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Short communication

Allelopathy at the land/sea interface: microalgae and Brazilian pepper

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Abstract

Microalgae are significant contributors to biodiversity and primary production in shallow coastal habitats. The invasive Brazilian pepper (*Schinus terebinthifolius*) can occupy former mangrove habitats in disturbed areas, and its allelopathic capability can affect growth rates of microalgae. The four microalgae tested against dilution extracts of *Schinus* fruits varied in their growth response, suggesting variable but unpredictable impacts on microalgal biodiversity, primary production, and community structure.

Keywords: microalgae; Brazilian pepper; *Schinus terebinthifolius*; diatom, Allelopathy; invasive species; land/sea interface

1. Introduction

The important role of estuarine microalgae, both benthic and planktonic, to ecosystem structure, primary productivity and coastal biodiversity is well established (Cahoon, 1999; Geider et al., 2001; Irgoien et al., 2005). In most coastal areas of the world, population and development pressures are modifying ecosystem structure and function (Selman et al., 2008; Vallega, 2000). The coasts of Florida are under particular stress from increases in coastal population, changes in land use, and various anthropogenic inputs to coastal waters. Historically mangrove forests and salt marsh communities have buffered the land/sea interface, but anthropogenic reduction of these buffer zones has often created niches into which invasive plants intrude, either as intentional or adventitious introductions. One such invader is the Brazilian pepper, *Schinus terebinthifolius* Raddi. It is particularly successful in invading disturbed mangrove habitats. This plant was introduced intentionally to the United States from South America for landscape purposes in the 19th century (Olmstead and Yates, 1984), and is now found in Florida, Louisiana, Texas, California, Hawaii and Puerto Rico (Ferriter, 1997). There is genetic evidence that introduction to Florida has taken place at least twice (Williams et al., 2007). Copious fruit production occurs in November to February in Florida: birds (especially the migratory *Turdus migratorious*) and various small mammals consume the berries, and have contributed substantially to its spread (Ferriter, 1997).

Brazilian pepper is in the family Anacardiaceae. As with many members of this family, its leaves, sap, and fruits are copious producers of a variety of terpenes, flavonoids, organic acids, and other complex organic chemicals (Lloyd et al., 1977; Morton, 1978; Skopp and Schwenker, 1986). Many of these metabolites are potentially allelopathic and, given its hardiness, the plant is effective at displacing native vegetation including mangroves. *Schinus terebinthifolius* has invaded most successfully in Florida, where portions of the Everglades have been seriously impacted by replacement of native flora by *Schinus* (Ferriter, 1997). Along the coastline in low energy wave environments *Schinus* is often found in disturbed areas down to the high water line and sometimes slightly below, depending on water table. When it fruits, the berries are found in great abundance on intertidal mud flats in environments such as the Indian River Lagoon system on Florida's east coast. Since these mudflats and the overlying shallow waters at the land-sea interface are home to a highly productive and biodiverse (but as yet incompletely identified) microalgal flora (Hargraves, 2002), it would be useful to evaluate the impact of this invasive plant at the land/sea margin on microalgae. Accordingly, controlled experiments of berry extracts against locally isolated microalgae were undertaken to examine

51 potential impact of allelopathic chemicals from *Schinus* berries on microalgal growth rates. This
52 is the first research to focus on the impact of *Schinus* on microalgae.

53

54 2. Methods

55

56 In Florida, Brazilian pepper flowers primarily in October and the fruits mature in
57 November to February (Ferriter, 1997). Ripe berries were collected at this time in 2006 from the
58 high tide line in the central Indian River Lagoon near Vero Beach, Florida, and frozen at -25C
59 until use. Concurrently, several benthic and planktonic microalgae were isolated from the IRL
60 into culture. The isolation medium was local seawater, 0.2µm filtered, and enriched with 'L'
61 medium nutrients (Guillard and Hargraves, 1983). All experiments used the same batch of
62 seawater base. Three diatoms and one dinoflagellate were chosen as test organisms.

63 *Amphidinium carterae* is a dinoflagellate that is mostly planktonic but is also common in
64 the benthos. It is photosynthetic, an active swimmer, reportedly found worldwide in temperate
65 and tropical waters (Steidinger and Tangen, 1997), though its real distribution may be more
66 restricted due to confusion with other *Amphidinium* species (Murray et al., 2004). It can be
67 responsible for fish kills and human toxicity, as it produces a number of toxic and bioactive
68 compounds (Hallegraeff, 1993; Echigoya et al., 2005).

69 *Palmerina hardmaniana* is a large, solitary, and distinctive planktonic diatom, confined
70 to the tropics, widespread but not often abundant. This apparently is the first time it has been
71 cultivated.

72 *Melosira nummuloides* is primarily an epiphytic diatom of widespread geographic
73 distribution in coastal waters, often adventitious in the plankton, forming uniseriate filaments of
74 several to dozens of cells.

75 *Pleurosigma salinarum* is a widely distributed but rarely abundant benthic diatom, from
76 coastal and estuarine waters, occasionally found in high conductivity fresh water, and
77 adventitious in the plankton. It is actively motile on the surface of sediments in shallow water in
78 temperate and tropical regions.

79 Thawed *Schinus* berries were macerated into 'L' medium (Guillard and Hargraves, 1983)
80 at concentrations of (W:V) 1:10, 1:100, and 1:1000, berry pulp filtered out, and dispensed as
81 50ml aliquots into 125ml Erlenmeyer flasks. Each treatment, plus septuplicate controls ('L'
82 medium, without *Schinus* berry extract) was replicated seven times for each microalgal species,
83 for a total of 112 flasks. Flasks were inoculated with log phase cells of each tested microalgal
84 species, and placed in a growth chamber at 22C, ~150µmol m⁻²s⁻¹ and 16:8 L:D cycle. Initial cell
85 concentrations for each species were 150+/-50 cells per ml. After 10 days, cells for each species
86 were counted. Growth rates were calculated as µ(day⁻¹) according to:

87

$$88 \mu = \ln N_1 / N_0 t$$

89

90 where N₀ and N₁ represent cell density at the start and the end of the growth period, and t is the
91 time between measurements in days. Preliminary growth rate measurements of the four
92 microalgae suggested that under these growth conditions, log phase growth would not end for 2-
93 4 weeks.

94 Growth rates and their significance were analyzed using Welch's t-test (Welch, 1947), an
95 adaptation of Student's t-test for use with samples having possibly unequal variances.

96

97 3. Results

98
99 Results of the growth experiments are summarised in Table 1.

100 (TABLE 1 INSERTED HERE)

101 The four test microalgae were individually distinctive in their growth response. *Melosira*
102 *nummuloides* was significantly inhibited by *Schinus* berry extract at 1:10 and 1:100 dilutions, but
103 growth was equally significantly enhanced at 1:1000 dilution. No unusual cytological
104 manifestations were seen at any of the treatments, although in two of the seven replicates in the
105 1:1000 treatment, auxospore formation was observed (not pictured). *Pleurosigma* XXXXXXXXX
106 grew at more than triple the rate of *Melosira*, and there was no difference between control
107 growth rate and the extract dilutions.

108 The growth rate of *Palmerina hardmaniana*, the only holoplanktonic test species, was
109 inhibited at all extract concentrations, though the results were not as definitive at the 1:100
110 dilution. As with *Melosira nummuloides*, growth rate was enhanced at the highest dilution level
111 tested. The dinoflagellate *Amphidinium carterae* had the highest mean growth rate, but growth
112 was inhibited at the more concentrated extract levels. With the 1:1000 dilution the difference
113 was not significant compared to control flasks.

114 115 116 4. Discussion

117 Allelopathy is a common influence on community composition and function in both
118 terrestrial (Scrivanti et al., 2003; Morgan and Overholt, 2005) and marine environments
119 (Legrand et al., 2003; Graneli and Hansen, 2006). In the most comprehensive study to date of
120 Brazilian pepper's potential allelopathic chemicals, 57 essential oils were identified in various
121 parts of *S. terebinthifolius*, mostly as terpenoids, that accounted for 4.65% W/W in ripe fruits
122 (Almeida Barbosa et al., 2007). The dominant constituents were alpha-cadinol, elemol, beta-
123 phellandrene and germacrene-D. These authors also found some variability in sesquiterpene
124 constituents among fruits, stems, and leaves (their Table 2), and among leaves over an annual
125 cycle, but only in minor constituents and in minor amounts. For this preliminary research, all
126 experiments were performed using ripe berries collected at one time at one location; thus growth
127 inhibition could be different using material from other locations and times. It is presumed that
128 these terpenoids have different solubilities in the seawater culture medium, and different
129 volatilities.

130 It is unremarkable that different microalgae have different responses. The microalgal
131 flora in this location exceeds 10^2 species, and may be as high as 10^3 species (Hargraves, 2002 and
132 pers. obs.), and consists of a mix of epibenthic, epiphytic, epipsammic, and planktonic taxa in
133 many different protistan divisions. Each species is likely to have specific environmental
134 preferences. Thus, *Pleurosigma*, an epibenthic species adapted to microenvironments where
135 water/sediment chemistry fluctuations can be frequent and rapid, is not significantly affected by
136 *Schinus* extracts. Conversely, *Melosira* (an epiphyte raised above the benthos on seagrasses) and
137 *Palmerina* (more common in open coastal loci) show inhibition at all dilutions tested. The
138 dinoflagellate *Amphidinium*, thriving equally as epibenthic and planktonic, has a mixed response.
139 It is noteworthy that the growth rates off both *Melosira* and *Palmerina* are significantly
140 enhanced relative to controls at the 1:1000 dilution. This may be due to a stimulatory effect of
141 very low concentrations of *Schinus* extract directly (non-terpenoid constituents are poorly
142 known), or perhaps to a chelating effect on some micronutrient in the seawater medium.

143 In Florida, Brazilian pepper is classified as a category I invasive species (effectively
144 disrupting terrestrial natural communities); its success is due jointly to allelopathic activity

145 (Bennett and Habeck, 1991; Morgan and Overholt, 2005) and environmental eurytolerance (Ewe
146 and Sternberg, 2007; Spector and Putz, 2006). Because it is now shown to have species-specific
147 impacts on microalgae at the land-sea interface, the possibility of a cascade effect on primary
148 productivity, biodiversity, and community structure needs evaluation. However, the
149 consequences of dilution by hydrodynamic forces may mitigate any substantial impact.

150

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152

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156 **5. References**

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Table 1. Growth rates [μ /day] of microalgae challenged with *Schinus terebinthifolius* extracts. Significance level (*) is the likelihood that differences in growth rate are by coincidence.

	control	1:10 dilution	1:100 dilution	1:1000 dilution
Melosira				
mean	0.35	0.23	0.23	0.46
range	0.29-0.41	0.20-0.27	0.20-0.33	0.40-0.54
significance*		<.01	<.05	<.05
Pleurosigma				
mean	1.29	1.31	1.49	1.24
range	1.0-1.6	1.1-1.5	1.2-1.8	1.0-1.4
significance*		NS	NS	NS
Palmerina				
mean	1.03	0.73	0.94	1.21
range	0.8-1.3	0.3-1.1	0.8-1.2	1.0-1.5
significance*		<.05	<.10	<.05
Amphidinium				
mean	1.43	1.23	1.36	1.32
range	1.2-1.6	1.1-1.5	1.1-1.4	1.0-1.8
significance*		<.05	<.05	NS

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