Comparative Sensitivity of Freshwater Algae to Atrazine

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Received: 18 August 2005/Accepted: 8 November 2005

Widespread use of environmentally persistent herbicides has led to increasing concern over their impact on aquatic ecosystems and nontarget organisms. The triazine herbicide atrazine (6-chloro-N-ethyl-N\(^{1}\)-[1-methyl-ethyl]-1,3,5-triazine-2,4-diamine) remains one of the most heavily used pesticides worldwide, and was the second most commonly used conventional pesticide in the U.S. agricultural market in 2001 (www.epa.gov/oppbud1/pestsales/01pestsales/usage2001_2.htm). Atrazine is an \(S\)-triazine herbicide that targets the photosynthetic process to control broadleaf weeds. Due to the moderate solubility (33 mg/L at 22°C) and relative persistence of atrazine in water (Solomon et al. 1996), contamination of surface waters as a result of non-point source inputs is a threat to lakes and streams. Atrazine contamination has been documented year-round in the Platte River in Nebraska (Nelson et al. 1999), and concentrations as great as 691 µg/L have been observed in first-order stream samples collected during post-planting storm events (Langan et al. 1993). The effect of surface-water contamination on nontarget organisms is especially important for freshwater algal communities, given their role as the predominant primary producers in most lotic environments (Herman et al. 1986). Moreover, freshwater algae form the base of aquatic food webs and changes in algal community structure and composition may have cascading effects on other components of the community due to changes in food availability and important community-level interactions.

A number of laboratory, microcosm and field studies have examined atrazine toxicity in freshwater algae (DeNoyelles et al. 1982; Herman et al. 1986; Solomon et al. 1996; Tang et al. 1997; Fairchild et al. 1998). From these studies, it is generally recognized that certain algal species are more sensitive to atrazine (DeNoyelles et al. 1982; Tang et al. 1997; Fairchild et al. 1998). Green algae generally are more susceptible to atrazine than diatoms (Tang et al. 1997). However, relatively few algal divisions and species have been tested for their response to atrazine exposure.

The overall objective of this research was to determine the differential sensitivity of atrazine to algal species common in freshwater ecosystems in the Midwest. Several of the previously described studies examined a small number of algal
species and divisions, focusing on green algae, cyanobacteria (also known as blue-green algae), and diatoms. We used a greater number and variety of species to define tolerance to atrazine over a broader range of freshwater algae. Additionally, cell size was examined as a possible determinant of differential toxicity to atrazine among algal species.

MATERIALS AND METHODS

Algal taxa representative of streams and rivers from across the agricultural Midwest were selected from culture collections maintained in our laboratory (Green algae: Ankistrodesmus falcatus (Corda) Ralfs, Chlorella vulgaris Beyerinck, and Staurastrum cristatum (Näg.) Arch; Diatoms: Cyclotella meneghiniana Kütz., and Nitzschia palea (Kütz.) W. Smith; Cryptomonad: Cryptomonas ovata Ehr. Additional cultures were obtained from Carolina Biological Supply (Cyanobacteria: Arthrospira sp. and Euglenoid: Euglena gracilis Klebs) and the University of Texas Culture Collection (Cyanobacteria: Synechococcus sp.). The diatoms, green algae, and cryptomonad were grown in WC freshwater medium (Nichols 1973; Tang et al. 1997), the cyanobacteria in Allen’s medium (Fogg et al. 1973), and the euglenoid in soil-water extract (Nichols 1973). Different media were used to achieve high growth rates for each species. All of the algae were grown under unialgal, axenic conditions in 25x150-mm glass culture tubes, and were transferred to fresh growth medium every 14 d to maintain high growth rates. Cultures were incubated at 21°C with an alternate 12:12 h light:dark cycle under cool white fluorescent lights (100 µmol m⁻² s⁻¹).

Technical grade atrazine (99% purity) was purchased from Chem Service (West Chester, PA, USA). A 1 mg/ml stock solution was prepared by dissolving atrazine in 100% ethanol which had been filter sterilized using a 0.22-µm Millex-GS filter unit (Milli-Q, Bedford, MA, USA). Serial dilutions (10:1) of the primary stock provided secondary atrazine stock solutions. These stock solutions were added to autoclaved growth medium at nominal concentrations of 0, 0.01, 0.1, 1.0, 10, 100, and 1000 µg atrazine/L. Atrazine concentrations were measured at the University of Nebraska Water Sciences Laboratory (0.20 µg/L detection limit) using gas chromatography-mass spectrometry (GC-MS) analysis (Nelson et al. 1999). Initial concentrations of atrazine were <0.20 (0.01), <0.20 (0.10), 0.97 (1.0), 10.44 (10.0), 106.2 (100), and 1159 µg/L (1000) (nominal concentrations in parentheses). This range of atrazine concentrations encompasses environmentally realistic levels reported in the literature for midwestern aquatic habitats (Jurgensen and Hoagland 1990; Thurman et al. 1991). The final concentration of ethanol did not exceed 0.001%. Ethanol was added to the control medium at the same concentration, to insure that effects were due to atrazine exposure. The atrazine-treated growth medium was dispensed into autoclaved culture tubes (39 ml) and subsequently inoculated with 1 ml of algal cell suspension (7-10 days old). Five replicate tubes were used for each herbicide concentration, providing