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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^{TM}, 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^{TM} 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^{TM} 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^{TM} 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