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Effect of biosolids-derived triclosan and triclocarban on the colonization of plant roots by arbuscular mycorrhizal fungi

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HIGHLIGHTS

- Examined whether triclosan or triclocarban affects arbuscular mycorrhizal fungi colonization
- No relationship between concentration of triclosan or triclocarban and colonization of roots
- Biosolids had no effect on the colonization of the roots of corn plants.
- Biosolids had a positive effect on the colonization of the roots of lettuce plants.

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with the majority of crop plants. AMF provide plants with nutrients (e.g., P), modulate the effect of metal and pathogen exposure, and increase tolerance to moisture stress. The benefits of AMF to plant growth make them important to the development of sustainable agriculture. The land application of biosolids is becoming an increasingly common practice in sustainable agriculture, as a source of nutrients. However, biosolids have been found to contain numerous pharmaceutical and personal care products including antimicrobial chemicals such as triclosan and triclocarban. The potential risks that these two compounds may pose to plant–AMF interactions are poorly understood. The current study investigated whether biosolids-derived triclosan and triclocarban affect the colonization of the roots of lettuce and corn plants by AMF. Plants were grown in soil amended with biosolids that contained increasing concentrations of triclosan (0 to 307 μg/g dw) or triclocarban (0 to 304 μg/g dw). A relationship between the concentration of triclosan or triclocarban and colonization of plants roots by AMF was not observed. The presence of biosolids did not have a significant ($p > 0.05$) effect on percent colonization of corn roots but had a significant, positive effect ($p < 0.05$) on lettuce roots. Biosolids-derived triclosan and triclocarban did not inhibit the colonization of crop plant roots by AMF.

1. Introduction

The majority of crop plants form a symbiotic relationship with arbuscular mycorrhizal fungi (AMF) (Jansa et al., 2006). As obligate symbionts, AMF require the roots of the host plant to complete their life cycle, and thus propagation of the AMF community within the soil (Smith and Read, 2008). The hyphae of AMF colonize the roots of plants and form arbuscules within the cells of the root cortex (Fig. 1). The formation of arbuscules allows for the exchange of nutrients that have relatively low mobility in the soil, e.g., phosphorus, zinc, and copper, from the AMF to the plant, and photosynthesis-derived carbohydrates from the plant to the AMF (Smith and Read, 2008). As well as facilitating the uptake of nutrients, colonization by AMF has also been shown to increase plant tolerance to various environmental stressors (e.g., toxic metals, pathogens, reduced soil moisture) and increase soil aggregation and stabilization (Auge, 2000; Dodd, 2000; Larsen et al., 2007; Linderman, 2000; Plenchette et al., 2005). Consequently, in many situations, colonization of plant roots by AMF has shown to increase crop yield and crop nutrient content (Cavagnaro et al., 2003, 2006; McGonigle, 1988; Plenchette et al., 1983). McGonigle (1988) reviewed 78 field studies that investigated the effect of inoculating soil with AMF on the yield of crop and pasture herb species. They found that in general inoculation with AMF resulted in a 37% increase in crop yield (McGonigle, 1988). Increasingly it is being recognized that AMF play an important role in sustainable agriculture due to their potential contribution to plant health and soil tilth (Hart and Trevors, 2005).

There are a number of practices employed in modern agriculture that have been shown to inhibit the colonization of crop roots by AMF

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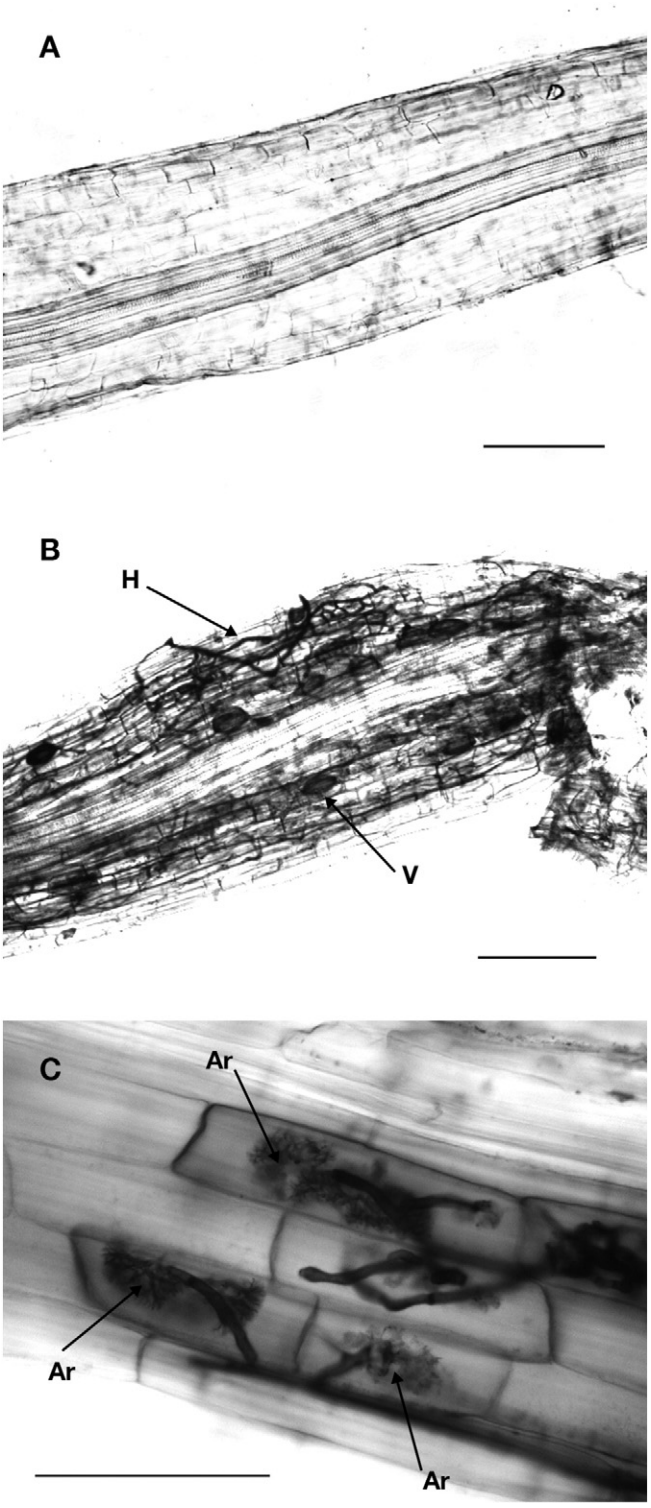

Fig. 1. A) A segment of corn root that is not colonized by AMF. B) A segment of corn root that is extensively colonized by AMF; H: hyphae, V: vesicle. C) A number of cells in the cortex of a corn root that contain an arbuscule; Ar: arbuscule. The bar in each picture has a length of 10 μm.

(e.g., tillage, application of inorganic fertilizer, use of pesticides) (Jansa et al., 2006). The effect of amending soil with biosolids on the colonization of crops by AMF has been well studied. Biosolids can improve crop yield and soil structure by providing a variety of micro- and macronutrients along with organic matter to soil (O'Connor et al., 2005; Singh and Agrawal, 2008). However, biosolids are known to possess a large number of contaminants (e.g., metals, polybrominated diphenyl ethers, alkylphenolics, synthetic fragrances, pharmaceuticals, personal care products) (CCME, 2010; USEPA, 2009a), the effect that these may have on AMF or plant-AMF interactions is largely unknown. Of particular concern with respect to AMF is the large number of pharmaceuticals and personal care products (PPCPs) found in biosolids that have antimicrobial properties.

Triclosan (TCS) and triclocarban (TCC) are two of the most commonly detected antimicrobial compounds in biosolids. Used in a wide variety of personal care and consumer products (e.g., soap, shampoo, deodorant, cosmetics, textiles, and kitchenware) that are disposed of down the drain, TCS and TCC are usually present in biosolids at greater concentrations than other PPCPs (CCME, 2010; Clarke and Smith, 2011; USEPA, 2009a,b). Surveys of wastewater treatment facilities in the United States and Canada have detected TCS and TCC in biosolids at concentrations ranging from 334 to 133,000 ng/g dry weight (dw) and 64 to 441,000 ng/g dw, respectively (CCME, 2010; USEPA, 2009a). The two compounds are also relatively persistent in soil with reported half-lives ranging from 12.7 to 193 days and 87 to >1000 days, respectively (Walters et al., 2010; Wu et al., 2009; Xu et al., 2009). As well as being present in relatively high concentrations and being relatively persistent in biosolids-amended soil, the mode of action of TCS and TCC could adversely affect AMF. TCS has been shown to have broad-spectrum antibacterial and antifungal properties through membrane disruption and inhibition of enoyl-acyl carrier protein reductase involved in fatty acid synthesis (McDonnell and Russell, 2001; McMurry et al., 1998; Russell, 2004; Stewart et al., 1999). The antimicrobial properties of TCC are elicited through membrane disruption; a specific mode of action has not been identified (Beaver et al., 1957; Hamilton, 1971; McDonnell and Russell, 2001). A combination of concentrations reported in biosolids, persistence in soil, and potential broad-spectrum antifungal properties justifies the concern that TCS and TCC may have an effect on AMF.

Few studies have investigated the effect of exposure to TCS on AMF, and to our knowledge no studies have investigated the effect of exposure to TCC. Hillis et al. (2008) exposed the AMF species *Glomus intraradices* to TCS at concentrations up to 1000 μg/L in carrot root-organ cultures to investigate the effect on root length, spore production, and hyphal length. Exposure to TCS at 1000 μg/L did not significantly affect any of the three endpoints (Hillis et al., 2008). Twanabasu et al. (2013a) observed significant inhibition of hyphal and arbuscule colonization in the roots of three wetland plants, i.e., *Eclipta prostrate*, *Hibiscus laevis*, and *Sesbania herbacea*, that were exposed to 0.4 and 4.0 μg/L of triclosan in a flow-through exposure system for 30 days. Static exposure of *G. intraradices* spores to a solution of TCS at 0.4 and 4.0 μg/L has also shown to significantly reduce spore germination, hyphal growth, and hyphal branching (Twanabasu et al., 2013b). However, no studies have employed an environmentally relevant exposure scenario to examine the effect of biosolids-derived TCS and TCC on the colonization of terrestrial plants by AMF. When attempting to understand the effect of TCS and TCC on plant–AMF interactions it is important to consider the role that physicochemical properties of the soil and the biosolids may play in modulating the exposure of AMF and plant roots to TCS and TCC. For example, TCS and TCC have relatively large soil organic carbon–water partition coefficients (log K_{OC}, TCS: 3.3–4.5 (Karnjanapiboonwong et al., 2010; Singer et al., 2002; Waller and Kookana, 2009; Wu et al., 2009; Xu et al., 2009) and TCC: 4.7–4.9 (Wu et al., 2009)), such that sorption of TCS and TCC to the organic matter present in biosolids (e.g., >13% dw) and soil will likely limit the exposure of AMF and plant roots. The objective of the current study, therefore, was to determine whether biosolids-derived TCS and TCC has an effect on the colonization of the roots of various plant species by AMF in biosolids-amended soil. The findings of the current study will provide insight into the effect that biosolids amendment and associated triclosan and triclocarban may have on AMF-plant interactions.

2. Methods and materials

2.1. Analysis of TCS and TCC

The method of analysis of TCS and TCC in soil and biosolids used in this study is described in R.S. Prosser et al. (2014). Soil from each treatment was sampled by pooling four soil cores of depth 15 cm and diameter 4 cm from each pot. Triplicate soil samples (~10 g ww) from each treatment at the initiation and conclusion of the test and four replicate sub-samples of biosolids (~1 g ww) were analyzed to determine the concentration of TCS and/or TCC. Samples were extracted using a Soxhlet apparatus with dichloromethane (HPLC grade, Caledon Laboratory Chemicals, Georgetown, ON). Extracts underwent solid phase extraction using Supelco Select HLB columns (12 mL, 500 mg) (Sigma Aldrich, Oakville, ON). Internal standards, TCS-$^{13}C_{12}$ and TCC-$^{13}C_{13}$ (Wellington Laboratories, Guelph, ON), were used to quantify the concentration of TCS and TCC in samples by isotope dilution. Agilent 1100 Series HPLC (Agilent, Mississauga, ON) with a Phenomenex Synergi Polar-RP column (4 μm, 150 × 4.60 mm) (Canadian Life Science, Peterborough, ON), and Applied Biosystem MDS Sciex API 4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, US) was used to quantify the concentration of TCS and TCC in the extracts.

The average relative recovery of TCS and TCC in biosolids samples was 87 ± 8% (±: range) and 89 ± 8%, respectively, and 91 ± 9% and 94 ± 10% in soil samples, respectively, run for quality control (TCS: n = 24; TCC: n = 21). The method detection limit (MDL) for TCS and TCC in soil was 1.7 and 1.1 ng/g dw, respectively, and was 2.1 and 1.5 ng/g dw in biosolids, respectively. The limit of quantitation (LOQ) for TCS and TCC in soil was 5.9 and 3.9 ng/g dw, respectively, and was 7.4 and 5.4 ng/g dw in biosolids, respectively.

2.2. Soil and biosolids

Soil with a loam texture was collected from an agriculture field in the Country of Wellington, Ontario, Canada. Pesticides and biosolids had not been applied to the field in the ten years prior to collection of soil for the current study. Anaerobically-digested dewatered biosolids were provided by the wastewater treatment facility operated by the City of Guelph. The physical and chemical properties of the soil and biosolids used in this study were measured by techniques outlined in R.S. Prosser et al. (2014) and are summarized in Table 1.

Table 1
Physical and chemical properties of soil from agriculture field in Wellington County, ON, Canada and anaerobically-digested dewatered biosolids provided by the wastewater treatment facility operated by the City of Guelph, ON.

Soil		Biosolids	
Texture	Loam	Dry matter	23.25%
Organic matter	3.3% dry	Organic matter	13.9%
pH	7.9	pH	7.8
Ammonium-N	10.2 mg/kg dry	Ammonium-N	6600 mg/kg dry
Nitrate-N	4.8 mg/kg dry	Nitrate-N	13.8 mg/kg dry
Phosphorus	14 mg/kg	Phosphorus	7852 mg/kg
Magnesium	300 mg/kg	Magnesium	944 mg/kg
Potassium	80 mg/kg	Potassium	200 mg/kg
Inorganic carbon	2.59%	Inorganic carbon	0.476% dry
Organic carbon	1.81%	Organic carbon	32.3% dry
Total carbon	4.40%	Total carbon	32.8% dry
CEC	14.1 cmol+/kg	Calcium	7477 mg/kg
Water holding capacity	49.1%	Sodium	392 mg/kg
		Conductivity	2.42 mS/cm
Arsenic	3.2 μg/g dry	Arsenic	1.7 μg/g dry
Cadmium	0.34 μg/g dry	Cadmium	0.73 μg/g dry
Chromium	25 μg/g dry	Chromium	85 μg/g dry
Cobalt	5.3 μg/g dry	Cobalt	6.4 μg/g dry
Copper	11 μg/g dry	Copper	690 μg/g dry
Lead	28 μg/g dry	Lead	27 μg/g dry
Molybdenum	1.3 μg/g dry	Molybdenum	7.0 μg/g dry
Nickel	14 μg/g dry	Nickel	23 μg/g dry
Zinc	130 μg/g dry	Zinc	1000 μg/g dry
Mercury	0.05 μg/g dry	Mercury	0.60 μg/g dry

Methods for determination of physical and chemical properties described in R.S. Prosser et al. (2014).

2.3. Experimental design

Corn (*Zea* mays var. saccharata), lettuce (*Lactuca sativa*), soybean (*Glycine max*), and spring wheat (*Triticum aestivum*) plants were grown in biosolids-amended soil containing increasing concentrations of TCS or TCC. Corn (Variety HZ982GT, Syngenta) plants were exposed in 1-L pots (6.4 cm × 36.0 cm) (Stuewe & Sons, Tangent, OR, USA) and lettuce (Hilde II Improved, William Dam Seeds Ltd, Dundas, ON, Canada), soybean (Variety S20-Z9, Syngenta), and spring wheat (Variety 5604, Syngenta) plants were exposed in 3-L pots (20.3 cm × 14.3 cm) (ITML, Brantford, ON).

The rate of amendment of biosolids for each pot was calculated using the NMAN3 software produced by the Ontario Ministry of Agriculture and Food (OMAF) (OMAF, 2012a,b; R.S. Prosser et al., 2014). This software is used to determine the amendment rate for non-agriculture source material (NASM), such as biosolids, in the province of Ontario, which is required to apply biosolids to agricultural fields (OMAF, 2012a). The rates of amendment determined by NMAN3 were 32, 26.5, 29, and 21 t wet weight (ww)/ha for corn, lettuce, soybean, and spring wheat plants, respectively. These rates of amendment corresponded to 13, 83, 91, and 66 g ww of biosolids per pot for corn, lettuce, soybean, and spring wheat plants, respectively. Biosolids were mixed into the top 15 cm of soil in each pot using a gloved hand for duration of 3 min. This procedure was meant to mimic tilling of the dewatered biosolids into the soil after spreading across the surface of the field, which is common practice for the application of dewatered biosolids in Ontario.

Plants were exposed to biosolids containing six different concentrations of TCS or TCC (i.e., B1 to B6). The B1 treatment contained 7569 (±314) and 3587 (±154) ng/g dw of TCS and TCC, which are the concentrations present in the biosolids when they were received from the wastewater treatment facility. Treatments B2 to B6 were produced by spiking biosolids with TCS or TCC in methanol to produce nominal concentrations of 17,000, 37,000, 77,000, 157,000, and 307,000 ng/g dw and 14,000, 34,000, 74,000, 154,000, and 304,000 ng/g dw, respectively. The chosen range of concentrations is reflective of the concentrations found in biosolids across jurisdictions (Clarke and Smith, 2011). The greatest exposure of TCS was twice as large as the greatest concentration of TCS measured in biosolids in the U.S. (i.e., 133,000 ng/g dw) and 6.6 times greater than the greatest concentration measured in biosolids in Canada (i.e., 46,600 ng/g dw) (CCME, 2010; USEPA, 2009a,b). The greatest exposure of TCC was larger than the 99th centile of concentrations measured in biosolids in the U.S. (i.e., 276,708 ng/g dw) and approximately 45 times greater than concentrations measured in biosolids in Canada (i.e., 6700 ng/g dw) (CCME, 2010; USEPA, 2009a,b). A control treatment consisting of only soil and a solvent control treatment were included in the experimental design. The solvent control treatment was produced by spiking a quantity of soil of equal weight to the biosolids added to the biosolids treatments (e.g., 13 g for corn) with the largest volume of methanol used to spike the biosolids treatments (i.e., 0.6, 14.1, 15.5, and 1.8 mL of methanol for corn, lettuce, soybean, and spring wheat plants, respectively, in the TCS experiment and 0.7, 8.1, 8.9, 1.1 mL, respectively, in the TCC experiment). Biosolids spiked with TCS and TCC and soil spiked with only methanol were left for 24 h after spiking to allow for methanol to evaporate and for equilibration of TCS and TCC with biosolids. Amended soil was left for 48 h before seeding. There were 5 replicate pots for each treatment that were seeded and 3 replicate pots for each treatment that were not seeded. The unseeded pots were used to determine the concentration of TCS and TCC at the initiation of the test.

Lettuce and wheat pots received 8 seeds, soybean pots received 6 seeds, and corn pots received 3 seeds. Corn and soybean seeds were sown to a depth of 50 mm, lettuce to a depth of 5 mm, and wheat to

depth of 30 mm. The initial soil moisture of the soil ranged from 14 to 23% and all lettuce, soybean, and wheat pots received 250 mL of water at seeding, and corn pots received 80 mL. All pots were covered with polyethylene plastic to maintain soil moisture while seeds germinated. Corn, lettuce, soybean, and wheat were randomly thinned to 1, 4, 3, and 5 plants per pot, respectively, after emergence. Pots were arranged randomly on the table in the growth chamber or greenhouse and repositioned randomly once every week. Lettuce plants were grown in the growth chamber (23 ± 1 °C day, 20 ± 1 °C night, 16:8 h day: night, 60 ± 10% relative humidity, and 299 ± 87 μmol photons/m^2•s) and corn, soybean, and wheat plants were grown in the greenhouse (19 to 31 °C, 32 to 93% relative humidity). Pots were irrigated daily with between 35 and 100 mL of DI water depending on the plant species and the moisture of the surface of the soil. All pots for a given plant species were given the same volume of water at each irrigation event. Corn, lettuce, soybean, and wheat plants were grown for 85, 55, 39, and 65 days. Percent emergence, fresh and dry root mass, fresh and dry shoot mass, and shoot height of plants from each treatment were measured and are reported in R. Prosser et al. (2014).

Initial tests were conducted with the field-collected soil to ensure it was suitable for use in the current study. Soybean and lettuce plants were grown in the soil to determine whether minimum percentages of emergence could be achieved and that the soil could support the growth of plants. Triplicate pots were prepared for each plant species. The roots of plants from all pots were examined to determine whether the soil contained a viable community of AMF. Mean percent colonization of roots was below 1%. This lack of colonization is likely due to the soil being stockpiled for ≥2 years before being used in this study. The AMF community is degraded over time if the soil is left bare, as there is no host plant present to provide nourishment and allow for spore production (Allen et al., 2001; Oliveira and Sanders, 1999; Thompson, 1987). Therefore, it was decided to inoculate soil with AMF (Micronized Endomycorrhizal Inoculant, BioOrganics™, New Hope, PA, US) for the larger experiment that incorporated exposure to TCS and TCC. The inoculant contained a minimum of 10 spores/cm^3 of *Glomus aggregatum*, *Glomus etunicatum*, *Glomus intraradices*, and *Glomus mosseae*, and 2 spores/cm^3 of *Glomus clarum*, *Glomus monosporus*, *Gigaspora margarita*, and *Paraglomus brasilianum*. Each lettuce, soybean, and wheat pot received approximately ~21.6 g of AMF inoculant and corn pots received approximately ~10.2 g. The inoculant was spread evenly over the surface of the soil and mixed into the soil with a stainless steel scoopula for 30 s to a depth of approximately 8 cm.

2.4. Quantifying AMF colonization

Five sets of roots were randomly sampled from five randomly chosen pots from each treatment for each plant species. Roots were thoroughly washed with deionized (DI) water to remove soil. The roots were stored in 70% ethanol at 4 °C until staining and mounting could occur. The method to stain roots used in the current study is based on Brundrett et al. (1996). Roots were placed in 10% KOH (e.g., 50 g of KOH pellets in 1000 mL) and autoclaved at 120 °C for 15 min. Roots were removed from 10% KOH and rinsed with DI water, then placed in white vinegar for ≥1 h at 23 °C. The roots were removed from vinegar and rinsed with DI water, then placed in a 0.03% chlorazol black E solution (e.g., 0.03 g in 100 mL of 1:1:1 ratio of glycerol, 80% lactic acid, water) for approximately 18 h at 23 °C. After staining, roots were stored in 1:1 DI water and glycerol solution at 4 °C for ≥3 days before being mounted.

Randomly chosen 2.5-cm segments of root taken from each replicate were mounted horizontally on microscope slides in light white corn syrup. Ten root segments were placed on each slide. A 24 × 50 mm cover slip was placed over the roots. Slides were left in the dark at 23 °C for ≥3 days before quantifying colonization under the microscope. Colonization of roots by AMF was quantified using the technique of McGonigle et al. (1990). Intersections of root and the vertical cross hair of the microscope eyepiece were analyzed at 200× magnification for the presence of hyphae, arbuscules, and/or vesicles. Colonization at a total of 100 intersections was quantified for each replicate. Percent colonization (presence of hyphae, arbuscules, and/or vesicles), percent colonization by arbuscules, and percent colonization by vesicles were calculated from the observed intersections for each replicate.

2.5. Data analysis

One-way ANOVA ($\alpha = 0.05$) was used to determine if there was a significant difference in percent colonization, percent colonization by arbuscules, and percent colonization by vesicles among treatments. Kruskal–Wallis one-way analysis ($\alpha = 0.05$) was performed upon failure of a test of normality and/or equal variance. A post hoc Tukey's test ($\alpha = 0.05$) was conducted if a significant difference between treatments was identified by the ANOVA or Kruskal–Wallis test. Linear regression ($\alpha = 0.05$) was conducted to determine if a significant trend between measured concentrations of TCS or TCC at day 0 and the endpoints related to colonization in the biosolids treatments. Statistical analysis was performed using Sigma Stat (Version 3.5, Systat Software, San Jose, CA, US).

3. Results and discussion

3.1. TCS and TCC in soil

The concentration of TCS and TCC in the soil of the control and solvent control treatment replicates were all below the MDL (i.e., <1.7 ng/g dw) (Supporting Information, Table S1 & S2). The difference between nominal and mean measured concentrations of TCS and TCC in the soil of the biosolids treatments at the initiation of the test ranged from 1.7 to 31.3% and 1.0 to 39.0%, respectively (Table S1 & S2). The mean concentrations of TCS and TCC in the soil of the biosolids treatments decreased from the initiation to the conclusion of the test by 80 to 93% and 21 to 57%, respectively (Table S1 & S2).

The greater dissipation of TCS relative to TCC is likely due to the pH of the biosolids-amended soil and irrigation of pots. The pKa value of TCS is 7.9 (Loftsson et al., 2005) and the pH of the soil and biosolids was 7.9 and 7.8, respectively (Table 1). Approximately 50% of the triclosan molecules present in the biosolids-amended soil solution would form an anion at these pH values. The anion has a greater solubility in water than the neutral form of the molecule. Therefore, daily irrigation may have contributed to the dissipation of TCS in the biosolids-amended soil. TCC has a pKa value of 12.7 (Loftsson et al., 2005), which means that TCC remains in the relatively insoluble neutral form at the pH of the biosolids-amended soil. An additional post hoc experiment was conducted to investigate the relative rapid dissipation of TCS in the biosolids-amended soil. It is described in R. Prosser et al. (2014) and the experiment confirmed that daily irrigation was likely responsible for the dissipation of TCS.

3.2. Colonization of AMF

AMF colonization of spring wheat and soybean plants was <2% across treatments so these species could not be used to determine whether exposure to biosolids-derived TCS or TCC affected colonization. Spore density is the most likely cause of reduced colonization of spring wheat, soybean, and lettuce plants relative to corn plants (Sanders and Sheikh, 1983). The loading rate of AMF inoculant was not the same for all four species. The AMF inoculant loading rates were greater in corn pots (i.e., 0.32 g/cm^3) compared to the other three species (i.e., 0.07 g/cm^3). The experiments using spring wheat, soybean, and lettuce plants were run simultaneously before the experiment with corn plants. The relatively low colonization in the initial three species resulted in the decision to increase the spore density by increasing the amount of AMF inoculant added to the soil for the experiment with corn plants.

In lettuce plants, the control had significantly less ($p < 0.05$) colonization in the roots of plants relative to B3 and B5 treatments exposed to TCS (Fig. 2). No other significant differences were observed across the endpoints in the TCS and TCC experiment (Fig. 2). The reduced colonization in controls is likely due to the significantly reduced ($p < 0.05$) growth in the roots of lettuce plants in the controls of both the TCS and TCC experiments (Fig. 2; Tables S10–S13 of R. Prosser et al. (2014)). The lettuce plants grew significantly better in the soil amended with biosolids compared to the control, which contained only soil. This resulted in significantly greater root development in the plants of the biosolids treatment relative to the control. The rate of root growth has been shown to influence the level of colonization of roots by AMF (Sanders and Sheikh, 1983; Smith and Walker, 1981; Sutton, 1973). There was no consistent trend in colonization of lettuce roots with increasing concentration of TCS or TCC in biosolids treatments (Fig. 2).

There were no significant differences ($p > 0.05$) in percent colonization and percent colonization by arbuscules in the corn roots exposed to TCS and TCC (Fig. 3). There were also no significant differences in percent colonization by vesicles in corn roots exposed to TCS but there were significant differences ($p < 0.05$) in corn roots exposed to TCC (Fig. 3). Colonization by vesicles was significantly greater in roots from the B5 treatment of the TCC experiment compared to the B2 and B3 treatments (Fig. 3). Colonization by vesicles in the B5 treatment was not significantly different from the control. The growth of roots of corn plants in the controls was not significantly different from the biosolids treatments in the TCS and TCC experiment (Tables S10–S13 of (R. Prosser et al., 2014)), which likely explains the greater colonization of control roots in corn relative to lettuce plants (Figs. 2 & 3). No consistent TCS or TCC concentration-dependent trend in colonization of corn roots by AMF was observed in the current study (Fig. 3).

No studies have been conducted that examine the effect of biosolids-derived TCS and TCC on the colonization of terrestrial plant roots by AMF. Germination, hyphal growth, and hyphal branching in spores of *G. intraradices* were inhibited when exposed to TCS at 0.4 μg/L on filter paper (Twanabasu et al., 2013b). Exposure of three wetland plant species at a concentration of 0.4 μg/L has also been shown to inhibit colonization of roots by hyphae and arbuscules from *G. intraradices* spores delivered in a liquid suspension (Twanabasu et al., 2013a). Arguably,

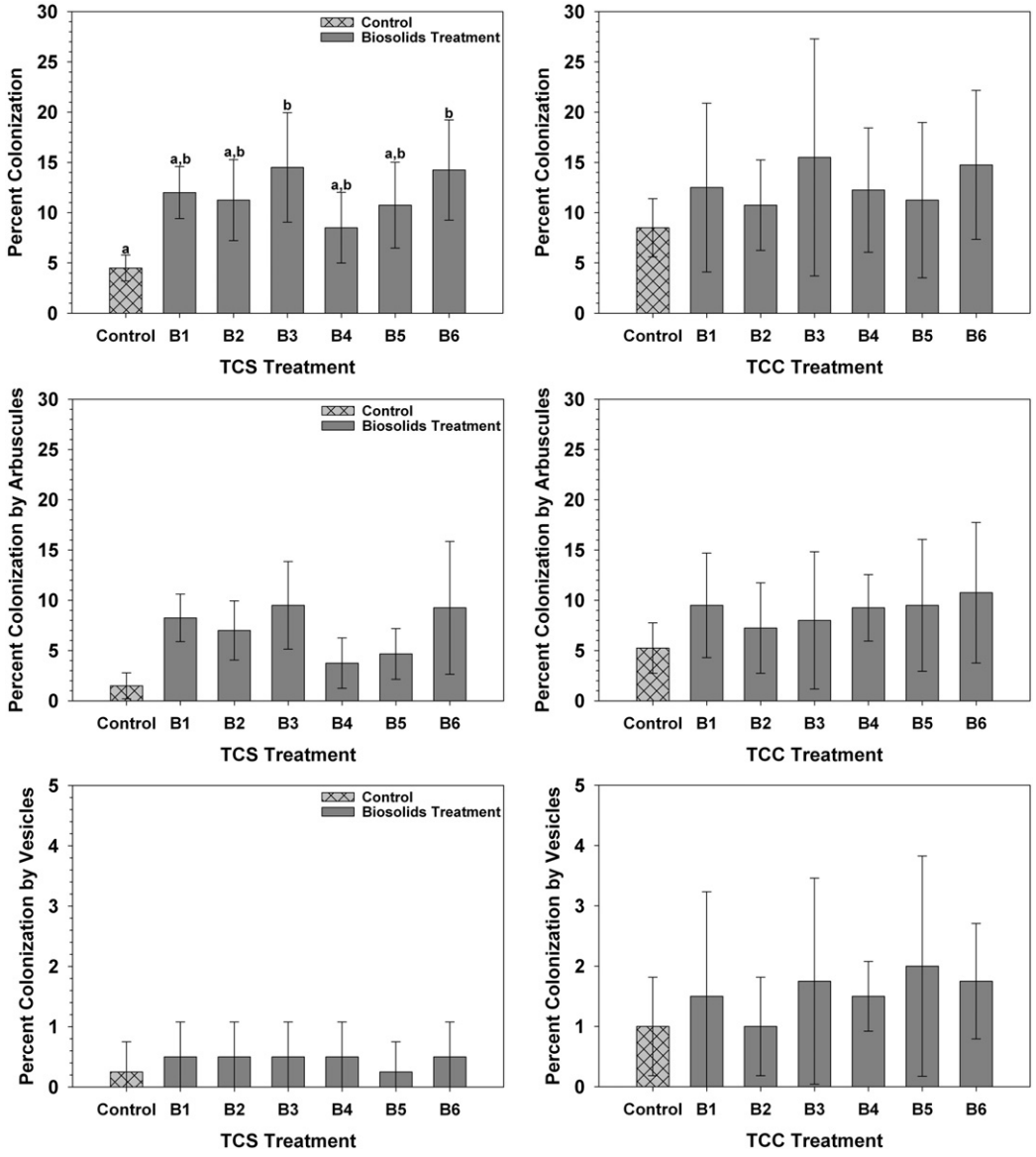

Fig. 2. Percent colonization, percent colonization by arbuscules, and percent colonization by vesicles of the roots of *lettuce plants* exposed to increasing concentrations of biosolids-derived TCS and TCC (B1 to B6) and in soil only (control). If significant differences were present, bars denoted with the same letter (a–b) were not significantly different ($p > 0.05$) according to the post-hoc Tukey test. Bars represent one standard deviation.

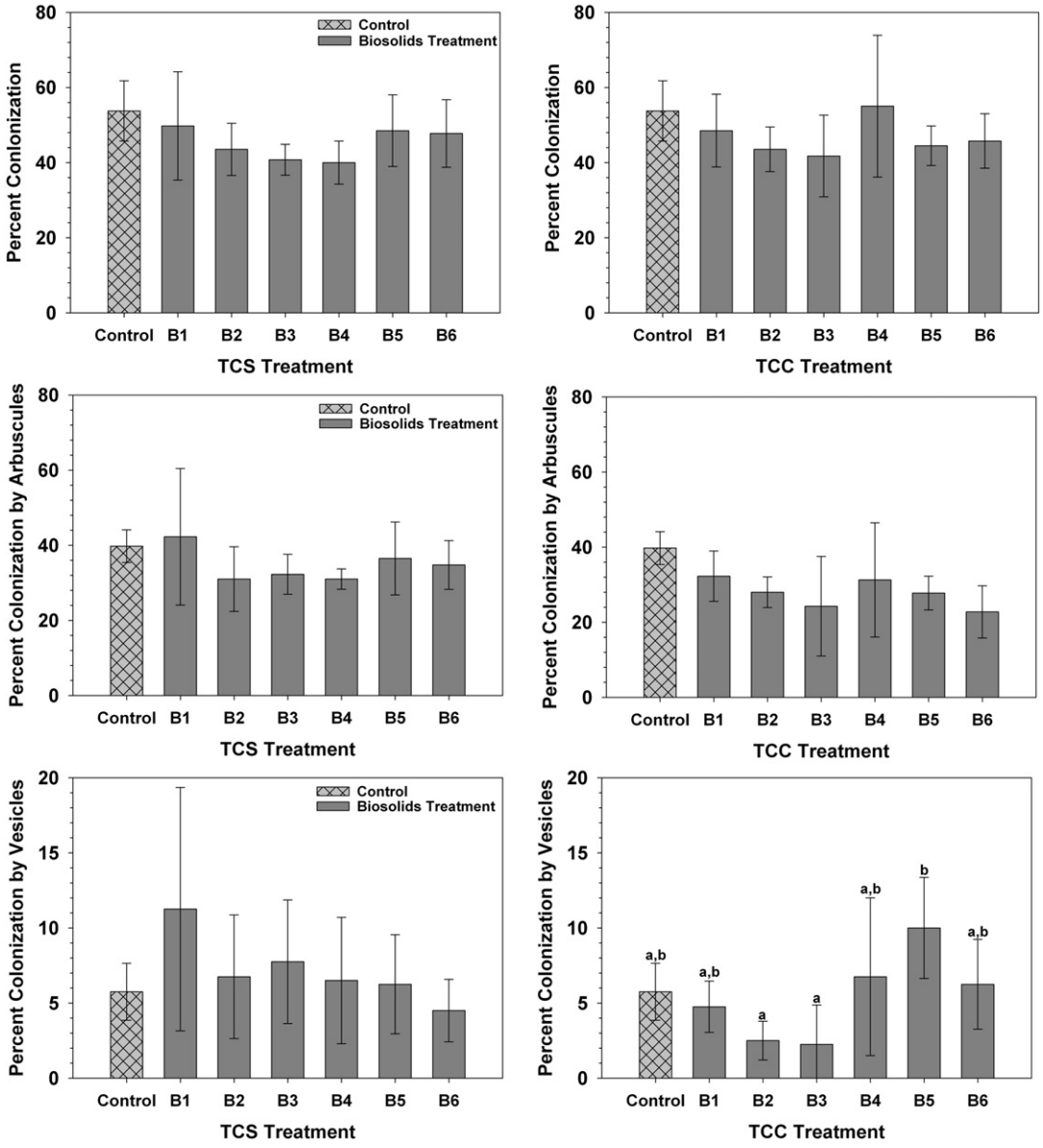

Fig. 3. Percent colonization, percent colonization by arbuscules, and percent colonization by vesicles of the roots of *corn plants* exposed to increasing concentrations of biosolids-derived TCS and TCC (B1 to B6) and in soil only (control). If significant differences were present, bars denoted with the same letter (a–b) were not significantly different ($p > 0.05$) according to the post-hoc Tukey test. Bars represent one standard deviation.

these data are not representative of terrestrial plants as roots were exposed directly to TCS through the surrounding water. However, the roots of terrestrial plants are exposed to TCS and other PPCPs through pore water in biosolids-amended soil. If the greatest concentration of TCS measured in biosolids (i.e., 133 μg/g dw (USEPA, 2009a)) and the upper range of biosolids amendment rates (i.e., 21 t/ha dw) are input into the Biosolids-Amended Soil Level IV (BASL4) model, the concentration predicted in pore water at the time of amendment is 2.84 μg/L (Table S3) (Hughes and Mackay, 2011). The pore water concentration predicted by BASL4 model for the greatest exposure of TCS to corn plants (i.e., B6) used in the current study was 2.45 μg/L. The predicted pore water concentration is greater than the lowest inhibitory concentration observed by Twanabasu et al. (2013a) and thus could be considered to represent a worst-case scenario of exposure in biosolids-amended soil. However, inhibition of colonization of the roots of corn and lettuce plants by AMF was not observed in the current study. The binding of TCS and TCC to organic matter in biosolids and soil and dissolved organic matter in pore water may reduce exposure of plants roots and propagules of AMF and explain the lack of inhibition, as both compounds have relatively large soil organic carbon–water partition coefficients (log K_{OC}, TCS: 3.3–4.5 (Karnjanapiboonwong et al., 2010; Singer et al., 2002; Waller and Kookana, 2009; Wu et al., 2009; Xu et al., 2009) and TCC: 4.7–4.9 (Wu et al., 2009)). The decrease in exposure over time in the current study (Tables S1 & S2), which has been shown to occur elsewhere (Wu et al., 2009), is also important to consider as a flow-through system was employed by Twanabasu et al. (2013a) to produce a constant exposure of TCS to the wetland plants. This exposure scenario for terrestrial plants is likely only to occur during irrigation of soil with wastewater, which has been shown to contain TCS and many other PPCPs, and could more closely mimic a constant concentration of TCS and TCC in soil. The effect of irrigating soil with wastewater on the colonization of plant roots by AMF has not currently been investigated but may be an important area of study.

Few studies have investigated the effect of biosolids amendment on the colonization of the roots of plants by AMF and studies that have been conducted report conflicting results. Barbarick et al. (2004) investigated the effect of amending shrubland and grassland with biosolids at rates ranging from 0 to 40 t/ha. The colonization of the roots of western

wheatgrass (*Pascopyrum smithii*) and blue grama (*Bouteloua gracilis*) by AMF was 33% and 23%, respectively, greater in biosolids amended plots compared to unamended plots six years after application (Barbarick et al., 2004). These results correspond with the current study, which found that biosolids did not significantly influence colonization of plant roots for corn and had a positive effect on the colonization of lettuce roots. However, Arriagada et al. (2009) observed a decrease in colonization of the roots of *Eucalyptus globulus* and decrease in the metabolic activity (measured by succinate dehydrogenase activity) of AMF in roots with an increase in the rate of biosolids amendment (i.e., 0 to 8 g/100 g soil). Biosolids amendment has also been shown to cause a significant decrease in a fatty acid biomarker (ester-linked fatty acid methyl esters; EL-FAMEs) specific to AMF (i.e., 16:1ω5*c*) at rates of biosolids amendment ranging from 2.5 to 30 t/ha, relative to a control, which did not receive biosolids (Sullivan et al., 2006). Lack of consensus across studies indicates that further works need to be done to examine the effect of biosolids on the colonization of plant roots by AMF.

Manure is an organic amendment that is more commonly used in agriculture relative to biosolids, and therefore a greater body of work exists for the effect of manure amendments on colonization of plants by AMF (Jansa et al., 2006). There is consensus in that manure amendment has less of an inhibitory effect on plant colonization by AMF compared to the application of inorganic fertilizers, particularly inorganic fertilizers high in phosphorus (Jansa et al., 2006). This relationship likely also exists for biosolids but has not been tested. Similar to biosolids, there is conflicting results on the effect of manure on the colonization of plant roots by AMF. Spore and hyphal densities and length of AMF mycelium in soil have been shown to increase in soil amended with manure (Gryndler et al., 2001; Picone, 2002), which corresponds to the enhanced colonization observed in lettuce roots grown in biosolids-amended soil made in the current study. However, Joner (2000) observed less colonization of roots by AMF in soil amended with manure relative to soil amended with fertilizer containing nitrogen and potassium but greater colonization relative to soil amended with fertilizer containing nitrogen, potassium, and phosphorus. Further work needs to be conducted to investigate the effect of organic amendments (i.e., biosolids, manure, compost) on the colonization of plant roots by AMF and the contribution that AMF make to plant growth when soil is amended with organic fertilizers.

4. Conclusions

Biosolids-derived TCS and TCC did not inhibit the colonization of corn and lettuce roots by AMF, even when exposed to biosolids that contained the upper range of concentrations of TCS or TCC measured in biosolids. Biosolids did not significantly affect the colonization of corn roots by AMF but they had a significant, positive effect on the colonization of lettuce roots likely due to improved root growth. Combined with our previous study on the effects of TCS and TCC on the growth of these four crop species (R. Prosser et al., 2014), the results of this study indicate that the concentrations of these compounds typically pose minimal risk to plant–AMF relationships in agro-ecosystems to which biosolids are applied. Further works need to be done to investigate the effect of biosolids amendment and biosolids-derived contaminants on AMF communities and the plant–AMF relationship in agricultural fields over the course of multiple years and/or biosolid application events.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.12.014.

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