

Phytoremediation of Persistent Organic Pollutants: Mechanistic studies and Field Application



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Persistent Organic Pollutants (POPs) in Soil

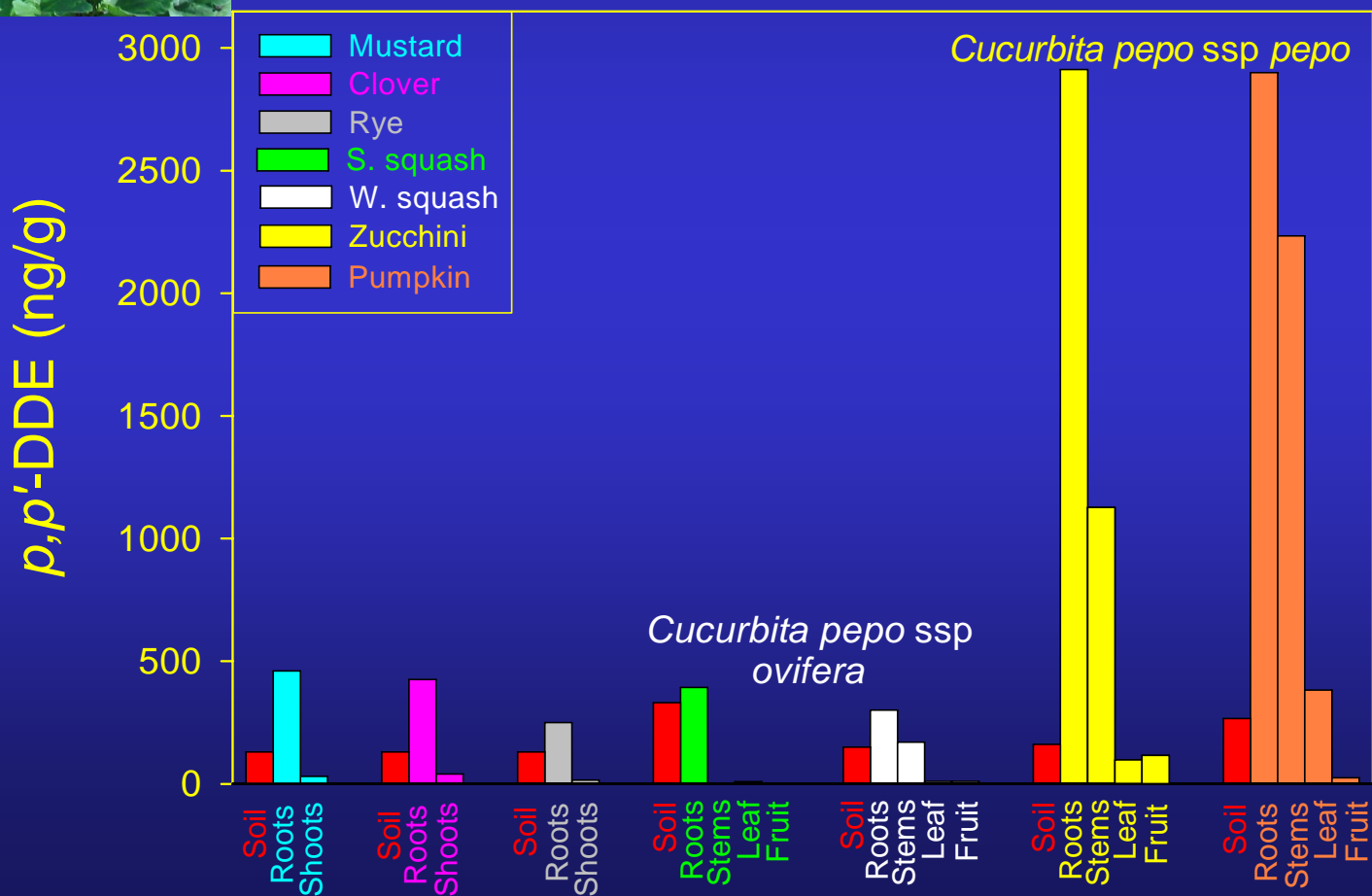
- They persist for decades
- Global distribution
- Likely mutagenic, estrogenic, carcinogenic effects
- Bioaccumulation, biomagnification
- Other remediation strategies are ineffective due to high degree of sequestration; plants should not facilitate their remediation

EPA Dirty Dozen

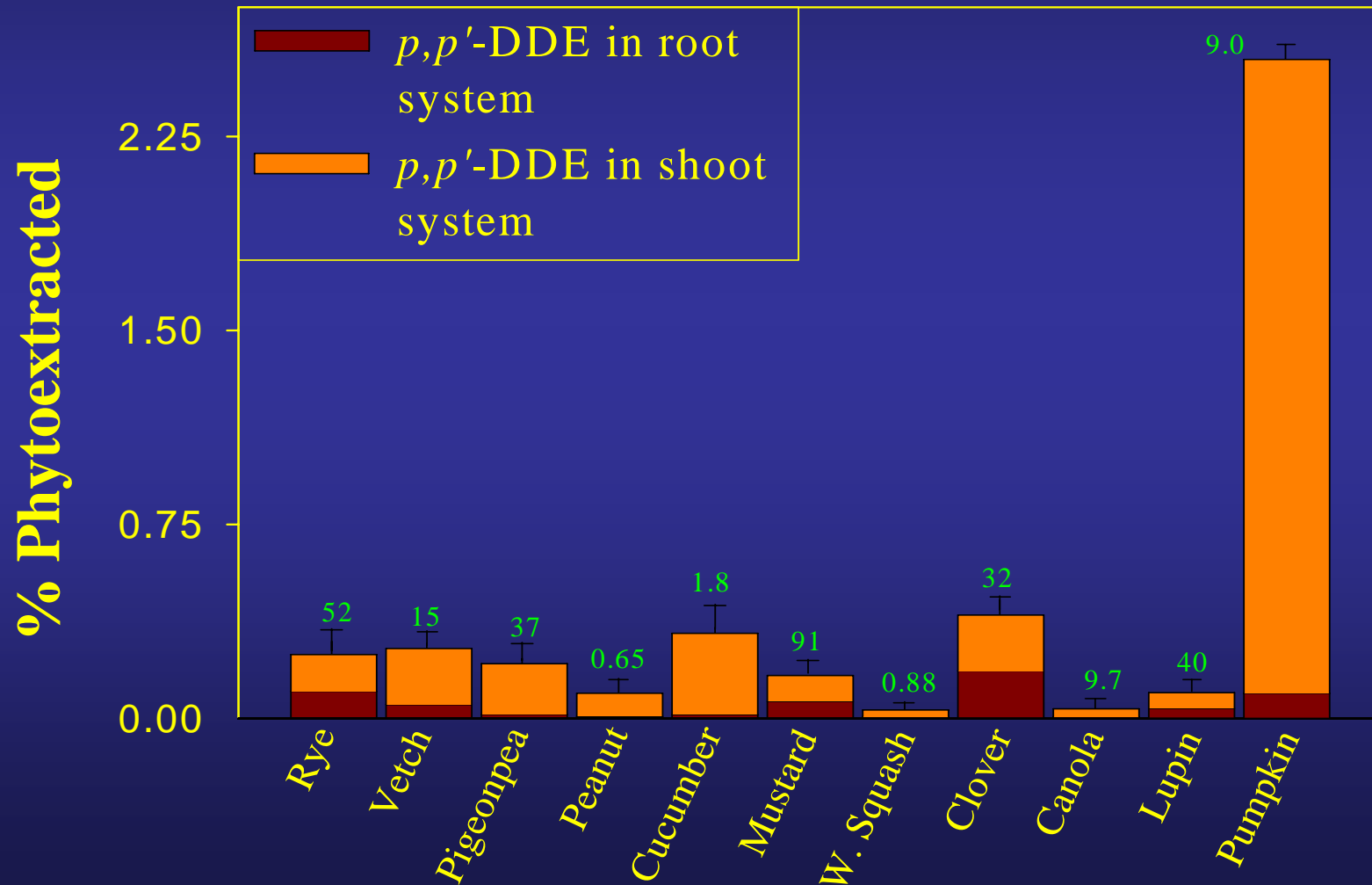
| | | |
|-----------------|-------------------------|-----------------------|
| <i>Aldrin</i> | <i><u>Chlordane</u></i> | <i><u>DDT/DDE</u></i> |
| <i>Dieldrin</i> | <i>Dioxins</i> | <i>Endrin</i> |
| <i>Furans</i> | <i>Heptachlor</i> | <i>Lindane</i> |
| <i>Mirex</i> | <i><u>PCBs</u></i> | <i>Toxaphene</i> |



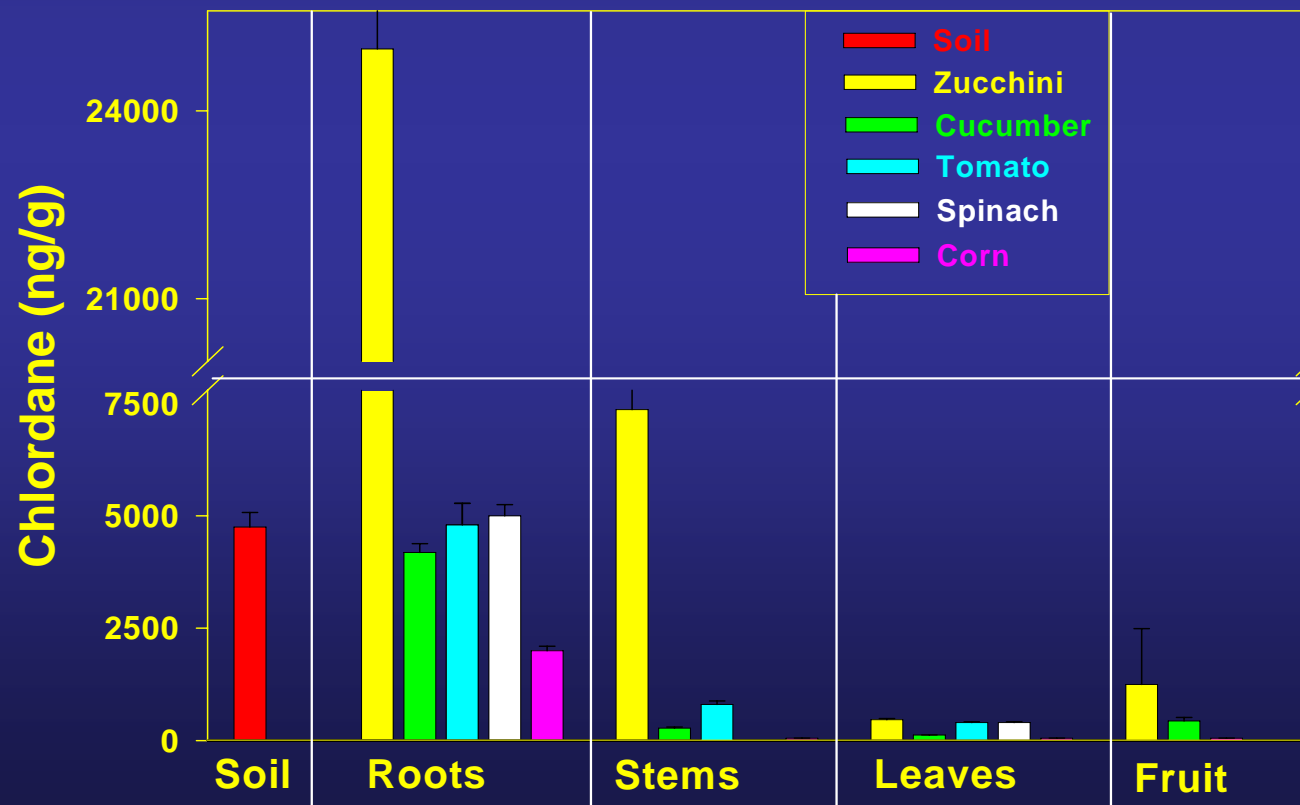
Uptake and translocation of weathered p,p' -DDE by selected plant species



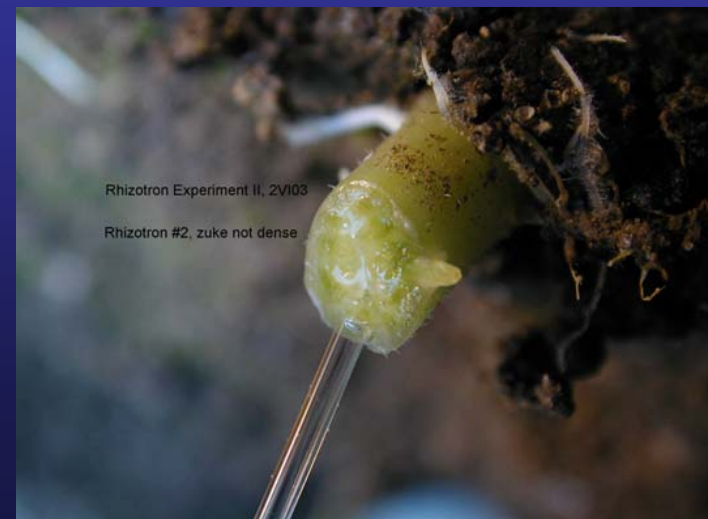
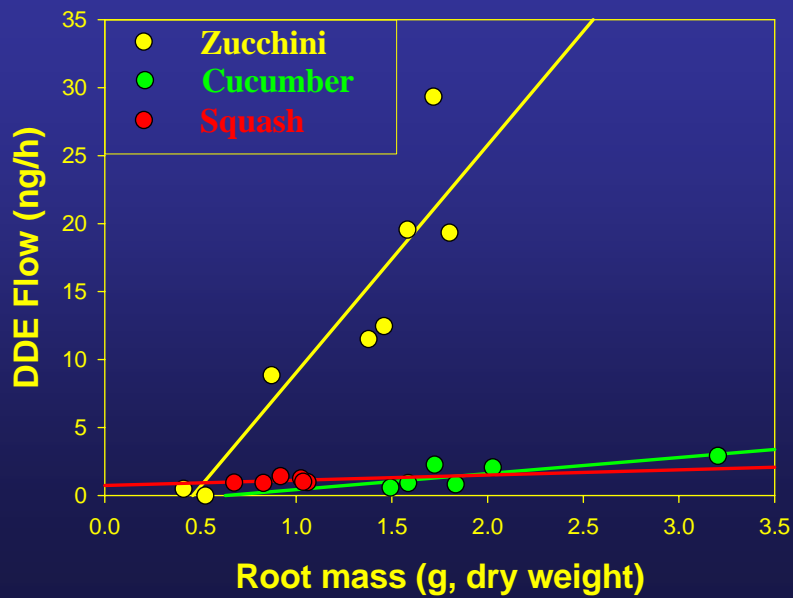
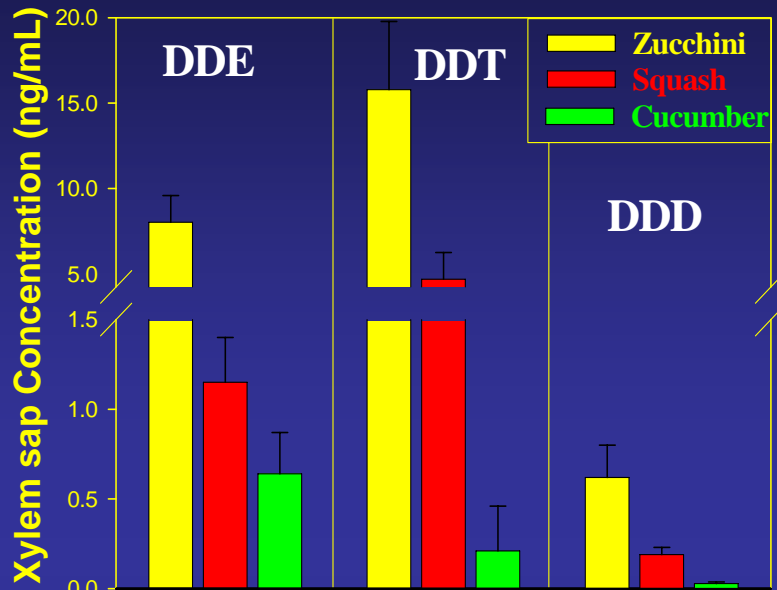
Phytoextraction (% removal) of weathered p,p' -DDE by plant species. Numbers in green indicate mass ratio of root to soil (values are 10^{-4})



Uptake and translocation of chlordane from soil by selected plant species



DDX in the xylem sap of 3 cucurbits



CAES Research on the *C. pepo* ssp *pepo* system

- **Focused on two separate lines of investigation:**

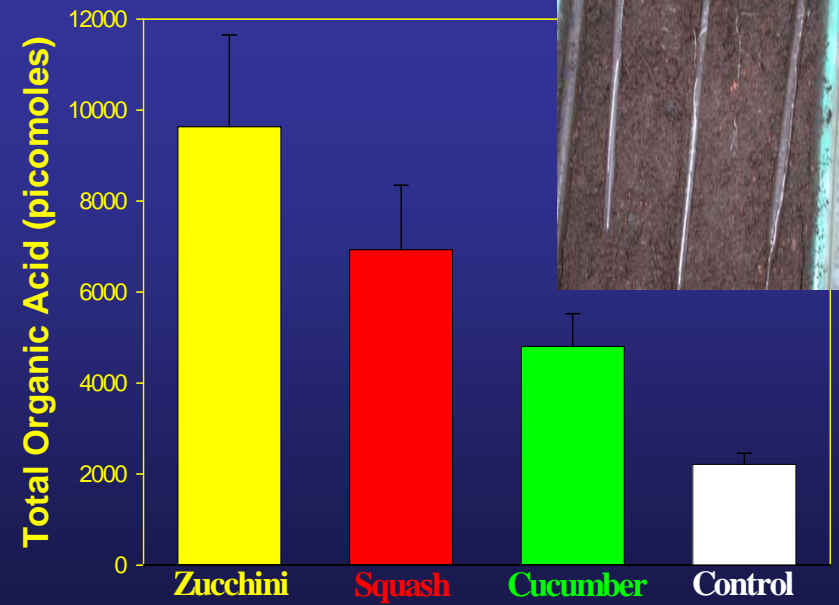
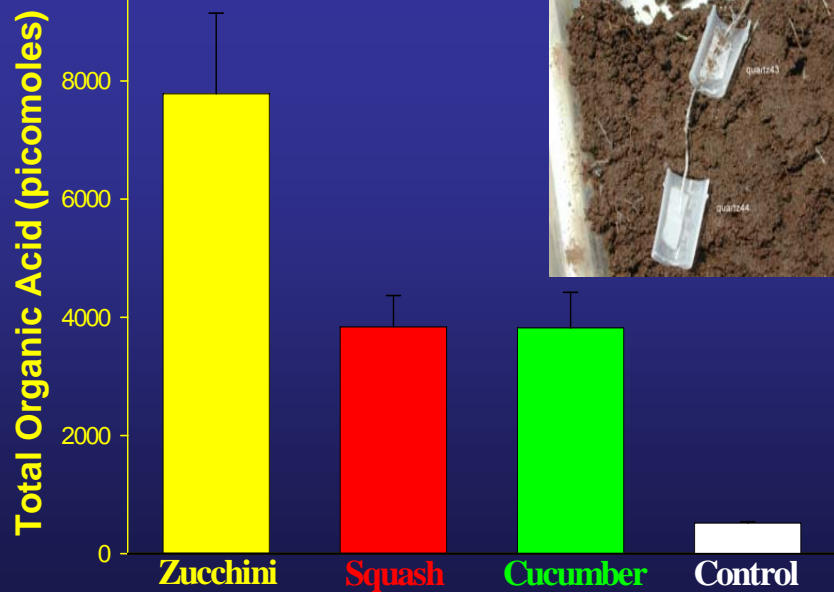
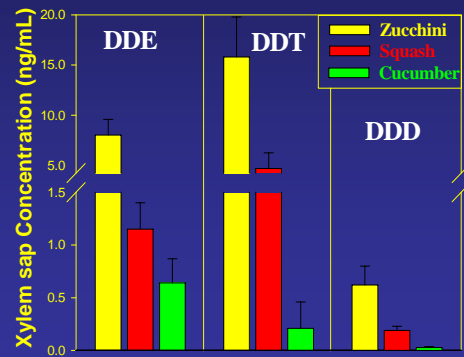
1. Optimizing remediation- Field/pot studies in an effort to further enhance contaminant uptake (cropping strategy, surfactants, organic chelators or TPAs, fungal inocula, intercropping)

2. Mechanistic studies- hydroponic, rhizotron, grafting, breeding, and molecular studies to elucidate governing processes.

The *C. pepo* ssp *pepo* system

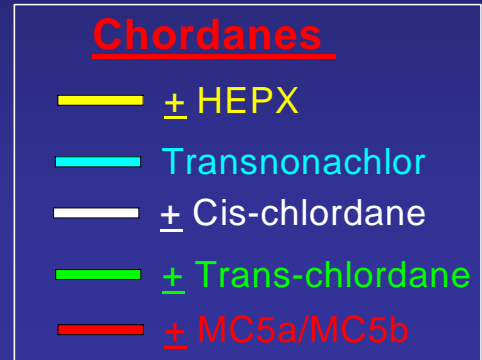
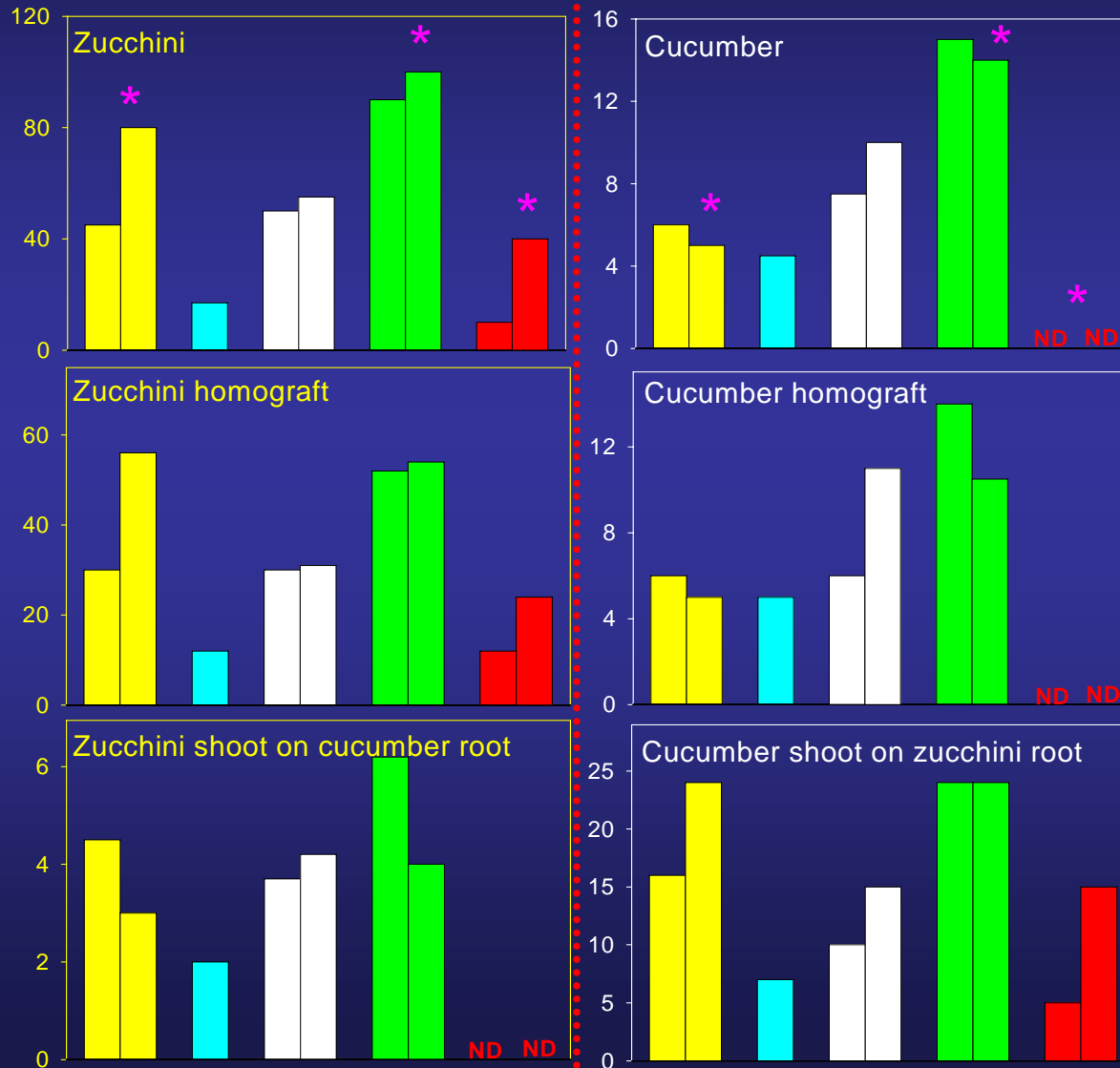
- Mechanistically, you need to consider two areas
- *Ex planta*- Release and uptake of a highly sequestered residue from soil to roots
 - Plant exudates are released to facilitate nutrient acquisition from soil. Species vary widely in exudation. Greater amounts of low molecular weight organic acids result in soil matrix deconstruction
 - This disruption in soil structure inadvertently increases the bioavailability of weathered POPs
- *In planta*- Translocation of a hydrophobic residue from roots to shoots.
 - Hydrophobic contaminants must move with water through root tissue and cross into the vascular cylinder. Should be minimal but some plants are clearly more effective than others.

Zucchini exude more organic acids, whether you look directly at the root surface (left) or in the rhizosphere pore water (right)



* Organic acids summed are citric, malic, succinic, tartaric, malonic

Accumulation of chlordanes components from soil by shoots of intact or grafted zucchini and cucumber plants (units are raw area counts)

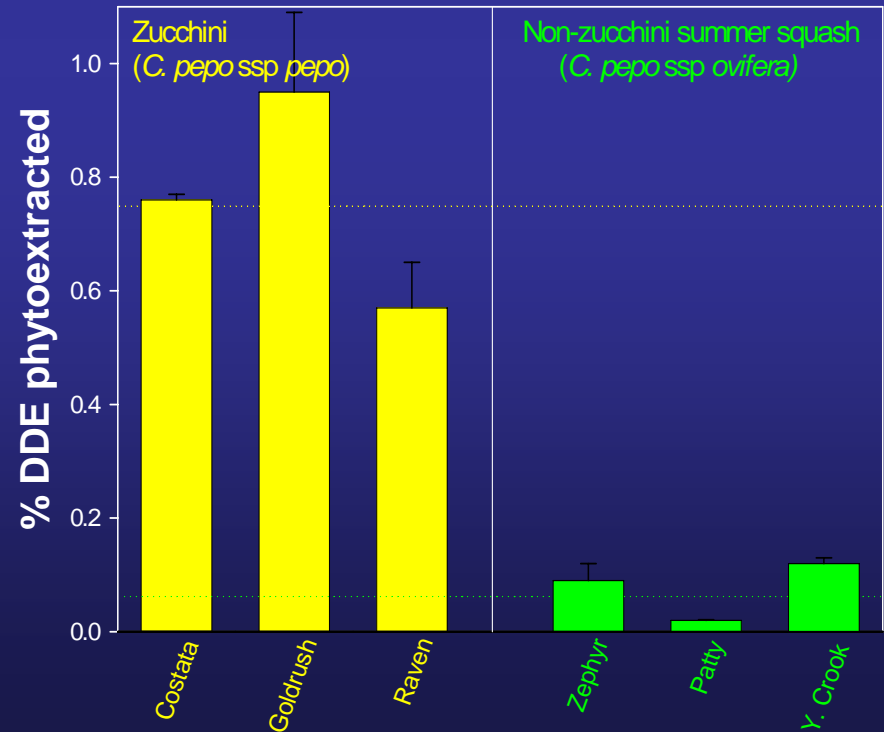
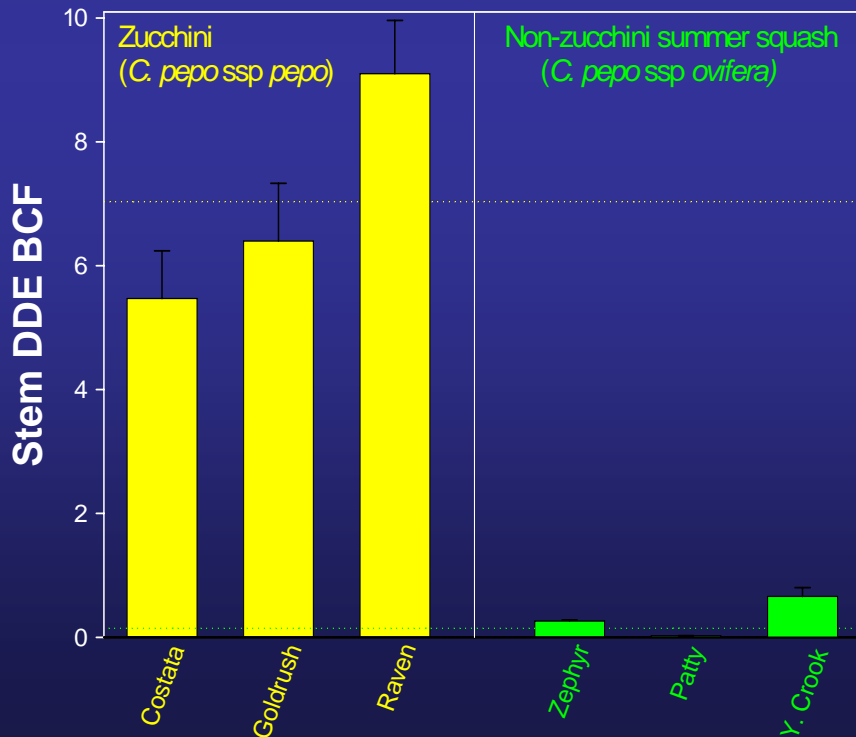


Mattina et al., 2007

Stem DDE bioconcentration factors and % DDE phytoextracted for 3 cultivars each of two *C. pepo* subspecies



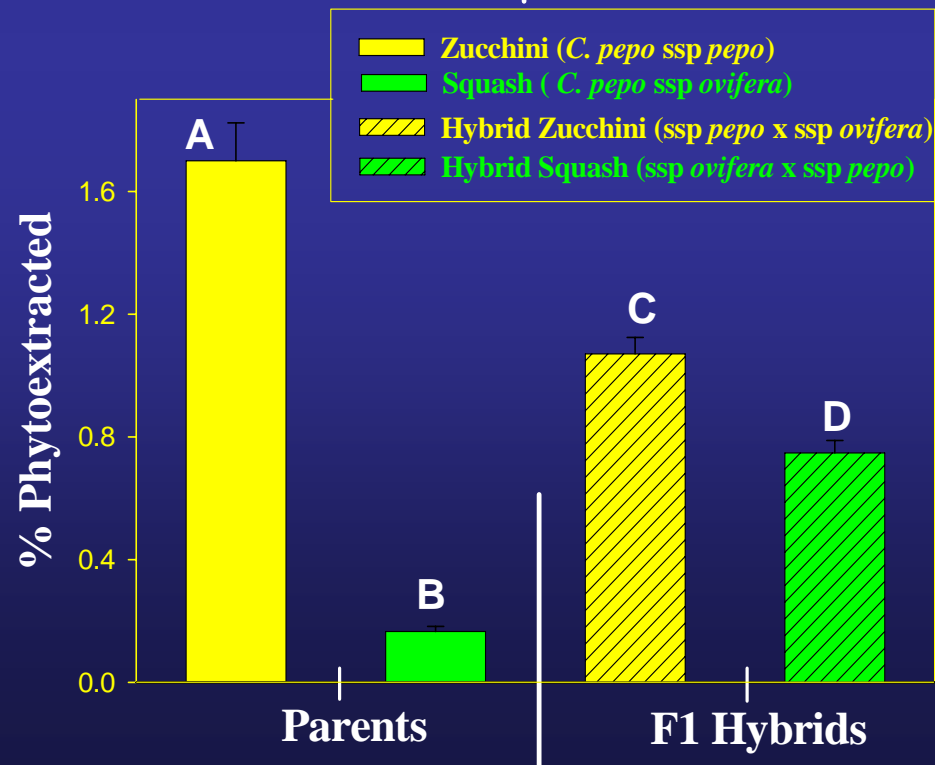
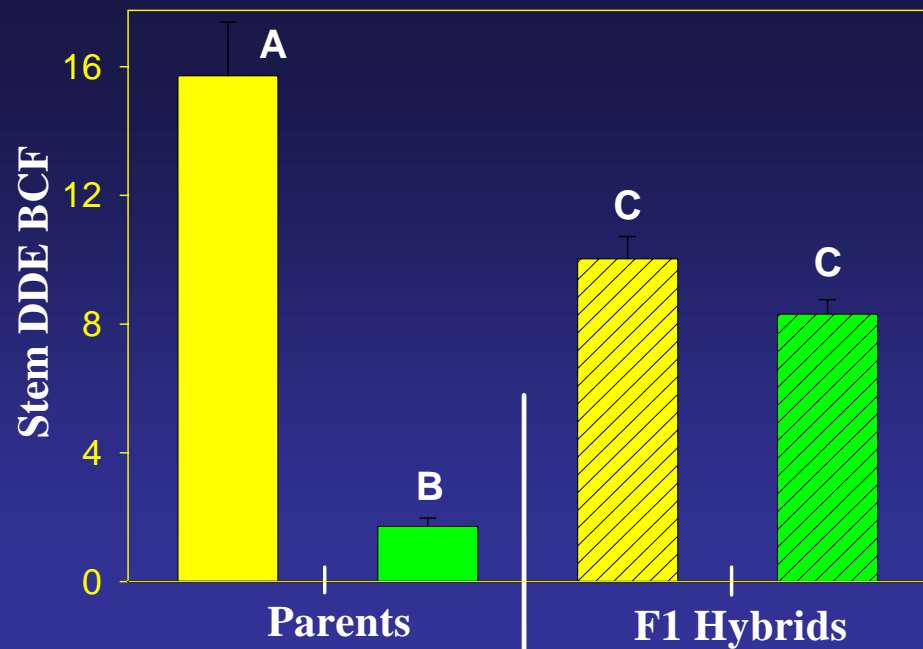
pepo subspecies



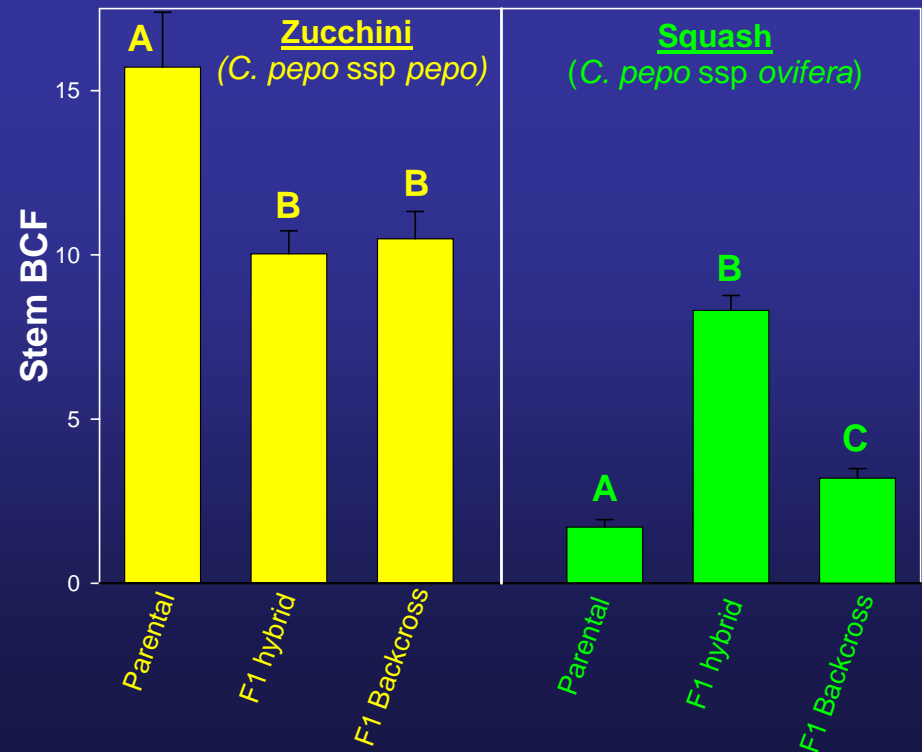
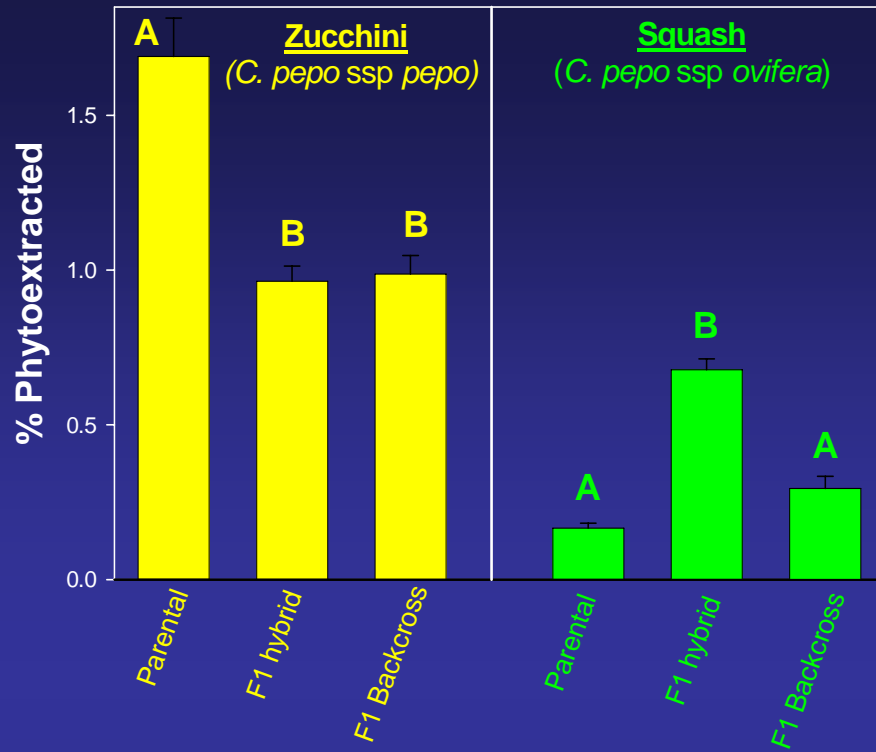
Traditional Breeding Studies- DDE-accumulating zucchini and non-accumulating squash are in the same subspecies.
What will be the abilities of the hybrids?



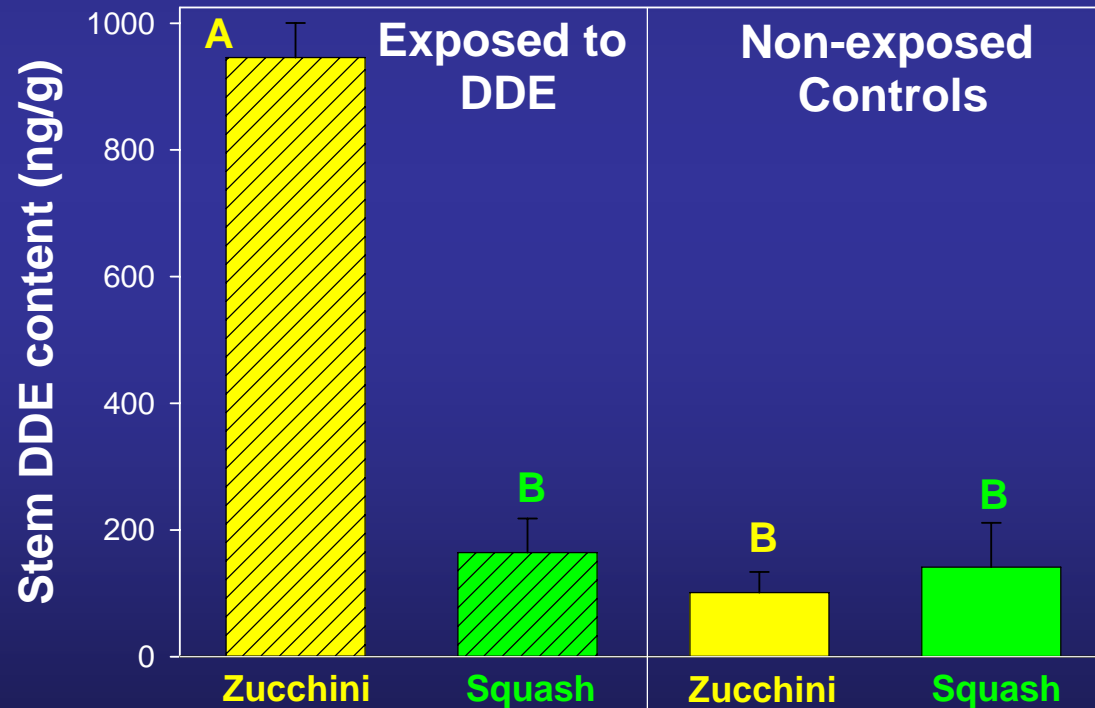
Stem bioconcentration factors (BCFs) and % DDE phytoextracted by parental and F1 hybrid zucchini and squash



% DDE Phytoextracted and Stem BCFs by parental, F1 hybrid, and F1 Backcrossed zucchini and squash (preliminary data)



Uptake of DDE by zucchini and squash exposed to 120 ng/mL DDE under batch hydroponic conditions for 96 hours



Zucchini and squash that were or were not exposed to DDE were subjected to differential transcript analysis and subtractive hybridization techniques. Subtracted cDNAs were cloned into a TA cloning vector and expression in *E. coli* via colony array analysis was employed (Fig. 1).

Figure 1.

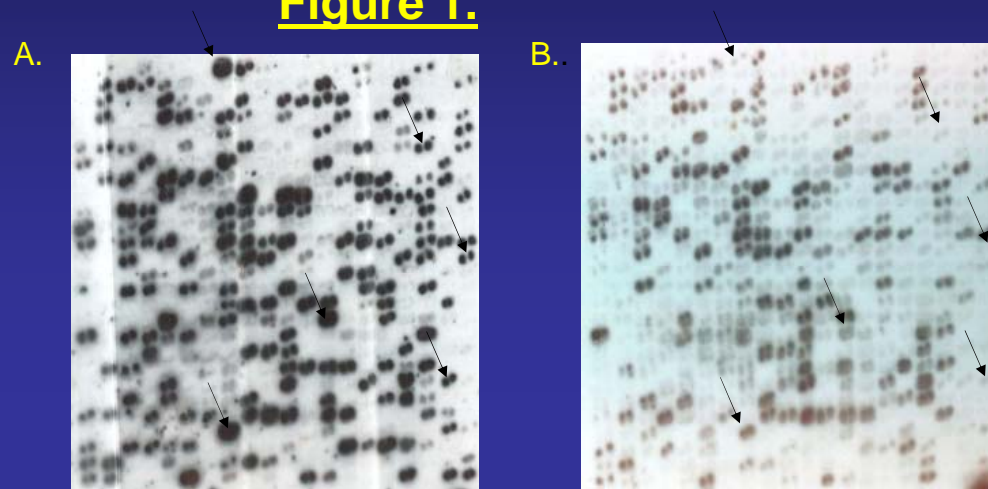
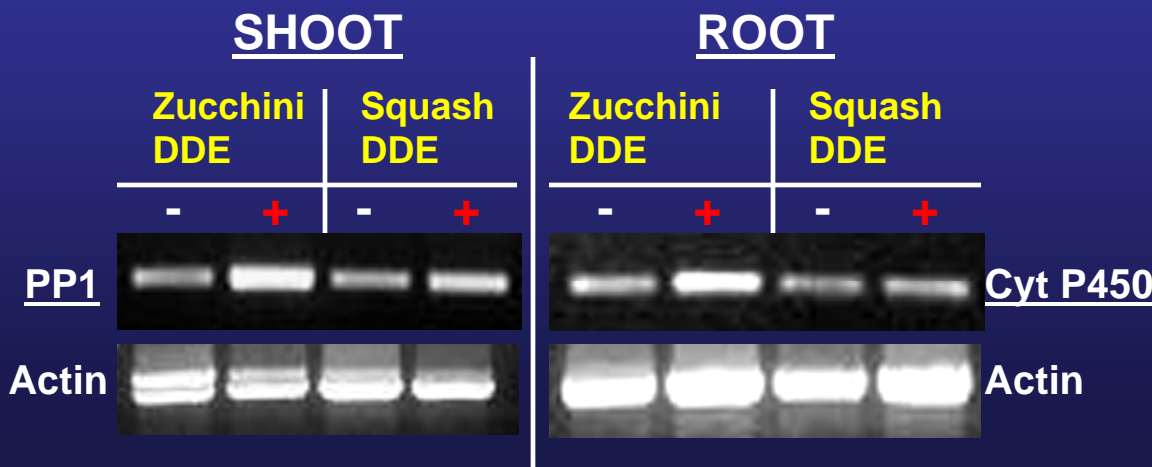


Figure 2.

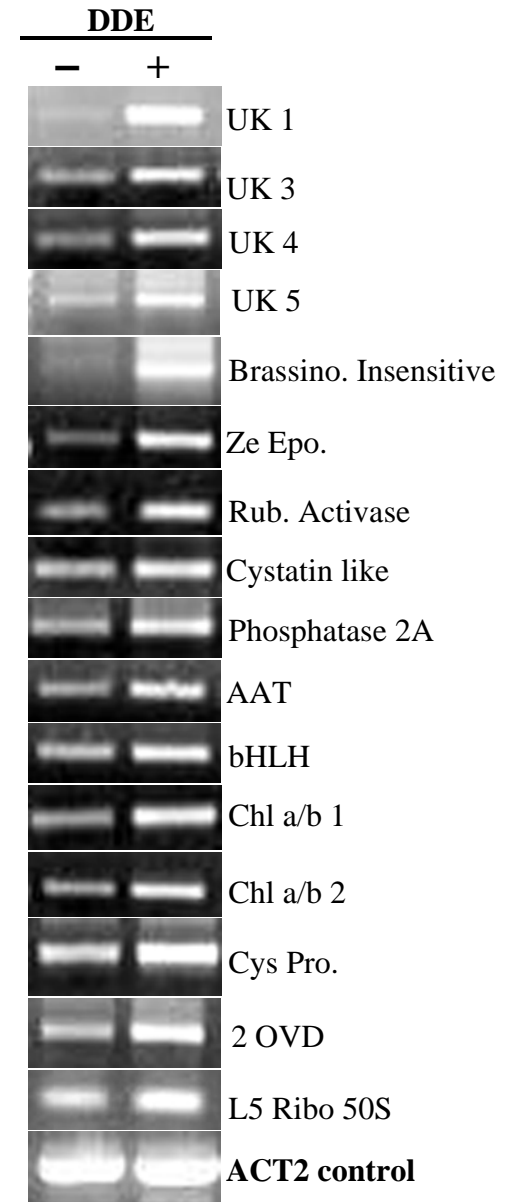


Blast search on isolated differentially expressed cDNAs includes sequence homology match to “phloem protein I” (PP1) and a cytochrome P450. Semi-quantitative RT PCR confirmed differential regulation (Fig. 2).

List of DDE induced subtracted cDNA clones from zucchini

| Gene Description | Accession Number | E value | Max % Identity | Functions |
|--|------------------|---------|----------------|--------------------------------|
| 2-Oxoisovalerate dehydrogenase (2 OVD) | NM_203027 | 3e-73 | 79% | oxidative stress |
| Aspartate aminotransferase (AAT) | XM_002515409 | 5e-108 | 85% | Amino acid metabolism |
| Brassinosteroid Insensitive 1 associated receptor kinase (Brassino. Inh) | XM_002532844 | 1e-104 | 80% | defense |
| Chlorophyll a/b-binding protein -1 | DQ471302 | 1e-76 | 84% | photosynthesis |
| chlorophyll a/b-binding protein -2 | XM_002284820 | 4e-97 | 83% | photosynthesis |
| Cystatin (Cystatin like) | ABF94587 | 3e-07 | 53% | cystein proteinase inhibitor |
| Cysteine Proteinase (Cys Pro) | AJ415386 | 3e-59 | 82% | signaling and abiotic stresses |
| DNA binding protein with bHLH domain (bHLH) | XP_002521010 | 3e-32 | 45% | unknown |
| Glucosylceramidase (Gluc. Cera.) | NM_124368 | 1e-09 | 81% | Lipid metabolism |
| Protein phosphatase-2a | XM_002513094 | 2e-60 | 84% | Involved in phosphorylation |
| 50S ribosomal protein L5 (L5 Ribo 50S) | XM_002511867 | 7e-51 | 79% | Unknown |
| Ribulose activase | EF506513 | 1e-122 | 82% | photosynthesis |
| Putative senescence-associated protein | BAB33421 | 4e-44 | 79% | Stress tolerance |
| Unknown protein 1 (UK 1) | XP_002527893 | 0.004 | 48% | Unknown |
| Unknown protein 2 (UK 2) | BT009458 | 8e-27 | 100% | Unknown |
| Unknown protein 3 (UK 3) | AY942801 | 4e-33 | 77% | Unknown |
| Unknown protein 4 (UK 4) | XM_002532526 | 3e-48 | 80% | Unknown |
| Unknown protein (predicted protein mahagunin like) (UK 5) | XM_002519497 | 1e-52 | 82% | Unknown |
| Unknown protein (predicted protein with chlorophyll a/b-binding) (UK 6) | U01964 | 1e-23 | 83% | Unknown |
| Unknown protein (Predicted protein with DNA binding domain) (UK 7) | XP_002531567 | 4e-09 | 49% | Unknown |
| Unkown predicted protein (UK 8) | XM_002309035 | 1e-31 | 78% | Unknown |
| Zeaxanthin epoxidase (Ze. Epo.) | DQ641126 | 2e-98 | 90% | abiotic stress tolerance |

Confirmation of upregulation of zucchini subtracted cDNAs by RT-PCR analysis



Conclusions

- *C. pepo* ssp *pepo* uniquely accumulates significant quantities of weathered POPs from soil
- POP accumulation is dependent on both contaminant properties and plant genotype
- Soil amendments such as organic acids, surfactants, and fungal inocula can increase POP extraction but effects are cultivar- and treatment-specific
- Although *C. pepo* ssp *pepo* has a unique organic acid exudation profile (as a nutrient acquisition strategy), the differences do not explain the observed POP accumulation
- Based on hydroponic, grafting, and molecular data, it appears that a unique transport protein in *C. pepo* ssp *pepo* tissue permits POP movement into the vascular cylinder
- Investigations elucidating the nature of this transport system are underway

Acknowledgements

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