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Lauris O. Hollis
Louisiana State Univ, Dept Oceanog & Coastal Sci, lholli8@lsu.edu

R. Eugene Turner
Louisiana State Univ, Dept Oceanog & Coastal Sci, euturne@lsu.edu

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The Tensile Root Strength of *Spartina patens* Varies with Soil Texture and Atrazine Concentration

Lauris O. Hollis[1] · R. Eugene Turner[1]

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Abstract

The widely used agricultural herbicide atrazine enters wetlands and may potentially affect wetland plants that provide critical reinforcement of soil strength and contribute to ecosystem stability in ways that may vary among soil types. We conducted greenhouse experiments using four levels of atrazine doses and three different soil textures to test for differences between control and experimental treatments and interactive effects of soil texture and atrazine exposure by using the tensile root strength of the coastal wetland emergent macrophyte *Spartina patens* as the response variable. The tensile root strength of *S. patens* was not affected after 50 days of atrazine exposure in an organic soil, but was 29–55% lower vs. controls in a 204-day experiment in sand-, clay-, and organic-dominated soil textures after atrazine exposure with the greatest decline in the sand-atrazine treatment. But there was no statistically significant difference in the distribution of the tensile root strength data of the main effects from individual treatments compared with the soil texture and atrazine combination treatments or any difference in the magnitude of the tensile root strength means. These results indicate that there were no synergistic interactive effects of soil texture and atrazine exposure on *S. patens* tensile root strength. However, the biogeochemical characteristics of soil texture may play an important role in the plant absorption of xenobiotics.

Keywords Tensile strength · Roots · Wetlands · Atrazine · Soil texture

Introduction

Atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a member of the s-triazine herbicide family that is widely used in agriculture to control broadleaf weeds by inhibiting photosynthesis (Kruger et al. 1993). It may be persistent in the environment and is present in surface and subsurface waters (Clay et al. 1988). Atrazine concentration was between 0.06 to 3.3 μg L^{-1} in all nine water quality stations in the lower Mississippi River watershed after the major flooding events in 1993 and 2011 (Goolsby et al. 1993; Welch et al. 2014).

Atrazine adsorption increases under acidic soil conditions and decreases under alkaline soil conditions (Harris and Warren 1964; McGlamery and Slife 1966; Laird and Koskinen 2008). Many studies have demonstrated that humic acids, fulvic acids, and organic matter can exert considerable influence on atrazine adsorption and desorption (Frissel 1961; Harris and Warren 1964; McGlamery and Slife 1966; Weber et al. 1969; Hayes 1970; Stevenson 1972; Senesi and Testini 1982; Borggaard and Streibig 1988; Laird et al. 1994; Senesi et al. 1995). The pH and typically high organic content of wetland soils might be expected to increase the affinity of organic matter for the herbicide. Meakins et al. (1995) investigated the mobility, partitioning, and degradation of atrazine in soil and water samples from salt marshes in the watershed of River Blackwater in Essex, UK, and found negligible adsorption of atrazine onto suspended solids with total suspended solid (TSS) concentrations as high as 4 g L^{-1}.

Atrazine degradation in aerobic mineral soils typically increases with high soil temperatures and moisture, whereas degradation decreases with depth, lower pH, lower temperature, and lower soil moisture (Laird and

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✉ Lauris O. Hollis
lholli8@lsu.edu

[1] Department of Oceanography and Coastal Sciences, Louisiana State University, 1195 Energy Coast and Environment Building, Baton Rouge, LA 70803, USA

Koskinen 2008). McCormick and Hiltbold (1966), for example, reported that the rate of atrazine decomposition doubled with each 10 °C increase in temperature from 10 to 30 °C. Meakins et al. (1995) found that the atrazine metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA) were detectable in both the vegetated marsh and mudflat soil cores eight days after atrazine treatments (between 5 to 10 ng g^{-1}) and that there was a greater degree of vertical migration of atrazine and its metabolites in mudflat soil cores than in the vegetated marsh soil cores. The movement of the metabolites in soil can be quite complex. Mersie and Seybold (1996), for example, characterized the adsorption and desorption of atrazine and its metabolites DEA, DIA, and hydroxyatrazine (HA) on a silt loam tidal wetland soil from the James River watershed in Virginia. The adsorption coefficients (K_f) indicated that HA was more strongly adsorbed (109 μmol L^{-1} kg^{-1}) than atrazine (38.6 μmol L^{-1} kg^{-1}), DIA (26.3 μmol L^{-1} kg^{-1}), or DEA (22.1 μmol L^{-1} kg^{-1}) and that the amount desorbed was greater for DEA (29%) than for atrazine (24%), DIA (23%), and HA (16%). Larsen et al. (2001) reported that less than 2% of atrazine was mineralized in sandy, sandy peat, and peat slurries that were created with groundwater under aerobic, denitrifying, sulfate-reducing, and methanogenic redox conditions. They concluded that the aromatic rings of atrazine were not severed under anaerobic conditions, which prevented subsequent atrazine degradation and complete mineralization. Atrazine introduced into anaerobic soils, therefore, is likely to persist longer than in aerobic soils.

The uptake of atrazine by *Sagittaria lancifolia*, *Typha domingensis*, and *Echinochloa pyramidalis* can occur within 10 min (Cejudo-Espinosa et al. 2009) and can pose risks to wetland plants in substantially anaerobic and organic-rich soils (Lee et al. 1995; USEPA 2016). Lytle and Lytle (1998), for example, exposed the wetland macrophytes *Spartina alterniflora* and *Juncus roemerianus* to three concentrations of atrazine (0.03, 0.25, and 3 mg L^{-1}) during a 5-week greenhouse experiment and found that the mean shoot growth of *J. roemerianus* was inhibited by the two highest atrazine concentrations, but *S. alterniflora* growth was curtailed only during the first week of the experiment. *S. alterniflora* was tolerant of high concentrations of atrazine, which would be lethal to *J. roemerianus*. *S. alterniflora* can tolerate atrazine doses as high as 3 mg L^{-1} after five weeks of exposure (Lytle and Lytle 1998).

Previous studies have demonstrated that atrazine may weaken the tensile root strength of emergent macrophytes. For example, Turner and Dickens (1987) applied three rates of atrazine treatment (0.6, 1.1, 2.2 kg ha^{-1}) to 1.5 × 4.5 m plots of *Eremochloa ophiuroides* (Centipedegrass) that were cultivated in sandy loam and silt loam soils under acidic conditions (pH 5.3–5.8) during a 3-year experiment. They found that the tensile strength of *E. ophiuroides* sod blocks decreased linearly with increasing rates of atrazine application and that visual evidence of injury to the grass was greater with the 2-week application interval than with the 4-week interval. A second study by Turner et al. (1990) involved measuring the tensile strength of *E. ophiuroides* sod blocks grown in a silt loam soil under acidic conditions (pH 5.2–5.6) in 1.8 × 3.7 m plots where atrazine was applied at rates of 2.2 and 3.4 kg ha^{-1}, and 2000 cm^2 blocks were harvested from each plot two, four, and eight weeks after atrazine treatment. They found no difference in the tensile strength of sod blocks extracted from the 2- and 4-week plots at both treatment levels and control for either year; however, there was a difference in tensile strength between the 1987 8-week, 2.2 kg ha^{-1} treatment plots and control (Table 2, Turner et al. 1990).

Plant roots interface directly with the soil containing this mixture of atrazine and its degradation products, and the results of these two non-wetland plant studies indicate that atrazine could potentially weaken the strength of the belowground biomass of emergent wetland macrophytes, but there are no previous studies of this nature of which we are aware. The belowground biomass structure holds the soil together against the erosive forces of wind and waves and the uplifting buoyancy from flooding and contributes to the vertical accumulation of marsh soil (Bodker et al. 2015; Turner 2011). When wetland macrophytes are "loaded" with these surface forces, the aboveground biomass may act as a lever and transmit torsional, compressional, and tensile forces to the belowground biomass (Niklas 1992; Niklas and Spatz 2012). Quantifying the tensile root strength of emergent wetland plants in different soil textures, therefore, is an important consideration for habitat conservation and management because tensile strength may be an effective metric to understand coastal wetland health, sustainability, and resistance to perturbations.

This study examined the effects of atrazine and different soil textures on the tensile root strength of the wetland macrophyte *Spartina patens* (Ait.) Muhl., which is an emergent macrophyte occupying a large proportion of coastal wetland plant communities in the eastern USA and Louisiana's coastal wetlands (Chabreck 1972), and it is exposed to atrazine after agricultural harvesting operations in the Midwest and the Mississippi River Delta. We report on the results from two experiments examining the effects of interactions between atrazine and different soil textures using the tensile root strength of *S. patens* as the main metric of response. These experiments tested the hypothesis that the interaction between atrazine and soil texture produces synergistic effects that reduce tensile root strength.

Materials and Methods

Experimental Design and Setup

Atrazine exposure experiments were conducted in greenhouses under natural light conditions. The Green Seasons Nursery (Tampa, FL) provided *S. patens* plugs from the Tampa Bay estuary and each plug consisted of seven to twelve stems growing from a 3.0 × 3.0 × 6.6 cm root mass and had no pre-experiment exposure to atrazine. The samples arrived in June 2015 and were immediately transplanted to 3.78 L glass jars containing various combinations of organic sphagnum peat, clay/silt, and sand according to requirements of each experiment. The sand, silt, and clay components were obtained by a Louisiana State University (LSU) greenhouse staff from soil in the Sterlington soil series (coarse-silty, mixed thermic Typic Hapludalfs) located in the Mississippi River floodplain in West Baton Rouge Parish, LA. The soil texture of clay/silt components was estimated by a texture-by-feel field technique and determined to be sandy clay loam. After transplantation, deionized water was added to the experimental units to replace water lost to evaporation until the soil was saturated at the surface. No nutrients were added. The transplants acclimated for six to eight weeks to adjust to greenhouse conditions. Glass pots were randomly assigned positions and rotated on a reverse-orientation basis after every treatment period (e.g., south to north, west to east) to reduce the variation in environmental conditions.

Fifty-Day Exposure Experiment with One Soil Type

The 3.78 L glass jars were filled with 3.0 L of a mixture (by volume) of 65% sphagnum peat (Premier Sphagnum Peat Moss; 100% Canadian peat moss, no added fertilizer or nutrients), 30% clay/silt, and 5% sand. The experimental units consisted of four levels of treatments of atrazine (0, 0.5, 1.5, and 3.0 μg L^{-1}), with four replicates each for a total of 16 experimental units. Atrazine treatments were added weekly in a 1 L deionized water solution. A 25 mg L^{-1} solution of atrazine was diluted to the volume required for each treatment level by the equation $C_1V_1 = C_2V_2$, where C is concentration and V is volume. The equation was solved for V_2, which determined the volume of atrazine to add to 1 L of deionized water to obtain the treatment concentration. Water levels were maintained 1.75 cm above the soil surface to ensure saturated soil conditions by adding deionized water to each unit. Soil temperature, pH, and redox potential were measured weekly, before the addition of atrazine treatments. A soil probe thermometer was inserted into each container to record temperature to the nearest 0.1 °Celsius (C). An improvised pore water sampler collected a 175 mL sample of soil pore water and dispensed it into a 250 mL amber glass bottle. This sampling instrument consisted of a Lisle vacuum pump (Lisle Corporation, Clarinda, IA) with an intake line composed of Teflon tubing that was secured to a 15 cm metal probe. The porewater pH was measured by a Hach HQ 40d multiparameter meter (Hach Industries Loveland, CO). The redox potential was measured in the soil after the method of Reddy and Delaune (2008) using a 45 cm-long standard platinum probes and a Corning calomel reference probe (Corning Inc. Corning, NY) that were connected to a Fluke 73 Multimeter (John Fluke Manufacturing, Everett WA). A correction of +244 mV was added to redox measurements (Reddy and Delaune 2008). The Hach HQ 40d was calibrated monthly according to the manufacturer's instructions, while the redox probes were calibrated bi-monthly with 1 g of 98% quinhydrone in a 100 mL pH 7 solution. The experiment lasted for 50 days from 12 August to 1 October 2015.

Two-Hundred Four-Day Exposure Experiment with Different Soil Types

The second experiment was a 3 × 4 × 4 factorial design with soil texture and atrazine exposure as the main effects with four replicates each (Appendix A). The soil textures of both main effects were an organic-, clay-, or sand-dominated mixture: (1) the clay-dominated texture was 65% clay, 30% organic, and 5% sand, (2) the organic-dominated texture was 65% organic peat, 30% clay, and 5% sand, and (3) the sand component was 65% sand, 30% organic peat, and 5% clay. Atrazine treatments were added monthly in a deionized water solution at 0, 1.0, 3.0, and 5.0 μg L^{-1}. These treatments were prepared and administered in the same manner as the previous experiment. There were four untreated controls with plants for each soil texture. There were two kinds of "disturbed controls" treated monthly with a 3.0 μg L^{-1} atrazine solution: (1) four controls with no plants and with equal amounts (33.3%) of each soil component and (2) four deionized water controls with no plants or soil. The concentration of atrazine in leaf, root, and soil porewater samples was measured using a high-performance liquid chromatography–mass spectrometry at the LSU Agricultural Chemistry Laboratory. The detection limit was 25 μg g^{-1} for leaf and root samples; however, the detection limit for soil porewater was 0.1 μg L^{-1}. Water levels were maintained 1.75 cm above the soil surface between treatments to ensure saturated soil conditions by adding deionized water to each unit. Soil temperature, pH, and redox potential were measured on a monthly basis as mentioned above. The experiment lasted for 204 days from 22 November 2015 to 15 June 2016.

Tensile Strength Testing

Tensile strength testing utilized live roots in only one of the five diameter size classes created by Hollis and Turner (2018). Only live roots were used because of the short growing period,

small belowground biomass, and the paucity of dead roots. In addition, the plant samples did not have sufficient time to produce fully developed fibrous root systems or to generate large numbers of dead roots. Roots between 0.5 to 1.0 mm in diameter were used because of their relatively high numbers and the increased probability of conducting successful tensile strength tests. Six tests were conducted for every successful tensile strength test. A successful test consisted of root samples that failed between the supports of the test stand, whereas roots that failed at the point of contact on the supports were considered unsuccessful tests and the data were considered invalid. Live roots and rhizomes were differentiated from dead roots by their white, turgid, and translucent appearance whereas dead roots were dark and flaccid (Darby and Turner 2008). However, many live roots were stained by soil deposits and they were separated from dead roots by the presence of turgor, bifurcations of fine roots, and their ability to float.

Five individual root metrics were measured: mass, length, diameter, cross-sectional area, and volume. Root length was measured to the nearest 0.1 mm with a Scale Master© Classic digital plan measure (Calculated Industries, Carson, NV, USA). The mean root diameter was measured to the nearest 0.1 mm with a Starrett digital IP67 micrometer. The measurements were taken at both ends and at the middle of each root and averaged. The cross-sectional area (mm^2) and volume (mm^3) were calculated from length and diameter measurements after tensile strength testing was performed. The fine root hairs of each test sample were trimmed to 0.16 cm (1/16 in.) with an X-acto© craft knife. Three roots were destructively sampled to determine the mass of the remaining 0.16-cm projections, which was a correction factor that was subtracted from initial measurement of root mass. Root samples were weighed on a scale to estimate individual mass to the nearest 0.1 milligram (mg).

We used a Mecmesin MultiTest 1-d motorized stand (Mecmesin Limited; Sinfold, West Sussex, UK) to measure tensile root strength in Newtons (N). Individual roots were secured to two support clamps that were perpendicular to the base of the test stand. The contact surfaces of the clamps provided 1.25 × 2.50 cm of area and were lined with a fine sandpaper to reduce or eliminate slippage. In addition, the support clamps were attached to a Mecmesin Basic Force Gauge load meter that was capable of measuring 1000 N of force with a precision of 0.1 N. After the test stand was activated, the top support was pulled upward by a vertical hydraulic piston until the root exhibited structural failure. The load that induced failure at that point, or breaking force, was recorded as tensile strength.

Statistical Analyses

The tensile root strength data did not meet two assumptions of an analysis of variance (ANOVA), which were normality and homoscedasticity; therefore, the standard one- or two-way ANOVA could not be used to analyze the data. As a result, we analyzed the variation in tensile root strength with a one-way Welch's ANOVA and differences between the tensile root strength means were determined using a Steel–Dwass nonparametric multiple comparison test. The data are reported as the mean ± 1 standard error of the mean (μ ± 1 SE) unless otherwise noted. Homoscedasticity and normality of residuals were determined with Brown-Forsythe and Shapiro-Wilks tests, respectively. Statistical significance among the soil temperature, redox potential, and pH parameter data were tested using a one-way ANOVA.

In the "50-Day Exposure Experiment with One Soil Type", we conducted a one-way Welch's ANOVA to test for significant differences in the mean tensile root strength between the 0, 0.5, 1.5, and 3.0 μg L^{-1} atrazine treatments.

In the "204-Day Exposure Experiment with Different Soil Types", the differences in the mean tensile root strength by soil texture and atrazine main effects and soil texture–atrazine combination treatments were detected using Welch's ANOVA. In lieu of a two-way ANOVA, the effects of the combination treatments were analyzed by creating data subsets by soil texture and conducting a one-way Welch's ANOVA by atrazine treatment.

To address the question of whether the effects of atrazine and soil texture on tensile root strength were interactive, we used the definition of an interaction promulgated by the Environmental Protection Agency (USEPA 1972, 1986). Based on this definition, if stress A reduces tensile root strength by 30% and stress B reduces tensile root strength by 20%, then, there is no interaction between A and B if the combined effect of A and B together is a 30% reduction of tensile root strength. According to this definition, the toxicity of a mixture of toxicants is determined by the single toxic substance present in the greatest relative amount. The presence of the other toxic substances does not matter because there is "no interaction". Alternatively, a two-way analysis of variance would conclude that there was no interaction between A and B if the combined effect of A and B together was a 50% reduction of tensile root strength. However, based on the EPA water quality criteria (USEPA 1972, 1986), A and B would be considered to interact in a "strictly additive" manner if the combination of A and B together reduced tensile root strength by 50%.

Because we used the EPA definition of "no interaction", the null hypothesis was that the combined effect of two or more stresses on the tensile root strength was the same as the effect of the greatest stress by itself. We used a Kruskal–Wallis (KW) test to test this null hypothesis because a KW test does not assume that the data are normally distributed with equal variances. We used a post hoc Steel–Dwass nonparametric multiple comparison test to find the nature of the differences between the treatments. The Steel–Dwass

nonparametric multiple comparison test eliminates the need to conduct a Bonferroni correction for Type I errors. We did not, however, test for interactive effects by determining if the effect of "A" depends on the level of "B" or the effect of "B" depends on the level of "A". We investigated the relationship between the tensile strength response variable and the root metrics using regression analyses. All statistical tests were performed in JMP v. 13 (SAS, Cary, NC) at a significance level of $p < 0.05$.

Results

Fifty-Day Exposure Experiment with One Soil Type

The mean soil temperature range for the 0.5, 1.5, and 3.0 μg L^{-1} atrazine treatments was between 27.6 and 27.9 °C (Table 1) and the same as the mean air temperature within the greenhouse. The mean pH for all treatments was between 6.9 and 7.1. The mean redox potentials for the 0, 0.5, 1.5, and 3.0 μg L^{-1} atrazine treatments were − 2.2, 8.3, − 12.7, and − 9.2 mV, respectively. A one-way Welch's ANOVA found no difference in tensile root strength between atrazine treatments and control after 50 days ($F = 1.0024$, $p = 0.39$; Fig. 1a). In addition, there was no difference in tensile root strength between the atrazine treatments. The tensile root strength grand mean between treatments and control was 4.6 ± 0.3 N.

Table 1 Results of a one-way ANOVA and summary of the soil parameter testing for atrazine in the 50-day experiment using one soil type

Parameter	Experimental units			
	0 μg L^{-1}	0.5 μg L^{-1}	1.5 μg L^{-1}	3.0 μg L^{-1}
Soil temperature (°C)				
Mean	27.6[a]	27.7[a]	27.6[a]	27.9[a]
Min	23.4	23.9	24.0	24
Max	34.4	34.8	33.0	34.9
Standard error	0.53	0.52	0.46	0.54
pH				
Mean	7.0[a]	7.1[a]	7.0[a]	7.1[a]
Min	6.8	6.9	6.9	7.0
Max	7.3	7.2	7.2	7.4
Standard error	0.03	0.02	0.02	0.02
Redox potential (mV)				
Mean	− 2.2[ab]	8.3[a]	12.7[a]	− 9.2[b]
Min	− 38.4	− 9.3	− 12.1	− 29.2
Max	24.7	27.2	61.5	3.1
Standard error	3.1	1.9	4.0	1.8

Statistical significance between the means is indicated by values with different letters

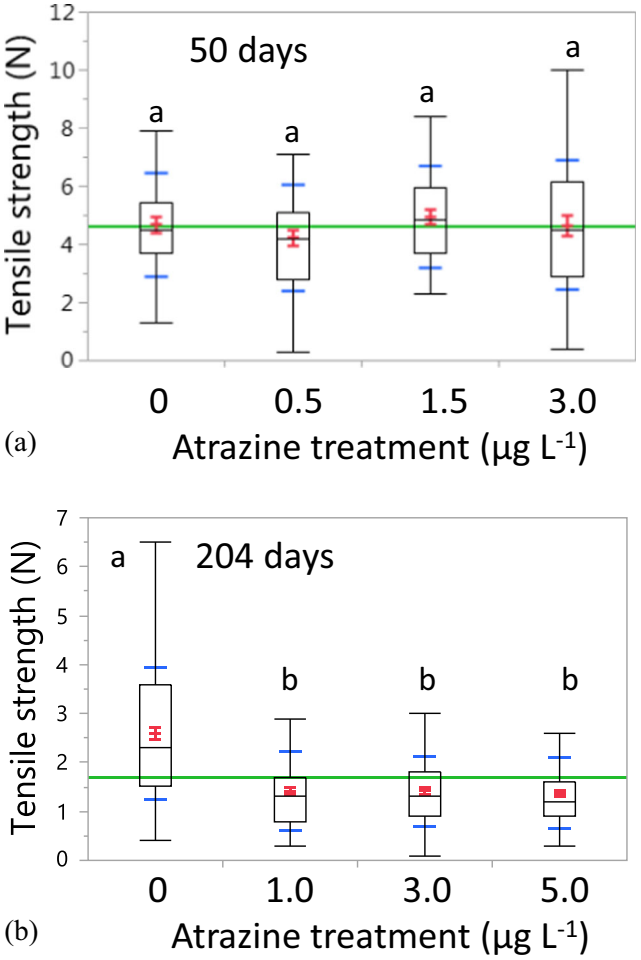

Fig. 1 Box and whisker plots of mean tensile root strength across atrazine treatments. (a) Tensile root strength with atrazine as the main effect for the 50-day atrazine exposure experiment. There was no difference between control and atrazine treatments or among atrazine treatments ($p = 0.39$). (b) Tensile root strength with atrazine as the main effect for the 204-day atrazine experiment. The tensile root strength in 0 μg L^{-1} was higher than in the 1.0 μg L^{-1}, 3.0 μg L^{-1}, and 5.0 μg L^{-1} atrazine treatments ($F = 17.9$, $F = 16.4$, $F = 15.9$, respectively; $p < 0.0001$). There were no differences between the atrazine treatments ($p = 0.78$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line is the grand mean for all groups. Box plots with different letters denote differences between treatments

Two-Hundred Four-Day Exposure Experiment with Different Soil Types

The mean soil temperature in the experimental units was 25.5 °C, and there were no differences between the soil temperatures among the three soil textures or between the soil texture controls and the disturbed soil control (Table 2, Appendix B, Fig. B.1). The pH of the experimental units was acidic throughout the experiment and ranged from 5.0 pH in the organic units to 6.2 pH in the sand units (Fig. B.2). The mean pH of the organic units were

Table 2 Results of a one-way ANOVA and summary of the soil parameter testing for the 204-day experiment using multiple soil types and atrazine doses

Parameter	Experimental treatments			Controls			
	Organic	Clay	Sand	Organic	Clay	Sand	No plant
Soil temperature (°C)							
Mean	25.4[a]	25.7[a]	25.7[a]	25.1[a]	25.5[a]	25.2[a]	25.7[a]
Min	23.4	23.4	23.6	23.4	23.6	23.4	23.8
Max	27.3	28.1	29.1	26.5	27.9	26.3	27.9
Standard error	0.21	0.21	0.26	0.19	0.21	0.15	0.19
pH							
Mean	5.1[a]	6.0[bc]	5.9[bc]	5.0[a]	6.0[c]	6.1[c]	5.5[d]
Min	5.0	5.7	5.8	4.7	5.6	5.9	5.1
Max	5.5	6.2	6.0	5.6	6.2	6.1	5.9
Standard error	0.02	0.02	0.01	0.04	0.02	0.01	0.04
Redox potential (mV)							
Mean	108.0[a]	54.5[b]	58.8[b]	113.0[a]	56.0[b]	62.1[b]	85.3[c]
Min	77.6	24.3	38.5	87.5	20.7	28.5	72.7
Max	140.0	72.1	84.2	148.6	80.6	83.1	96.5
Standard error	3.1	2.2	2.2	3.0	2.7	2.9	1.4

Statistical significance between the means is indicated by values with different letters

significantly different from the pH of the clay and sand units (Table 2). The pH of the organic units remained consistently below 6.0 pH, while the clay and sand units fluctuated above and below a pH of 6.0. Also, there were significant differences in soil pH among the soil texture controls and the disturbed soil control (Table 2). The mean redox potential was highest in the organic compared with the sand and clay soil types, but the maximum difference was 57 mV (Table 2, Fig. B.3). The redox potentials of the control units were similar in range and magnitude with the experimental units of the same soil type, but there were significant differences among controls and the disturbed soil control (Table 2).

A one-way Welch's ANOVA revealed lower tensile root strength between all atrazine treatments compared with the control after 204 days ($F = 28.5$, $p < 0.0001$; Fig. 1b, Table 3). The grand tensile root strength mean was 1.70 ± 0.14 N, and there were no differences in tensile root strength among the three atrazine treatments, and no differences in tensile root strength among the three soil texture treatments ($p = 0.997$ data not shown).

The results from a one-way Welch's ANOVA of the soil texture–atrazine combination treatments also revealed that the tensile root strength of the three atrazine treatments in the organic, clay, and sand subsets were lower than that in the control (Fig. 2a–c; $F = 15.0$, $p < 0.0001$; $F = 4.5$, $p = 0.026$; $F = 15.2$, $p < 0.0001$). There were no differences in the tensile root strength among the soil texture–atrazine combination treatments and the mean tensile root strength were 1.42 ± 0.14 N, 1.37 ± 0.15 N, and 1.36 ± 0.14 N in the organic, clay, and sand soil texture subsets, respectively. However, the high atrazine treatment–sand soil texture treatment reduced the tensile root strength by 55% versus the sand control (i.e., from 2.88 to 1.31 N) and 36% vs. the clay control (i.e., from 2.02 to 1.36 N).

The results of a Kolmogorov–Smirnov goodness–of–fit test indicated that there was no difference between the tensile root strength data distribution between the greater of either atrazine and soil texture main effects and the atrazine–soil texture combination treatments. Additional Kruskal–Wallis tests indicated that that there was no difference between the tensile root strength means of the atrazine and soil texture main effects and the atrazine–soil texture combination treatments. Furthermore, Steel–Dwass nonparametric multiple comparison tests indicated that the tensile root strength means of the atrazine–soil texture combination treatments were not significantly higher than the greater of either the atrazine or soil texture main effects. Consequently, we concluded that there were no synergistic interactive effects of the atrazine doses and soil types. Analyses of the regression residuals found no significant relationship between tensile root strength and any of the five root metrics ($p > 0.05$, data not shown).

Neither atrazine nor any of its primary metabolites were detected in the roots or leaf samples in any of the treatments. However, atrazine was detected in the soil porewater of the disturbed controls (control-no plant, CNP) at a concentration of 0.28 μg L^{-1} while deisopropylatrazine (DIA)

and deethylatrazine (DEA) were detected at 0.1 μg L^{-1}. In addition, atrazine and DEA were detected in the deionized water controls at mean concentrations of 6.96 and 1.60 μg L^{-1}, respectively.

Discussion

Fifty-Day Exposure Experiment with One Soil Type

Weekly atrazine treatments at 0.5, 1.5, and 3.0 μg L^{-1} did not change the tensile root strength of *S. patens* after 50 days. *S. patens* may have exhibited tolerance to the doses of atrazine that were administered. The plant may have successfully metabolized the herbicide before it could harm the plant. Lytle and Lytle (1998) demonstrated that *S. alterniflora* could tolerate atrazine doses as high as 3 mg L^{-1} over a 5-week period. It is unknown if *S. patens* also possesses this ability, but the results of this study suggest that this species has some level of tolerance to atrazine exposure. The duration of the experiment, as well as the amount and rate of atrazine exposure, may not have been sufficient to induce biomechanical changes within the plant that would be manifested as declining the tensile root strength.

Two-Hundred Four-Day Exposure Experiment with Different Soil Types

The results from the 204-day experiment revealed reductions in the tensile root strength between the three atrazine doses and control and between the various soil texture–atrazine combination treatments and the controls that ranged from 29 to 55%. The 1.0 μg L^{-1} concentration was the lowest effective dose, or lowest observed adverse effect concentration (LOAEC), but no additional effects were initiated by the higher doses (3.0 and 5.0 μg L^{-1}) because they did not meet an unknown threshold of a higher concentration effect. However, there were no synergistic interactive effects of soil texture and atrazine concentration on the tensile root strength of *S. patens*. The reasons for the changes, or not, are dependent on various factors, including soil textures, atrazine exposure amounts and duration, plant absorption, and plant attributes. A similar decline in percent tensile root strength loss after enhanced nutrient addition and atrazine exposure was

Table 3 Summary statistics of the tensile root strength (Newtons) for the atrazine exposure main effect and combination treatment subsets testing for interactive effects in the 204-day experiment using multiple soil types and atrazine doses

Source	N	Max	Min	Mean	Group mean	Grand mean	SE	SD	*p* value
Atrazine	160	N/a	N/a	N/a	1.40	1.70	N/a	N/a	< 0.0001
0 μg L^{-1}	40	6.5	0.4	2.60[a]	N/a	N/a	0.09	1.35	N/a
1.0 μg L^{-1}	40	4.0	0.3	1.37[b]	N/a	N/a	0.09	0.73	< 0.0001
3.0 μg L^{-1}	40	3.7	0.1	1.42[b]	N/a	N/a	0.09	0.71	< 0.0001
5.0 μg L^{-1}	40	4.2	0.3	1.41[b]	N/a	N/a	0.09	0.79	< 0.0001
Atrazine (organic)	160	N/a	N/a	N/a	1.42	1.79	N/a	N/a	< 0.0001
0 μg L^{-1}	40	5.7	0.4	2.90[a]	N/a	N/a	0.14	1.39	N/a
1.0 μg L^{-1}	40	4.0	0.5	1.28[b]	N/a	N/a	0.14	0.62	< 0.0001
3.0 μg L^{-1}	40	3.2	0.7	1.47[b]	N/a	N/a	0.14	0.63	< 0.0001
5.0 μg L^{-1}	40	3.4	0.3	1.52[b]	N/a	N/a	0.14	0.72	< 0.0001
Atrazine (clay)	160	N/a	N/a	N/a	1.37	1.55	N/a	N/a	0.0265
0 μg L^{-1}	40	5.0	0.6	2.02[a]	N/a	N/a	0.15	1.18	N/a
1.0 μg L^{-1}	40	3.9	0.5	1.41[b]	N/a	N/a	0.15	0.78	0.0229
3.0 μg L^{-1}	40	3.7	0.1	1.36[b]	N/a	N/a	0.15	0.87	0.0102
5.0 μg L^{-1}	40	4.2	0.3	1.40[b]	N/a	N/a	0.15	0.88	0.0200
Atrazine (sand)	160	N/a	N/a	N/a	1.36	1.76	N/a	N/a	< 0.0001
0 μg L^{-1}	40	6.5	1.1	2.88[a]	N/a	N/a	0.14	1.33	N/a
1.0 μg L^{-1}	40	3.5	0.3	1.43[b]	N/a	N/a	0.14	0.78	< 0.0001
3.0 μg L^{-1}	40	3.0	0.4	1.43[b]	N/a	N/a	0.14	0.60	< 0.0001
5.0 μg L^{-1}	40	3.9	0.4	1.31[b]	N/a	N/a	0.14	0.77	< 0.0001

N is the total number of tensile root strength tests conducted for the treatment in bold. Max and min denoted the maximum and minimum tensile root strength, respectively. Group mean is the mean of the treatment levels sans the control. Grand mean is the mean of all treatment levels, including the control. Statistical significance between mean values and control is indicated by different superscripts and *p* values < 0.05

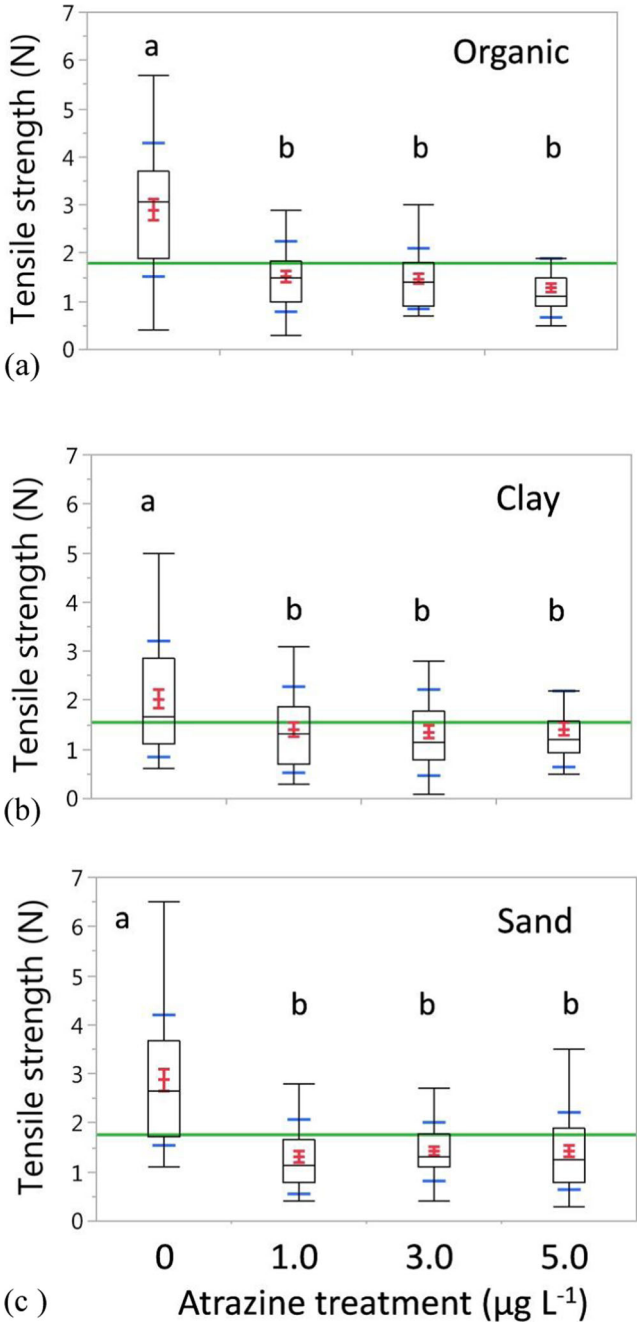

Fig. 2 Box and whisker plots of mean tensile root strength across combination treatments. (a) Organic soil texture data subset. (b) Clay soil texture data subset. (c) Sand soil texture data subset with atrazine as the main effect for the 204-day exposure experiment to test for interactive effects between atrazine treatment and soil texture. Tensile root strength in the controls (0 µg L^{-1}) were higher than in 1.0 µg L^{-1}, 3.0 µg L^{-1}, and 5.0 µg L^{-1} atrazine treatments for organic, clay, and sand subsets (F = 15.0, F = 4.5, F = 15.2, respectively; $p < 0.0001$). There were no differences among the combination treatments ($p = 0.55$). The box plot whiskers represent the range; the blue horizontal lines denote ± 1 standard deviation; the center horizontal red lines represent the group mean ± 1 standard error of the mean. The horizontal green line across the plot is the grand mean for all groups. Box plots with different letters denote differences between treatments

documented for the same plant grown under greenhouse conditions (Hollis and Turner 2019).

The uptake of atrazine may have been affected by soil texture, but pH, temperature, redox potential, and species-specific adaptations may render the plant resistant to the herbicide. These factors will either constrain or enhance the plant's ability to assimilate any available atrazine. Atrazine inhibits photosynthesis by preventing the transfer of electrons from photosystem II to photosystem I, which disrupts the ability of the plant to fix carbon dioxide (USEPA 2016). As the leaves succumb to atrazine exposure, transpiration and stomatal conductance may be affected and the soil-plant-water continuum could break down (USEPA 2016). The loss of water potential may eventually affect the roots' ability to acquire water and nutrients from the soil. In addition, the loss of turgor pressure will directly affect the tensile strength and structure of the roots by changing the orientation of macrofibrils within the cell walls (Niklas 1992). Therefore, the resultant loss of photosynthate due to the herbicide-induced disruption of photosynthesis, combined with the loss of water and nutrients, could have a negative effect on the physiology of the belowground biomass, which may cause a reduction in the tensile root strength. A few observations of tensile strength reduction after atrazine exposure have been observed in other plants. Sharpe et al. (1989) reported a loss in the tensile strength of *Cynodon dactylon* (Bermuda grass) sod cultivated in Dothan loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults). They found a 50% reduction in the tensile strength after eight weeks.

The primary influence of soil texture on tensile root strength is partly because of its effect on the availability of the herbicide for plant absorption. The soil of the organic treatments was comprised of 65% sphagnum peat, which was nearly three times the amount of the organic matter fraction that was utilized by others. We did not anticipate that the 1.0 µg L^{-1} monthly dose would produce discernible effects because of the expected binding of atrazine to organic matter. However, the mean tensile root strength in the organic treatments was not different from that in either the clay or sand treatments. The soil pH in the organic treatments remained moderately acidic and the redox potentials were moderately anaerobic. These three conditions (high organic matter, low pH, and low redox potentials) seemed "ideal" for atrazine adsorption. In addition, the soil temperature ranged from 23.4 to 29.1 °C, which was within the temperature range that McGlamery and Slife (1966) reported was conducive to atrazine adsorption. Harris and Warren (1964), however, found no difference in atrazine adsorption onto an organic soil at either 50 or 0 °C. Laird and Koskinen (2008) reviewed numerous atrazine studies and concluded that soil temperature could increase, decrease, or have no effect on atrazine adsorption due to the high number of permutations of soil component combinations.

Atrazine entry into the plant can be rapid. Pillai et al. (1977), for example, reported that 90% of atrazine absorbed by *S. alterniflora* was present in shoots within the first 48 h of their experiment, and Cejudo-Espinosa et al. (2009) found atrazine in the roots of three emergent macrophytes in less than 10 min. Also, Cejudo-Espinosa et al. (2009), Collander (1959), and Hance (1988) found that there were two stages of atrazine uptake by plants: a rapid initial stage, primarily driven by interstitial diffusion, followed by a slower second stage facilitated by membrane transport. Multiple kinds of interactions of atrazine and dissolved organic matter are possible which could increase its mobility, storage, and later release. As a result, the instantaneous rate of atrazine desorption could exceed the adsorption rate. Furthermore, the composition of the organic peat may have had a lower affinity for atrazine. Laird and Koskinen (2008), for example, reported that humic substances are highly heterogeneous in nature and that assumptions cannot be made about their interaction with atrazine.

Some of the adsorbed parent compound atrazine may be transformed to its primary metabolites before being absorbed by the plants to cause toxic effects. The atrazine metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA) were detected at 0.1 μg L^{-1} in the soil porewater and both metabolites have been reported to be as phytotoxic as the parent atrazine compound (Belluck et al. 1991; Meakins et al. 1995).

Conclusions

The effect of atrazine exposure on *S. patens* roots was significant at levels below those that are routinely detected in streams and rivers and the effects were dependent upon plant absorption of the herbicide, the duration of atrazine exposure, and the attributes of the soil texture. Soil texture may cause either positive or negative feedbacks, depending upon environmental factors such as soil temperature, pH, and redox potential. The numerous potential soil texture permutations of sand, silt, clay, and organic matter can confound interpretation of previous studies, as demonstrated by the range of results concerning the soil parameters in these experiments. This may explain the effects of the soil texture–atrazine treatment combinations on the tensile root strength of *S. patens*, which was affected by exposure to atrazine doses greater than or equal to 1 μg L^{-1} in sand-, clay-, and organic-dominated soils. In addition, the structure and type of soil components can have an effect on herbicide adsorption. Atrazine did not appear to undergo photodegradation and the primary metabolites DEA and DIA were detected in the soil porewater. This suggests that these compounds could have produced an additional effect on the tensile root strength when they are transported into the rhizosphere. The lack of visible injury to the plants suggests that *S. patens* tolerates some exposure to atrazine. Importantly, the results of this study indicate that the LOAEC of atrazine for *S. patens* may be much lower than previously observed for other species of emergent macrophytes. The lowest dose for these experiments was well below the ambient levels of atrazine that have been recorded in the Mississippi River (Welch et al. 2014). When transported in streams and rivers, atrazine may not be subjected to photodegradation because of its molecular structure, the turbidity of the water, and possible adsorption to suspended sediment. Knowing the concentration of atrazine in soil porewater, rather than in surface water, may be far more relevant to the health of coastal marshes.

The herbicide may be prone to hysteresis, which is the dynamics of adsorption and desorption of atrazine in organic matter mediums, and not much is known about these processes in wetlands that possess conditions suitable to be a sink for atrazine (Singh and Cameotra 2013). Louisiana coastal wetlands are exposed to atrazine inputs from adjacent agricultural fields and these fluxes, although infrequent, can be an order of magnitude greater than the atrazine concentrations that were used in this study (Selim et al. 2000). These "secondary sources" of atrazine may have a considerable additive effect when combined with the atrazine from the major tributaries and distributaries of the Mississippi River watershed. The results of this study indicate that extensive field experiments in areas without atrazine exposure are needed to ascertain the effect of atrazine on the tensile root strength of *S. patens* and other coastal emergent macrophytes that play a pivotal role in reducing and/or preventing coastal land loss as sea level rises and more frequent tropical cyclones occur. It is important to understand the factors that compromise tensile root strength to protect the sustainability of these ecosystems.

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