Harmful Algae 11 (2011) 106–116

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Allelopathic inhibition of competing phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium fundyense*: Evidence from field experiments, laboratory experiments, and bloom events

Theresa K. Hattenrath-Lehmann, Christopher J. Gobler *

Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, NY 11968, USA

ARTICLE INFO

Article history:
Received 4 April 2011
Received in revised form 6 August 2011
Accepted 6 August 2011
Available online 16 August 2011

Keywords:
Alexandrium
Allelopathy
North American strains
PSP
Red tide
Brown tide
Harmful algal blooms
Aureococcus anophagefferens

ABSTRACT

The role of allelopathy in bloom formation by the paralytic shellfish poisoning (PSP) dinoflagellate, *Alexandrium fundyense*, was examined using five strains isolated from across the latitudinal PSP-toxicity gradient found along the North American East Coast. We specifically present bi-algal laboratory experiments, field experiments using cultured *A. fundyense* and natural phytoplankton communities, and the temporal dynamics of plankton assemblages during *A. fundyense* blooms within Northport Bay, NY, USA. Culture experiments demonstrated that all *Alexandrium* strains (from NY, CT, ME, and Canada) were capable of inhibiting the growth of the cryptophyte, *Rhodomonas salina*, as well the diatoms, *Thalassiosira pseudonana* and *Thalassiosira weissflogii*. This allelopathic effect was density dependent for both donor and target species as well as strain specific with the NY strain having the largest allelopathic effect (up to 100% reduction) on *R. salina*, followed by the ME, Canadian and CT strains. During field experiments all five strains caused significant decreases in autotrophic nanoflagellate and diatom abundances and significant increases in dinoflagellate densities. Consistent with these experimental results, *Alexandrium* bloom events were accompanied by significant declines in autotrophic nanoflagellate and diatom populations. Finally, density dependent inhibition of another harmful alga, the pelagophyte *Aureococcus anophagefferens*, was observed when *Alexandrium* filtrate was administered to water from Quantuck Bay, NY, during a brown tide bloom. Collectively, these results suggest that allelopathic inhibition of competing phytoplankton promotes *Alexandrium* blooms in North America.

1. Introduction

Blooms of the dinoflagellate *Alexandrium* are common to coastal regions around the world and are particularly harmful because they produce saxitoxins, the suite of toxins that cause the potentially fatal human health syndrome, paralytic shellfish poisoning (PSP; Anderson, 1994, 1997; Glibert et al., 2005). *Alexandrium* blooms along the northeast coast of the United States occur as both large-scale coastal events (Anderson, 1997; Anderson et al., 2005a,b; Townsend et al., 2005) as well as regional events in estuaries and coastal embayments (Anderson and Morel, 1979; Anderson, 1997; Hattenrath et al., 2010) and are often associated with substantial economic losses due to the closure of shellfish beds (Anderson et al., 2000; Jin and Hoagland, 2008; Jin et al., 2008). North American blooms exhibit a well-established north to south PSP toxicity gradient with northern strains (e.g. Canada, Maine, and Massachusetts) predominately synthesizing the more potent carbamate toxins and the southern strains (e.g. Connecticut and New York) containing a higher ratio of the less potent N-sulfocarbamoyl toxins (Maranda et al., 1985; Anderson et al., 1990, 1994; Bricelj and Shumway, 1998).

The ability of *Alexandrium* blooms to persist and attain high cell densities has been attributed to a number of factors including cyst bed distribution and cyst abundance (Anderson, 1997; Anderson et al., 2005a,b,d, 2008), deterrence of zooplankton grazing (Teegarden, 1999; Teegarden et al., 2008), and anthropogenic nutrient loading (Hattenrath et al., 2010). Another factor that is likely important for the development and persistence of *Alexandrium* blooms is the production of allelochemicals. Studies have established that potent allelochemicals produced by *Alexandrium* are capable of inhibiting the growth and/or lysing a diversity of cultured phytoplankton and that PSP toxins are not the chemical that elicits these effects (Tillmann and John, 2002; Fistarol et al., 2004b; Tillmann and Hansen, 2009; Tillmann et al., 2009; Yang et al., 2010). Investigations of the allelochemical potency of *Alexandrium* spp. have almost exclusively focused on strains from Europe, Asia, South America and New Zealand (Arzul et al., 1999; Tillmann and John, 2002; Fistarol et al., 2004a,b; Tillmann et al.,

* Corresponding author.
E-mail address: cgobler@notes.cc.sunysb.edu (C.J. Gobler).

1568-9883/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2011.08.005

2007, 2008, 2009; Yang et al., 2010) with only one study examining two strains of the North American ribotype (Tillmann and John, 2002). Furthermore, a majority of studies have explored the allelopathy of *Alexandrium* using bi-algal cultures (Arzul et al., 1999; Tillmann and John, 2002; Fistarol et al., 2004b; Tillmann et al., 2007, 2008, 2009; Yang et al., 2010) with only two investigating the effects of allelopathy on natural phytoplankton assemblages in Europe (Fistarol et al., 2004a,b). Moreover, of all the studies conducted on *Alexandrium* in general, few studies have examined the temporal dynamics of *Alexandrium* blooms in parallel with the phytoplankton community composition (Anderson et al., 1983; Penna et al., 2002). As such, the allelopathic effects of *Alexandrium* on phytoplankton in the field during bloom events have not been well characterized.

Here we report on the role of allelochemicals in *Alexandrium* bloom formation using multiple strains of *Alexandrium* spanning the latitudinal PSP toxicity gradient found along the North American East Coast. We present experiments using natural phytoplankton communities from Northport Bay, an area that has experienced chronic shellfish bed closures due to PSP (Hattenrath et al., 2010; pers. obs.), as well as controlled laboratory experiments with multiple species of diatoms and dinoflagellates as well as the widely used target alga, *Rhodomonas salina*. We also report on the natural dynamics of *Alexandrium* and the phytoplankton community during multiple bloom events that support the hypothesis that allelopathic inhibition of competing phytoplankton occurs during blooms.

2. Materials and methods

2.1. Cultures and culturing conditions

Five *Alexandrium fundyense* strains spanning the latitudinal PSP toxicity gradient found along the North American East Coast were used as donor species during our investigation of allelopathy. Two high toxicity strains (with regard to saxitoxin production), one from the Bay of Fundy (CCMP 2304; herein BoF) and the other from the Gulf of Maine (CCMP 1719, synonymous with GTCA28; herein GoM) were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP; Maine, USA). Two low toxicity strains, ATNPD7 and NPB8 (herein NY), both isolated from Northport Bay, NY were generously provided by Dr. Donald M. Anderson's Lab and Dr. Hans Dam's Lab (isolation by Hayley Skelton), respectively. Finally, a non-toxic strain (GTCN-16; herein CT) isolated in Mumford Cove, CT was also obtained from the Dam lab. The cryptophyte, *R. salina* (CCMP 1319; isolated from CT waters) which has often been used as a model target species to test the potency of allelochemicals (Fistarol et al., 2004b; Tillmann et al., 2007, 2008, 2009; Ma et al., 2009) was used as our target species in laboratory experiments. Other target species investigated included the diatoms, *T. pseudonana* (CCMP 1335; isolated from NY waters) and *T. weissflogii* (CCMP 1336; isolated from NY waters), and the dinoflagellates, *Prorocentrum minimum* (CCMP 696; isolated from NY waters) and *Heterocapsa arctica* (MS5, isolated from NY by Dr. YZ Tang; species identity confirmed via sequencing of the large subunit of the ribosome; Tang et al., 2010).

Algal cultures were grown in sterile *f*/2 medium (Guillard and Ryther, 1962) with a salinity of 32 PSU, made with boiled and 0.2 μm-filtered seawater, at 18 °C in an incubator with a 12:12 h light:dark cycle, illuminated by a bank of fluorescent lights that provided a light intensity of ~100 μmol quanta m^{-2} s^{-1} to cultures. Antibiotics (stock solution, Thermo Scientific HyClone Penicillin (10,000 U/ml) Streptomycin (10,000 μg/ml) in 0.85% NaCl) were added to all cultures at a final concentration of 1% by volume to discourage microbial contamination. Cultures used in field experiments were maintained as described above but were gradually adjusted to a salinity of 25 PSU to match the salinity of the field study site, Northport Bay.

2.2. Laboratory experiments using algal monocultures

2.2.1. Whole cell addition experiments

To assess the allelopathic properties of *Alexandrium* strains from the North American East Coast, laboratory experiments were conducted by creating a mixture of a single donor species and a single target species. Donor species included the above described strains of *Alexandrium* from NY (NPB8), CT, GoM and BoF, whereas our target species was the widely used model organism, *R. salina* (CCMP 1319; Fistarol et al., 2004b; Tillmann et al., 2007, 2008, 2009; Ma et al., 2009). Dose–response experiments were conducted by: (1) keeping the cell densities of donor species (*Alexandrium* (NY, CT, GoM and BoF) = 400 cells ml^{-1}) constant and varying densities of the target organism (*Rhodomonas* = 100, 1000, 7000, 10,000, 14,500 and 29,000 cells ml^{-1}) and (2) varying the cell densities of donor species (*Alexandrium* (NY) = 5, 10, 50, 60, 80, 100, 200 and 400 cells ml^{-1}) and keeping the target organism densities fixed (*Rhodomonas* = 7000 cells ml^{-1}). All experiments were conducted in sterile, autoclaved 250 ml Erlenmeyer flasks. Stock cultures of target and donor species in exponential growth phase were mixed to appropriate experimental densities using sterile, 0.2 μm filtered *f*/2 media to a total volume of 50 ml with 1% antibiotic solution. All experiments were incubated as described above for 72 h. At the end of each experiment aliquots were preserved with Lugol's iodine and cells were enumerated with either a 0.1 ml or 1 ml Sedgewick-Rafter counting chamber using a compound microscope. Nutrient samples were obtained from experiments and all experiments had nitrate concentrations >40 μM by the end of the experiments, significantly above the half-saturation constant of diatoms, dinoflagellates, and flagellates (Smayda, 1997). Furthermore, the difference in pH values between controls and treatments were always <0.3 units, and always within the range typically found in target algal cultures, suggesting that the observed effects were not due to differences in pH.

2.2.2. Filtrate experiments

The effects of filtrate from the NY strain (NPB8) and CT strains of *Alexandrium* were assessed using environmentally realistic densities of several target organisms: the diatoms, *T. pseudonana* (CCMP 1335; 4000 cells ml^{-1}) and *T. weissflogii* (CCMP 1336; 4000 cells ml^{-1}), and the dinoflagellates, *P. minimum* (CCMP 696; 1000 cells ml^{-1}) and *H. arctica* (MS5; 1000 cells ml^{-1}). *Alexandrium* cultures in exponential growth phase were diluted to concentrations of 400 cells ml^{-1} with *f*/2 and cell-free *Alexandrium* medium was obtained by gentle filtration (<5 psi) through sterile 0.2 μm Millipore Steritop filters. Experiments were conducted in 7 ml borosilicate vials adding 2.5 ml of either *f*/2 (control), NY filtrate (final concentration = 200 cells ml^{-1}), or CT filtrate (final concentration = 200 cells ml^{-1}) to 2.5 ml of the above mentioned target organisms (final densities of target organisms are noted above). Experimental cultures were supplemented with *f*/2 to ensure any treatment effect was due to allelochemicals and not nutrient limitation. Vials were incubated for 72 h as described above and at the end of the experiment Lugol's iodine was added to each vial and cells were enumerated.

2.3. Natural phytoplankton community experiments

To assess the potential role of allelopathy in promoting *Alexandrium* blooms several field experiments were conducted over a two-year period (2009 and 2010) in which cultured *Alexandrium* (whole cells and filtrate) was added to a natural phytoplankton assemblage from Northport Bay, NY, an embayment that has