominant in the stems but in the fruit, the –TC enantiomer is more abundant. These findings agree well with Mattina *et al.* (2004), where similar +/-TC patterns were observed with a different zucchini cultivar ("Black beauty"). For CC, the change in EF is less dramatic; although +CC is the dominant enantiomer in both the soil and roots, there is a significant increase in the fraction of –CC upon entry into the root tissue. Similar to the CR values, differential translocation of chlordane enantiomers to the stem tissues is observed for the zucchini and squash. Zucchini stems contained significantly greater quantities of +TC and +CC, as well as correspondingly lower levels of the –TC/-CC enantiomers, than does squash. These findings agree well with Mattina *et al.* (2006), where the amounts +TC/+CC in zucchini shoots were 9–25% greater than the levels in squash. In the fruit, the subspecies difference in TC enantiomers disappears but zucchini still contain significant greater quantities of +CC than does squash.

Table 3 Chlordane component ratio (CR) and enantiomer fraction (EF) values for the root, stem and fruit tissues of the parental generation (P) cucurbits. TN = trans-nonachlor, CC = cis-chlordane, TC = trans-chlordane.

Parameter	Zucchini[a] (n)	Squash[b] (n)
Roots		
Component Ratio[c]		
TN	0.171 A[e] (11)	0.161 A (12)
TC	0.300 A (11)	0.290 A[a] (12)
CC	0.528 A (11)	0.548 A[b] (12)
Enantiomer Fraction[d]		
TC	0.544 A (11)	0.535 A (12)
CC	0.551 A (11)	0.549 A (12)
Stems		
Component Ratio		
TN	0.150 A (12)	0.125 B (10)
TC	0.302 A (12)	0.270 B (10)
CC	0.548 A (12)	0.605 B (10)
Enantiomer Fraction		
TC	0.531 A (12)	0.488 B (10)
CC	0.540 A (12)	0.505 B (10)
Fruit		
Component Ratio		
TN	0.116 A (6)	0.142 B (5)
TC	0.325 A (6)	0.350 A (5)
CC	0.558 A (6)	0.509 B (5)
Enantiomer Fraction		
TC	0.440 A (6)	0.461 A (5)
CC	0.640 A (6)	0.570 B (5)

[a]Values represent averages of Costata and Raven cultivars.

[b]Values represent averages of Zephyr and Patty cultivars.

[c]Component ratio (CR) is the relative amount of a given component (TN, TC, or CC) expressed as a fraction of the total chlordane present (TN + TC + CC).

[d]The enantiomer fraction (EF) is the amount of a given (+) enantiomer expressed as a fraction of the total amount of the given component (sum of the two enantiomers).

[e]Within a row (across subspecies), average values followed by different letters are significantly different (One way ANOVA with a Student Newman Keuls MCT; $p < 0.05$).

[f]Values are significantly different at $p = 0.088$.

[g]Values are significantly different at $p = 0.061$.

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Hybrid Generations

The uptake and translocation of weathered chlordane and DDX was determined in 18 F1 hybrids and 18 F1 BC plants created from the original four parental cultivars. The average total wet mass of the F1 hybrid zucchini and squash were 18,120 and 13,350 g, respectively (not significantly different). The masses of the hybrid F1 and F1 BC zucchini and squash roots, stems, leaves and fruit are shown in Table 1. The only significant difference between the F1 hybrid plant types was in the fruit mass; zucchini hybrids were significantly greater than the squash hybrids (one way ANOVA with SNK MCT). There were no biomass differences between the F1 BC zucchini and squash. In relation to the parental generation, there were no significant differences in total or individual tissue masses between the squash parents, F1 or F1 BC hybrids (Table 1). Conversely, the total mass, as well as the fruit, leaf, and stem masses, were significantly greater for the zucchini F1 hybrids than for the parental or F1 BC generations (one way ANOVA with Student Newman Keuls MCT) (Table 1).

The reason for the significantly larger F1 zucchini generation is not known. White (2010) noted that although there were no differences in total plant biomass, F1 hybrid zucchini stems and leaves were of less mass than the corresponding backcrosses. In that same study, squash F1 BC leaf and stem masses were significantly larger than the corresponding F1 hybrids or parents. The practical significance of these differences in morphology is likely minimal and may simply be the result of inherent variability in biological systems.

Similar to the parental generation, the highest concentrations of chlordane and DDX in the F1 and F1 BC plants were found in the roots, with decreasing amounts in the stems and fruit. All chlordane and DDX tissue concentrations for F1 hybrids were expressed as BCFs. Similar to the parental generation, the chlordane root BCFs for the plant types were not significantly different. Chlordane BCFs for the F1 zucchini and squash were 41.6 and 36.9, respectively (Table 2). However, the DDX root BCF values were 9.58 and 5.87, respectively; these values are significantly different. Unlike the parental generation, the F1 stem BCF values for chlordane and DDX are not significantly different between the hybrid zucchini and squash. However, when the parental squash are crossed with zucchini, the stem BCF values for the hybrid squash increase by 4.15 and 2.81 times, respectively (one way ANOVA on Ranks with a Dunn's MCT). Although chlordane and DDX stem BCFs for the parental and F1 zucchini do not differ, the TF values for the F1 hybrids did decrease relative to the parents. The decrease in the DDX TF was statistically insignificant but the chlordane TF decreased by 28% upon zucchini hybridization with squash (one way ANOVA on Ranks with a Dunn's MCT). The fruit chlordane content did not differ significantly between plant types or across generations. The tissue BCF values for the F1 BC zucchini and squash are also shown in Table 2. The root chlordane and DDX BCFs of the F1 BC hybrid zucchini are 30.7 and 8.37, respectively, and the F1 BC squash values are 22.6 and 5.28, respectively. Within a contaminant type, these values are not significantly different between the plant types (F1 BC zucchini and squash) or across generations (P, F1, BC). The same trend in the data holds true for the fruit compartment; BCF values for both contaminants do not differ significantly between plant type or across generation. The stem BCF values for the F1 BC hybrid squash are less than the corresponding F1 BC zucchini; for chlordane the difference is only significant at a p value of 0.147 but for DDX, the level of significance is $p = 0.015$.

The component ratios and enantiomer fractions of the F1 and F1 BC hybrid zucchini and squash are shown in Table 4, as well as Figures 1 and 2. Similar to the parental generation, all F1 and F1 BC hybrid tissue CR and EF values were significantly different from that in the soil. For the F1 hybrids, the root and fruit CRs for both plant types (zucchini and squash) do not differ significantly from each other or from the respective parental generations (Table 4). A similar trend of statistical insignificance is evident for the fruit CRs of the F1 BC plants. However, the TC and CC fraction in the roots of F1 BC squash are significantly different than the corresponding values in the backcrossed zucchini. Across generations but within a plant type, the root CRs of the zucchini and squash did not differ significantly across their respective parental, F1 or F1 BC generations. In the fruit compartment, the TN component of the F1 hybrid zucchini (0.138) was significantly greater than both the parental value (0.116) and the F1 BC (0.124) (one way ANOVA with SNK MCT). More importantly, the stem CR values for the parental, F1 and F1 BC zucchini and squash are shown in Figure 1. For each of the three components, a clear and significant statistical pattern is evident in the data. The parental generation CR values differ significantly among the plant types (zucchini vs squash) but when crossed, the resulting F1 CR values in the stems become statistically indistinguishable. Upon backcrossing with the

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Table 4 Chlordane component ratio (CR) and enantiomer fraction (EF) values for the root and fruit tissues of the F1 and F1 BC cucurbits. TN = trans-nonachlor, CC = cis-chlordane, TC = trans-chlordane. The number in parentheses is n.

Parameter	F1 Zucchini[a]	F1 Squash[b]	F1 BC Zucchini	F1 BC Squash
Roots				
Component Ratio[c]				
TN	0.161 A (18)	0.166 A (16)	0.170 A (18)	0.157 A (16)
TC	0.294 A (18)	0.285 A (16)	0.289 A (18)	0.273 B (16)
CC	0.545 A (18)	0.549 A (16)	0.541 A (18)	0.570 B (16)
Enantiomer Fraction[d]				
TC	0.523 A (18)	0.538 A (16)	0.548 A (18)	0.517 B (16)
CC	0.550 A (18)	0.544 B (16)	0.552 A (18)	0.544 A (16)
Fruit				
Component Ratio				
TN	0.138 A (9)	0.136 A (8)	0.124 A (10)	0.134 A (9)
TC	0.348 A (9)	0.331 A (8)	0.340 A (10)	0.339 A (9)
CC	0.514 A (9)	0.532 A (8)	0.537 A (10)	0.528 A (9)
Enantiomer Fraction				
TC	0.474 A (9)	0.475 A (8)	0.445 A (10)	0.462 A (9)
CC	0.604 A (9)	0.578 A (8)	0.636 A (10)	0.593 B[f] (9)

[a]Values represent averages of Costata and Raven hybrid cultivars.

[b]Values represent averages of Zephyr and Patty hybrid cultivars.

[c]Component ratio (CR) is the relative amount of a given component (TN, TC, or CC) expressed as a fraction of the total chlordane present (TN + TC + CC).

[d]The enantiomer fraction (EF) is the amount of a given enantiomer (+) expressed as a fraction of the total amount of the given component (sum of the two enantiomers).

[e]Within a row (across subspecies), average values followed by different letters are significantly different (One way ANOVA with a Student Newman Keuls MCT; $p < 0.05$).

[f]Values are significantly different at $p = 0.094$.

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original parent, the F1 BC generation returns to the statistically distinct CR patterns for the zucchini and squash.

The EF values for the F1 and F1 BC zucchini and squash are also shown in Table 4. Similar to the parental generation, the EF values for the hybridized zucchini and squash tissues differ significantly from those in the soil. As shown in Table 4, the differences in root and fruit EF between the F1 and F1 BC zucchini and squash are not extensive. In the roots, only the F1 BC TC values differed significantly between zucchini and squash; in the fruit, only the CC values differed significantly. Across generations within a plant type, the fruit EF in both plant types and the root EF in the squash did not differ significantly among the parental, F1 and F1 BC generations. Similarly, for zucchini the CC EF did not differ across generations, although the TC EF of the F1 zucchini (0.474) was significantly greater than the parental (0.440) and F1 BC (0.445) generations (one way ANOVA with SNK MCT). The stem EF values for the parental, F1 and F1 BC generations are shown in Figure 2. Similar to the CR values, the stem EFs show a clear and statistically distinct pattern across the three generations. For both chiral components, the parent stems are statistically different but upon hybridization, the F1 individuals become statistically indistinguishable. Upon backcrossing to the parent, the statistically distinct chiral pattern among the zucchini and squash is recovered.

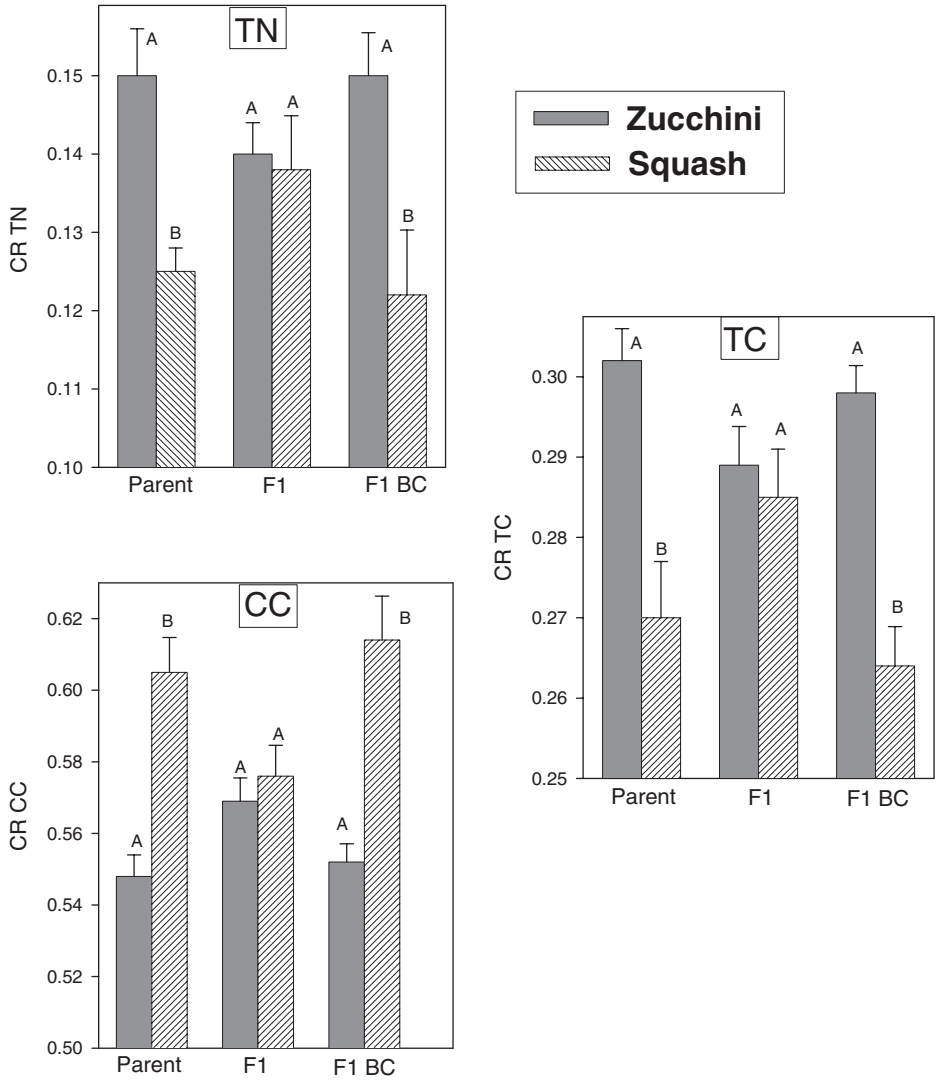

Figure 1 Chordane component ratios (CR) in the stems of parental, F1 hybrid, and F1 BC squash. TN = trans-nonachlor, TC = trans-chlordane, CC = cis-chlordane. Within a component type and generation, bars with different letters are significantly different (One way ANOVA with a Student Newman Keuls Multiple Comparison Test; $p < 0.05$)

The inheritance and trait segregation pattern observed for both component ratios and enantiomer fractions in the parental, F1 and F1 BC zucchini and squash tissues, particularly the stems, clearly suggests single gene or single locus control. These findings agree with White (2010) where nearly identical results for DDE bioaccumulation were observed. In the current study, the overall trends in total chlordane and DDX bioaccumulation were in general agreement with White (2010), although significant replicate variability confounded some of the statistical analyses. The reasons for this variability and the observed minor differences in POP distribution among the hybridized cultivars from the previous study are

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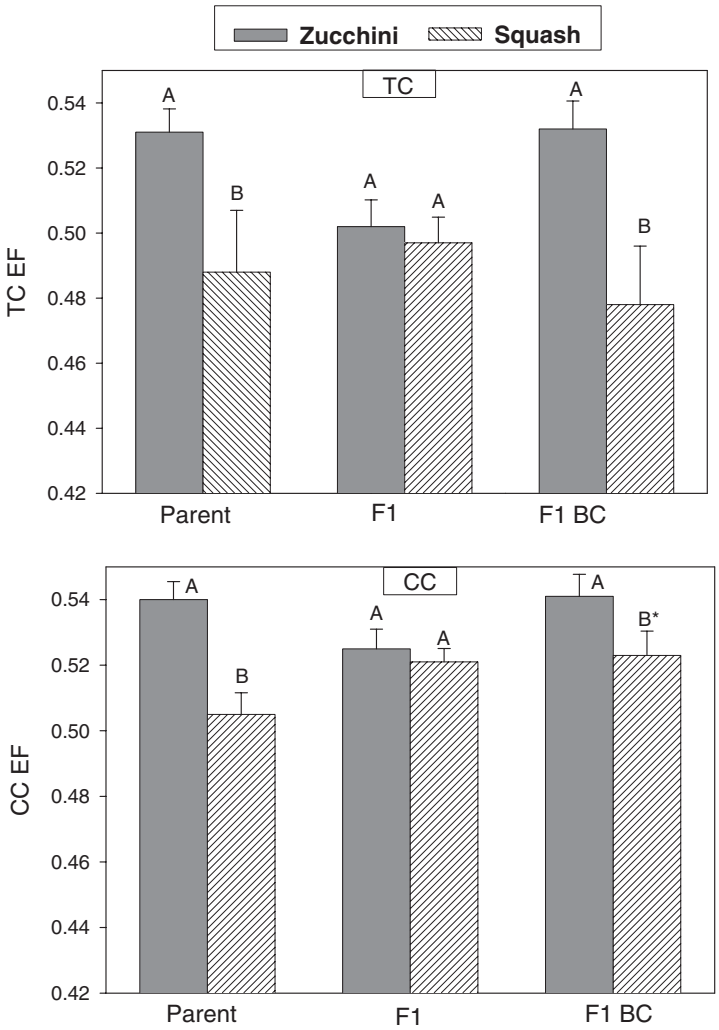

Figure 2 Chordane enantiomer fraction (EF) values in the stems of parental, F1 hybrid, and F1 BC squash. Within a component type and generation, bars with different letters are significantly different (One way ANOVA with a Student Newman Keuls Multiple Comparison Test; $p < 0.05$). F1 BC CC EF values are significantly different at $p = 0.094$.

unknown but likely a function of different site location and soil type, as well as contaminant profile and concentration.

The genetic basis in zucchini for its unique POP bioaccumulation ability is currently unknown. In previous grafting studies using POP contaminated soils (Mattina *et al.* 2007; Isleyen and Sevim 2012), rootstock was the critical component in determining DDX content. For example, when watermelon (*Citrullus lanatus*) scions were grafted on cucurbit rootstock (*C. maxima* × *C. moschata*), the stem DDX content increased by 19–140 times relative to the intact (ungrafted) or homografted melon. Similarly, Isleyen *et al.* (2012) observed that upon grafting watermelon onto *C. pepo* rootstock, xylem sap DDE content increased from 0.49 μg/l to 71 μg/L. These findings agree with an earlier small pot study by Otani and

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Seike (2006) which showed similar trends in the uptake of endrin and dieldrin by cucumber (*Cucumis sativus*) grafted onto *Cucurbita* sp. rootstock. Taken together, these data clearly demonstrate that the key component controlling POP accumulation potential resides in the rootstock, which does include a small portion of the stem tissue.

Chhikara *et al.* (2010) exposed zucchini and squash to DDE under hydroponic conditions and used a differential transcript analysis technique followed by semi-quantitative RT-PCR to compare the resulting expression profile to non-exposed plants. Upon DDE exposure, 22 genes in zucchini were significantly upregulated, and although several were of unknown function, the majority of the genes were linked to abiotic stress, signaling pathways, lipid metabolism or photosynthesis. Interestingly, when DDE-exposed zucchini were directly compared to exposed squash, the zucchini shoots and roots exhibited significant upregulation of genes with high sequence homology to a phloem loading protein (PP1) and a cytochrome P-450, respectively, but expression of these genes in the squash was unaffected. The PP1 finding is of particular interest given the observed high degree of POP translocation in *C. pepo* and related plants. Zhang *et al.* (2010) described the unique and functionally discrete two part phloem system of cucurbits. Murano *et al.* (2010b) noted that when zucchini and cucumber were grown in dieldrin contaminated soil, the shoots of these plants contained significantly greater levels of the organochlorine than did other species. When xylem sap was applied to C8 columns containing sorbed dieldrin, cucurbits leached measurable amounts of the contaminant from the stationary phase whereas other species had no effect. The authors speculate that a protein-based material in the sap and/or root exudates of select cucurbits may be responsible for increased dieldrin uptake and transport.

The widespread contamination of soil with low to moderate concentrations of weathered persistent organic pollutants is among the most intractable environmental problems we face. The current toolbox of remedial options consists of techniques that are either ineffective or prohibitively expensive. The unique abilities of *C. pepo* with regard to contaminant accumulation and translocation may represent a viable remediation system but obtaining a mechanistic understanding of *C. pepo* physiology and genetics is a critical step. The findings of the current study indicate that single gene or locus control for POP uptake ability. Also, enantiomer-specific changes upon hybridization implicate a chirally sensitive mechanism such as a transport protein. Work of this type will permit the successful development of a low cost, plant-based system for the long-term remediation POP-contaminated soils can occur and be of great benefit to both ecosystem and human health.

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