The relative effects of the herbicide atrazine on selected microalgae

By

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Abstract

Cyanobacterial blooms are often associated with eutrophication of lakes and waterbodies which degrade the water quality due to chronic and episodic inputs of nutrients, water stratifications and climatic changes. Increasing terrestrial application of photosynthesis-inhibiting herbicides that enter water bodies during/after heavy rain, can affect the photosynthetic capacity and growth of phytoplankton at sub-lethal concentrations. As herbicide sensitivity of phytoplankton varies among species, their presence can alter phytoplankton community structure to favour more tolerant species, or particular groups such as cyanobacteria which are considered more tolerant of photosynthesis-inhibiting herbicides. This study examined the potential for photosynthesis-inhibiting herbicides to promote cyanobacterial blooms in temperate lakes and waterways. The most commonly applied triazine herbicide, atrazine, was used due to its solubility, mobility and persistence in temperate environments. The relative effects of atrazine on the growth of selected planktonic green algae and cyanobacteria (primarily bloom-forming *Anabaena* species) were investigated using laboratory mono-cultures and two-species competition cultures.

In the second chapter, the relative tolerance to atrazine of some common freshwater green algae (*Selenastrum capricornutum, Desmodesmus asymmetricus* and *Chlorella protothecoides)* and cyanobacteria of the genus *Anabaena*, particularly *Anabaena circinalis* were compared in single-species assays using *in-vivo* fluorescence estimation of growth rates. While the green algae species examined displayed higher intrinsic growth rates than *Anabaena* strains, their relative tolerance to atrazine (50 – 250 µg L^{-1}) expressed as EC_{50} was of similar magnitude and range (72-140 µg L^{-1}) compared to the seven *Anabaena* strains (59 - 111 µg L^{-1}) under light and temperature conditions typical of temperate mid-latitude summer conditions. However, atrazine tolerance varied significantly among the 10 species examined but there was no significant difference in mean atrazine tolerance between the two groups, the cyanobacteria and green algae indicating that the selective effects of atrazine operate at a species/strain level rather than more generally favouring cyanobacteria over green algae.

The third chapter adapted and tested a high through-put microplate-based approach as a rapid and reliable phytoplankton herbicide sensitivity assay that could be used to examine the influence of herbicides on the growth of green algae and cyanobacteria in two-species competition cultures. The assay was based on *in-vivo* fluorescence quantification of chlorophyll a and phycocyanin. Minimum detection limits and correlations of cell concentration and fluorescence were established for two species of eukaryotic green algae and seven *Anabaena* strains. Calibration curves were established for the seven species examined and the detection limits and ranges were sufficient for reliable detection and simultaneous estimation of cyanobacteria and green algal growth rates in two-species competition laboratory cultures. Two-species competition culture experiments were carried out using *A. circinalis* grown with the green algae *Selenastrum capricornutum* or *Desmodesmus asymmetricus*. The growth rate of *A. circinalis* strains showed a 20% increase in exponential growth rate compared to mono-culture controls, whereas the green algal species growth rate was reduced by 13-17%, indicating that allelopathic interactions may alter the selective effects of herbicides on phytoplankton community structure.

In the fourth chapter, relative inhibition of the green alga, *Desmodesmus asymmetricus* and the cyanobacterium *A. circinalis* by atrazine was examined at different combinations of light (high = 100, low =30 µmole photon m^{-2} s^{-1}) and temperature (high = 24°C ±1 and low= 18±1°C) when grown separately or in two-species competition cultures. When grown separately, *A. circinalis* showed similar or higher tolerance (EC_{50}) to atrazine as *D. asymmetricus* and maintained an increasingly higher growth rate with increasing atrazine concentration under all conditions, except at low light and high temperature where the growth rate of *D. asymmetricus* was higher at atrazine concentrations >150 µg L^{-1}. When grown in competition, *A. circinalis* was favoured in the presence of atrazine under high light conditions regardless of temperature, and *D. asymmetricus* was favoured by the presence of atrazine (or equally tolerant) under low light regardless of temperature. Overall, the presence of atrazine favoured *A. circinalis* at high light with the largest relative effect at low temperature. This may explain how temperate mid-latitude summer blooms of *Anabaena circinalis* can maintain their relative community dominance during declining autumn temperatures in lakes and rivers.

The fifth chapter used two-species competition cultures with different relative starting concentrations of *D. asymmetricus* and *A. circinalis* to determine whether the outcome of green algae/cyanobacteria growth competition could be reversed by atrazine starting from scenarios of different relative dominance (4:1, equal, or 1:4 starting concentration of each species). In the absence of atrazine, *D. asymmetricus* dominated 10 day growth competition experiments from scenarios from both dominant and equal starting concentration, whereas *A. circinalis* dominated only in cultures in which it started with 1:4 dominance. In the presence of low concentrations of atrazine (10-60 µg L^{-1}), *A. circinalis* dominated over *D. asymmetricus* regardless of the species dominance at the start of the experiment. The relative patterns of growth in the experiments suggested that the dominant factor during exponential growth phase (first 5-6 days) was inhibition of both species by atrazine but more severe inhibition for *D. asymmetricus*. After day 5 inhibition of *D. asymmetricus* by the allelopathic activity of *A. circinalis* became the dominant factor. These experiments show that the allelopathic activity of *A. circinalis* and low concentrations of atrazine (10µg L^{-1}) combine reverse growth competition outcomes even from a position of green algal dominance, and indicate a mechanism by which low concentrations of herbicides can shift algal communities toward cyanobacterial dominance in temperate mid-latitude lakes and rivers.

The influences of photosynthetic-inhibiting herbicides in combination with other adaptive physiological strategies/mechanisms that promote cyanobacterial blooms are also discussed.

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