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Tolerance of *Oscillatoria limnetica* Lemmermann to Atrazine in Natural Phytoplankton Populations and in Pure Culture: Influence of Season and Temperature

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Abstract. The responses of algae to herbicides depend on the sensitivity of each species, but competition within algal communities may be an important regulator of the effects of herbicides on aquatic systems. The impact of herbicides on algae also depends on abiotic factors like nutrients, light, and temperature. We examined the tolerance of the alga *Oscillatoria limnetica* Lemmermann to the photosystem II inhibitor herbicide atrazine under different culture conditions to assess those interactions between herbicides and biotic and abiotic factors. The density of the cyanobacterium *O. limnetica* was determined in natural phytoplanktonic assemblages and in unialgal cultures in medium containing 10 μg/L atrazine. Experiments (total of 13) were conducted in spring and early and late summer, during which the effect of atrazine varied in nature and intensity. The growth of the cyanobacterium was always inhibited in cold experiments, whereas it was stimulated in warm experiments within the natural phytoplankton assemblage, but unaffected in pure culture. Laboratory experiments with unialgal culture showed that the sensitivity to atrazine increased as the temperature decreased. Phytoplankton community structure, interactions between species, and environmental parameters (*e.g.,* temperature) are important factors controlling the responses of cyanobacteria to the herbicide. These interactions between sensitivity to herbicides and environmental factors may reduce or emphasize the effects of pollution in aquatic systems. Thus, the ecotoxicological relevance of herbicides in aquatic systems is quite complex and cannot be assessed by single-species short-term laboratory toxicological tests.

The herbicide atrazine is widely used and has contaminated many aquatic environments. Because it inhibits photosynthesis (Photosystem II [PS II] inhibitor), it can directly influence ecophysiology and the dynamics of algal populations (Buser 1990; Huber 1993; Solomon *et al.* 1996). The response of algae to PS II inhibitors differs depending on the sensitivity of the species (or strain) and the effective herbicide concentration. Because organisms vary in their tolerance or resistance, selection pressure may result in susceptible organisms being replaced by more resistant ones. In this way, PS II inhibitors in the environment could affect the structure of algal communities (de Noyelles *et al.* 1982; Hamilton *et al.* 1988; Molander and Blanck 1992; Hoagland *et al.* 1993; Thompson *et al.* 1993; Caux and Kent 1995; Kasai and Hanazato 1995; Bérard *et al.* 1999). Those few studies that have used repeated outdoor experiments on natural communities indicate that the responses of algae to the herbicide vary from experiment to experiment (Herman *et al.* 1986; Lampert *et al.* 1989; Guasch *et al.* 1997). For example, our ecotoxicological experiments on outdoor phytoplankton microcosms showed that the responses to atrazine of some species of algae varied (Bérard and Pelte 1996). The fraction of the cyanobacterium *Oscillatoria limnetica* in phytoplankton communities growing in contaminated microcosms was reduced at the end of some experiments, whereas this cyanobacterium grew well under the same atrazine concentration during other experiments (Bérard and Pelte 1996). All of these outdoor experiments on natural phytoplanktonic communities were performed with the same concentration of atrazine (10 μg/L), but at different periods of the year, and environmental factors such as community structure and abiotic factors (*e.g.,* nutrients, temperature, light) varied from one experiment to another.

The physiology and growth of algae depend on abiotic factors, and competition within algal communities is an important influence on community structure (*e.g.,* Reynolds 1984; Sommer 1986). The effects of these biotic and abiotic factors on phytoplankton suggest that there is a link between herbicide toxicity for algae and environmental factors.

We therefore set out to identify selected environmental factors that influence the sensitivity of *O. limnetica* to atrazine under our experimental conditions. We investigated the effect of seasonal environmental factors and, more precisely, the effect of interactions between species and temperature on the sensitivity of this cyanobacterium to atrazine. The specific objectives of this study were: (1) to compare the tolerance of *O. limnetica* to atrazine in outdoor microcosms experiments performed at different seasons; (2) to compare the tolerance of *O. limnetica* to atrazine in outdoor microcosms, among natural phytoplankton communities, and in pure culture; and (3) to compare the

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tolerance of *O. limnetica* to atrazine in laboratory pure culture at two temperatures.

Material and Methods

Three kinds of experiments were carried out: One with natural phytoplanktonic communities cultivated in microcosms, a second with an *O. limnetica* monoculture cultivated under the same physicochemical conditions (microcosms), and a third with *O. limnetica* pure cultures cultivated in the laboratory at two temperatures. A total of 13 microcosms experiments were carried out during the spring and early and late summer in 1995, 1996, 1997, and 1998.

Outdoor Microcosm Systems

Material: The microcosms were made with 5-L Pyrex bottles. Each treated-bottle contained 10 μg/L at the beginning of the experiment, and three to five replicates for tests and controls were performed, depending on the experiment. Water samples containing phytoplankton were removed through a silicon cap and glass and silicon tubing. The material and installation are described in Bérard *et al.* (1999). The experiments ran for 10–21 days and stopped when the relative growth rate of control cultures declined, just before the stationary phase of phytoplankton growth.

Natural Samples: Nine experiments were carried out during 1995, 1996, 1997, and 1998. The water for the microcosms was taken from the centre of Lake Geneva. Blanc *et al.* (1998) reported atrazine concentrations of 0.03–0.04 μg/L in the euphotic layer of the whole lake, indicating a low, stable pollution with this herbicide during 1995–1997. Each 5-L jar contained 2.5 L of phytoplankton suspension, plus 2.5 L of 0.8 μm–filtered (polycarbonate filter) natural lake water from the same location (Bérard *et al.* 1999). The concentrations of the various nutrients and chlorophyll *a* at the beginning of the experiments are given in Tables 1 and 2, respectively. Water temperature was continuously recorded *in situ* with an Hexaparameter (Ponselle) device, whereas surface irradiance was provided by a local meterological equipment.

The phytoplankton biomass was estimated daily by *in vivo* fluorescence measurements using a Turner fluorimeter (Sunnyvale, CA) to monitor the growth of the phytoplankton. Phytoplankton community structure was determined by counting the cells and filaments in samples at the beginning and at the end of the experiment, by using a Utermöhl inverted microscope technique at a magnification of 200. A minimum of 400 individuals of each dominant species (more than 100 individuals per ml) were counted, giving a counting accuracy of approximately ±10% (Lünd *et al.* 1958). Phytoplankton identity were determined with the help of the key figures and nomenclature system of Bourrelly (1966–70). The changes in the cell density of each species in the phytoplankton community were recorded.

The diversity (H′) of the algal communities was estimated at the beginning of each experiment using the Shannon-Weaver equation. The specific characteristics of the algal innoculum were analyzed by cluster analysis with the ADE software (Thioulouse *et al.* 1997). The inoculum was described in the data matrix by the percentages of the total biomass of the algal classes (except for *Bacillariophyceae,* which was separated into pennate and centric diatoms). The density of each dominant species in the contaminated communities was compared to the control at the end of experiments using the Mann-Whitney nonparametric test.

In situ *Microcosms with Pure Cultures of* O. limnetica

Four experiments were carried out during the spring and summer of 1997 in parallel with the natural phytoplankton microcosm experiments (March, April, June, and August 1997) and at the same place and under the same conditions. Monocultures of *O. limnetica* were placed in 5-L Pyrex microcosms filled with 5 L lake water (culture medium) filtered through 0.8-μm membrane filter. The culture medium used to grow monocultures of *O. limnetica* was taken from the same site as the water for the natural phytoplankton microcosms experiments.

O. limnetica (strain CCAP 1459/18 isolated from unpolluted aquatic ecosystems) in exponential growth was used as inoculum. The experiments performed in March and April 1997 were inoculated with *O. limnetica* grown at 12°C, whereas those carried out in June and August 1997 were inoculated with cells cultivated at 20°C to avoid temperature stress. Each 5-L bottle containing natural medium was inoculated with 33-ml aliquots of cyanobacterium suspension, which provided an initial biomass of 2.14 μg/L chl *a* (comparable to the mean chl *a* of the natural phytoplankton inoculi of the March, April, June, and August 1997 experiments). Experiments ran for 7–22 days and were terminated when the relative growth rate declined. *O. limnetica* biomass was estimated daily by measuring optical density (OD) (Kontron Uvikon 710, Rotkreuz, Switzerland) at 650 nm. OD_{650} was used because it closely correlated with the number of *O. limnetica* filaments (data not shown, $R^2 = 0.93$). The effect of atrazine on changes in cyanobacterium biomass was analyzed using the student's *t* test ($p = 0.05$).

Microcosms: Temperature and Irradiance Conditions

Mean temperatures were generally below 15°C during the spring experiments (March, April, and May) and above 15°C during the summer and autumn experiments (June, July, August, and September) (Figure 1). The irradiance was highest during the summer experiments (June and July) and lowest during the fall experiments (September). The spring experiments (March and April) received intermediate irradiance (Figure 1).

Toxicants

Atrazine (98%, Greyhound/Chem Service, UK) was dissolved in double-distilled water with continuous stirring for 48 h (10 mg/ml stock solution). No organic solvent was used to avoid any possible side effects (Bérard 1996). The effective concentration of the herbicide solution was checked by HPLC. The microcosms contained 10 μg/L atrazine at the beginning of each experiment (three or five replicates for tests and controls), and this concentration is equivalent to severe herbicide contamination, which occasionally occurs in some aquatic systems during runoff (de Noyelles *et al.* 1982; Caux and Kent 1995; Solomon *et al.* 1996).

In vitro *Experiments with Pure Cultures of* O. limnetica

Two sets of experiments were carried out with the *O. limnetica* CCAP 1459/18 in 250-ml batch cultures filled with Z medium, specific for cyanobacteria (Table 3). The inoculum concentration was the same as for the cyanobacterium microcosm experiments. Cells were cultured in a geometric nominal concentration series of 22 atrazine concentrations ranging from 1.153 to 125 μg/L, with three control cultures. Experiments were conducted at 13 ± 1°C or 20 ± 1°C (cold and warm experiments). Illumination was provided by cool white fluorescent lamps set on a 16:8 light:dark photoperiod with an irradiance of 39 μmol quanta/m^2/s.

Growth was monitored by measuring OD_{650} immediately after adding cyanobacterium inoculum and herbicide, and then daily (days 5–9 for the cold experiments and days 3–6 for the warm experiments) during exponential growth (Hersh and Crumpton 1987). The percent inhibition of growth was calculated by comparing OD values for each

Table 1. Nutrient concentrations in the microcosms at the beginning of each experiment

Experiment	March 97	April 96	April 97	May 98	June 95	June 97	July 95	July 98	Aug 97	Sept 95	Sept 96	Sept 98
Orthophosphates (mg P/L)	0.006	0.002	0.012	0.003	0.002	0.003	0.004	0.001	0.001	0.001	0.004	0.000
Inorganic nitrogen (mg N/L)	0.55	0.43	0.52	0.42	0.35	0.38	0.58	0.037	0.023	0.27	0.24	0.156
Silicium (mg SiO_2/L)	0.12	0.34	0.81	0.12	1.30	0.70	1.21	0.54	1.63	<0.02	0.34	0.14

Table 2. Biomass (expressed as chl *a*) and diversity (Shannon-Weaver diversity index) of the inoculum of the nine natural communities used in the microcosms experiments

Experiment	March 97	April 96	May 98	June 95	July 95	July 98	Sept 95	Sept 96	Sept 98
Chlorophyll *a* (μg/L)	4.70	3.54	3.23	0.48	1.83	2.01	2.61	3.20	6.83
Diversity index (H′: bits/individual)	0.88	1.73	1.14	1.95	1.70	2.94	2.63	2.42	1.90

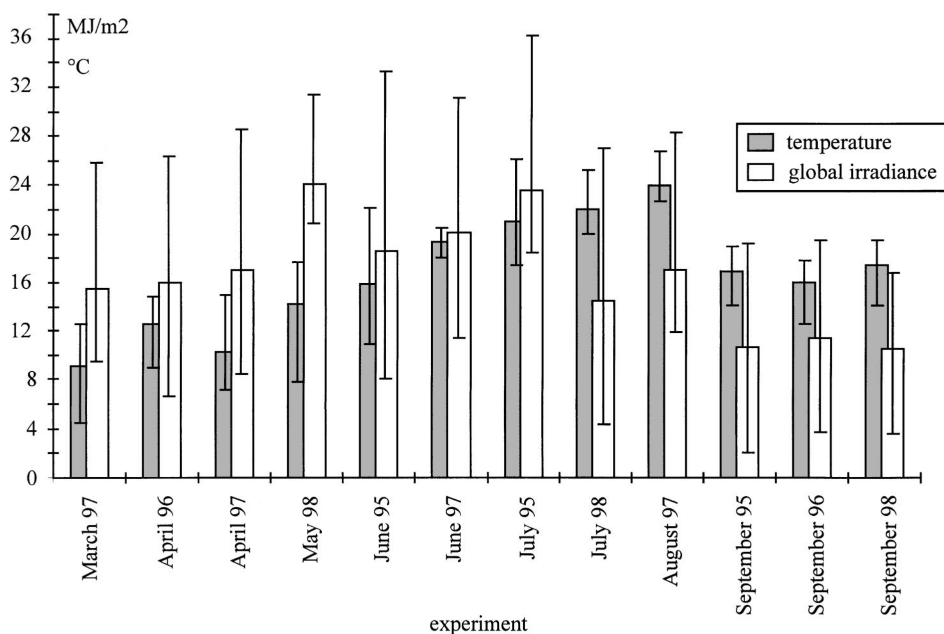

Fig. 1. Mean temperature and global irradiance conditions of microcosm cultures. Bars represent minimum and maximum values recorded during each experiment

atrazine concentration to the mean OD values of the controls on days 7, 8, 9, and 10 for cold experiments and on days 4, 5, 6, and 7 for warm experiments. The EC_{50} values and confidence intervals (95%) were estimated from the regression lines of percentage inhibition of growth against the log dose of atrazine. EC_{50} values were calculated from 10–12 atrazine concentrations lying within the portion of the curve where the changes in percentage inhibition of growth were linear. Calculations were carried out using Splus software. The day-8 EC_{50} value for cold experiment and day-5 EC_{50} value for warm experiment were the minimal EC_{50} values calculated for each experiments; they were used to assess the influence of temperature on the sensitivity of *O. limnetica* to atrazine.

Results

Microcosms: Initial Composition of Natural Communities

Since the nine microcosm experiments involving cultivation of natural phytoplankton communities were initiated at different periods during the 4 years, we describe the phytoplanktonic communities on the date when atrazine was added. Cyanobacteria were not the dominant class at the beginning of all experiments. *O. limnetica* was always the dominant species among the cyanobacteria in the microcosms.

The characteristics of the inocula using cluster analysis of specific composition are shown in Figure 2. The initial communities in spring experiments (March, April, and May) were dominated by centric diatoms (*Stephanodiscus minutulus, Stephanodiscus noeastrae,* and *Cyclotella* sp.), cryptophytes (*Rhodomonas minuta, R. minuta* var. *nannoplanctica, Cryptomonas* sp.), and some pennate diatoms (*Asterionella formosa, Fragilaria crotonensis,* and *Melosira varians*). The June 1995 experiment started during a "clear water" phase with a low biomass inoculum dominated by chlorophytes (*Chlamydomonas* sp., *Chlorella vulgaris, Elakatothrix gelatinosa,* and some colonial cells as *Eudorina elegans*), and cryptophytes. The July 1995–1998 experiments started at the end of the clear water phase. The inoculum was an early summer phytoplankton

Table 3. Z culture medium composition

Component	Concentration
$NaNO_3$	467 mg/L
$Ca(NO_3)_2 \cdot 4H_2O$	59 mg/L
K_2HPO_4	31 mg/L
$MgSO_4 \cdot 7H_2O$	25 mg/L
Na_2CO_3	21 mg/L
$Cl_3Fe \cdot 6H_2O$ in HCl 0.1 M and EDTA 0.1 M	10‰ (v/v)
Trace elements[a]	0.08‰ (v/v)

[a] Final concentrations in culture medium: H_3BO_4 248 μg/L; $MnSO_4 \cdot 4H_2O$ 178.4 μg/L; $NaWO_4 \cdot 2H_2O$ 2.64 μg/L; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 7.04 μg/L; KBr 9.52 μg/L; KI 6.64 μg/L; $ZnSO_4 \cdot 7H_2O$ 22.96 μg/L; $Cd(NO_3)_2 \cdot 4H_2O$ 12.32 μg/L; $Co(NO_3)_2 \cdot 6H_2O$ 11.68 μg/L; $CuSO_4 \cdot 5H_2O$ 10 μg/L; $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$ 15.84 μg/L; $Cr(NO_3)_3 \cdot 7H_2O$ 2.96 μg/L; $V_2O_4(SO_4)_3 \cdot 16H_2O$ 2.8 μg/L; $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$ 37.92 μg/L)

assemblage dominated by pennate diatoms, chrysophytes (*Ochromonas* sp. and *Dinobryon* sp.), cryptophytes (*R. minuta* var. *nannoplanctica*), desmids (*Staurastrum* sp.), cyanobacteria (*O. limnetica*), and dinoflagellates. The September experiments started at the end of the summer successions when chlorophytes (*Chlamydomonas* sp. and *Coelastrum* sp.), dinoflagellates (*Ceratium hirundinella*), chrysophytes (*Dinobryon* sp.), cyanobacteria (*O. limnetica*), and filamentous zygnematophyceae (*Mougeotia gracillima*) were dominant.

Microcosms: Atrazine Effects on O. limnetica

The growth of *O. limnetica* in the natural phytoplankton microcosms containing atrazine was inhibited (compared to the controls, Mann-Whitney tests) during spring experiments (March, April, and May) and stimulated (compared to the controls, Mann-Whitney tests) during summer experiments, except for the September 1996 and July 1998 experiments, when the cyanobacterium showed no significant reaction to atrazine (Table 4).

O. limnetica in microcosm monocultures was less abundant in treated microcosms at the end of the spring experiments (student test, 95% confidence, n = 3), but grew well in treated microcosms during the summer experiments (Figure 3).

In vitro *Experiments with* O. limnetica *Monoculture*

The EC_{50} values estimated for *O. limnetica* were 24.2 μg/L atrazine (95% confidence interval: 23.7–24.7) when cultivated at 13°C and 52.3 μg/L atrazine (95% confidence interval: 51.1–53.6) when cultivated at 20°C. These experiments were performed twice and results were similar.

Discussion

Temperature Dependence of O. limnetica *Sensibility to Atrazine*

The percent *O. limnetica* in the phytoplanktonic communities was significantly replaced at low temperature (<15°C), and increased or unaffected at higher ones (p = 0.0041, n = 9, two-way test since the 15°C threshold was choosen after the test); the growth of *O. limnetica* in pure cultures was inhibited at low temperature and unaffected at higher temperature (p = 0.06, n = 4, one-way test since the 15°C threshold was fixed before the experiments). Mean irradiance had no significant influence on sensitivity to atrazine, but higher light levels do enhance the sensitivity of benthic algae to atrazine (Guasch and Sabater 1998). The cyanobacteria were not inhibited by atrazine at the end of summer, when irradiance was lower than during early summer and spring. In the same way, *O. limnetica* reactions to atrazine were not linked to the initial nutrient concentrations in the media. Temperature was therefore examined in laboratory cultures of *O. limnetica.* The cyanobacterium was twice as sensitive to atrazine at low temperature (which is similar to the means observed in the microcosms during spring) than at high temperature (comparable to the means measured in the microcosms during summer experiments). This temperature-dependent sensitivity could be due to the turnover rates of the D1 protein, which is the specific target of atrazine; turnover could contribute to the recovery of PSII activity after atrazine binding. For example, Ross and Vincent (1998) have shown that the repair of UV-damaged D1 protein in an Antarctic cyanobacteria is less effective at lower temperatures. These are clear signs that detoxification and/or recovery process depend on temperature, rather than the toxic effect itself.

Influence of Other Species on the Effect of Atrazine on Oscillatoria

The initial composition of the natural communities in the microcosms led us to consider the interactions between species during experiments. The species compositions of spring inoculum experiments (Figure 2) (March, April, and May) were similar, and quite different from the initial phytoplankton compositions of early and late summer experiments. This specific composition is characteristic of spring bloom assemblages in great alpine lakes with pioneer species found within poorly diversified (Table 2) and robust communities, whereas summer inocula have mature, stable, more diversified communities (Reynolds 1984; Frontier 1985; Sommer *et al.* 1986; Gawler *et al.* 1988).

The growth of *O. limnetica* was significantly inhibited by atrazine, both in natural community and in microcosm monocultures (Table 4, Figure 3). This suggests that the growth of the cyanobacterium is directly inhibited by the herbicide under spring environmental conditions. The spring bloom species (diatoms and cryptophytes) did not react to atrazine, or their growth in the contaminated natural communities was stimulated (Table 4). Herman *et al.* (1986) obtained similar results for periphyton in atrazine-treated (100 μg/L) limnocorrals during the early spring. Cyanobacteria, which were dominated by *Oscillatoria* sp., almost completely disappeared from contaminated limnocorrals, although it had no effect on diatoms. These different reactions to the pollutant suggest that there are interactions between algal species, *e.g.,* competition between diatoms and cyanobacteria in spring with atrazine contamination. Diatoms are more competitive than cyanobacteria under winter and spring conditions (Løvstad 1984), and cyanobacteria develop most during the late summer succession rather than

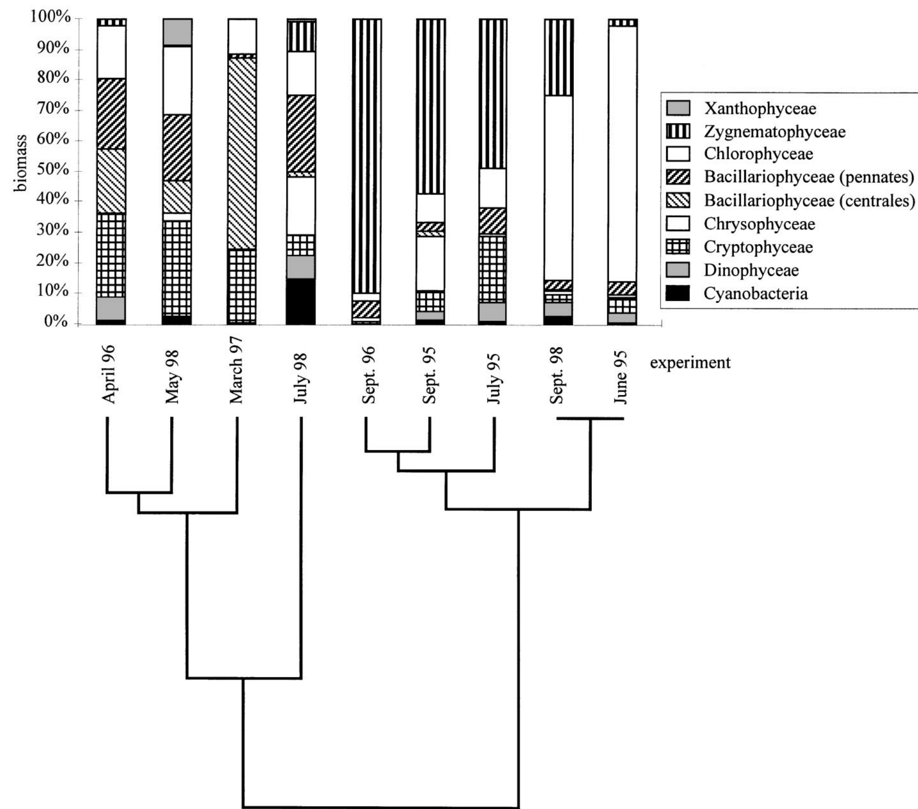

Fig. 2. Algal class composition of microcosms in the beginning of each microcosm experiment. Cluster analysis on the algal class community structure (Manhattan distance; Chessel *et al.* 1995)

Table 4. Response of the cyanobacterium *Oscillatoria limnetica* and the other dominant species to 10 µg/L atrazine in microcosms with natural phytoplankton communities (Mann-Whitney nonparametric test at the end of each experiment)

		Atrazine Effect								
Class	Species	March 97	April 96	May 98	June 95	July 95	July 98	Sept 95	Sept 96	Sept 98
Cyanobacteria	*Oscillatoria limnetica*	− (95%)	− (97%)	− (95%)	+ (90%)	+ (90%)	0	+ (90)	0	+ (98%)
	Pseudanabaena galeata								− (95%)	0
Cryptophyceae	*Rhodomonas minuta*			0			0			0
	Rhodomonas minuta var *nanno.*	0	+ (97%)	0	+ (90%)		0	+ (97%)		+ (97%)
Chrysophyceae	*Ochromonas* sp	+ (95%)	+ (90%)	0		0	0	0	0	
	Desmarella brachycalyx	+ (95%)				0	+ (95%)			0
	Dinobryon sp							0		
	small flagellate cells	+ (95%)		0			− (99%)			+ (99%)
Xanthophyceae	*Tribonema ambiguum*			0						0
Bacillariophyceae	*Stephanodiscus minutulus*	0		0			− (95%)			
	Asterionella formosa		+ (94%)			0		+ (90%)		
	Diatoma elongatum			0			0	+ (98%)	0	0
	Fragilaria crotonensis		0	0	+ (90%)	0	0	0	+ (95%)	
	Nitzschia acicularis				+ (90%)	0		+ (97%)		
	Nitzschia sp			0					0	
	Synedra acus		0			0	0	0	0	0
	Synedra acus var *angustissima*		+ (89%)					+(85%)	0	
Chlorophyceae	*Chlamydomonas* spp	− (85%)	0	0			0			0
	Chlorella vulgaris	− (95%)	− (97%)	0	− (90%)	0	− (95%)	− (99%)	0	0
	small flagellate cells		− (97%)	0	+ (90%)		0	0		
Zygnematophyceae	*Staurastrum* sp					+ (90%)				
	Mougeotia gracillima						0	0	0	− (85%)

⟨⟨+⟩⟩: greater density of individuals than in controls
⟨⟨−⟩⟩: fewer individuals than in controls
⟨⟨0⟩⟩: no significant effect
▒: not present (<100 individuals/ml)

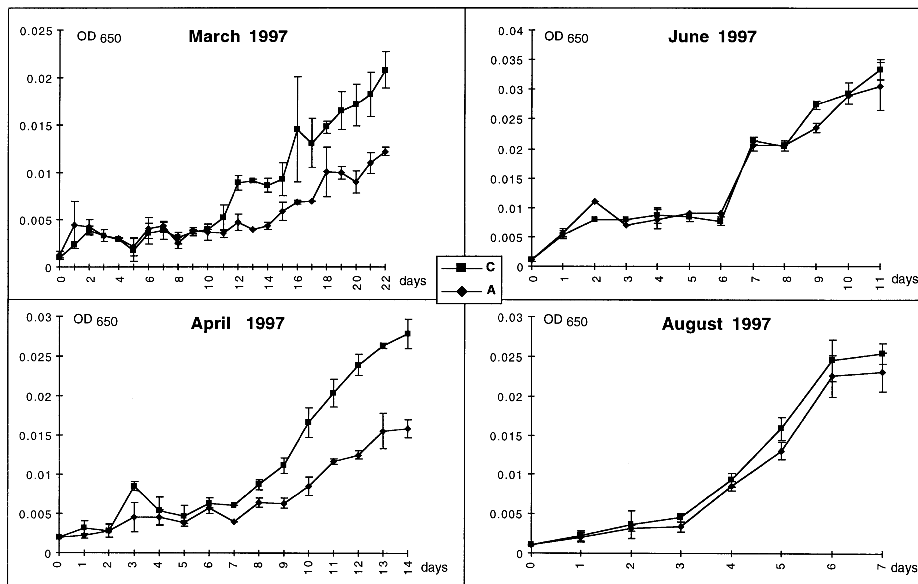

Fig. 3. Growth of *Oscillatoria limnetica* in pure culture microcosm during 1997 experiments, measured by OD_{650}. ■: control. ♦: contaminated by 10 μg/L atrazine. Error bars represent confidence interval (n = 3; p = 0.05)

early spring (Sommer *et al.* 1986). Diatoms and cryptophytes are also known to be more tolerant to PS II inhibitors than are other algae (de Noyelles *et al.* 1982; Herman *et al.* 1986; Hamilton *et al.* 1988; Gurney and Robinson 1989; Molander and Blanck 1992; Hoagland *et al.* 1993; Shehata *et al.* 1993).

The densities of *O. limnetica* were higher in the contaminated microcosms in most of the summer multispecies experiments. The cyanobacterium was not only tolerant but was stimulated by the herbicide (Table 4). However, the growth of *Oscillatoria* in the monocultures microcosms contaminated by atrazine was not enhanced in the summer experiments of 1997 (Figure 3). This enhanced growth of contaminated communities in summer could also be a side effect of interspecies relationships. The herbicide may particularly inhibit sensitive species like chlorophyta, which are numerous in summer and known to be more sensitive to PS II inhibitors than other algae (de Noyelles *et al.* 1982; Hamala and Kolligt 1985; Herman *et al.* 1986; Hoagland *et al.* 1993). This could allow enhanced growth of the more tolerant species (*e.g., O. limnetica*), as suggested by Bryfogle and McDiffett (1979), de Noyelles *et al.* (1982), Goldsborough and Robinson (1986), Pratt *et al.* (1988), and Shehata *et al.* (1993).

Studies on algal assemblages have shown that the effects of PS II inhibitors depends on factors, such as exposure time and concentration, but also on the initial composition and state of phytoplankton communities and environmental factors (Herman *et al.* 1986; Mayasich *et al.* 1987; Stay *et al.* 1989; Guasch *et al.* 1997). We suggest that the response of *O. limnetica* to atrazine depends on the environmental conditions and the concurrent species.

It is therefore difficult to identify the specific factor (community structure or temperature) that influences the toxicity of atrazine on *O. limnetica* in natural assemblages. Though our single species results and other studies indicate that if environmental factors may influence directly the sensitivity of algae to the toxicant (Mayasich *et al.* 1986; Millie *et al.* 1992; Fournadzhieva *et al.* 1995; Guasch and Sabater 1998), these same factors may also influence competition among algae (Tilman 1977; Løvstad 1984; Sommer 1986; Wehr 1993; Spijkerman and Coesel 1996), changing the structures communities and so indirectly acting on the sensitivity of a specific alga to the herbicide.

Conclusion

Community structure and environmental parameters (*e.g.,* temperature) are important factors controlling the responses of algae to toxicants. *O. limnetica* appears to be inhibited when growing in spring phytoplankton communities contaminated by 10 μg/L atrazine. In contrast, summer favors the development of this cyanobacterium in phytoplankton assemblages (Sommer *et al.* 1986; McKnight *et al.* 1990), even those polluted by PS II inhibitors, which might sometimes enhance the extent of this bloom. This may be of ecological importance. The effects of herbicides in aquatic systems are complex. The responses to atrazine in natural phytoplankton communities differ from those of single-species tests because of the influence of a range of biotic and abiotic factors. Single-species tests may fail to predict indirect or system responses to toxicants, such as changes in competition and succession. Multispecies tests can include ecologically important interactions, evaluate effects on complex processes, and may better predict the ecotoxicological risk of toxicants in aquatic systems (Cairns 1983, 1986; Lampert *et al.* 1989).

Seasonal change in the species of algae in communities (successions) occurs in response to changing environmental factors by interactions between species and physicochemical parameters (Reynolds 1984). Therefore, atrazine may be an extra factor disturbing algal successions in lakes (de Noyelles *et al.* 1982; Neugebaur *et al.* 1990; Caux and Kent 1995; Jüttner *et al.* 1995). However, as this study illustrates, interactions among species, phytoplankton successions, and environmental parameters (*e.g.,* temperature) also affect the response of phytoplankton communities to the pollutant. Time of year must be taken into account when monitoring polluted aquatic systems because of the seasonal variation in the response to algae to PS II inhibitors, as well as seasonal variations in the contamination of

the water bodies by these herbicides from watersheds (Lakshminarayana *et al.* 1992; Detembeck *et al.* 1996; Solomon *et al.* 1996; Müller *et al.* 1997). We must now carry out experimental studies that take into account the natural fluctuations in population and ''pulses'' of herbicides into the systems to further investigate these interactions between sensitivity to herbicides and biotic and abiotic factors. In addition these studies must be complemented with *in situ* studies to provide more reliable predictions of the safety of herbicides in aquatic environments.

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