

Effects of glyphosate on lignicolous freshwater fungi of Hong Kong

Clement K.M. Tsui*, Kevin D. Hyde & I. John Hodgkiss

Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity,
The University of Hong Kong, Pokfulam Road, Hong Kong

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The effect of herbicide glyphosate on the biomass production of four freshwater fungi, *Annulatascus velatisporus*, *Camposporium antennatum*, *Massarina* sp. and *Helicosporium griseum*, isolated from submerged wood was investigated. At 500 mg l⁻¹, inhibition from herbicide ranged between 19 and 79%. At 50 mg l⁻¹, glyphosate stimulated the biomass production of *C. antennatum* and *H. griseum* by about 14%. The degree of inhibition varied among species.

Keywords: ascomycetes, glyphosate, hyphomycetes, lignicolous, liquid culture.

Glyphosate, [N-(phosphonomethyl)glycine], is a water-soluble, non-selective post-emergence herbicide which has a wide application in weed control and is increasingly used internationally (Lévesque & Rahe, 1992). It is known to control 95% of the worst weeds. The effects of glyphosate on fungal communities have previously been investigated (Estok & al., 1989; Wardle & Parkinson, 1990, 1992; Wardle & al., 1994). The mycelial growth of three species of ectomycorrhizal fungi was completely inhibited at about 5,000 mg l⁻¹ in agar (Estok & al., 1989). The biomass production of another five ectomycorrhizal fungi was significantly reduced when they were exposed to concentrations above 36 mg l⁻¹ (Chakravarty & Chatarpaul, 1990). Nevertheless, in the same study, glyphosate did not reduce the soil fungal and bacterial colonies on agar or carbon dioxide evolution in the long term. Similarly, Wardle & Parkinson (1990) noted that the number of fungal colonies in soil samples was unaffected by the application of glyphosate ranging from 2 to 200 µg g⁻¹. Even though there have been contradictory results concerning the effect of glyphosate on fungal communities, this herbicide was thought to influence species interactions (Wardle & Parkinson, 1992). Interspecific competition between fungi on agar became more or less intense as glyphosate concentrations increased, and glyphosate was probably acting as a fungal nutrient (Wardle &

Parkinson, 1992). In fact, some fungal strains have been shown to utilize glyphosate as a source of phosphorus or carbon (Krzysko-Lupicka & Orlik, 1997).

Glyphosate has been introduced into the aquatic environment indirectly while controlling the terrestrial weeds (Bowner, 1982). The effects of glyphosate on aquatic insects, fishes and aquatic bacteria have been extensively reviewed (Franz & al., 1997). Several studies have been conducted on the effects of various pesticides mainly on aquatic hyphomycetes (Bärlocher, 1992), but the impact from glyphosate is poorly known. Freshwater fungi including ascomycetes and hyphomycetes inhabiting woody substrates are very diverse in Hong Kong (Goh & Hyde, 1999; Tsui & al., 2000), and the production of lignin-degrading enzymes demonstrates their role in freshwater ecosystems (Wong & al., 1998). Glyphosate is a widely used herbicide in horticulture in Hong Kong (Tsui, unpublished data) and caused a reduction in aquatic bacterial load in the Lam Tsuen River at 200 mg l⁻¹ during field studies (Chan & Leung, 1986). In this preliminary study, the effect of glyphosate on biomass production of lignicolous freshwater fungi is investigated.

Materials and methods

Culture and medium

Four species (*Annulatascus velatisporus* K. D. Hyde, *Camposporium antennatum* Harkn., *Helicosporium griseum* Berk. & M. A. Curtis, and *Massarina* sp.) were chosen because they are common in Hong Kong freshwater habitats (Tsui & al., 2000). All cultures were obtained from the Hong Kong University Culture Collection (HKUCC), and isolates were maintained on potato dextrose agar (PDA) at room temperature (ca 25 C). The experiment was conducted in a defined liquid medium consisting of: glucose, 20 g l⁻¹; KH₂PO₄, 1 g l⁻¹; MgSO₄·7H₂O, 0.5 g l⁻¹; CaCl₂, 0.1gl⁻¹; ammonium tartrate, 0.22 g l⁻¹; vitamin solution, 1ml; trace element solution [ferric citrate, 4.8 g l⁻¹; ZnSO₄·7H₂O, 2.64 g l⁻¹; MnCl₂·4H₂O, 2.0 g l⁻¹; CoCl₂·6H₂O, 0.4 g l⁻¹; CuSO₄·5H₂O, 0.4 g l⁻¹], 1 ml. All isolates were grown in the defined medium amended with filter sterilized glyphosate ("Ronall", 544 g l⁻¹ in aqueous solution) of different concentrations: 0, 50, 100, 200 and 500 mg l⁻¹. The pH of the media was maintained at about 7, which roughly corresponds to the value of stream water.

Biomass production in liquid media

A 5 mm mycelial disc aseptically cut from the growing edge of the mycelium of each species was added to a flask containing autoclaved defined media amended with different concentrations of

glyphosate. Three replicates were maintained for each treatment (4 species \times 5 concentrations). Cultures were grown for 28 days before filtering the mycelium through pre-dried and weighed filter paper. The mycelium retained was freeze-dried and the weighed. The pH of the media was checked weekly throughout the experiment.

The biomass production of each isolate was subjected to linear regression analysis. Percent inhibition or percent increment of biomass production was determined by the following equation (Chandrashekar & Kaveriappa, 1989):

$$I = \frac{100 (C-T)}{C}$$

Where I = inhibition of biomass production, C = biomass production in control, and T = biomass production in flask with glyphosate.

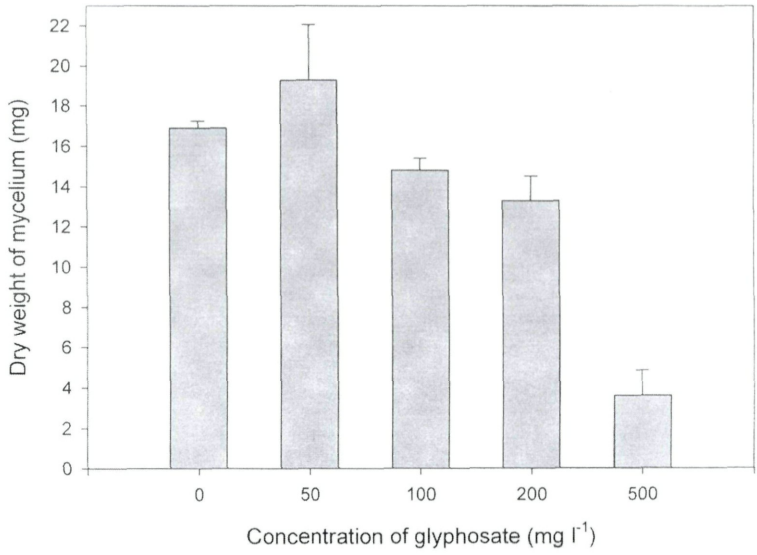
Results and discussion

As expected, the biomass production of all isolates decreased with increasing concentration of glyphosate but the degree of inhibition varied among the four isolates. There was a slight increase in biomass production by isolates of *Camposporium antennatum* and *Helicosporium griseum* at 50 mg l⁻¹ (Fig. 1). Also *C. antennatum* grew quite well as its biomass production was not severely inhibited below 500 mg l⁻¹ (Fig. 1). With the exception of *H. griseum* ($r^2 = 0.333$, $P = 0.0242$), there were high linear relationships between the reduction in biomass production in *C. antennatum*, *Annulatascus velatisporus* and *Massarina* sp. and the entire concentration range (Figs. 1, 2; Tab. 1).

At 200 mg l⁻¹, the inhibition of biomass production in *A. velatisporus* was over 50% and in *C. antennatum* and *Massarina* sp. were about 21% and 37% respectively (Tab. 1). Of the four isolates tested, *H. griseum* was the most tolerant species to glyphosate because the percent inhibition was about 19% at 500 mg l⁻¹, while the inhibition of biomass production was over 50% for the other three species (Tab. 1).

The responses of freshwater fungi towards various pesticides have been investigated (Bärlocher, 1992). The mycelial growth of three aquatic hyphomycetes species, *Flagellospora penicillioides* Ingold, *Lunulospora curvula* Ingold and *Phalangispora constricta* Nawawi & J. Webster was completely inhibited by the fungicides Mancozeb and Captafol at concentrations between 500 mg l⁻¹ and 1000 mg l⁻¹ on plate (Chandrashekar & Kaveriappa, 1989). In the same study, the two herbicides paraquat and 2,4-dichlorophenoxy-

Camposporium antennatum



Helicosporium griseum

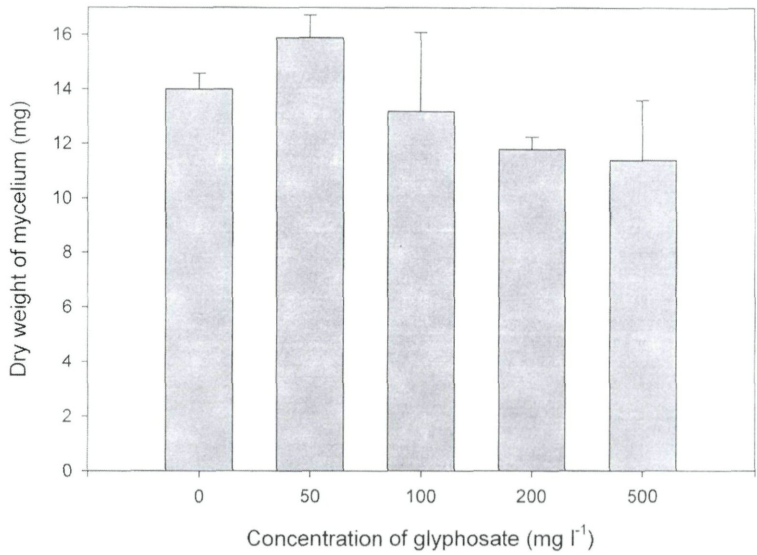
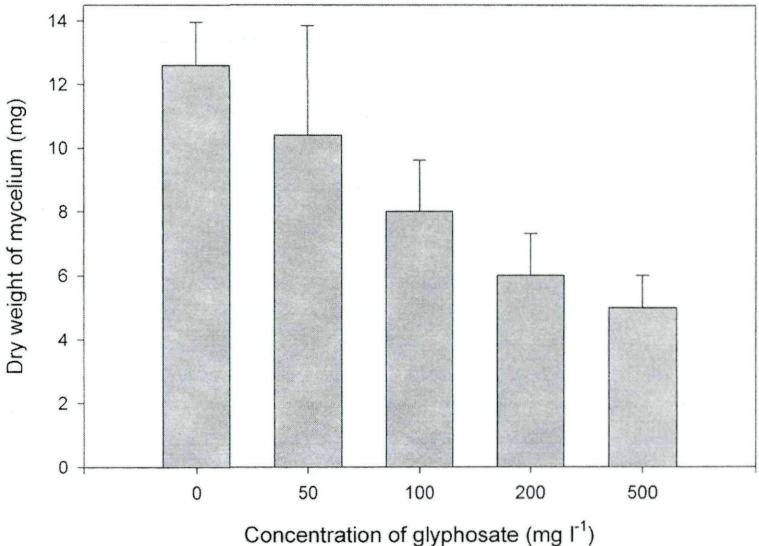


Fig. 1. – Effect of glyphosate on the biomass production of *Camposporium antennatum* and *Helicosporium griseum* (mean + standard deviation).

Annulatascus velatisporus



Massarina sp.

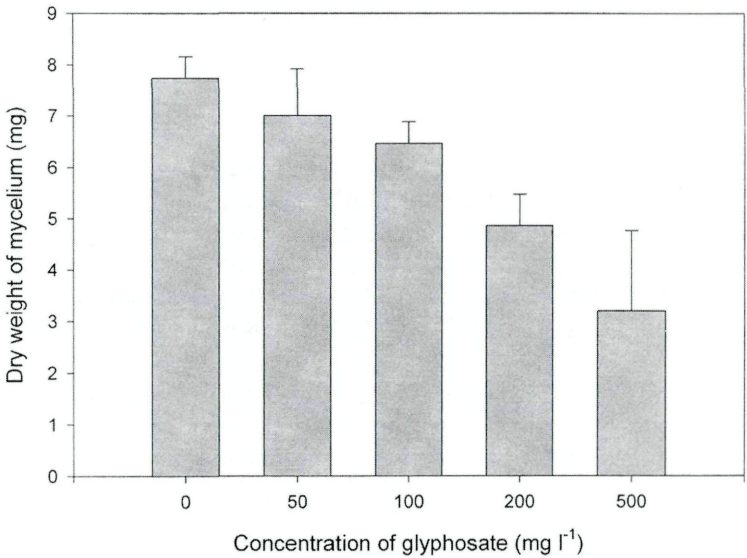


Fig. 2. – Effect of glyphosate on the biomass production of *Annulatascus velatisporus* and *Massarina* sp. (mean + standard deviation).

Tab. 1. – Inhibition (increment) in biomass production of 4 species treated with different concentrations of glyphosate (**P<0.05; ***<0.001). Value in parentheses indicate increment in biomass production.

	Concentration (mg l ⁻¹)					r ²	
	0	50	100	200	500		
<i>Annulatascus velatisporus</i>	0	17.5	36.5	52.4	57.1	0.551	**
<i>Camposporium antennatum</i>	0	(14.2)	12.5	21.4	78.7	0.895	***
<i>Massarina</i> sp.	0	0	13.2	36.8	57.9	0.793	***
<i>Helicosporium griseum</i>	0	(14.3)	5.7	15.7	18.6	0.333	**

butyric acid had a weaker effect and only reduced the growth by only 50% at 5000 mg l⁻¹. Mecoprop caused 50% reduction in biomass production of *Heliscus lugdunensis* Sacc. & Therry at concentrations between 10–100 mg l⁻¹ (Bermingham & al., 1998). Dalton & al. (1970) found that concentrations of DDT between 2 and 60 mg l⁻¹ increased the growth of four aquatic hyphomycete species, *Heliscus submersus* H. J. Huds., *Tetracladium setigerum* (Grove) Ingold, *Varicosporium elodeae* W. Kegel and *Clavariopsis aquatica* De Wild. This showed some resemblance to the performance of *C. antennatum* and *H. griseum* in our study and suggests that freshwater fungi may utilize trace amount of pesticides as nutrient sources. In general the degree of tolerance in freshwater fungi varied with chemicals, and the EC₅₀ concentrations (effective concentration which inhibits growth by 50%) seems to be relatively lower in liquid than in agar culture. Our results, however, have to be considered as preliminary because of the small number of species used and the failure in some cases of producing dose response curves over adequate concentration ranges.

Freshwater fungi seemed to be less sensitive to glyphosate than mycorrhizal fungi. Biomass production of the latter in liquid media was 50% inhibited at about 36 mg l⁻¹ (Chakravarty & Chatarpaul, 1990). Mycelial growth (colony area on agar) was inhibited by 70% at about 100 mg l⁻¹ (Estok & al., 1989), while the species tested in this study were mostly inhibited at 500 mg l⁻¹. It is difficult, however, to make direct comparisons between these studies due to different media.

In contrast to inhibitory and stimulatory responses reported from laboratory experiments, there were no apparent differences in species composition of aquatic hyphomycetes between streams treated with and without the insecticide methoxychlor (Suberkropp & Wallace, 1992). Similarly, the number of fungal propagules in the water column and sediments of a pond treated with 2,4-dichlorophenoxyacetic acid (herbicide) were not drastically affected (Sherry, 1994). The amount of glyphosate in natural waters

was only in traces and had not been determined. Although its impact on aquatic fungal communities has not been investigated, the decreased biomass production and germination rates (Tsui, 1999) may affect the ability of these fungi to colonize new substrates and thus their ecological functions.

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