Non-Target Effects of Glyphosate on Soil Microbes

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Introduction

Glyphosate is among the most popular herbicides registered for forest use in California. Noted for its broad effectiveness on competing vegetation, mild effect on conifers, rapid inactivation in soil, and low mammalian toxicity (DiTomaso 1997), glyphosate is an integral component of conifer release programs and has led to improvements in the growth of intensively managed forests (Powers and Reynolds 1999). Benefits of herbicide use must be viewed cautiously, however. Public concerns about environmental risks makes their forestry use controversial. Policy makers and forest managers thus need scientific documentation of the ecological effects of herbicides that go beyond the toxicological requirements met during product registration. In particular, our knowledge of the effect of glyphosate on non-target organisms in forest ecosystems is incomplete.

Soil microorganisms are an ideal community to evaluate non-target effects because they are affected both directly and indirectly by glyphosate. Direct, toxic effects result from inhibition of amino acid synthesis via the shikimic acid pathway (Grossbard and Atkinson 1985). Microorganisms and higher plants are the only organisms known to utilize this pathway, and thus are intolerant of glyphosate. All others lifeforms, including mammalian and avian species, lack the shikimic acid pathway and are unaffected by glyphosate (for example: LD_{50} for rats > 5000 mg/kg). Indirect effects of glyphosate may also be a driving force influencing the microbial community. Long-term control of understory vegetation can reduce soil organic matter and nitrogen content (Busse et al. 1996), both vital resources for microbial activity. Vegetation control can also regulate microbial activity by modifying microclimate, soil temperature, and soil moisture (Shainsky and Radosevich 1986). The choice of soil microorganisms as model organisms is further warranted by their ecological role. Soil microorganisms are responsible for essential processes in forests: decomposing organic matter, cycling nutrients, degrading toxic materials, and contributing to disease occurrence and suppression. Our objective was to determine whether soil microbial communities are adversely impacted by the non-target effects of glyphosate. This paper summarizes our preliminary findings. Complete details will be presented in a later paper (Busse et al., in preparation). Mention of any trade product does not imply Forest Service endorsement.

Experimental Approach

Direct and indirect responses of soil organisms to glyphosate were tested at three "Garden of Eden" study sites (Powers and Ferrell 1996). Briefly, the Garden of Eden study is a classic comparison of vegetation control, insect control, and fertilization across a range California's Westside ponderosa pine plantations. For our purposes, a subset of treatments was compared: (1) 9 years (minimum) of understory vegetation control using repeated glyphosate applications, (2) no vegetation control. Three sites were selected among common soil types to provide a gradient of low (Elkhorn), medium (Whitmore) and high (Feather Falls) site productivity, and treatments were replicated three times per site. Understory-control plots have been maintained weed free since plantation establishment, whereas control plots were densely covered (> 70%) with shrubs at Whitmore and Feather Falls (Powers and Reynolds 1999) and moderately covered with shrubs (~ 25%) at Elkhorn by the 9-11th growing season.

Direct effects of glyphosate. Soil from the upper surface horizon (0-15 cm depth) was collected randomly from control plots at each site and used in the following studies.

(1) *Toxicity in soil-free media.* Bacterial and fungal communities were extracted from soil using physiologically-buffered saline and grown on both liquid and solid media containing increasing concentrations of glyphosate as its commercial formulation, Roundup, at 0, 0.5, 1, and 10 times the recommended spray-solution concentration (50 mM). Measurements of short-term, toxic responses were made, including bacterial and fungal viability, bacterial growth rate, and functional diversity of bacteria.

(2) *Toxicity in soil.* Glyphosate was added to soil samples at 0, 5, 50, 500, and 5000 mg/kg. Subsamples were taken 1, 3, 7, and 30 days after glyphosate incorporation and tested for total microbial biomass, bacterial biomass, fungal biomass, and bacterial diversity using the C-utilization method and phospholipid fatty-acid signatures. Microbial activity was estimated by CO_2 release during the initial 9-day period following glyphosate incorporation.

Indirect effects of glyphosate. Seasonal changes in microbial community size and function were compared for the vegetation-removal and control treatments at the Garden of Eden sites using soil samples collected from the surface 0-15 cm during the 1998 growing season. Spring, summer, and fall samples were analyzed for microbial biomass, total bacteria, fungal hyphal length, bacterial diversity, respiration, and mineralizable nitrogen.

Findings

Glyphosate was lethal to bacteria and fungi when added to soil-free media. At the recommended sprayer concentration of 50 mM, glyphosate reduced bacterial viability 1000-fold (from 10^7 to 10^4 cells/g soil) on solid media, and completely eliminated fungal growth. Increasing glyphosate to 500 mM stopped all bacterial growth. Bacterial growth rate in liquid media also declined following additions of glyphosate (Figure 1). Results were consistent for all Garden of Eden sites, confirming that glyphosate is directly and indiscriminantly toxic to bacteria and fungi when added to soil-free media.

Contrary to the toxic response in soil-free media, glyphosate stimulated microbial growth and activity when added directly to soil. Microbial respiration, a standard measure of activity, increased with increasing levels of glyphosate (Figure 2). The response was minor at 5 and 50 mg/ha, the estimated concentration range in the upper horizon of mineral soil following field application, and greatest at highest application rate. Again, the results were consistent for all sites. Increases in total and viable bacteria were found at the highest rate of glyphosate addition, with *Psuedomonas*, *Arthrobacter*, *Xanthomonas*, and *Bacillus* spp. increasing in population dominance. Fungal population size remained relatively unchanged regardless of glyphosate application rate.

How can glyphosate kill microorganisms in soil-free media, yet stimulate growth when added directly to soil? This riddle is answered by recognizing herbicide chemistry and mobility in soil. Glyphosate is a polar compound that binds rapidly with soil colloids (clay, organic matter, aluminum and iron oxides), precluding uptake by microbial cells or roots. In effect, glyphosate is unavailable for biological activity once in contact with soil. By comparison, glyphosate remains active and unbound in soil-free media and can penetrate cellular membranes, disrupt protein synthesis, and ultimately kill microorganisms. This alone explains the differences we observed between soil and soil-free media. However, it does not clarify why microbial activity increased following glyphosate additions to the Garden of Eden soils (see Figure 2). Again, this observation can be attributed to herbicide chemistry. Glyphosate is a simple amino acid ($C_3H_8NO_5P$), capable of supplying energy (carbon) and nutrients (nitrogen, phosphorus) for microbial growth when bound to soil particles. Follow-up experiments have identified carbon as the major limiting factor for microorganisms in these soils, and implicate a beneficial role of glyphosate as an available energy source for microorganisms.

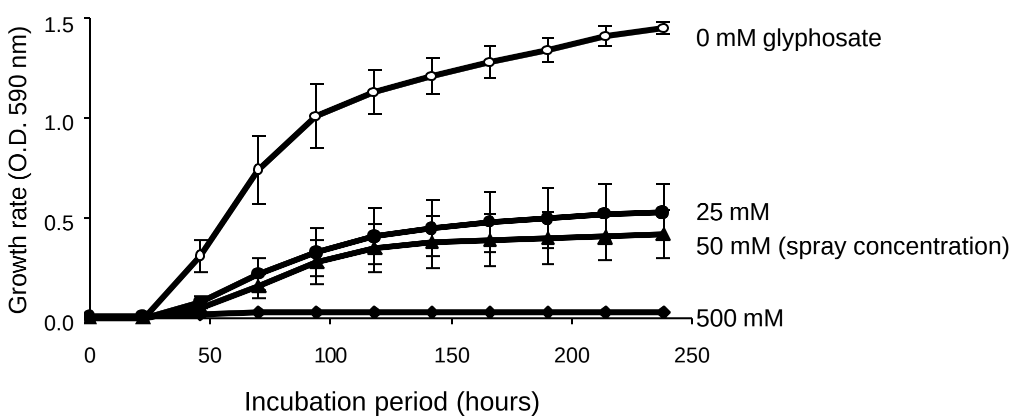

Figure 1. Inhibition of bacterial growth in culture media containing increasing concentrations of glyphosate. Bacteria were extracted from the Whitmore soil and their growth rate was determined by optical density using the average well-color development on Biolog plates. Similar results were found for Elkhorn and Feather Falls soils. Bars indicate one standard error of the mean.

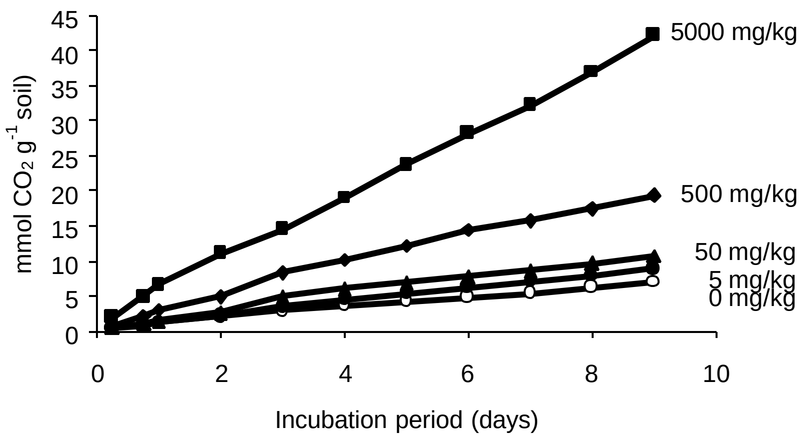

Figure 2. Stimulation of microbial respiration following addition of glyphosate to Whitmore soil. Between 5 and 50 mg/kg is the estimated concentration of glyphosate in soil following field application.

Glyphosate use, and weed control practices in general, prompt an additional ecological question regarding non-target organisms: does removal of understory vegetation and its associated functions modify the soil microbial community? We hypothesized that eliminating understory vegetation for a minimum of 9 years would affect microbial characteristics by reducing organic carbon input from roots and litter and modifying soil temperature and moisture. When monitored throughout the 1998 growing season, however, no differences in soil biological properties were found between glyphosate and control plots. All measures of microbial community size, diversity, and function were statistically equivalent between treatments. Further, no differences in soil carbon content or moisture availability were found between treatments.

Conclusion

Glyphosate had an inconsequential toxic affect on microorganisms from several ponderosa pine plantation soils. Microbial community size and activity were unaltered at the range of concentrations in soil anticipated following application. In fact, application of 100-times the normal soil concentration stimulated microbial activity and bacterial numbers by supplying supplemental energy for growth. Glyphosate toxicity was found in soil-free media, although the absence of glyphosate-adsorptive material (such as organic matter, iron and aluminum oxides, clay) in culture media is misrepresentative of soil conditions and should be considered an artifact. These findings agree with results from agricultural studies that show glyphosate does not have a toxic effect when added to soil due to its strong adsorption to soil colloids (e.g. Wardle and Parkinson 1992). Are these preliminary findings applicable to other forest types or soils? We suggest that the answer is yes. Lack of glyphosate toxicity to non-target microorganisms can be expected based on results from numerous studies plus recognition of glyphosate as a non-mobile and inactive compound in soil. Field results also indicated an inconsequential indirect effect of continuous understory vegetation control on microbial characteristics during the 1998 growing season at the Garden of Eden sites. Microbial communities were insensitive to 9 years of vegetation control, even though dramatic changes in shrub cover, tree growth, and tree nutrition were found (Powers and Ferrell 1996; Powers and Reynolds 1999).

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