

# Hexazinone Effects on Soil Biota and Processes: Preliminary Findings

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## Abstract

Direct effects of hexazinone on soil biota were assessed at several ponderosa pine plantations in Northern California. Response variables included microbial biomass, soil respiration, nitrogen mineralization, and soil arthropod assemblages. Measurements were taken a minimum of three times during the first growing season following hexazinone application at the recommended field rate. Hexazinone and control treatments were implemented in plots with no understory vegetation to avoid confounding, indirect effects from removal of vegetation and input of decaying organic material. Microbial community size, activity, and function were unaffected by hexazinone. There were some minor shifts in arthropod assemblage structure, but all appear to be transitory and none were statistically significant. Our results suggest that hexazinone treatment does not disrupt microbial communities or soil arthropod assemblages.

## Introduction

Hexazinone is one of the most widely used herbicides in California forests, following only glyphosate and triclopyr in terms of numbers of acres treated per year (Calif. Dept. Pesticide Regulation 2000). A triazine compound, hexazinone is an effective inhibitor of photosynthesis by herbaceous and woody plants. Since the majority of soil biota are non-photosynthetic, however, the herbicide's mode of action should theoretically have little effect on soil biological processes. Several, but not all, past studies confirm this premise (Maynard 1993, Chakravarty and Chatarpaul 1990, Rhoades et al. 1980). In contrast, a series of studies conducted on the coastal plains of North Carolina indicated that hexazinone was responsible for a reduction in soil microbial biomass that led to elevated nitrate levels in soil solution (Vitousek and Matson 1985, Andariese and Vitousek 1988).

Concern has been expressed about the general effects of herbicides on non-target biota, especially soil microorganisms and arthropods that are key to such crucial below ground processes as decomposition and nutrient mineralization. To address these concerns, we have initiated a series of laboratory and field studies on the direct (toxic) and indirect (removal of vegetation) effects of herbicides. Our initial study found minimal changes in soil organisms and their processes resulting from the indirect effect of continuous vegetation control (Busse et al. 2000, Busse et al. in press). Here we report preliminary results from the second of these studies, focusing on the direct effects of hexazinone.

## Materials and Methods

*Study sites.* The experiment was conducted at three long-term study sites in Northern California, part of the complex of "Garden of Eden" study sites established between 1986

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and 1988 to assess the effects of nutrient deficiency, competing vegetation, and insect herbivory on pine productivity (Powers and Ferrell 1996). The following plantations were selected to provide a range of site productivity:

<u>Plantation</u>	<u>Level of Productivity</u>	<u>Location</u>
Elkhorn Ridge	Low	California Coast Range, Mendocino County
Whitmore	Medium	Westside Cascades, Shasta County
Feather Falls	High	Westside Sierra Nevada, Plumas County

*Treatment design.* Three replicate, paired plots (70 m<sup>2</sup>) were installed at each plantation. Treatments (hexazinone vs. control) were then randomly assigned to the paired plots. Each set of paired plots was located within a larger plot that had been maintained free of understory vegetation for a minimum of 12 years. No herbicides had been applied, however, since 1995. Hexazinone (Velpar) was applied at the recommended field rate of 3 kg active ingredient per hectare in the spring of 2000. All plots received a minimum of 5 cm of precipitation within 1 to 2 weeks following hexazinone application. Mention of any trade name does not imply Forest Service endorsement.

*Microbial sampling.* Litter and mineral soil (0-15 cm depth) were collected on day 1, 7, 30, 100, and 191 following the initial precipitation after hexazinone application. Samples were analyzed for microbial biomass, basal respiration, and utilization of 95 carbon compounds (Biolog). Two additional, *in situ* measurements were made on each sampling date: (1) net nitrogen mineralization at 0-15 cm mineral soil depth using the core-replacement technique (Raison et al. 1987), (2) surface CO<sub>2</sub> efflux as a measure of soil respiration.

*Arthropod sampling.* We installed three polyethylene pitfall traps (Lemieux and Lindgren 1999), using propylene glycol as a preservative, on each plot. Pitfall trap placement was randomly assigned. Arthropod samples were collected at monthly intervals for three months post-treatment. Specimens were collected and then strained from the propylene glycol, identified, and sorted into broad taxonomic categories. We report here the results for mites, spiders, beetles, and springtails in part because they were the most abundant. Furthermore, mites and springtails are arguably the most important arthropods in terms of soil processes because they are the most important forest floor microbivores, stimulating microbial turnover and regulating the balance between bacterial and fungal biomass (Seastedt 1984). Spiders are universally predatory, and can serve as important indicators of prey base populations. Beetles, on the other hand, are more trophically diverse, serving as microbivores (e.g. featherwing beetles), carrion feeders (e.g. rove beetles), and predators (e.g. carabids).

## Results

Responses of soil biota to hexazinone were generally similar at all sites. As a consequence, results will be shown only for the Whitmore plantation (medium site productivity). Complete findings for all sites will be presented in a subsequent publication.

*Microbial analyses.* Hexazinone had little or no measurable effect on microbial community size, activity, or function. For example, microbial biomass, a standard measure of community size, was virtually identical between hexazinone and control plots throughout the growing season in both litter and mineral soil (Figure 1). The only treatment effect was a slight

depression of microbial biomass in the mineral soil at the final sample date. This was considered inconsequential, however, since no significant main effect or treatment x time interaction were found in repeated measures analysis. Community-level indices of soil activity (basal respiration, *in situ* respiration) were supportive of these results, showing no significant herbicide effects. Further, nitrogen availability, indicative of microbial functioning, was unaffected by hexazinone (Figure 2).

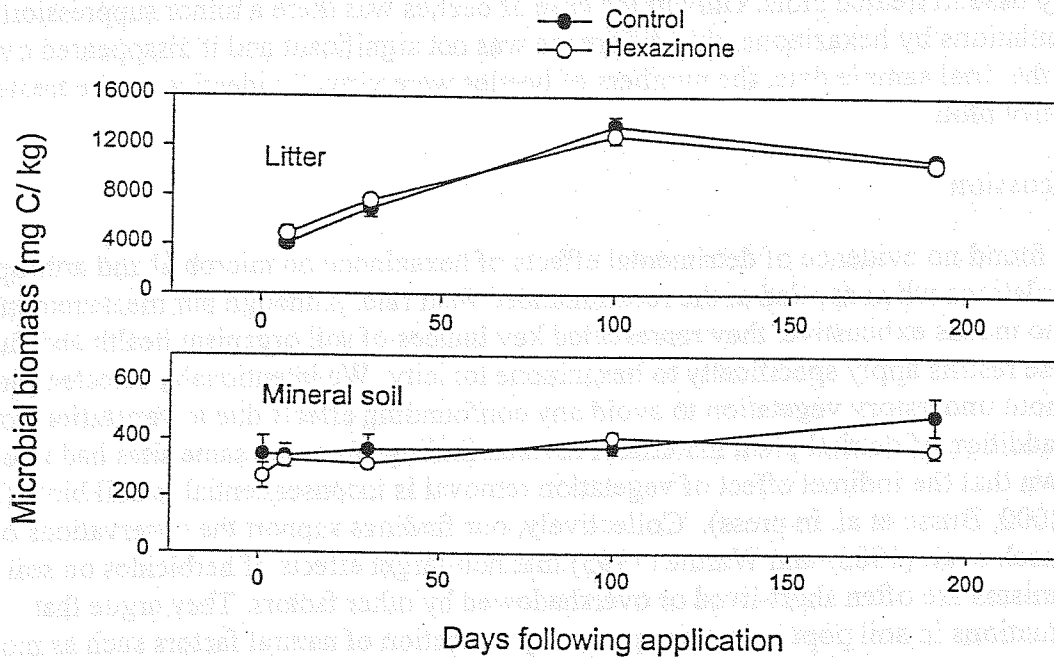


Figure 1. Effect of hexazinone on microbial biomass of litter and mineral soil at Whitmore (medium site productivity). Error bars represent standard error of the mean.

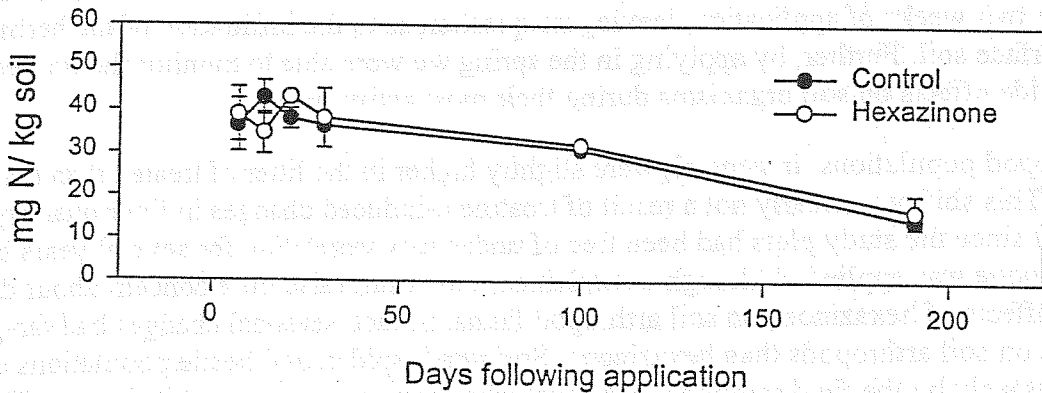


Figure 2. Effect of hexazinone on available nitrogen in the surface mineral soil at Whitmore (medium site productivity). Error bars represent standard error of the mean.

*Arthropod analyses.* There were no significant differences in numbers of mites, spiders, beetles, or springtails between hexazinone and control treatments for any of the sampling intervals. In fact, for most groups and most sampling intervals, the numbers of arthropods collected in pitfall traps were actually slightly greater (although not significantly so) in treated plots than in control plots (Figure 3). For both mites and springtails – functionally, the most important groups of arthropods -- the numbers were always slightly greater in treated plots than in control plots. The same is true for spiders, which probably reflects the higher prey base in treated plots. Only in the case of beetles was there a minor suppression of populations by hexazinone; this difference was not significant and it disappeared over time. By the final sample date, the numbers of beetles were virtually identical in the treated and control plots.

## Discussion

We found no evidence of detrimental effects of hexazinone on microbial and arthropod populations when applied at the recommended field rate. Although our measurements were by no means exhaustive, they represented key indices of soil organism health and function. These results apply specifically to hexazinone toxicity. We intentionally selected plots without understory vegetation to avoid any confounding effects due to vegetation control and the addition of detrital plant material. Previous findings from the same sites had already shown that the indirect effect of vegetation removal is inconsequential to soil biota (Busse et al. 2000, Busse et al. in press). Collectively, our findings support the observations of Domsch et al. (1983) and Wardle (1995) that non-target effects of herbicides on soil organisms are often short-lived or overshadowed by other factors. They argue that fluctuations in soil populations are primarily a function of natural factors such as moisture, temperature, pH, organic matter availability, and the additive effects of time and disturbance.

Hexazinone is a soil active herbicide, typically applied in the fall to ensure adequate leaching into the rooting profile from winter precipitation. By applying hexazinone in the spring, our results may not reflect typical practice. However, hexazinone was applied at each site immediately prior to a large storm event. Rainfall totals were 5 cm minimum and occurred within two weeks of application, leaving no question as to the infiltration of the herbicide in the surface soil. Further, by applying in the spring we were able to monitor the immediate herbicide effects on soil organisms during their most active period.

Arthropod populations, in general, were slightly higher in the litter of treated than control plots. This shift was clearly not a result of treatment-induced changes in litter quantity or quality since the study plots had been free of understory vegetation for several years before hexazinone was applied. Although unexplained, this result raises little concern about direct toxic effects of hexazinone on soil arthropod fauna. In fact, seasonal changes had far-greater effects on soil arthropods than hexazinone. Springtail, spider, and beetle populations dropped precipitously by the final sampling in August when soil moisture was at its lowest. The mite population was less sensitive to the summer conditions, as is predicted by their physiological adaptations to the environment (*K* strategists), such as thick exoskeleton and relatively low reproductive rate.

There was some indication that litter arthropod populations, especially the microbivorous mites and springtails, responded in tandem with litter microbial biomass. For the first 90 days post-treatment, both microbial and microbivore populations were slightly higher in treated than in control plots. We did not see a similar relationship between arthropod populations and mineral soil microbial biomass, but that is to be expected since soil arthropods do not feed extensively in the mineral soil layer. Most soil arthropod fauna feed primarily in the litter fraction of the forest floor where nutritional resources and physical structure provide optimum habitat. In contrast, the mineral soil layer is used by arthropods primarily as a refuge from heat and desiccation during extremes of summer heat and wildfire.

In conclusion, the results of this study raise no concern about direct toxic effects of hexazinone for the soil organisms and processes that were measured.

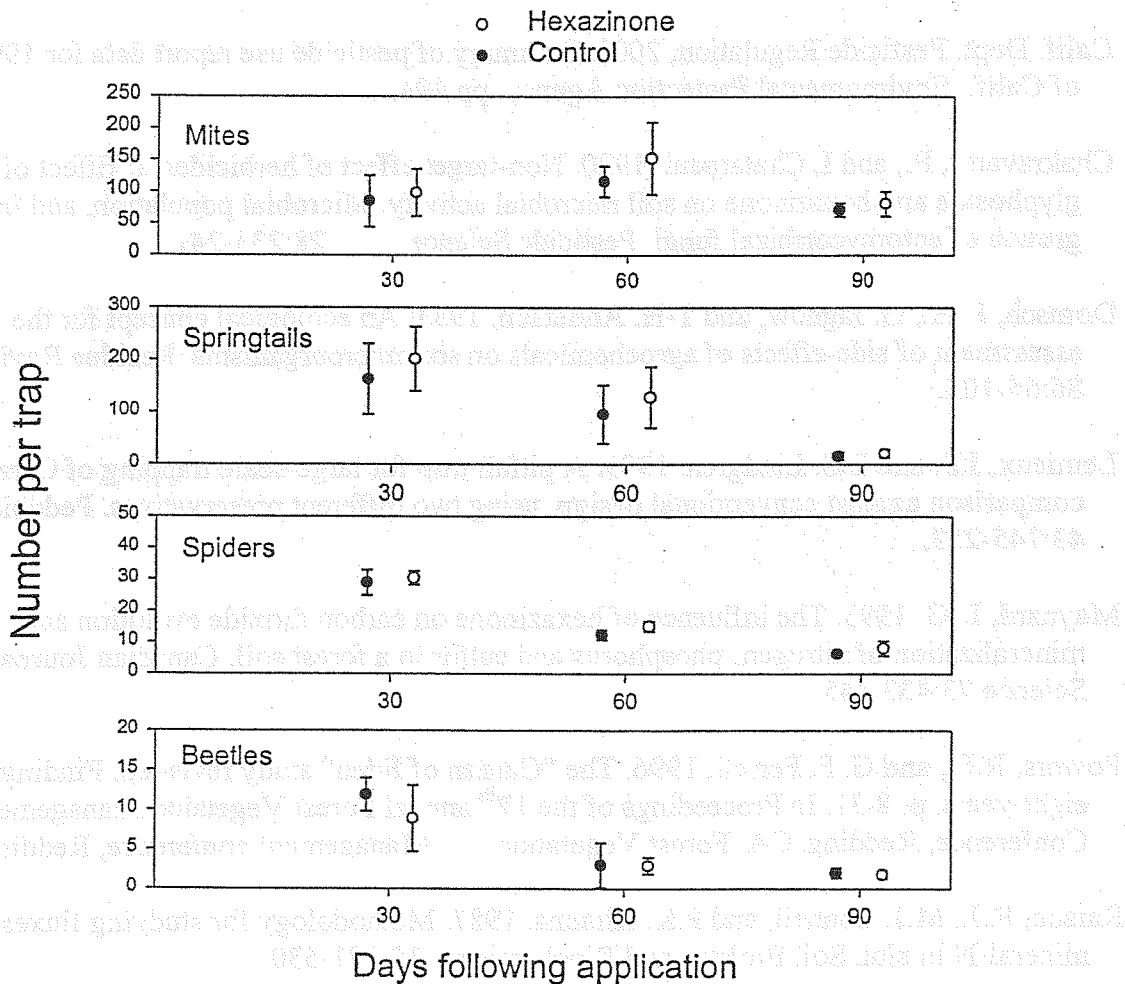


Figure 3. Effect of hexazinone on mites, springtails, spiders, and beetles at Whitmore. Values are means and standard deviation.

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