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# Role of allelopathy of *Phragmites australis* in its invasion processes

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ABSTRACT

Allelopathy is one of the mechanisms that help to explain the invasion success of some plant species. Invasion of *Phragmites australis* through allelopathy is not robust enough to provide reliable information that could integrate the existing knowledge to sound on-ground reality. This study analysed the chemical characteristics of soil and water and monitored both over four seasons taking into consideration the phenological cycle of *P. australis* in the field. A series of bioassays has been conducted to test phytotoxicity using field concentrations of allelochemicals on different plant species in laboratory. Significant changes in soil and water chemistry were observed in invaded area compared to uninvaded area. Soil-water (rhizosphere and surface) and whole plant-leachate significantly inhibited germination and α-amylase activity of *Lactuca sativa* as well as the adventitious root formation of *Phaseolus aureus*. Seasonal impact on allelopathic interference of *P. australis* in terms of germination and growth of *L. sativa*, *Melaleuca ericifolia*, and *Poa labillardierei* showed distinct but inconsistent variation. Soil biota played an important role in reducing the phytotoxicity in natural soil. In addition, *P. australis* infested soil showed a lower arbuscular mycorrhizal fungus inoculum potential in terms of *Zea mays* roots colonization. A synthesis from field studies and associated laboratory experiments may provide a more logical understanding towards invasion mechanisms of *P. australis* through allelopathy, leading to more realistic management decisions.



## 1. Introduction

Biological invasion by non-native species is a worldwide phenomenon that threatens to dramatically change communities and ecosystems (Alvarez and Cushman, 2002; Mack et al., 2000). Allelopathy, the release of phytotoxins by plants, has been proposed as an alternative theory for the success of many invasive plants (Callaway and Aschehoug, 2000; Callaway et al., 2008; Donnelly et al., 2008; Ens et al., 2009). There are some important methodological aspects of allelopathy which are crucial to be addressed in determining the impacts. One aspect of allelopathy presently not generally addressed is phytotoxicity assessment in relation to field concentration of phytotoxins released from the allelopathic plants. Most of the research works performed on phytotoxic assessments are carried out in the laboratory and greenhouse using only plant extracts that makes difficult to show functional importance in nature (Mallik, 2000; McNaughton, 1968). In this background, more ecological realistic experiments are needed to understand the allelopathic functional activities occurred in natural ecosystems though due to complexity in natural system it is nearly impossible to demonstrate allelopathy (Harper, 1975). In addition, field chemistry associated with soil and water seem to be the key in determining the qualitative and quantitative availability of allelochemicals in vicinity of neighbouring species and is critically important to provide the better understanding in allelopathy (Callaway et al., 2004; Inderjit, 2001, 2005) and suppression of germination, growth and establishment of the associated species in fields (Lambers et al., 2008). As interactive effects of plants, water, and soil are not independent in natural ecosystems, it is essential to integrate them in experiments on biological invasion of any wetland invasive species through considerations of natural allelochemical concentration in bioassays for allelopathy (Inderjit and Nilsen, 2003). With these complex interactions, these studies were designed to find out the role of allelopathy in invasion of *Phragmites australis* using field concentrations of allelochemicals that almost reflects the complexity of the 'natural' ecosystem.

A ubiquitous wetland plant, *Phragmites australis*, has been considered one of the most invasive species in the world (Fell et al., 1998; Uddin et al., 2012), however, the origin and original distribution of the species is still unclear (Plut et al., 2011). It is a perennial graminaceous plant, 3 to 4 m tall, which reproduces mainly through rhizomes and, at low frequency, through seeds. This large wetland grass grows in all temperate zones of the world, and is especially common in North

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America, Europe and Australia (Hocking et al., 1983; Kulmatiski et al., 2011; Morris et al., 2008). The distribution and abundance of *P. australis* has expanded over the last 150 years and in most areas it forms dense monocultures (Saltonstall et al., 2005). Due to *P. australis* invasions, habitats have been diminished or altered significantly with direct and indirect impacts on flora and fauna causing loss of biodiversity and ecosystem functions (Silliman and Bertness, 2004; Warren et al., 2001). Interestingly, some chemicals produced by residue decomposition of *P. australis* may be responsible for die-back of *P. australis* itself (Armstrong and Armstrong, 1999) and associated plant species (Uddin et al., 2014c). Photo-degradation of secreted phytotoxins by *P. australis* can cause severe phytotoxicity to other plant species (Rudrappa et al., 2009). Some previous studies established that *P. australis* achieves its inference success by inhibiting the germination, growth, physiology and establishment of certain plant species through water-soluble compound synthesis (Uddin et al., 2012, 2014a), root exudations (Rudrappa et al., 2007; Uddin et al., 2014b), and residue decomposition (Uddin et al., 2014c). Gallic acid, an important inhibitor, has been identified as a major contributor in the invasion process of *P. australis* (Rudrappa et al., 2007).

In addition to ecological impacts, *P. australis* may cause substantial economic damage or loss. An estimated cost about $25 billion has been spending each year in the USA for management of invasive species (Pimentel et al., 2005). Many multi-million dollar projects have been undertaken in wetland restoration initiatives with particular reference to *P. australis* invasion in wetlands throughout the world, including the USA, Europe, Canada and Australia (Silliman et al., 2009; Streever, 1997; Van der Putten, 1997). It is generally acknowledged that those restoration projects that involve control of invasive plant such as *P. australis* might be limited due to lack of specific knowledge of the species regarding allelopathy (Walker et al., 2007). Restoration of ecosystems after the removal of invasive species, particularly those that are allelopathic, relies heavily on a sound knowledge of biochemical processes (Siemens and Blossey, 2007).

The literature of allelopathy involved in *P. australis* invasion processes is not robust enough to provide reliable information that could integrate the existing knowledge to sound on-ground reality. Because some studies of *P. australis* has clearly shown its evidence of allelopathy (Rudrappa et al., 2007), and Bains et al. (2009) but however, other studies such as Weidenhamer et al. (2013) has contradicted the findings. This complexity directs ample opportunity to do more allelopathy research related to the *P. australis* invasion process considering the effects in more ecologically realistic ways. Within this context, the studies were designed through two distinct methods: observational and experimental studies in the field and laboratory respectively. Measuring the chemical changes in the soil and water of invaded wetlands allows the comparison of the chemistry of invaded and uninvaded *P. australis* habitat and thereby, may assist to identify potential effects of an invading species. Integrating the field studies with the laboratory studies allow for a more logical explanation for invasion of *P. australis* than would be able to be achieved by field study alone.

## 2. Methods

### 2.1. Study site and sample collection

The studies were conducted on natural stands of *P. australis* adjacent to Cherry lake (37° 51′ 30″S, 144° 50′ 5″E). Cherry lake is a part of the coastal wetlands at Altona, a suburb of Melbourne, Australia. It covers an area of 60 ha within a large reserve of 176 ha. Prior to European settlement it was a low-lying and seasonally flooded swampy area. Subsequently, it has been partly drained and modified due to pressure of urban development. The water regime is highly variable and the soil consists of sand, gravel, clay, and coal. Among the vegetation of European settlement, the *P. australis* occupied a small portion of that area which has been expanding vigorously. As a result, it changes the floristic composition by invading the wetland and ultimately makes other native population more vulnerable. The species in invaded zone was only *P. australis* but the uninvaded zone occupied with *Eleocharis acuta*, *Bolboschoenus caldwellii*, *Atriplex hastata*, *Rumex* spp. and *Melaleuca ericifolia*. The salinity level in water varied from 0.200‰ to 0.236‰ (Uddin et al., 2014c) and there is no significant effect on germination (Robinson et al., 2006). All plant, soil materials and water were collected seasonally on basis of phenological cycles of the plant from areas considered visually homogeneous with respect to shoot density and age. All the sites (invaded and uninvaded) sampled had the same water level, soil texture class and subjected to similar environmental conditions.

### 2.2. Seasonal impact on rhizosphere water and soil surface water

This study was conducted to determine the impact of seasonal variation on chemistry of rhizosphere water and soil-surface water of *P. australis* invaded zones. Sampling was done in four different seasons, namely spring (September to November), summer (December to February), autumn (March to May), and winter (June to August). Generally, rhizosphere water was extracted by centrifugation (3000 rpm for 30 min) of wet soil collected from field followed by sterilization with microfiltration (0.22 μm) but exception of summer sample. The summer soil was extracted with addition of distilled water at saturation level as these samples were almost dry. In addition, soil surface water was collected during the spring and winter season from the same populations considering the 'invaded' and 'uninvaded' zone. These samples were processed in the laboratory followed by sterilization with microfiltration (0.22 μm). All samples were preserved in freezer at −80 °C for chemical analysis and bioassay experiments. Rhizosphere water and soil-surface water were also collected from *P. australis* nearby uninvaded zone. Only summer samples (full-strength) were diluted with distilled water during bioassay and they were referred as half-strength and quarter-strength. Electrical conductivity (EC) and pH were measured with conductivity meter (TPS Digital conductivity meter, 2100, TPS Pty Ltd., Australia) and a pH meter (Pocket digital pH meter, 99559, Dick-smith electronics, Australia) respectively. Osmotic potential (OP) was calculated using the equation ($OP = EC * -0.36$) according to McIntyre (1980). Dissolved organic carbon (DOC) was measured using a TOC analyser (TOC-V with TN detector, Shimadzu, Kyoto, Japan). Water soluble phenolics (WSP) were measured according to Singleton and Rossi (1965) with gallic acid used as standard.

### 2.3. Germination with α-amylase activity bioassay by whole plant leachate and rhizosphere water

Whole plants collected at mature stage (summer) were dried at room temperature, and chopped into small pieces (<2 cm long). To formulate leachate, 5 g dried plant was submerged in 100 mL distilled water (5%) and agitated for 24 h on an orbital shaker (Orbital Mixer, EOM5, Ratek Instruments Pty. Ltd., Vic-3155, Australia) at room temperature. The leachate was filtered through cheese-cloth, centrifuged at 10,000 rpm for 10 min (Beckman Avanti 30 High Speed Compact Centrifuge, 364105, Beckman Coulter Inc., USA) and sterilized (microfiltration with 0.22 μm pore filter). The pH and OP of the leachate were measured. During experiment set-up, the 5% base concentration (full-strength) was diluted with distilled water to obtain concentrations of 2.5% (half-strength), and 1.25% (quarter-strength). All extracts were buffered with 0.01 M sodium phosphate buffer and pH values of the extracts including distilled water (control) were adjusted to 6.5 with either 1 N NaOH or 1 N HCl. The OPs of all concentrations were in the range of −0.076 to −0.264 bar that could not significantly affect germination and growth of the test species (Uddin et al., 2014a).

Seeds of *Lactuca sativa* were sterilized with 1.5% (v/v) sodium hypochlorite for 1 min and subsequently washed. Five milliliter of each solution (plant and soil) with different concentrations (distilled water as