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Phenolic content of invasive and non-invasive emergent wetland plants

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ABSTRACT

Secondary chemical production is a potential mechanism of invasive plant success, but little is known about the prevalence of these compounds in wetland species. High concentrations of phenolic compounds, with their diverse functionality, may confer an advantage to plants in response to environmental conditions. We surveyed 19 plant species from ten sites in New York State and explored the relationship between foliar phenolic content (FPC), soil nitrogen, soil phosphorus, herbivory, neighboring plant cover, and sampling date. We also evaluated the responsiveness and variability of FPC between *Typha latifolia* and *T. angustifolia* by manipulating nutrient availability and herbivore pressure in the field. We found no consistent difference in FPC between invasive and non-invasive species and no relationship with environmental factors for non-invasive plants. However, invasive plants differed significantly among sites, suggesting spatial variability is influenced by local environmental factors. Sampling date, soil nutrients and herbivory were among the most important predictive factors for FPC in invasive populations of *T. latifolia, T. angustifolia, L. salicaria* and *P. arundinacea,* implying plasticity for some invaders. Although nutrient availability was negatively associated with FPC for some *Typha* species, we failed to reproduce this effect in our manipulative experiment. Generalities regarding intra- and interspecific differences in phenolic compound production in wetland plants and their role in invasion success remain elusive. However, our work provides a comprehensive accounting of relative FPC in wetland plants and its relationship to environmental variation that serves as a foundation for future manipulative studies.

1. Introduction

The invasion success of some terrestrial plant species has been linked to environmentally-responsive production of phytotoxins (e.g. Callaway and Ridenour, 2004; Cappuccino and Arnason, 2006; Gibson et al., 2011; Kim and Lee, 2011). However, plant biochemistry is understudied with respect to wetland invasion ecology (Gross and Bakker, 2012). Due to the complexity of biotic-abiotic interactions in wetlands, the relationship between environmental factors and phenolic compound production remains unclear and warrants further investigation (Inderjit, 2001; Cronin and Lodge, 2003; Ervin and Wetzel, 2003; Jarchow and Cook, 2009; Gross and Bakker, 2012; Iason et al., 2012).

Phenolic compounds are among the most common and diverse plant chemicals, and include phenolic acids, flavonoids, tannins, lignins, and coumarins (Dai and Mumper, 2010). Among their many functions, these compounds provide pathogen resistance, deter herbivory, and attract pollinators (Dai and Mumper, 2010; Li et al., 2010). Phenolic compounds also may foster allelopathic effects that negatively affect growth, nutrient acquisition, or susceptibility to herbivores or parasites of neighboring plants, thus making nutrients and space more accessible for an invader (Callaway and Aschehoug, 2000; Gibson et al., 2011; Kim and Lee, 2011). Based on the benefits of phytotoxin production to terrestrial plant invasion success, we hypothesized that invasive plants of wetlands may exhibit similarly responsive production of phenolic compounds that may confer benefits to the plant.

A variety of biotic (herbivore presence, phenology, presence of other plant spp.) and abiotic environmental factors (nutrients, light availability, season etc.), in addition to plant genetic characteristics, and growth form (aquatic or terrestrial) influence phenolic compound production by plants (Jones and Hartley, 1999; Smolders et al., 2000; Cronin and Lodge, 2003; Boege, 2005; Li et al., 2010;

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Rejmánková, 2015). Thus, phenolic compound concentrations are highly variable within and among species (Kim and Lee, 2011).

The diverse functions of phenolic compounds and their sensitivity to environmental cues has been widely documented. This variability is poorly understood with respect to invasive species of wetlands. Among the many hypotheses developed to explain the variability or responsiveness of phenolic compound production, some of the most relevant suggest that phenolic concentrations may be related to herbivore pressure (Blossey and Nötzold, 1995), species origin and coevolution (Callaway and Ridenour, 2004), resource availability (Coley et al., 1985), nutrient availability (Rejmánková, 2015), biomass (Koricheva, 1999), and genetic disposition combined with growth requirements (Jones and Hartley, 1999).

Cattails (*Typha*) are among the most aggressive invaders in freshwater wetlands of North America, particularly those subject to recent disturbance (e.g., Apfelbaum, 1985, Galatowitsch et al., 1999). Invasive cattails rapidly form monotypic stands that produce copious amounts of litter and decrease biodiversity by displacing noninvasive species. These interactions simultaneously alter nutrient cycling and trophic interactions (e.g. Angeloni et al., 2006; Tuchman et al., 2009).

Examining the concentrations and environmental response of foliar phenolic compound production across taxa may provide additional insight regarding differences in secondary compound production between native and exotic invasive plants. Given the need for a better understanding of wetland plant invasions and the paucity of information on secondary metabolite production in wetland plants we set out to (1) document foliar phenolic content between noninvasive and invasive wetland plants compared between multiple sites, (2) assess the relationship between FPC and biotic and abiotic environmental factors, and (3) investigate experimentally how variability in environmental cues influences FPC between congeneric species.

2. Methods

2.1. Survey of wetland vegetation phenolic compound concentrations across sites

The relationship between biotic and abiotic environmental factors and foliar phenolic content was assessed for six invasive and 13 noninvasive plants in ten palustrine emergent wetlands throughout Central and Western New York State (Table 1; Appendix A in Supplementary material). Our focal invasive taxa were members of the genus *Typha* (cattails). We consider a species invasive when it has become aggressively dominant and also causes negative economic and ecological impacts (Mack et al., 2000), regardless of its geographic origins (Carey et al., 2012). *Typha* contains taxa that can be classified as an "invasive native" (*T. latifolia*), an "exotic invader" (*T. angustifolia*), and a hybrid invader (*T.* × *glauca*) that currently occupy a similar geographic range. Taxonomic details of the sampled species are included in Appendix B-1 in Supplementary material.

2.1.1. Study sites

Appendix A in Supplementary material provides detailed site information. At three sites (Rice Creek Field Station [RCFS], Rochester Institute of Technology [RIT], and High Acres Nature Area [HANA]) we sampled all wetland plants within a 5 m radius of each randomly selected sampling point (Table 1; $n = 5$ replicates). At the remaining seven sites, only *Typha* spp. were sampled due to their dominance in regional wetlands. The RCFS site (Oswego, NY), is comprised of the Fallbrook emergent marsh adjacent to Rice Creek (established 2012) and the littoral marsh surrounding Rice Pond 1 km downstream (established circa 1965). RIT, (Henrietta, NY), and HANA (Perinton, NY), were created in 2007 and 2009, respectively. The seven *Typha* spp. sampling sites, with the exception of Tinker Nature Park (Henrietta, NY) and E. River and Bailey (Henrietta, NY), were all presumed to be natural wetlands since the historical satellite imagery was consistent with current site conditions (1941–1958 – USGS EarthExplorer). The wetlands at Tinker Nature Park were created in 1994 and Bailey formed sometime between 1994 and 2002.

2.1.2. Plant sampling

Plant community composition was variable among sites necessitating sampling of different species at each of the three primary sites. The majority of the six invasive and 13 noninvasive species sampled were perennial monocots (except *Eutrochium maculatum, Mimulus ringens,* and *Lythrum salicaria*) and had a forb/herb or graminoid growth habit (except *L. salicaria).* See Appendix B-1 in Supplementary material for details. Five individuals of each species (except RCFS – *Carex lupulina* [$n = 4$], and RIT – *Sagittaria latifolia* [$n = 2$]) were randomly selected and a 1 m^2 quadrat was centered over the plant of interest. Plant characteristics (number of leaves, plant height), and herbivore damage (snail radulations, broken and damaged leaves or stems) were recorded. To quantify herbivore damage, we counted the total number of snail radulations (scrape marks made by snail radula) and beetle-inflicted grazing holes on all leaves and normalized the damage to the number of leaves per plant. These ratios (radulations/leaf and holes/leaf) were then used to calculate z-scores specific to the type of herbivore damage. The z-scores were then combined to create a single variable representative of herbivory (see Appendix C in Supplementary material for calculations). Mature, non-senescent leaves were collected from each plant and stored at −80 °C prior to FPC analysis. Rhizospheric soil samples (10 cm diameter x 15 cm deep) were collected using a hand trowel, placed in ziploc bags, homogenized, and stored at −20 °C prior to subsampling for moisture and nutrient analyses.

2.1.3. Chemical analyses

Total FPC was determined by pooling multiple leaves from individual plants, freeze-drying leaf tissue with liquid N and grinding it into a fine powder with a mortar and pestle. Ground plant tissue (0.1 g) was extracted in 60% acetone (10 mL) for 48 h in the dark at room temperature. Total phenolic compound concentrations were measured using a procedure adapted from Ainsworth and Gillespie (2007). Gallic acid (Sigma-Aldrich) was dissolved in 60% acetone and used to make standards between 0.00 and 0.75 mM. Extractants and gallic acid standards were plated into a 96-well microplate. Folin-Ciocalteu reagent (Sigma-Aldrich, 0.2 M – 1:10 v/v) and NaOH (700 mM) were added and the absorbance was measured immediately at 765 nm using a Thermo Scientific Varioskan Flash Spectral Scanning Multimode Reader. All phenolic compound concentrations were calculated from the gallic acid standard curve and are reported in gallic acid equivalents (GAE). All extractions were performed in duplicate. To assess the extraction efficiency of the method, a second extraction was performed on select samples; this second extraction typically yielded phenolic compound concentrations below the detection limits of the assay, suggesting that the first extraction yielded 95–100% of extractable phenolic compounds (Harrison unpub. data).

Soil samples were thawed and roots and rhizomes removed prior to subsampling for soil moisture and nutrient analyses. Rhizospheric soil moisture content was determined gravimetrically after oven-drying 5 g soil at 105 °C for 48 h, and calculating the percent mass lost as water (Topp et al., 2008). Inorganic N was extracted by shaking 5 g moist soil with 50 mL 2 M KCl for 16 h. Samples were then centrifuged, the supernatant decanted, filtered (0.45 μm), placed into whirl-paks and frozen at −20 °C until analy-