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**Effect of glyphosate, hexazinone and triclopyr
on in vitro growth of five species of ectomycorrhizal fungi**

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Abstract

In vitro growth tests with glyphosate (Roundup^R), hexazinone (liquid Velpar L.^R and granular PrononeTM5G^R) and triclopyr (Garlon^R) on five species of ectomycorrhizal fungi (*Hebeloma crustuliniforme*, *Laccaria laccata*, *Thelephora americana*, *T. terrestris* and *Suillus tomentosus*) showed varied species sensitivity to different concentrations of herbicides. Fungal growth was significantly ($P = 0.05$) reduced particularly at concentrations above 10 ppm. Garlon with triclopyr as a. i. was the most toxic of the four herbicide formulations.

1 Introduction

The need for herbicides and pesticides has been rapidly increasing for the effective management of forests in North America. Microorganisms, particularly mycorrhizae, form an important component of forest ecosystems. Their response to herbicides needs to be investigated to evaluate the total impact of herbicide usage in forest management. Failures in afforestation trials and reduction in forest productivity have been attributed to the absence of suitable mycorrhizal fungi in some managed forest sites (IYER and WILDE 1965; MIKOLA 1970; MARX 1980). Several other studies conducted on the effects of biocides on mycorrhizal fungi, arrived at varied conclusions (FONTANA and LUPPI 1975; FONTANA et al. 1976; ILOBA 1975, 1977; DASILVA et al. 1977; KELLEY and SOUTH 1980; TRAPPE et al. 1984; HARVEY et al. 1985). Most herbicides, though they inhibit fungal growth at high concentrations, are stimulatory to growth of mycorrhizal fungi at low concentrations (TRAPPE et al. 1984).

Three herbicides, glyphosate (Roundup^R), hexazinone (liquid Velpar L.^R and granular PrononeTM5G^R) and triclopyr (Garlon^R) have recently been identified to be the most suitable for conifer release from broad leaf competition in North American forests (SASSMAN et al. 1984). At present in Canada, only two herbicides (2,4-D and glyphosate) are fully

registered for use in forestry. The other prospective forestry herbicides include hexazinone and triclopyr. The effect of these herbicides on ectotrophic mycorrhizae are not known. The present study was undertaken to investigate the effect of glyphosate, hexazinone and triclopyr on *in vitro* of five species of mycorrhizal fungi.

2 Materials and methods

Five species of mycorrhizal fungi were used in this study. *Hebeloma crustuliniforme* (Bull ex St. Amans) Quel and *Laccaria laccata* (Scop. ex Fr.) Berk and Br. were isolated from fruiting bodies collected from lodgepole pine and white spruce forests, Rocky Mountain House, Alberta, Canada whereas, *Suillus tomentosus* (Kauf.) Snell, Singer and Dicks was collected from Jasper National Park, Alberta, Canada. Cultures of *Thelephora americana* Lloyd ex Corner and *T. terrestris* (Ehrh.) Fr. were obtained respectively from M. CASTELLANO, Forestry Sciences Lab., Oregon, USA and D. H. MARX, Forestry Sciences Lab., Athens, Georgia, USA. Isolates of all the mycorrhizal fungi were maintained on Modified Melin Norkrans (MMN) nutrient agar medium (MARX 1969). Four commercial formulations of three active herbicidal ingredients (glyphosate, hexazinone and triclopyr) were used in the study. The formulations were Roundup^R (35.9 % a.i. glyphosate), liquid Velpar L.^R (25 % a.i. hexazinone), granular PrononeTM5G^R (5 % a.i. hexazinone) and Garlon^R (48 % a.i. triclopyr). Hereafter, herbicides will be referred to by their commercial names, however, concentrations will mean the concentration of the active ingredient (a. i.) in the formulation. Each of these was tested at concentrations (a. i.) of 0, 0.1, 1, 10, 50, 100, 500 and 1000 ppm on the five species of mycorrhizal fungi in liquid culture (MMN). Twenty five ml of MMN liquid medium in 200 ml Erlenmeyer flasks was autoclaved for 15 min at 121°C. Then each flask was kept in a water bath at 50°C. Non sterilized 25 ml of each aqueous solution of herbicide concentrations were mixed with 25 ml of the sterile liquid MMN. The control substrate was a mixture of 25 ml of liquid MMN and another 25 ml sterile distilled water. All substrate solutions were replicated ten times, aseptically inoculated with pure cultures of mycorrhizal fungi, shaken vigorously for 30 seconds and then incubated at 25°C in dark for 30 days. The locations of flasks in the incubator were re-randomized every 5 days. After the 30 days incubation period, the mycelium was harvested on a Whatman No. 1 filter paper. The filter paper with mycelium was then oven-dried at 70°C for 48 hr and weighed to the nearest 0.5 mg and the dry wt. of mycelia was calculated. The results were statistically analysed by ANOVA procedures for a factorial design using BMDP statistical software (DIXON 1981) on a VAX-11/750 system. The individual means were compared using Duncan's New Multiple Range Test (STEEL and TORRIE 1960).

The relative sensitivity of the mycorrhizal fungi to herbicides and the relative toxicity of herbicides to fungal species were evaluated based on: 1) the lowest concentrations of a herbicide at which there was a significant ($P = 0.05$) reduction in mycelial growth of a species, and 2) the degree of reduction (% of control) in their growth. For evaluating the relative sensitivity of a species to the herbicides a Relative Sensitivity Index (RSI) was calculated as $\sum_i \log C$ (where $i, H =$ no. of herbicides, $C =$ concentration at which a significant reduction in growth occurred). The species with highest RSI value was considered as the least sensitive and vice versa. Similarly Toxicity Index (TI) of each herbicide was calculated as $\sum_i \log_e C$ ($i, S =$ no. of species). The herbicide with greatest TI value was the least toxic and vice versa.

3 Results and discussion

The effect of the four herbicides on *in vitro* growth of five species of mycorrhizal fungi are presented on Table 1 and Fig. 1. The ANOVA analyses concluded that the effects due to herbicides, species, concentrations and all interactions were significant ($P = 0.05$). In most species/herbicide combinations, PrononeTM5G, Velpar L. and Roundup had no adverse effect on the *in vitro* mycelial growth upto a herbicide concentration of 10 ppm. In fact, PrononeTM5G and Roundup at 0.1 ppm were marginally stimulatory to the growth of *H. crustuliniforme* and *S. tomentosus* respectively whereas, Velpar L. at 1 ppm was slightly stimulatory to *L. laccata* (Table 1, Fig. 1). Reduction in growth of all the species at 10–20 ppm concentrations of Velpar L., PrononeTM5G and Roundup was less than 10 % and 17 % respectively of the control. Garlon was much more toxic to all the fungal species than the other 3 herbicides and reduced the growth in five species of fungi at concentrations of 0.1 ppm and above (Table 1, Fig. 1).

Table 1

Effect of different concentrations of herbicide on the *in vitro* mycelial growth of five species of mycorrhizal fungi

| Mycorrhizal fungi/Herbicide | Mycelial dry wt. (mg) at different concentrations (ppm) | | | | | | | |
|---|---|------|------|-----|-----|-----|-----|------|
| | 0 | 0.1 | 1 | 10 | 50 | 100 | 500 | 1000 |
| <i>Hebeloma crustuliniforme</i> : | | | | | | | | |
| Velpar L. (a. i. hexazinone) | 48a | 46a | 46a | 46a | 30 | 19 | 12 | 9 |
| Pronone TM 5G (a. i. hexazinone) | 49a | 50a | 49a | 48a | 37 | 28 | 20 | 17 |
| Roundup (a. i. glyphosate) | 49a | 48a | 47a | 48a | 37 | 24 | 15 | 6 |
| Garlon (a. i. triclopyr) | 49a | 48a | 45 | 42 | 28 | 19 | 1 | 6 |
| <i>Laccaria laccata</i> : | | | | | | | | |
| Velpar L. (a. i. hexazinone) | 48a | 48a | 49a | 45 | 24 | 19 | 11 | 8 |
| Pronone TM 5G (a. i. hexazinone) | 47a | 48a | 47a | 46a | 39 | 29 | 19 | 14 |
| Roundup (a. i. glyphosate) | 48a | 49a | 47a | 47a | 38 | 30 | 16 | 7 |
| Garlon (a. i. triclopyr) | 48a | 47a | 47a | 37 | 19 | 16 | 10 | 6 |
| <i>Telephora americana</i> : | | | | | | | | |
| Velpar L. (a. i. hexazinone) | 47a | 47a | 47a | 45a | 32 | 20 | 12b | 10b |
| Pronone TM 5G (a. i. hexazinone) | 48a | 47ab | 46ab | 45b | 36 | 33 | 24 | 18 |
| Roundup (a. i. glyphosate) | 47a | 47a | 46a | 45a | 33 | 27 | 19 | 9 |
| Garlon (a. i. triclopyr) | 48 | 45 | 39 | 29 | 20 | 15 | 6a | 5a |
| <i>T. terrestris</i> : | | | | | | | | |
| Velpar L. (a. i. hexazinone) | 45a | 43a | 45a | 44a | 33 | 24 | 20 | 12 |
| Pronone TM 5G (a. i. hexazinone) | 46a | 46a | 46a | 44a | 38 | 29 | 24 | 19 |
| Roundup (a. i. glyphosate) | 47a | 46a | 46a | 46a | 29 | 19 | 8 | 5 |
| Garlon (a. i. triclopyr) | 46a | 46a | 45a | 39 | 19 | 14 | 7b | 6b |
| <i>Suillus tomentosus</i> : | | | | | | | | |
| Velpar L. (a. i. hexazinone) | 55a | 54ab | 52b | 52b | 33 | 25 | 21 | 17 |
| Pronone TM 5G (a. i. hexazinone) | 55a | 55a | 54a | 50b | 49b | 48b | 38 | 30 |
| Roundup (a. i. glyphosate) | 55a | 56a | 52b | 50b | 47 | 38 | 21 | 5 |
| Garlon (a. i. triclopyr) | 54a | 53a | 50 | 33 | 20 | 16 | 7b | 5b |

1. Concentrations are ppm of a. i. of a herbicide in the liquid MMN (MARX 1969).
2. Values in rows followed by the same letters are not significantly ($P = 0.05$) different from each other. All other values are significantly different from each other.
3. Each value is an average of ten replicates.

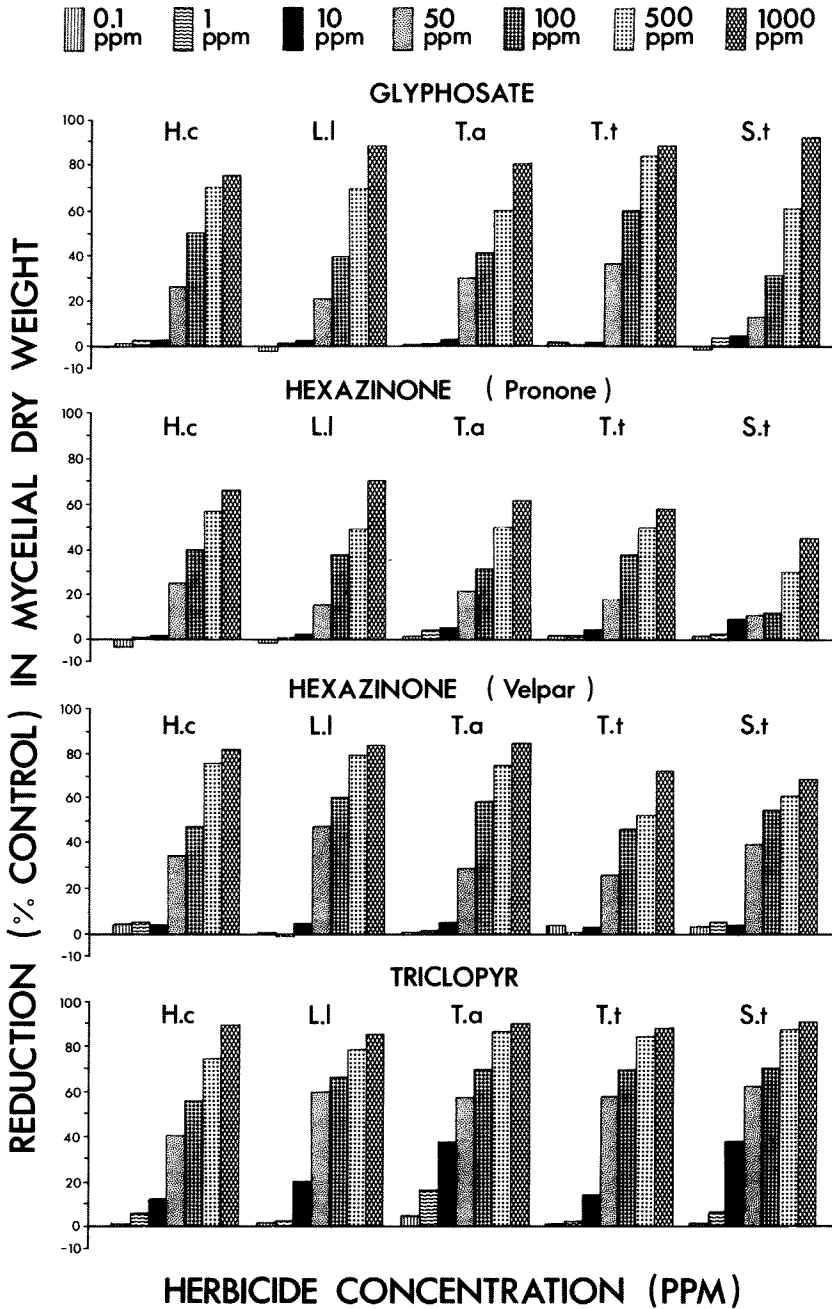


Fig. 1. Reduction in mycelial dry weight of mycorrhizal fungi at various concentrations of 3 active ingredients (glyphosate, hexazinone and triclopyr) in four herbicide formulations (Roundup, Pronone™ 5G, Velpar L. and Garlon). H.c = *Hebeloma crustuliniforme*; L.l = *Laccaria laccata*; T.a = *Thelephora americana* T.t = *T. terrestris*; S.t = *Suillus tomentosus*

Table 2

Relative Sensitive Index (RSI) of mycorrhizal fungi and Toxicity Index (TI) of herbicides.Values are Log_e concentration at which significant reduction in growth occurred.

| Mycorrhizal fungi | Herbicides | | | | R.S.I. |
|---------------------------|---------------------------------|--|-----------------------------|-----------------------------|--------|
| | Velpar L. (a. i. hexazinone) | Pronone TM 5G (a. i. hexazinone) | Roundup a. i. glyphosate | Garlon (a. i. triclopyr) | |
| <i>H. crustuliniforme</i> | 3.9 | 3.9 | 3.9 | 0.0 | 11.7 |
| <i>L. Laccata</i> | 2.3 | 3.9 | 3.9 | 2.3 | 12.4 |
| <i>T. americana</i> | 3.9 | 2.3 | 3.9 | -2.3 | 7.8 |
| <i>T. terrestris</i> | 3.9 | 3.9 | 3.9 | 2.3 | 14.0 |
| <i>S. tomentosus</i> | 0.0 | 2.3 | 0.0 | 0.0 | 2.3 |
| T.I. | 14.0 | 16.3 | 15.6 | 2.3 | |

The relative sensitivity of fungal species to herbicides and the relative toxicity of herbicides were evaluated by calculating their RSI's and TI's respectively (Table 2). Based on RSI, the 5 species range in order of their decreasing sensitivity to herbicides as follows:

S. tomentosus > *T. americana* > *H. crustuliniforme* > *L. laccata* > *T. terrestris* (Table 2). The relative rating for the toxicity of the 4 herbicides was: PrononeTM5G < Roundup < Velpar L. < Garlon (Table 2).

Herbicides, in general, are known to inhibit *in vitro* growth of mycorrhizal fungi. KELLEY and SOUTH (1980) reported that 14 herbicides, including hexazinone, had stimulatory, inhibitory or no effect on the growth of mycorrhizal fungi *in vitro*. DASILVA et al. (1977) reported similar findings with other herbicides. In our study we found low concentrations of Velpar L. (a. i. hexazinone), PrononeTM5G (a. i. hexazinone) and Roundup (a. i. glyphosate) to promote growth and concentration higher than 10 ppm strongly inhibited the growth of 5 species of mycorrhizal fungi.

The stimulatory effects of herbicides on growth of mycorrhizal fungi are very similar to the effects of very low concentration of pesticides such as BHC, DDT and Demephion reported by KISS (1965) and ILOBA (1974); of Simazin by UHLIG (1966), and of 2,4-D and MCPA reported by DASILVA et al. (1977).

Toxicity of forestry herbicides and their biodegradability by microorganisms are both important to evaluate their environmental impact and efficacy. The four herbicides used in our study are all decomposed by microbes in the soil (WSSA 1983). Phytotoxicity of herbicides may be enhanced under sterile soil conditions. In a preliminary test with PrononeTM5G (a. i. hexazinone), we observed higher phytotoxicity to white spruce and lodgepole pine seedlings under sterile than under non-sterile soil conditions. This suggests a rapid microbial degradation of hexazinone. The field application of Roundup, Velpar L. and PrononeTM5G result in low concentration of the herbicide in the soil. FENG and CAMPBELL (1985) reported hexazinone concentrations upto 4.85 µg/g soil at 0-15 cm depth after an aerial application of Velpar L. at a rate of 4 kg a. i./ha. In another field application, PRASAD and FENG (1985) reported hexazinone concentration of 1.12 ppm (µg a. i./g soil) after 7 days of application of Velpar L. at a rate of 1.2 kg a. i./ha. The recommended rate of application of 1-4 kg a. i./ha of Roundup, PrononeTM 5G or Velpar L. could result in a maximum calculated concentration of 4-18 ppm (a. i. w/w) in soil (0-5 cm depth) soon after greenhouse or field applications. Comparing the response of mycorrhizal fungi to various concentration of these herbicides in Table 1 and Fig. 1, there is a strong indication that the application of Roundup, Velpar L. and PrononeTM 5G at 1-4 kg a. i./ha would not have greater than 4 to 17 % reduction in growth of mycorrhizal fungi. The real effects would, however, vary with the bulk density of the soils, microbial populations and the mycorrhizal fungal species, and time and rate of application of herbicides.

The results of the present study are not absolutely applicable to field conditions where a complex of environmental factors are operating simultaneously in determining the effects of herbicides and pesticides (LAIHO and MIKOLA 1964; STOLL 1964; ILOBA 1975). However, these results do indicate the trends of possible effects of herbicides usage on mycorrhizal species in the field. Further *in vitro* field and greenhouse studies are in progress.

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Summary

Effects of glyphosate (Roundup^R), hexazinone (liquid Velpar L.^R and granular PrononeTM 5G^R) and triclopyr (Garlon^R) on the *in vitro* growth of five species of mycorrhizal fungi, *Hebeloma crustuliniforme*, *Laccaria laccata*, *Suillus tomentosus*, *Thelephora americana* and *T. terrestris* were determined over a 30 days period on Modified Melin Norkrans' liquid medium amended with herbicides at rates of 0, 0.1, 1, 10, 50, 100, 500 and 1000 ppm. Considerable variation in effect was observed among the herbicides for mycorrhizal fungi. At concentrations above 10 ppm, growth of all the mycorrhizal fungi were significantly suppressed. The toxicity index of herbicides were as Garlon (a. i. triclopyr) > Velpar L. (a. i. hexazinone) > Roundup (a. i. glyphosate) > PrononeTM 5G (a. i. hexazinone). Relative Sensitive Index of mycorrhizal fungi were *S. tomentosus* > *T. americana* > *H. crustuliniforme* > *L. laccata* > *T. terrestris*.

Résumé

Effet du glyphosate, de l'hexazinone et du triclopyr sur la croissance *in vitro* de cinq espèces de champignons ectomycorhiziens

Les effets du glyphosate (Roundup^R), de l'hexazinone (Velpar L.^R liquide et PrononeTM 5G^R granulé) et du triclopyr (Garlon^R) sur la croissance *in vitro* des cinq champignons mycorrhiziens: *Hebeloma crustuliniforme*, *Laccaria laccata*, *Suillus tomentosus*, *Thelephora americana* et *T. terrestris*, ont été étudiés sur une période de 30 jours en milieu liquide de Melin Norkrans modifié. Les herbicides sont additionnés aux doses de 0, 0.1, 1, 10, 50, 100, 500 et 1000 ppm. Des variations considérables d'effet sont observées parmi les herbicides. Le Pronone et le Glyphosate à 0.1 ppm et le Velpar à 1 ppm sont légèrement stimulants pour certaines espèces. Au-dessus de 10 ppm, la croissance de tous les mycorrhiziens est significativement réduite. Le classement des herbicides pour leur toxicité est: Garlon > Velpar > Roundup > Pronone et celui des champignons pour leur sensibilité est *S. tomentosus* > *T. americana* > *H. crustuliniforme* > *L. laccata* > *T. terrestris*.

Zusammenfassung

Die Wirkung von Glyphosat, Hexazinon und Triclopyr auf das *in vitro*-Wachstum von 5 Ektomykorrhiza-Pilzen

Es wurde die Wirkung der Herbizide Glyphosat (Roundup^R), Hexazinon (Velpar L.^R, flüssig + PrononTM 5G^R-Granulat) und Triclopyr (Garlon^R) nach 30 Tagen auf das *in vitro*-Wachstum von *Hebeloma crustuliniforme*, *Laccaria laccata*, *Suillus tomentosus*, *Thelephora americana* und *T. terrestris* ermittelt, wobei ein modifiziertes Melin-Norkrans Flüssigkeitsmedium mit Herbizidzusätzen von 0; 0,1; 1; 10; 50; 100; 500 und 1000 ppm Verwendung fand.

Es wurde eine erhebliche Streubreite in der Wirkung der genannten Herbizide auf die Mykorrhizalpilze beobachtet. In Konzentrationen oberhalb 10 ppm wurde das Wachstum aller Arten signifikant unterdrückt. Der Toxizitäts-Index der Herbizide nahm von Garlon (aktive Komponente = Triclopyr) über Velpar (Hexazinon) und Roundup (Glyphosate) bis zum PrononTM 5G (Hexazinon) ab. Die relative Rangfolge im Empfindlichkeitsindex heißt für die Mykorrhizalpilze: *S. tomentosus* > *T. americana* > *H. crustuliniforme* > *L. laccata* > *T. terrestris*.

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