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Triclosan inhibits arbuscular mycorrhizal colonization in three wetland plants

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HIGHLIGHTS

- The effects of triclosan (TCS) on arbuscular mycorrhizal associations were assessed.
- We used a continuous flow-through exposure system and three wetland plant species.
- At 0.4 μg/L and 4.0 μg/L TCS, arbuscular and hyphal colonization levels were significantly reduced.
- 0.4 μg/L TCS is within the range of values obtained for North American surface waters.
- TCS may impair arbuscular mycorrhizal associations in North American wetlands.

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ABSTRACT

In terrestrial ecosystems, plant growth, plant community structure, and ultimately the ecosystem services provided by plants are dependent on the presence and composition of below ground arbuscular mycorrhizal (AM) fungal communities. AM fungi form obligate symbioses with plants providing nutrients to their host plants in exchange for photosynthates. While AM have been found in most wetland ecosystems, the effects of urban contaminants on AM associations are largely unknown. Triclosan (5-chloro-2-[2,4-dichlorophenoxy]phenol; TCS) is a widespread contaminant found in surface waters throughout North America and in addition to antimicrobial properties is purported to have antifungal properties. To determine the effects of TCS on arbuscular mycorrhizal associations, we exposed AM inoculated wetland plant species (*Eclipta prostrata*, *Hibiscus laevis*, and *Sesbania herbacea*) to TCS at concentrations of 0.0, 0.4 and 4.0 μg/L in a continuous flow-through exposure system. TCS exposure caused significant reductions in hyphal and arbuscular colonization while no significant effect was detected for vesicular colonization. Across all species, hyphal colonization was significantly higher in controls (18.58 ± 1.84%) compared to 0.4 and 4.0 μg/L (10.20 ± 1.34% and 9.86 ± 1.32% respectively) TCS treatments. Similarly, arbuscular colonization was significantly higher in the controls (4.58 ± 0.75%) compared to 0.4 μg/L (2.20 ± 0.38%) and 4.0 μg/L (1.22 ± 0.24%) TCS exposures. Since our lowest effect concentration, 0.4 μg/L, lies within the range of concentrations found in North American streams it is plausible that AM colonization has been impacted in streams receiving WWTP effluent. Further studies are required to understand the mechanism of TCS inhibition of mycorrhizal colonization in wetland plant species as well as the potential ecological consequences that a decline in the AM colonization levels may represent.

1. Introduction

Triclosan (5-chloro-2-[2, 4-dichlorophenoxy] phenol; TCS) is a widely used antibacterial found in pharmaceuticals and personal care products (PPCPs) ranging from soaps and detergents to clothing and kitchen aids (Dann and Hontela, 2011). As a result of the many consumer products containing TCS and their usage, TCS is considered a "down the drain" compound. Consequently, the primary source of TCS input to the environment is via wastewater treatment plant (WWTP) effluent (Oulton et al., 2010). Although TCS concentrations can be reduced by up to 98% of influent water depending on WWTP processing (Lishman et al., 2006; Thompson et al., 2005), effluent concentrations of up to 0.36 μg/L (Lee et al., 2005) and 2.7 μg/L (McAvoy et al., 2002) have been found in Canadian and US studies, respectively. Additionally, runoff from agricultural soils receiving sewage sludge as a soil amendment provides a second route of TCS entry into the environment (Macherius et al., 2012). TCS in sewage sludge from two North American studies was found at concentrations of 28.2 mg/kg in Canada (Lee and Peart, 2002) and 15.6 mg/kg (Chu and Metcalfe, 2007) in US, while TCS in agricultural soil amended with biosolids has been measured at the range of 0.160 to 0.960 mg/kg (Kinney et al., 2008).

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Triclosan is among the most widely detected PPCPs in surface waters with reported North American concentrations ranging from below detection levels to 2.3 μg/L (Dann and Hontela, 2011; Kolpin et al., 2002). TCS toxicity has been exhibited in a range of taxa including benthic invertebrates (Orvos et al., 2002), crustaceans (Tatarazako et al., 2004), fish (Ishibashi et al., 2004), algae (Wilson et al., 2003), duckweed (Fulton et al., 2009), and wetland macrophytes (Stevens et al., 2009). Algae have been identified as particularly sensitive to TCS exposure with a no observed effect concentration (NOEC) of 0.69 μg/L (Orvos et al., 2002); a value less than current TCS concentrations in some North American watersheds (Dann and Hontela, 2011). Wetland vascular plants may share a similar sensitivity to TCS exposure. Stevens et al. (2009) found that root development of three emergent vascular plants was inhibited by measured TCS concentrations of approximately 0.6 μg/L, the lowest concentration tested. The effects of TCS on soil fungi have been largely neglected despite the significant role that they play in nutrient cycling, soil stability and maintaining plant community structure.

The term "mycorrhiza" describes an association that develops between plant root systems and specific soil fungi. The association is widespread throughout the plant kingdom; more than 90% of terrestrial plants are estimated to form mycorrhizal associations (Strack et al., 2003). Arbuscular mycorrhizal (AM) fungi are the most abundant mycorrhizal fungi, colonizing root cortical cells and forming specialized structures including hyphae, arbuscules, and vesicles within root systems (Brundrett et al., 1996; Smith and Read, 2008). Through their accession of, and translocation of nutrients normally unavailable to the plant, primarily phosphorus and nitrogen, AM improve plant nutrient uptake. In exchange, the heterotrophic fungus, an obligate symbiont, is provided with host-produced photosynthates.

It is well recognized that AM play significant roles in terrestrial ecosystems due to their impacts on nutrient cycling, improvement in soil quality, carbon transport (Brundrett et al., 1996), providing food for soil invertebrates (Fogel, 1988) and limiting erosion due to the mechanical aggregation of soil particles (Andrade et al., 1998). More recently, AM have been shown to influence plant community structure by mediating competitive interactions (Hartnett and Wilson, 1999; John and Coleman, 1983), influencing soil microbial community structure, and altering host plant physiology (Rillig, 2004). The importance of AM to wetland plant communities and their role in wetland ecosystem services are largely unknown. AM were long thought absent in wetland plants (Khan and Belik, 1994), however, they have been found in many major wetland ecosystems including cypress swamps (Kandalepas et al., 2010), bottomland hardwood forests (Stevens et al., 2010a), nutrient poor fens (Cornwell et al., 2001), tropical river flood plains (de Marins et al., 2009), and tropical marshes (Radhika and Rodrigues, 2007). AM have been shown to affect the growth and development of several wetland plant species (e.g. *Cladium jamaicense* (Lin et al., 2011), *Lythrum salicaria* (Stevens et al., 2002), *Oryza sativa* (Solaiman and Hirata, 1997), *Leersia hexandra* and *Panicum hemitomon* (Miller and Sharitz, 2000), *P. hemitomon* and *Typha latifolia* (Ipsilantis and Sylvia, 2007)). However, not all species are affected to the same degree by the presence of AM fungi indicating that the magnitude of the AM effect is species specific (Stevens et al., 2010a; Daleo et al., 2008). Consequently, any perturbation that alters AM associations in wetlands may have a differential effect on wetland species and substantial repercussions in terms of wetland plant community dynamics and wetland ecosystem functions.

Triclosan disrupts fatty acid synthesis (FAS) by inhibiting the enoyl-acyl carrier protein reductase activity encoded by the *fabI* gene during Type II FAS (Heath et al., 1999; Newton et al., 2005); a pathway shared between bacteria and plants. In contrast, animals and fungi undergo Type I FAS (Lee et al., 2006) and should, therefore, be unaffected by TCS. TCS is, however, listed by the EPA as a fungicide and fungistat (Jones et al., 2000). The single study to date that examined TCS exposure on AM hyphal growth and spore production found no significant effects at concentrations of up to 1000 μg/L TCS (Hillis et al., 2008). This study utilized a static non-renewal exposure system with TCS dissolved in the agar media, and transformed carrot roots as the host organism. Consequently, this study may not reflect exposure dynamics in water bodies receiving WWTP effluent or responses of more typical wetland vegetation. Given the importance of AM in structuring and maintaining ecosystem services and lack of information regarding TCS impacts on AM associations, our goal was to assess the effects of TCS on early development of AM associations in three emergent wetland plant species (*Eclipta prostrata*, *Hibiscus laevis*, and *Sesbania herbacea*) utilizing a continuous flow-through exposure system.

2. Methods and materials

2.1. Plants

Based upon a preliminary assessment of the AM status of wetland plants in North Central Texas and their abundance in local wetlands, three rooted emergent wetland plant species were selected for this study: *E. prostrata* (L.) L., false daisy, in the family Asteraceae; *S. herbacea* (Mill.) McVaugh, big pod sesbania, in the family Fabaceae; and *H. laevis* All, halberd leaf rosemallow in the family Malvaceae (taxonomy follows Diggs et al., 1999).

2.2. Chemicals

Neat native TCS (Irgasan) was purchased from Fluka Laboratories (Buchs, Switzerland). The internal standard, $^{13}C_{12}$ TCS, was obtained from Wellington Laboratories (Guelph, ON, Canada). Analytical grade hexane (HEX), ethyl acetate (ETAC), chloroform (CHLF), and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were purchased from Fisher Scientific (Houston, TX, USA).

2.3. Flow-through exposure system

A flow-through exposure system was established in the Institute for Applied Sciences, Environmental Greenhouse at the University of North Texas, Denton, TX. Exposure solutions (0.0, 0.4 and 4.0 μg/L) were obtained by dissolving neat TCS in deionized (DI) water without the use of carrier solvents. Exposure solutions were mixed in 22 L high-density polyethylene (HDPE) reservoirs and replenished after 36 h. Nutrients were added to obtain 1/64 strength Long Ashton nutrient levels (Hewitt, 1966) in the exposure solution. This concentration of nutrients resulted in a phosphorus level comparable to the level present in the Trinity River, Denton, TX, and is a level previously found to promote mycorrhizal associations in native Texas wetland plants (Stevens et al., 2010b). Controls received 1/64th strength Long Ashton nutrients. Exposure solutions were delivered to non-draining plastic potting trays (54×28×6 cm, Summit Plastic Company) via a 12 channel peristaltic cassette pump (12/6 Thermo Scientific, Barrington, IL) at a constant flow rate of 2.5 mL/min. Four channels on the pump were utilized for each treatment. Seedling growth inserts (4×6×6 cm; Dillen Products, Rochester, NY) were placed in the trays. Each insert was filled with approximately 115 g of commercial sand (Sakrete Natural Sand, Bonsal American, Charlotte, NC, USA) and the sand surface was covered with light impenetrable fabric to inhibit algal growth. A small opening in the fabric permitted the shoots to pass through. To prevent algal growth in the 0.55 mm ID PTFE microbore tubing (Cole-Palmer, Vernon Hills, IL), delivery tubes from each peristaltic pump cassette were inserted into 1 cm diameter black tubing. All reservoirs and the peristaltic pump were shielded from the light by a shade tent made from light impenetrable fabric.

Seeds of experimental plants were germinated in Petri dishes on the surface of filter paper moistened with DI water. Immediately after radical emergence, seedlings were transplanted to the seedling growth inserts and inoculated with approximately 1 mL of *Glomus intraradices* spores in liquid suspension (BioSyneterra Solutions Inc., Quebec, Canada). One