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Effect of mycorrhizal fungi on the phytoextraction of weathered *p*,*p*-DDE by *Cucurbita pepo*

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Abstract

Field experiments were conducted to assess the impact of inoculation with mycorrhizal fungi on the accumulation of weathered *p*,*p*′-DDE from soil by three cultivars of zucchini (*Cucurbita pepo* spp. *pepo* cv Costata Romanesco, Goldrush, Raven). Three commercially available mycorrhizal products (BioVam, Myco-Vam™, INVAM) were inoculated into the root system of the zucchini seedlings at planting. In agreement with our previous findings, plants not inoculated with fungi accumulated large but variable amounts of contaminant, with root bioconcentration factors (BCFs, ratio of *p*,*p*′-DDE, on a dry weight basis, in the root to that in the soil) ranging from 10 to 48 and stem BCFs ranging from 5.5 to 11. The total amount of contaminant phytoextracted during the 62 day growing season ranged from 0.72–2.9%. The effect of fungal inoculation on the release of weathered *p*,*p*′-DDE from soil and on the subsequent uptake of the parent compound by zucchini appeared to vary at the cultivar level. For Goldrush, fungal inoculation generally decreased tissue BCFs but because of slightly larger biomass, did not significantly impact the percent contaminant phytoextracted. Alternatively, for Costata, BioVam and Myco-Vam™ generally enhanced *p*,*p*′-DDE accumulation from soil, and increased the amount of contaminant phytoextracted by up to 34%. For Raven, BioVam reduced contaminant uptake whereas Myco-Vam™ and INVAM increased contaminant phytoextraction by 53 and 60%, respectively. The data show that fungal inoculation may significantly increase the remedial potential of *C. pepo* ssp. *pepo*. The apparent cultivar specific response to mycorrhizal inoculation is unexpected and the subject of ongoing investigation.

Keywords: Phytoremediation; Phytoextraction; *Cucurbita pepo*; Zucchini; Persistent organic pollutants

1. Introduction

Chemicals such as dichlorodiphenyltrichloroethane (DDT) and its primary metabolites DDE/DDD are classified as persistent organic pollutants or POPs; a group that includes other contaminants such as dieldrin, polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) [1]. Due to their widespread occurrence, POPs present a significant environmental concern with regard to human and non-human exposure and risk. POPs are extremely hydrophobic, with octanol–water partition coefficients ($\log K_{ow}$) approaching 6.0–7.0. Consequently, these contaminants sorb strongly to soil/sediment organic matter and become progressively less bioavailable with time due to a process known as weathering or sequestration [2]. However, POPs will partition rapidly into fatty tissues or lipids of exposed organisms [3]. Because of their synthetic production and resulting unique molecular structure, biological systems generally lack the enzymatic potential to degrade POPs. In fact, contaminants such as DDT and its metabolites not only have half-lives in soil that are measured in decades but also have non-linear disappearance curves that level off, yielding a highly resistant fraction of the pollutant that is extremely difficult to

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remediate [2]. This overall recalcitrance, taken with the potential to accumulate in both natural solids and biological organisms, makes the investigation of novel POP remedial systems not only warranted but necessary.

Phytoremediation is the use of vegetation to remove organic and inorganic pollutants from contaminated natural media [4]. Organic pollutants may be degraded in the rhizosphere either by exuded enzymes or by the enhanced microbial community associated with plant roots. Alternatively, moderately hydrophilic organic compounds ($\log K_{ow}$ 1.5–3.0) may cross the plant root barrier with the flow of water, subsequently being degraded, volatilized, or stored in vegetative tissues [4]. Given the extreme hydrophobicity and general recalcitrance of POPs to degradation, plants are not expected to have a significant impact on the fate of these contaminants in soil, and in fact, much data exists in support of that contention [4–6]. However, Hülster et al. [7] reported in 1994 that certain cucurbits, specifically zucchini, could accumulate significant amounts of weathered dioxins from soil via a soil-to-plant transport mechanism. Subsequently, our group has shown that zucchini and pumpkin (both *Cucurbita pepo* spp. *pepo*) have a unique ability to remove and accumulate a range of persistent organic pollutants in both their root and shoot systems, including chlordane [5,8], *p*,*p*′-DDE [9,10], certain PCBs [11], and select polycyclic aromatic hydrocarbons (PAHs) [12]. The amount of each particular contaminant that is accumulated in the vegetation is variable, dependent not only on plant phylogeny but also on the physical/chemical characteristics of the pollutants [8].

Recent field experiments have shown that in spite of the unique ability of *C. pepo* ssp. *pepo* to accumulate weathered POPs from soil, low contaminant bioavailability still limits pollutant removal [10]. Thus, investigations focusing on treatments to enhance pollutant availability for subsequent phytoextraction will serve to maximize the remedial potential of this system. One such approach involves the use mycorrhizal fungi; eukaryotic microorganisms that form symbiotic associations with the roots of many plant species. In exchange for a suitable habitat and ready supply of complex high energy carbohydrates, the mycorrhizae utilize their unique hyphal structure and enzymatic potential to impact the soil structure and significantly increase the supply of available inorganic nutrients [13]. There are two main types of mycorrhizal fungi; ectomycorrhizal (EMF) and arbuscular mycorrhizal fungi (AMF). EMF are generally host specific and form a mycelial sheath around the plant root. AMF are generally not specific and physically penetrate the root with arbuscules, subsequently emitting their hyphae from within the root to the soil [14]. Although much work has been done on the effect of fungal species on the remediation of various organic and inorganic contaminants [15–19], the impact of these organisms on the rather unique *C. pepo*-POP phytoextraction process is unknown. Given the sequestered nature of the POPs and the rather intimate association of the fungal hyphae with the soil matrix, we hypothesize that the presence of these organisms in the rhizosphere may initially enhance contaminant bioavailability and subsequently increase the amount of pollutant removed from the soil. Thus, the current study assesses the impact of three commercially available AMF products on the phytoextraction of weathered *p*,*p*′-DDE by three cultivars of zucchini.

2. Materials and methods

2.1. Study site

Experimental plots were established at The Connecticut Agricultural Experiment Station's (CAES) Lockwood Farm (Hamden, CT) in areas contaminated with weathered *p*,*p*′-DDE residues at levels ranging from 50 to 800 ng/g dry weight [9]. The soil has an organic carbon content of 1.4%, pH of 6.7, and is classified as a fine sandy loam (56% sand, 36% silt, 8% clay). The experimental plot was covered with 400 m^2 of black polyethylene sheeting to limit the growth of unwanted vegetation and to aid in water retention. Prior to planting, 30 cm^2 squares were excised from the plastic at 3.0-m intervals (in all directions); each 30 cm^2 square served as a single replicate mound of vegetation.

Three cultivar varieties of *Cucurbita pepo* ssp. *pepo* ("Raven," "Goldrush," and "Costata Romanesco;" all are true zucchini) were acquired from Johnny's Selected Seeds (Albion, ME). Seeds were pre-germinated and the seedlings were planted in the field in May 2005. For the first 2–3 weeks of growth, the seedlings were protected from local fauna with row covers. Individual mounds of vegetation contained four plants. Each cultivar variety was grown in eight separate mounds; duplicates for each of the four treatments (three fungal inocula and the non-inoculated control plants) described below. Fruits were harvested throughout the summer. All plants were grown for approximately 62 day, with destructive harvest beginning in August 2005.

2.2. Fungal inocula

Three separate commercially available mycorrhizal inoculants were obtained. The first mycorrhizal root inoculant, called BioVam, was purchased from T&J Enterprises (Spokane, WA). The product contains vesicular arbuscular mycorrhizae (VAM) as a primary constituent and is recommended for use to enhance the growth and survivability of a range of agricultural and non-agricultural plant species. Specifically, the material consists of endomycorrhize (40–100 spores/cm^3), ectomycorrhizae (100–500 spores/cm^3), two *Trichoderma* species (up to 10,000 cells/cm^3), as well as the following bacteria (total of 20,000 cells/cm^3): *Arthrobacter globiformis*, *Bacillus subtillis*, two *Azobacter* species, and four *Pseudomonas* species. The second inoculant was called Myco-Vam™ and was purchased from Helena Chemical Company (Collierville, TN). This product contains three species of vesicular arbuscular mycorrhizal fungi; *Glomus intraradices* (minimum of 75 spores/cm^3), *Glomus aggregatum* (minimum of 13 spores/cm^3), and *Glomus mosseae* (minimum of 13 spores/cm^3). The final product was a mixture of four species obtained from Dr. Joseph Morton's INVAM collection at West Virginia University (Morgantown, WV). The spore counts are not known but the species present

are *Glomus intraradices*, *Glomus mosseae*, *Glomus etunicatum*, and *Glomus clarum*.

At planting of the three *C. pepo* ssp *pepo* cultivars (duplicate mounds for each of the 3 treatments and controls; for a total of 24 mounds), the inoculants (approximately 15 cm^3) were placed into the soil around the root system of each individual seedling.

2.3. Soil extraction

Soil cores (2.5 cm diameter, 6–10 cm depth) were collected prior to planting and were solvent extracted as described previously [9]. Eight separate soil cores were collected from each set of duplicate mounds; four cores from each of the corners of the replicates. Air dried and sieved through 2-mm sieve size, soil samples (3.0 g) from each treatment were amended with 1 μg (in 100 μl trimethylpentane) of *o*,*p*-DDE (as an internal standard) and 15 ml of hexanes prior to heating at 70 °C for 5 h. Particulates were removed by passing 1 ml of the supernatant through a glass microfiber filter (0.2 μm, Laboratory Science Inc., Sparks, NV) prior to collection in a chromatography vial. The average *p*,*p*′-DDE content in the soil of each set of duplicate mounds for all cultivar and treatment combinations was determined.

2.4. Vegetation extraction

At destructive harvest, a 1.0 m × 1.0 m × 0.25 m volume of soil that contained the root system of individual mounds of four plants was excavated. After careful removal of the roots from the soil, the fresh mass of plant tissues (fruit, leaf, stem, and root) was determined in the field. In the laboratory, the plant material was washed thoroughly with water to remove attached particles. The vegetation was then separated by treatment, cultivar, and tissue type; the biomass was then finely chopped and archived in 250-ml amber glass jars with Teflon-lined caps. A portion of the homogenized root systems of all plant/treatment combinations was stored at 4 °C in a separate 250-ml jars containing methanol; these roots were stained to quantify mycorrhizal colonization (described below). The procedure for extracting *p*,*p*′-DDE from vegetation is described in White et al. [9]. Briefly, the vegetation was mixed in an explosion-proof blender (Fisher Scientific, Springfield, NJ) with 2-propanol (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) amended with 1 μg of *o*,*p*-DDE (internal standard, in 100 μl of trimethylpentane) for 30 s prior to the addition of petroleum ether (Ultra-Resi-Analyzed, J.T. Baker, Phillipsburg, NJ) and subsequent blending for 5 min. After filtering and several water rinses, the petroleum ether was collected in a graduated cylinder containing 10 g of anhydrous sodium sulfate and allowed to sit for 2 h. The extracts were then filtered through florisil as follows. After conditioning 4 ml florisil cartridges (200 mg) (Alltech, Deerfield, IL) with 5 ml of petroleum ether that was discarded, 1 ml of the vegetation extract was loaded onto each cartridge. The cartridges were then eluted with 6 ml of 6% diethyl ether in petroleum ether. The volume of each extract was reduced to 1 ml under nitrogen on a heating block at 35 °C prior to analysis.

2.5. Quantifying mycorrhizal root colonization

Roots were removed from the methanol, air dried for 5 min, and weighed. For each cultivar/treatment combination, a 2–10 g (fresh weight) sub-sample of the fine roots were separated and weighed into Fisherbrand Histosette II tissue cassettes (Fisher Scientific, Springfield, NJ). The staining procedure is that of Giovannetti and Mosse [20]. All root-containing cassettes were placed in 10% KOH at approximately 90 °C for 1 h. The roots were removed and placed in tap water for 2 min prior to acidification in 2.3% HCl for 20 min. The roots were then placed in 3% hydrogen peroxide at 90 °C for 4 min. The roots were stained in 0.05% aniline blue in glycerol and lactic acid (v/v/v) for 8 min. After staining, the cassettes were opened and the roots were transferred to Petri dishes containing tap water and were stored at 4 °C.

When ready for analysis, water was drained from the Petri dish and the root sample was placed in a second dish that was marked with a grid matrix (0.25 cm^2). The root sample was spread out over the grid and a dissecting microscope was used to observe both the number of root-line intersections and the number of root-line intersections where mycorrhizal colonization was evident (fungal intersections). For each Petri dish, the number of fungal intersections occurring at every 60 root-line intersections was determined and expressed as percent colonization.

2.6. Chemical analysis

The *p*,*p*′-DDE content in the soil or tissue extracts was determined on a Agilent (Avondale, PA, USA) 6890 gas chromatograph (GC) with a ^{63}Ni micro-electron capture detector (ECD). The column (30 m × 0.53 mm × 0.5 μm) contained a SPB-1 film (Supelco, Bellefonte, PA) and the GC program was 175 °C initial temperature ramped at 3.5 °C/min to 225 °C, then ramped at 25 °C/min to 250 °C with a hold time of 4.71 min. A 2-μl splitless injection was used, and the injection port was maintained at 250 °C. The carrier gas was He, and the make-up gas was 5% CH_4 in Ar at 60 ml/min. The electron capture detector was maintained at 325 °C.

Crystalline *p*,*p*′-DDE and *o*,*p*′-DDE were acquired from the EPA National Pesticide Standard Repository (Fort Meade, MD). Portions of *p*,*p*′-DDE were transferred to trimethylpentane and were diluted to prepare calibration standards at 10, 25, 50, 100, 150, 250, and 500 ng/ml. One hundred ng/ml *o*,*p*-DDE was added to each calibration level as an internal standard. Concentrations of *p*,*p*′-DDE in the various vegetative tissues and soils were determined by internal standard calibration.

2.7. Statistical analysis

Soils from each cultivar/treatment combination were extracted in quadruplicate. The three separate cultivars of *C. pepo* ssp. *pepo* were designed to be replicates of each other; i.e., three zucchini replicates per treatment. In addition, vegetative tissues of each cultivar/treatment combination were extracted in triplicate. Amounts of *p*,*p*′-DDE in the tissues were determined

for the three cultivars with the different mycorrhizal inocula and were analyzed statistically by a two way ANOVA. The percent root colonization by mycorrhizal fungi was similarly analyzed for the three cultivars with the various inocula.

3. Results and discussion

3.1. Soil p,p′-DDE content

The concentration of weathered *p*,*p*′-DDE residues in the soil ranged from 48.7 ng/g (dry weight; "Goldrush" control) to 225 ng/g ("Costata" control). These values agree with previously reported contaminant concentrations and are indicative of historical DDT usage in agricultural areas [9].

3.2. Mycorrhizal colonization of roots

The percentage of colonization on the root systems of plants not receiving any fungal inoculum was 23%, as compared to 28, 35, and 24% for plants receiving BioVam, Myco-Vam™, and INVAM, respectively. Of these colonization values, only Myco-Vam™ is significantly greater than that present in the control root systems (one way ANOVA with Dunns multiple comparison test). Table 1 shows the percent mycorrhizal colonization for each of the cultivars. For all three cultivars, at least two of the three fungal inoculations resulted in significantly greater colonization than present in the controls but Myco-Vam™ was the only product that consistently increased mycorrhizal presence on the root system of these plants.

The methods for quantifying AMF colonization on plant roots are varied but fall into the general categories of visual observation (post-staining) or biochemical methods. The biochemical methods include quantitation of fungal constituents such as chitin, ergosterol, and fatty acid-sterols. Chitin is not specific to AMF and ergosterol rapidly degrades after cell death, in addition to be extremely sensitive to light and temperature. The biochemical detection of fatty acid-sterols is a novel and promising approach but the methodologies are relatively new and involved. Among the visual techniques, Giovannetti and Mosse [20] reported that the grid-line intersect method used in the current study produced the most consistent and reliable quantitation.

Kapulnik and Douds [13] report that nearly 80% of all plant families form symbiotic relationships with AMF species and that the origins of this mutualism extend 400 million years into the past. Thus, it is not particularly surprising to find 20–27% AMF colonization on the roots of our non-inoculated plant species. Table 1 seems to suggest that inoculation frequently but not consistently resulted in increased mycorrhizal colonization. It should be noted that variable response may be in part due to the method by which colonization was quantified. The primary weakness in the grid-line intersect method, as well as many of the other techniques mentioned above, is that they do not distinguish the species present. For example, there were several instances were AMF inoculation did not result in significantly greater colonization but it is entirely possible that the AMF community was completely different in the treated roots. Also, in instances where overall colonization was significantly increased by inoculation, there is no way to differentiate among the native species present in the soil or on the root and those that were present because of the direct introduction. Additional investigations are necessary to elucidate the fungal interactions and colonization at the species level.

Table 1
Percent mycorrhizal colonization as determined by a grid overlay method of *C. pepo* ssp. *pepo* roots for non-inoculated plants and for plants amended with three commercially available mycorrhizal fungal inocula at planting

Cultivar	Control[a]	BioVam	Myco-Vam™	INVAM
Costata	27A[b]	34B	35B	25A
Goldrush	23A	25A	32B	32B
Raven	20A	24B	37C	19A

[a] Controls are the three cultivars grown without mycorrhizal inoculation.
[b] Within a cultivar (row), values followed by different letters are significantly different (one way ANOVA with a Dunns Multiple Comparison test).

3.3. Vegetation p,p′-DDE content

Measurable levels of *p*,*p*′-DDE were detected in all vegetative tissues analyzed, and without exception, the highest concentration was found in the roots, followed by decreasing levels in the stems, leaves, and fruit. The INVAM treatment of Raven had the highest root *p*,*p*′-DDE content at 6900 ng/g and the highest level of contaminant in stem tissue was 1420 ng/g in Raven with Myco-Vam™. The concentration of *p*,*p*′-DDE in the remaining aerial tissues was 1–2 orders of magnitude less and ranged from 11–23 ng/g in the leaves and 9.0–43 ng/g in the fruit. Direct comparison of the contaminant concentrations in the tissues of the various cultivars is complicated by the differing amounts of *p*,*p*′-DDE in the soil compartment. This confounding factor is controlled by converting all data to bioconcentration factors or BCFs; the dry weight ratio of contaminant concentration in the tissue (root, stem, leaf, or fruit) to the *p*,*p*′-DDE level present in the soil of that particular cultivar/treatment combination. We have previously shown that contaminant accumulation over a concentration range of less than an order of magnitude (as in the current study) is linear and thus, BCF comparisons among different plant species and/or tissues is legitimate [10]. Another value of interest is the translocation factor or TF, which is defined as the stem BCF divided by the root BCF. Lastly, one can calculate the overall percent of *p*,*p*′-DDE removed or phytoextracted from the soil. This value is obtained by first calculating the absolute amount of contaminant in the plant; tissue concentration of the pollutant multiplied by biomass. The volume of soil impacted by each mound of vegetation is determined at destructive harvest (1.0 m × 1.0 m × 0.25 m) and converted to a mass by the pre-determined soil density of 1.14 g/cm^3. This mass of soil corresponds to 290 kg, which can then be used to calculate the absolute amount of *p*,*p*′-DDE in the soil compartment. A simple ratio of total contaminant in the plant over total contaminant in the soil yields percent contaminant phytoextracted.

The total plant biomass, tissue BCFs, and percent contaminant phytoextraction averaged across the three zucchini cultivars is shown in Table 2. For the non-inoculated plants, the average

Table 2
Effect of fungal inoculation on total plant biomass, tissue BCFs, and total percent contaminant phytoextracted averaged over the three zucchini cultivars

C. pepo ssp. *pepo*	Control	BioVam	Myco-Vam™	INVAM
Biomass[a]	1200 (490)	970 (510)	1500 (430)	1700 (540)
Root BCF[b]	30 (19)	19 (6.1)	24 (0.70)	33 (25)
Stem BCF[b]	7.8 (2.6)	8.4 (1.3)	4.7 (0.67)	5.1 (2.6)
Leaf BCF[b]	0.17 (0.12)	0.22 (0.07)	0.13 (0.04)	0.13 (0.05)
Fruit BCF[b]	0.22 (0.13)	0.19 (0.06)	0.12 (0.02)	0.15 (0.06)
%Phytoextracted[c]	1.6 (1.1)	1.1 (0.43)	1.6 (0.46)	1.3 (0.83)

Standard deviations are shown in parenthesis.
[a] Average total dry mass of the plants in the duplicate mounds.
[b] BCF or bioconcentration factor (dry weight ratio of tissue *p,p'*-DDE content to soil *p,p'*-DDE content).
[c] Ratio of total DDE mass in the plant to that present in the estimated 290 kg of soil containing the root system.

biomass was 1200 g (dry weight), with 98% of the mass being in the aboveground tissues and the fruit compartment representing 43% of the plant weight. The root and stem BCFs were 30 and 7.8, respectively, with the leaf and fruit compartments being at least an order of magnitude below the stems. On average, the zucchini cultivars removed 1.6% of the contaminant from the 290 kg of soil containing the plants and approximately 88% of the *p,p′*-DDE was present in the aerial tissues. Treatment with fungal inoculation did not significantly impact any of the parameters shown in Table 2. The reasons for this overall lack of effect are directly related to the large error associated with each term; the standard deviations were typically 40% of the average value.

Although the individual zucchini cultivars were designed to be replicates of *C. pepo* ssp. *pepo*, it was evident that under the various control and treatment regimes, the cultivars responded differently as indicated by a number of parameters (Table 3). For example, with the non-inoculated plants, the average biomass for Costata and Goldrush were 1400 and 1700 g (dry weight), whereas Raven was only 680 g. With only duplicates of each cultivar, statistical power is weak but by a simple Student's *t*-test, Goldrush is significantly greater than Raven. Similarly, the root and stem BCFs ranged from 10 to 34 and 5.3 to 11, respectively. One problem with isolating the replicate cultivars is the relatively weak statistical power upon comparative analysis but regardless, some interesting results are evident (Table 3). Here, a two way ANOVA was used to determine cultivar and treatment (fungal inocula) effects, although interactions could not be assessed due to the experimental design. Although the total plant biomass was unaffected, the relative mass of the fruit compartment or harvest index varied significantly by cultivar when analyzed over all treatments. The root, stem, and fruit BCFs, varied significantly with cultivars or treatments. Similarly, the translocation factor (stem BCF/root BCF) ranged from 0.13 (Raven INVAM) to 0.65 (Costata BioVam), varying significantly by both cultivar and treatment ($p < 0.001$).

The effect of fungal inoculation on the percent of *p,p′*-DDE phytoextracted from soil by each the three zucchini cultivars is shown in Fig. 1. For the non-inoculated or control plants, the amount of contaminant extracted agrees with previous reports, ranging from 0.72 to 2.9%. The effect of fungal inoculation varied greatly, but overall, the trends generally matched the observations made with the bioconcentration factors. In terms of statistical significance (two way ANOVA), percent phytoextraction varied significantly with cultivar when averaged over all treat-

Table 3
Effect of fungal inoculation on total plant biomass, harvest index, and tissue BCFs for the individual replicate zucchini cultivars

Cultivar	Treatment	Biomass[a]	Harvest index[b]	Root BCF[c]	Stem BCF[c]	Leaf BCF[c]	Fruit BCF[c]
Costata	Control	1400	0.43	10	5.3	0.10	0.12
	BioVam	1100	0.42	13	8.6	0.23	0.14
	Myco-Vam™	1400	0.36	25	7.1	0.18	0.14
	INVAM	1600	0.41	6.3	2.3	0.07	0.08
Goldrush	Control	1700	0.38	48	11	0.31	0.19
	BioVam	1300	0.54	20	7.0	0.16	0.17
	Myco-Vam™	1700	0.41	24	5.9	0.11	0.13
	INVAM	2200	0.41	34	5.8	0.15	0.14
Raven	Control	680	0.46	34	7.4	0.10	0.37
	BioVam	510	0.50	25	9.6	0.29	0.26
	Myco-Vam™	1500	0.59	24	7.0	0.11	0.10
	INVAM	1200	0.63	57	7.3	0.17	0.21
Significance[d]	Cultivar	ns	0.003	0.002	0.071	ns	0.002
	Treatment	ns	ns	0.046	0.030	0.059	0.060

[a] Average total dry mass of the plants in the duplicate mounds.
[b] The harvest index is the relative mass of the fruit compartment.
[c] BCF or bioconcentration factor (dry weight ratio of tissue *p,p′*-DDE content to soil *p,p′*-DDE content).
[d] Level of significance (probability or *p* value) as determined by a two way ANOVA; ns, not significant.

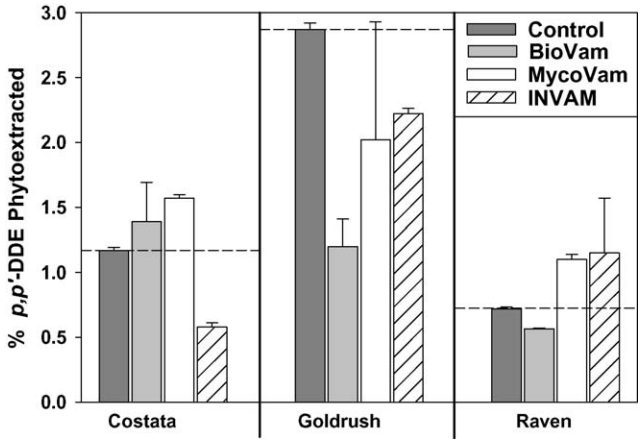

Fig. 1. Effect of mycorrhizal inoculation on the percentage of weathered *p*,*p*′-DDE phytoextracted from soil by three zucchini cultivars.

ments ($p < 0.001$). Although our experimental design prevented a statistical evaluation of the cultivar–treatment interactions, some trends in the data are evident. For example, with Costata, Myco-Vam^{TM} seemed to increase *p*,*p*′-DDE uptake whereas INVAM decreased contaminant accumulation. For Goldrush, mycorrhizal inoculation generally decreased the amount of contaminant removed from soil. For Raven, both Myco-Vam^{TM} and INVAM promoted *p*,*p*′-DDE phytoextraction whereas BioVam repressed contaminant uptake.

The data on *p*,*p*′-DDE content in the zucchini cultivars again confirms the unique abilities of this subspecies of *C. pepo* to accumulate and translocate weathered persistent organic pollutants. For example, White et al. [6] assessed *p*,*p*′-DDE uptake by 10 separate plant species (all non-cucurbits) within several different families and reported average stem BCFs, root BCFs, and percent contaminant extracted of 0.17, 1.9, and 0.12%, respectively. In the current study, the average stem BCF, root BCF, and percent contaminant phytoextracted across the three zucchini cultivars was at least an order of magnitude greater at 7.8, 30, and 1.6%, respectively. White et al. [9] specifically compared *p*,*p*′-DDE uptake in zucchini cultivars to that of other squash in *C. pepo* and reported 6.5-fold greater contaminant accumulation in the zucchini. Clearly, *C. pepo* ssp. *pepo* is unparalled in its abilities to phytoextract weathered POPs.

In the current study, the data suggest large variability in the parameters of interest among the three the *non-inoculated* zucchini cultivars. For example, dry biomass varied by a factor of 2.5, with fruit production being approximately 40% of the total mass and ranging from 310 to 640 g. The accumulation of *p*,*p*′-DDE also differed among the non-inoculated cultivars; root and stem BCFs varied by 4.8- and 2.1-fold, respectively. Similarly, percent phytoextracted varied by a factor of 4 across the three cultivars. These findings agree with those of White et al. [9] where 10 cultivars of *C. pepo* ssp. *pepo* (all zucchini) were compared. In that study, the average root BCF, stem BCF, and percent contaminant extracted were 7.2, 5.4, and 0.30, respectively, but these values varied by a factor of 6.7 across all cultivars and parameters. Comparing the data in White et al. [9] and to that in the current study, it is clear that a similar magnitude of variation for contaminant phytoextraction also exists at the cultivar level within the zucchini subspecies.

Given the previous discussion, it is not surprising to see that the effects of mycorrhizal inoculation on contaminant uptake and translocation also varied among the zucchini cultivars. The data in the current study agree with the findings of White et al. [10] where as part of a preliminary field study on *p*,*p*′-DDE uptake, BioVam was inoculated into the rhizospheres of five cultivars of *C. pepo* ssp. *pepo*, including Costata, Goldrush, and Raven. In that study, BioVam inoculation did not significantly impact plant biomass and the effect on contaminant uptake was cultivar specific, ranging from non-significance (Goldrush bioconcentration factors) to 6-fold increase in contaminant phytoextracted by a pumpkin cultivar. Similar to the previous study, Goldrush was again the least responsive cultivar in the current work, with the three fungal inocula either having no effect on uptake or actually decreasing the amount of *p*,*p*′-DDE in the plant. In both the previous and current experiments, Costata seemed to be the most responsive cultivar to inoculation, with two of the three commercial mycorrhizae promoting *p*,*p*′-DDE uptake. The reasons for this highly specific response of *C. pepo* ssp. *pepo* to mycorrhizal inoculation are unknown but may be the result of complex biochemical and physiological interactions between the inoculated fungi and the plant roots, as well as competition with other rhizosphere microorganisms. Experiments are currently being designed to elucidate some of these processes.

As mentioned previously, the vast majority of plant species form symbiotic associations with mycorrhizal fungi as a means to facilitate nutrient acquisition from soil. The potential mechanisms of this enhanced nutrient availability include increased hyphal/root surface area with associated greater soil contact, the extracellular production of lignin and humic acid degrading enzymes to disrupt soil structure, and the release of H^+ to enhance the solubilization of elements into the pore water [15,16]. Initial interest in the use of fungi for contaminant remediation focused on the white rot fungi (WRF); their excessive production of lignolytic enzymes promotes the degradation of complex organic material in soil but also in turn co-metabolizes a range of organic pollutants, including select PAHs, PCBs, and explosives [15,17]. However, WRF are difficult to culture and have a relatively short life span in soil; both features making them non-ideal organisms for bioremediation. However, Meharg and Cairney [15] noted the large degree of similarity between the WRF and EMF, and speculated that the slightly reduced degradative range of the EMF was more than compensated for by their broad phylogenetic diversity (6000 species) and widespread presence in the rhizosphere of many plant species. The primary shortcoming of the EMF is their host specificity; a major limitation when one considers the rather plant/contaminant specific relationships that have been observed in the phytoremediation literature. It is noteworthy EMF and AMF likely share many physiological characteristics of interest but that the arbuscular species are generally not host specific. However, the role of AMF in contaminant remediation remains largely unexplored. Oudeh et al. [18] observed increased Cd and Zn uptake by leek when AMF were present. Nelson and Khan [19] reported that AMF increased atrazine uptake by corn and more recently, Joner and

Leyval [16] observed increased degradation of certain PAHs in the rhizosphere of rye and clover. However, little work has been done with more recalcitrant contaminants such as POPs.

In spite of the impressive enzymatic potential of mycorrhizal fungi, the highly recalcitrant nature of *p*,*p*′-DDE and similar POPs makes contaminant biodegradation unlikely. We specifically note that the *C. pepo* system is not degrading the organic contaminants but in a process analogous to the uptake of heavy metals, unaltered parent compound is accumulating in the vegetation by unique mechanisms. We have shown that the bioavailability of weathered persistent organic pollutants is a limiting factor with regard to the *phytoextraction* abilities of *C. pepo* ssp. *pepo* and that amendments enhancing POP release/desorption from soil may subsequently promote contaminant accumulation by these species [10]. In fact, data suggests that the exudation of low molecular weight organic acids by zucchini cultivars is greater than for other cucurbits and the resulting soil matrix disarticulation that results from the chelation of inorganic soil elements by the acid carboxyl groups may result in enhanced POP desorption [21,22]. Thus, it is not the potential AMF-driven degradation of POPs that is of interest but it is the AMF's intimate association with, and potentially alteration of, the soil structure that is relevant. Through hyphal-induced disruption of the sequestering soil matrix, the subsequent increase in POP availability may enhance the zucchini-based phytoextraction of these contaminants. However, microbial interactions in the rhizosphere are extremely complex and competing AMF-driven process may have confounded the results. For instance, many of the AMF species used in the commercial formulations are in the *Glomus* genus. In addition to possessing the generic physiological processes described above for mycorrhizae, *Glomus* species are characterized by the exudation of a glycoprotein known as glomalin that, along with the extensive hyphal biomass, will promote the formation and stabilization of soil microaggregates [13]. In this case, glomalin-driven microaggregation could *further limit* the bioavailability of weathered contaminants and may explain the instances where *p*,*p*′-DDE accumulation by the plants was reduced after inoculation. Clearly, the interactions between mycorrhizal species, other rhizosphere microorganisms, and the host vegetation are extremely complex but must be understood prior to successful use of AMF species in POP phytoremediation strategies.

4. Conclusions

Persistent organic pollutants such as *p*,*p*′-DDE are among the most difficult and costly contaminants to remediate from soil. In spite of the fact that plant species are predicted to accumulate negligible quantities of weathered POPs from soil, this work again demonstrates the unique capacity of zucchini cultivars (*Cucurbita pepo* ssp. *pepo*) to phytoextract percent-level amounts of the pollutant. However, previous work has shown that the low bioavailability of weathered POPs is still a limiting factor for zucchini cultivars and that enhancing contaminant availability in soil will subsequently promote greater pollutant accumulation in the plant. One potential mechanism to enhance contaminant release from the soil is to inoculate the root zone of the cucurbits with arbuscular mycorrhizae; these symbiotic eukaryotic microorganisms are known for their enzymatic abilities to alter soil matrix structure so as to promote nutrient availability for the plant. In the current study, three commercially available mycorrhizal formulations were inoculated into the root zones of three zucchini cultivars and the effect on the phytoextraction of weathered *p*,*p*′-DDE was determined. The effects on contaminant accumulation were highly variable, ranging from a 2.5-fold *increase* in root BCFs for one cultivar to a nearly 2.8-fold *decrease* in phytoextraction for a second cultivar. The mechanisms driving the apparent cultivar- and inocula-specific responses are unknown but are likely related to the highly complex interactions between the various microorganisms in the rhizosphere and critical physiological processes of the plants such as root exudation and amenability to infection.

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References

[1] F. Wania, D. Mackay, Tracking the distribution of persistent organic pollutants, Environ. Sci. Technol. 30 (1996) 390A–396A.
[2] M. Alexander, Aging, bioavailability, and overestimation of risk from environmental pollutants, Environ. Sci. Technol. 34 (2000) 4259–4265.
[3] B.C. Kelly, F.A.P.C. Gobas, Bioaccumulation of persistent organic *pollutants* in lichen-caribou-wolf food chains of Canada's central and western arctic, Environ. Sci. Technol. 35 (2001) 325–334.
[4] J.L. Schnoor, Phytoremediation of soil and groundwater, Tech. Eval. Rep. 02-01 Ground Water Remediation Technologies Analysis Center, Pittsburgh, PA, USA, 2002.
[5] M.I. Mattina, W. Iannucci-Berger, L. Dykas, Chlordane uptake and its translocation in food crops, J. Agric. Food Chem. 48 (2000) 1909–1915.
[6] J.C. White, Z.D. Parrish, M. Isleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, M.I. Mattina, Uptake of weathered *p*,*p*′-DDE by plant species effective at accumulating soil elements, Microchem. J. 81 (2005) 148–155.
[7] A. Hülster, J.F. Muller, H. Marschner, Soil-plant transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (*Cucurbitaceae*), Environ. Sci. Technol. 28 (1994) 1110–1115.
[8] M.I. Mattina, M. Isleyen, B.D. Eitzer, W. Iannucci-Berger, J.C. White, Uptake by Cucurbitaceae of soil-borne contaminants depends upon plant genotype and pollutant properties, Environ. Sci. Technol. 40 (2006) 1814–1821.
[9] J.C. White, X. Wang, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, N.P. Schultes, M. Arienzo, M.I. Mattina, Subspecies-level variation in the phytoextraction of weathered *p*,*p*′-DDE by *Cucurbita pepo*, Environ. Sci. Technol. 37 (2003) 4368–4373.
[10] J.C. White, Z.D. Parrish, M. Iseleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, M.I. Mattina, Soil amendments, plant age, and intercropping impact DDE bioavailability to *Cucurbita pepo*, J. Environ. Qual., in press.
[11] J.C. White, Z.D. Parrish, M. Iseleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, J.W. Kelsey, M.I. Mattina, Influence of citric acid amendments on the availability of weathered PCBs to plant and earthworm species, Int. J. Phytoremed. 8 (2006) 63–79.
[12] Z.D. Parrish, J.C. White, M. Iseleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, J.W. Kelsey, M.I. Mattina, Accumulation of weathered poly-

cyclic aromatic hydrocarbons (PAHs) by plant and earthworm species, Chemosphere, in press.
[13] Y. Kapulnik, D.D. Douds (Eds.), Arbuscular Mycorrhizas: Physiology and Function, Kluwer Academic Publishers, Dordrecht, Netherlands, 2000.
[14] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, second ed., Academic Press, London, UK, 1997.
[15] A.A. Meharg, J.W.G. Cairney, Ectomycorrhizas—extending the capabilities of rhizosphere remediation? Soil Biol. Biochem. 32 (2000) 1475–1484.
[16] E.J. Joner, C. Leyval, Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhizal, Environ. Sci. Technol. 37 (2003) 2371–2375.
[17] J. Dittman, H. Wolfgang, H. Bucking, Biodegradation of aromatic compounds by white rot and ectomycorrhizal fungal species and the accumulation of chlorinated benzoic acid in ectomycorrhizal pine seedlings, Chemosphere 49 (2002) 297–306.
[18] M. Oudeh, A. Khan, J. Scullion, Plant accumulation of potentially toxic elements in sewage sludge as affected by soil organic matter level and mycorrhizal fungi, Environ. Pollut. 116 (2002) 293–300.
[19] S.D. Nelson, S.U. Khan, Uptake of atrazine by hyphae of glomus vesicular arbuscular mycorrhizae in the root system of corn, Weed Sci. 40 (1992) 161–170.
[20] M. Giovannetti, B. Mosse, An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots, New Phytol. 84 (1980) 489–500.
[21] B.D. Eitzer, M.I. Mattina, M. Isleyen, W. Iannucci-Berger, J.C. White, Impact of low molecular weight organic acids exuded from Cucurbitacea roots on the release of soil-sequestered contaminants, Environ. Tox. Chem., submitted for publication.
[22] J.C. White, M.I. Mattina, W.-Y. Lee, B.D. Eitzer, W. Iannucci-Berger, Role of organic acids in enhancing the desorption and uptake of weathered *p,p′*-DDE by *Cucurbita pepo*, Environ. Pollut. 124 (2003) 71–80.