Archives

NODA · NODA · EDNO

CANADA
ONTARIO
Northern Ontario
Development Agreement
Entente de développement
du nord de l'Ontario
Forestry • Foresterie

FILE REPORT 62

Potential of Bialaphos and Glufosinate-Ammonium as Silvicultural Herbicides:
I. Introduction, Literature Review and Conifer Tolerance

G.R. Stephenson and P.A. Turner

Natural Resources Canada
Canadian Forest Service

Ressources naturelles Canada
Service canadien des forêts

Ministry of Natural Resources
Ministère des Richesses naturelles
Ontario

This file report is an unedited, unpublished report submitted as partial fulfilment of NODA/NFP Project #4004, "The development of bialaphos and glufosinate-ammonium as silvicultural herbicides".

The views, conclusions, and recommendations contained herein are those of the authors and should be construed neither as policy nor endorsement by Natural Resources Canada or the Ontario Ministry of Natural Resources.

This file report is an unedited, unpublished report submitted as partial fulfilment of NODA/NFP Project #4004, "The development ~~Potential~~ of bialaphos and glufosinate-ammonium as silvicultural herbicides" ~~: I. introduction, literature review and conifer tolerance"~~.

The views, conclusions, and recommendations contained herein are those of the authors and should be construed neither as policy nor endorsement by Natural Resources Canada or the Ontario Ministry of Natural Resources.

NODA/NFP TECHNICAL REPORT

POTENTIAL OF BIALAPHOS AND GLUFOSINATE-AMMONIUM AS SILVICULTURAL HERBICIDES: I. INTRODUCTION, LITERATURE REVIEW AND CONIFER TOLERANCE

Gerald R. Stephenson and Patricia A. Turner
Department of Environmental Biology
University of Guelph
Guelph, Ontario, Canada N1G 2W1
Phone 519-824-4120
FAX 519-837-0442

and

James E. Wood, Robert A. Campbell and Dean G. Thompson
Natural Resources Canada, Canadian Forest Service
1219 Queen Street East, P.O. Box 490
Sault Ste. Marie, Ontario P6A 5M7
Phone 705-949-9461
FAX 705-759-5712

Funding for this report has been provided through the Northern Ontario Development Agreement's Northern Forestry Program

ABSTRACT

A large multi-disciplinary project was conducted to evaluate the silviculture potential of the natural weed control agent, bialaphos, and its synthetic analogue, glufosinate-ammonium. In this first of four parts of the project, the tolerance of several conifer species to these herbicides was assessed. Both outdoor studies and growth room studies indicated that most conifer species are likely to be less tolerant to these herbicides than to glyphosate. However, white spruce [*picea glauca* (Moench) Voss] displayed equal tolerance to bialaphos or glyphosate at rates as high as 2 kg/ha. There was some indication that tolerance of all conifers increased with increasing maturity of new growth.

POTENTIAL OF BIALAPHOS AND GLUFOSINATE-AMMONIUM AS SILVICULTURAL HERBICIDES:
I. INTRODUCTION, LITERATURE REVIEW AND CONIFER TOLERANCE

1.0 PROJECT OVERVIEW

To scientifically examine the silvicultural potential of the microbial herbicide, bialaphos and it synthetic analogue, glufosinate-ammonium, a large multi-disciplinary project was initiated and funded under the Northern Ontario Development Agreement-Northern Forestry Program(NODA/NFP). The project consisted of three distinct studies of bialaphos and glufosinate-ammonium: the examination of factors that influence conifer tolerance under controlled and semi-controlled environmental conditions; the evaluation of their silvicultural effectiveness under field conditions; the determination of their persistence and fate in a boreal forest soil and in an aquatic system; and the determination of their soil mobility. The project was initiated in 1993.

In the conifer tolerance studies, treatment differences were related to herbicide, dosage, coniferous species and application time. The experiments were conducted under controlled growth room conditions and in irrigated outdoor facilities. Evaluations of field efficacy and silvicultural efficacy were carried out at two locations near Thunder Bay, Ontario. *Calamagrostis canadensis* (Michx. Beaur) was the primary competing species on the first site, while red raspberry(*Rubus ideaus* L.var.strigosus(Michx.)) was the

(2) Potential of bialaphos and glufosinate-ammonium as silvicultural herbicides. II Efficacy on *Calamagrostis canadensis*(Michx. Beaur) and *Rubus ideaus* L.

(3) Potential of bialaphos and glufosinate-ammonium as silvicultural herbicides. III Silvicultural efficacy.

(4) Potential of bialaphos and glufosinate-ammonium as silvicultural herbicides. IV. Persistence and impact in soil and water.

In addition to the above-mentioned Technical Reports, a series of NODA-Technical Notes will be released to highlight the most significant findings in the project.

2.0 INTRODUCTION AND LITERATURE REVIEW

Synthetic herbicides can offer substantial benefits to forest management. However, public resistance to synthetic pesticide use in forestry is increasing. In most jurisdictions, natural pesticides would be preferred if they were available and proven to be safe and effective. In this context, naturally produced phytotoxins could be more acceptable to the public than synthetic herbicides while at the same time providing the forest manager with a cost-effective and efficient silvicultural tool. One such product, bialaphos, is currently being used as a herbicide in Japan. Bialaphos is a natural weed control agent that is the fermentation product derived from *Streptomyces viridochromogenes* and is produced by Meiji Seika Kaisha, Japan.(Meiji.,1984; Misato and Yamaguchi,1984) The active phytotoxic ingredient in bialaphos is phosphinothricin. Hoechst AG, Frankfurt, Germany is now marketing a synthetic phosphinothricin, which has the common name of glufosinate-ammonium(Hoechst.,1994). Glufosinate-ammonium has promising activity as a non-selective plant desiccant and it could also fill a need in forestry for a vegetation desiccant prior to prescribed fire. Because both of these potentially useful silvicultural herbicides have the same active ingredient, an applied research program that examined the potential of bialaphos and glufosinate-ammonium for use in siliviculture was proposed. Glufosinate-ammonium is very non-persistent in agricultural soils with a half-life of 7 days or less(Gallina and Stephenson, 1992).

Thus, in addition to being biorational or natural compounds, glufosinate-ammonium and/or bialaphos are both potentially much less persistent than glyphosate, one of the major synthetic herbicides currently used in silviculture.

Although biologically produced herbicides may have greater public acceptance for use on forest lands than synthetic herbicides, the environmental profile of biological herbicides must still be assessed to ensure that they are effective silvicultural tools as well as environmentally benign. In addition, biologically produced herbicides must be assessed to determine how to use them most effectively. Unlike most silvicultural tools, herbicides and other pesticides (synthetic or natural) must be registered before they may be used operationally. It was the purpose of this program to assess both the silvicultural efficacy and the environmental impact of both bialaphos and glufosinate-ammonium.

2.1 Economic Rationale

In Ontario, in 1990, over 235,000 ha of forested land were harvested(Deloitte and Touche, 1991). A survey of provincial silviculturalists found that 60% of the total area harvested required some form of vegetation management to ensure adequate survival and growth of crop trees. In general, chemical weed control is the safest method for controlling competition for both the forest worker and the crop tree. It is also the most cost-effective method, especially for large remote areas. However, public concerns over the use of synthetic pesticides in the forest continues to have an impact on forestry practices and forestry research priorities. In 1991, the Forestry Research Advisory Council of Canada listed finding better pest-management techniques, including the control of competing vegetation as one of its highest priorities for forestry research. Unquestionably, the Canadian public is concerned about the preservation of their forested lands and their forestry industries. It is equally certain that the public would prefer alternatives to the use of synthetic pesticides in forestry. However, synthetically produced herbicides continue to be the most practical method of controlling competing vegetation in new forest plantings. The effectiveness of these products is reflected in the dramatic increase in chemical stand tending in the province of Ontario and in Canada as a whole. For example in 1980, 30,100 ha of reforested land were treated with synthetic herbicides for conifer release and by 1990, the size of the release program had increased to 93,800 ha(Deloitte and Touche, 1991). If a more publicly acceptable biological herbicide could be registered, then the potential exists to expand the current provincial program to approximately 150,000 ha/yr. Site preparation for areas to be reforested often involves prescribed burning and about one third of the areas are first "browned or desiccated" with synthetic herbicide treatment before the prescribed burn. In Ontario, this amounts to at least 30,000 ha of synthetic herbicide use in a typical year. More rapidly effective, non-selective chemical desiccants could also expand the use of this effective silvicultural practice. Any attempt to reclaim portions of the unreforested backlog for sustainable forestry operations would also be aided immensely by effective and environmentally acceptable silvicultural herbicides.

2.2 Currently Available Silvicultural Herbicides

In Canada, there are currently only five herbicides registered for forestry use: glyphosate, 2,4-D, hexazinone, simazine and triclopyr(Campbell,1991). None of these herbicides are naturally derived. Because 2,4-D does not control grass or wild raspberry, once registered, the non-selective herbicide, glyphosate, became the predominant silvicultural herbicide. In 1988, glyphosate and 2,4-D accounted for more than 80% and 10% of total herbicide use in Canadian forestry, respectively(Campbell, 1991). For the purposes of this study, we chose to make all comparisons of glufosinate-ammonium and/or bialaphos with glyphosate, since it is the current standard for the industry(Table 1).

Glyphosate was developed by Monsanto(St.Louis, Missouri,USA) in the early 1970's(Duke, 1988). Glyphosate is a non-selective, systemic herbicide that is highly toxic to most herbaceous or woody plants(Table 1). Although it is moderately persistent in soil, with half-lives during the growing season of 1-2 months, it has little or no herbicidal activity in soil(Sprankle et.al, 1975). Because of these well defined properties, it has been developed to have a very diversified use pattern: For perennial weed control before and after most crops and landscape plantings, as a directed spray for weeds in orchards, landscapes and plantations, and for conifer release and site preparation in forestry. Some formulations also have uses for aquatic weed management. Glyphosate owes its highly systemic herbicidal activity to its chemical properties as a weakly acidic amino acid which becomes confined but readily mobile as an anion within living plant cells(Duke, 1988). It is not a contact herbicide. Root injury is usually apparent before the first symptoms of foliar injury can be observed. Toxic effects in plants are due to its inhibition of an enzyme, 5-enolpyruvyl- shikimate-3-phosphate synthase(PEP synthase)which has pivotal importance in the production of aromatic amino acids in plants (Duke, 1988) Because this enzyme is unique to plants, glyphosate is relatively non-toxic in most animal systems(Table 1).

2.3 Bialaphos and Glufosinate-Ammonium

The discovery of bialaphos (Table 1) occurred in 1971, when Bayer et. al.(1972) found a microbe in Camaroon which produced a biologically active compound consisting of two alanine moieties and the amino acid, L-phosphinothricin (PPT). In independent investigations, carried out at about the same time, microbiologists from Meiji Seika Kaisha Ltd. in Japan reported the discovery of a similar microbe in Japanese soil. The tripeptide, PPT, is a fermentation product of the soil actinomycetes, *Streptomyces viridochromogenes*(Bayer et. al.,1972), *S. hygroscopicus*(Kondo et. al.,1973) *Kitasatosporia phosalacinea*(Omura et. al. 1984a). PPT was found to have antibiotic activity against gram negative and gram positive bacteria as well as herbicidal activity against algae and higher plants (Wakabayashi et. al. 1978). It was registered in 1984 by Meiji Seika Kaisha Ltd. of Japan as the herbicide, Meiji Herbiace, with the common name of bialaphos(Table 1). It is the first herbicide in the world to be produced, biologically, by fermentation technology. However, it is not yet marketed in any country other than Japan. The synthetic analogue of bialaphos, glufosinate-ammonium was produced by Hoechst AG in Frankfurt, West Germany in 1975 (Willms, 1989). It now has a number of registered product names including the product Ignite which is marketed in Canada by AgrEvo, Regina, Saskatchewan (Table 1). Glufosinate-ammonium is the ammonium salt of PPT (Table 1). Since both glufosinate-ammonium and bialaphos have the same active ingredient, glufosinate-ammonium can be regarded as a "close to natural" or a biorational herbicide. Like glyphosate, both bialaphos and glufosinate-ammonium have relatively non-selective toxicity to plants with little or no soil activity (Table 1). Bialaphos is reported to have a half-life in soil of 20 to 30 days(Misato and Yamaguchi, 1984) and some investigators(Sekizawa and Takematsu, 1983)have reported that glufosinate-ammonium may be more persistent than bialaphos. However, Gallina and Stephenson(1992), using radiolabelled glufosinate-ammonium, reported a half-life in an agricultural soil of 7 days. There are no reported studies from forestry environments and more research is needed. However, under most soil conditions, it is expected that both bialaphos and glufosinate-ammonium could be less pesistent than glyphosate. Also similar to glyphosate, most available evidence indicates that there is little metabolism of glufosinate-ammonium in plants(Mersey et. al. 1990). Toxic effects of glufosinate-ammonium and bialaphos in plants are due to an inhibition of the enzyme, glutamine synthase, by their common active ingredient, phosphinothricin(PPT) which is a mimic of L-glutamic acid(Tachabana et. al. 1986a). Since glutamine synthase is also unique to plants, these herbicides are also likely to have low toxicity in animal systems. Compared to glyphosate, the shoot to root translocation of glufosinate-ammonium, bialaphos, or PPT appears to very limited (Haas and Muller, 1987; Shelp and Da Silva, 1990). The limited translocation and high contact activity of bialaphos and especially glufosinate-ammonium is thought to be due to the rapid toxic effects of ammonia which accumulates to high concentrations with the inhibition of glutamine synthase(Tachibana et.al.,1986; Sauer et.al.,1987). Both bialaphos and glufosinate-ammonium have registered uses as directed sprays to control a broad spectrum of broadleaved weeds and grasses in nursery and orchard crops(Table 1). Although they are likely to be less effective on some root suckering perennials than glyphosate, which is more systemic, they are very promising as pre-harvest desiccants.

2.4 Transgenic Development of Glufosinate-Ammonium Tolerant Crops

Like glyphosate, glufosinate-ammonium and bialaphos are relatively non-selective phytotoxins because few plants can metabolize or detoxify them. However, recent advances in the regeneration of whole plants from tissue cultures of embryonic plant cells, now permit the transgenic engineering of plants that are highly resistant to herbicides(Vasil et.al., 1994). This is particularly true for the phosphinothricin(PPT) containing herbicides, bialaphos and glufosinate-ammonium. Since PPT is synthesized by the soil microbes, *S. viridochromogenes* (Bayer et.al., 1972) and *S. hygroscopicus*(Kondo et.al.,1973), it is not surprising that these same organisms possess the phosphinothricin acetyltransferase(PAT) enzyme to degrade it(Vasil et. al., 1994). Geneticists have now employed the microbes, *Agrobacterium tumefaciens* or *A. rizogenes* and improved plant-tissue culture techniques to transfer the gene for PAT from *S.Viridochromogenes* or *S.hygroscopicus* to numerous crop plant species(Newmark, 1987; DeGreef et. al., 1989; Beard, 1993 and Vasil et.al., 1994). Using similar transgenic techniques, one tree species, *Populus tremula* X *Populus alba*, has now been engineered to be tolerant to glufosinate-ammonium by equiping it with the PAT gene(Devillard, 1992). Thus, it is very likely that important conifer species could also be engineered for tolerance to PPT containing herbicides by this transgenic biotechnology.

3.0 CONIFER TOLERANCE TO BIALAPHOS AND GLUFOSINATE-AMMONIUM

3.1 Introduction

Jobidon(1991a) was the first to suggest that bialaphos could have potential uses in silviculture. He found that bialaphos provided excellent control of wild red raspberry and Kalmia(*Kalmia angustifolia* L.) at rates of 2.0 and 2.5 Kg a.i./ha, respectively. He also observed promising tolerance for bialaphos in black spruce (*Picea mariana*(Mill.)B.S.P.)(Jobidon, 1991b). In subsequent studies, Sy et.al.(1994) also observed promising tolerance to bialaphos in black spruce, white spruce[*Picea glauca*(Moech)Voss, Norway spruce(*Picea abies*(L.)Karst.) and red pine (*Pinus resinosa* Ait.). In a study that compared applications at various times during the season, Sy et. al.(1994) observed greatest tolerance with applications in August.

To develop a better understanding for the potential use of phosphinothricin chemistry in silviculture, our first objective was to investigate the influence of both application time and application rate on the tolerance of important conifer species to bialaphos and glufosinate. In subsequent studies, biochemical mechanisms of phosphinothricin toxicity as well as physiological factors involved in conifer tolerance were also investigated.

3.1 Methodology

3.1.1 Outdoor studies of white spruce, black spruce and jack pine (*Pinus banksiana* Lamb) tolerance to glyphosate, bialaphos and glufosinate-ammonium

White spruce and black spruce seedlings were bare-root and were obtained from the Ontario Ministry of Natural Resources(OMNR) nursery at Swastika, Ontario. Containerized jack pine seedlings were obtained from Blazeck Greenhouses in Cochrane, Ontario. All seedlings were shipped to Sault Ste. Marie, Ontario and held in cold storage(5 C) until transferred to a sand/artificial soil (Pro-Mix BX) mixture(1/3, v/v) in outdoor plastic pots in early June. The potted trees were kept in irrigated, outdoor growth facilities at Sault Ste. Marie and were watered and fertilized as needed. All three herbicides were applied at rates of 0, 0.25, 0.5, 1.0, asnd 2.0 kg ae/ha(active phosphinothricin acid or glyphosate). Glyphosate and glufosinate-ammonium were also applied at 4.0 kg/ha. Bialaphos was not applied at the 4.0 kg rate because the formulation(Herbiace) was very viscous and was developed for application at very high spray volumes(e.g. 1400 L/ha). As a result, the 4.0 kg/ha rate could not be mixed and applied at spray volumes that were comparable with the other treatments. Except for bialaphos(which did not arrive from Japan until early August), the herbicides were compared at four different application times: June 24, July 22, August 19, and September 16. The herbicides were applied using a low volume laboratory sprayer that was designed to simulate aerial forestry herbicide applications(Campbell et.al.,1994). To achieve consistency among spray patterns, it was necessary to vary the spray volume with the herbicide and rate applied. For example, all of the glyphosate treatments and the glufosinate-ammonium treatments up to and including 2.0 kg/ha were applied at spray volumes of 60 L/ha, the 4.0 kg/ha rate of glufosinate-ammonium was applied at 120 L/ha and all of the bialaphos rates were applied at spray volumes of 180 L/ha. Trees were moved into the greenhouse from the outside compound the day prior to treatment. Prior to treating the trees, the soil surface in the plastic pots was covered with vermiculite. Once the spray had dried on the conifer foliage, the vermiculite was removed and the trees were returned to the outside compound. Visual assessments of needle necrosis(Frans et.al., 1986) in the October after treatment and measurements of height growth during the subsequent season were employed to compare differences in herbicide tolerance among the three species.

3.1.2 Growth room studies of conifer tolerance to bialaphos and glufosinate-ammonium at different stages of growth

One year-old white spruce, jack pine, white pine and red pine seedlings were obtained from the OMNR nursery at Midhurst,Ontario during early May. The seedlings were transported to the University of Guelph and kept in cold storage(2C)until they were transplanted to 2L pots containing an artificial soil(Pro-Mix BX)/silica sand mixture(2/1, v/v). The tree seedlings were then transferred to controlled environment growth rooms, programmed to mimic typical growing conditions for late May through September at Timmins, Ontario. Daylengths and day/night temperatures were adjusted weekly to conform with reported daylengths at 48^0 latitude(16 hrs in June to 10 hrs in October, Russelo et.al.,1974) and 10-year average temperatures[17-32 C(day), 10C(night)] for Timmins, Ontario, respectively. Light banks were kept 30 cm above the plants to provide a light intensity of 400 $\mu E/m^2/S$. Relative humidity was kept between 80 and 95%. The trees were watered every three days, alternately, with tap water or nutrient solution(20-8-20, pH 6.0). Bialaphos or glufosinate-ammonium were applied as foliage sprays with a moving-nozzle cabinet sprayer calibrated to deliver a spray volume of 200L/ha at a pressure of 250 kPa and nozzle speed of 3.4 km/hr. An application rate of 0.5 kg(PPT)/ha was selected since preliminary tests had shown it to be marginally toxic to conifer seedlings under growth room conditions. The treatments were applied to separate groups of five replicate trees of each species at eight different growth stages(May 31, June 16, 30, July 14, 28, August 11,25, September 8) separated by approximately 2 weeks each. Because of limitations in numbers of available seedlings for the different species, glufosinate-ammonium vs. bialaphos comparisons were only possible on white spruce and Jack pine. However, all four species were compared for tolerance to glufosinate-ammonium at the eight different growth stages. Visual estimates of needle necrosis in October were employed to assess the impact of growth stage on herbicide injury.

3.1.3 Physiological action of bialphos and glufosinate-ammonium in conifers

Foliar uptake of glufosinate-ammonium. Black spruce seedlings, 4 months in age, in tube culture, were transferred from Aidie Creek Nurseries, Kirkland Lake, Ontario to growth rooms at the University of Guelph. Glufosinate-ammonium-^{14}C(spa 15.5 mCi/mmol) was applied as a spray at the rate of 1.0 Kg(PPT)/ha to the spruce foliage and stems with an Aztck 300S Airbrush kit at 133 kPa. The individual spruce seedlings were placed in a glass cylinder(17cm in diameter x 70cm in height) and the pressurized spray nozzle (Tourquise No. 9306) was inserted through a slit in the parafilm cover on the top of the cylinder. Treatments were applied in volumes of 1ml per tree(approximately 440L/ha). After each treatment, a 200ml rinse of the glass cylinder, the parafilm cover and the filter paper cover at the tree base were frozen and later assessed for recoverable radioactivity by sample oxidation(R.J. Harvey OX 300 Biological Oxidizer) and/or liquid scintillation techniques(Beckman LS 6000SC Liquid Scintillation counter). The treated trees were returned to the growth rooms(16 hr day,200 uE/m /S, 22 C and 8 hr night, 17 C). At 12, 24, and 48hrs after treatment, rinses(100ml deionized water) of the needles, needles, stems and root portions of the spruce seedlings were frozen, lyophilized(Labconco 4.5 Freeze Dryer) and oxidized to assess the uptake and distribution of radioactivity in control seedlings as well as in seedlings pretreated with the growth regulator, paclobutrazol[2RS, 3RS-1-(4-chlorophenyl)-4,4(1,2,4-triazol-1-yl)pentan-3-ol].

Metabolism of glufosinate-ammonium in white spruce. To assess the capacity of white spruce shoot tissue to metabolize glufosinate ammonium-^{14}C, spruce seedlings were grown for 8 months under greeen house conditions(22 C with supplemental lighting between August and March) at the University of Guelph. Entire shoots were removed and then vacuum infiltrated with glufosinate-ammonium-^{14} C in a mannitol solution (3.02 umol or 46.9 uCi glufosinate-ammonium-^{14}C in 200 ml 0.35M mannitol) according to the methods of Stephenson et.al.(1971). The infiltrated seedlings(approximately 2.5g, fresh weight) were then incubated in a growth cabinet(16 hrs continuous light, 200 uE/m /S, 22 C) on moist filter paper in petri dishes for prior to rinsing(3X 10mls, optima water), freezing with liquid nitrogen, grinding to a powder and extraction by shaking(1 hr) in 20ml, 20% ethanol. The extracts were then vacuum filtered and concentrated to 5ml, adjusted to pH 3.5(H3PO4), centrifuged and filtered(0.22 um nylon mesh). The filtered supernatants were then analyzed for radiolabelled glufosinate-ammonium or metabolites by high pressure liquid chromatography(Shimadzu HPLC equipped with a Canberra-Packard radiomatic detector).

3.1.4 Statistical methods

All outdoor and indoor conifer tolerance studies and most laboratory studies were arranged in a completely randomized block design and data were analyzed using N.C.S.S. 6.0(Number Cruncher Statistical Systems) Software(Hintze, 1995). Significant differences were determined using a Student-Newman-Keuls Multiple Comparison Test(Kuehl, 1994). Correlations between first year foliar injury and second year height growth were determined using Sigma Stat(Fox et.al. 1994).

3.2 RESULTS

3.2.1 Outdoor studies of white spruce, black spruce and jack pine tolerance to glyphosate, bialaphos, and glufosinate-ammonium

On the basis of foliar injury during the season of treatment, and regardless of species, glufosinate-ammonium was clearly the most toxic, glyphosate was the least toxic, and bialaphos was intermediately toxic to white spruce, black spruce and jack pine($p<0.05$, Figures 1, 3 and 5). Most of these differences were still apparent in the effects on height growth of the trees during the year after treatment(Figures 2, 4 and 6). One major exception to the above, was the very equal tolerance of white spruce to August or September applications of either glyphosate or bialaphos(Figures 1 and 2).

Bialaphos was not available for the June or July applications in this outdoor study(Figures 1, 3 and 5). However, with respect to glufosinate-ammonium, both first-year foliar injury and second-year height growth assessments, indicated that white spruce was most tolerant to the August treatments(Figures 1 and 2). Second-year height measurements indicated that both black spruce and jack pine were most tolerant to glufosinate-ammonium applied in July(Figures 4 and 6). Greatest tolerance to glyphosate was observed with the later season applications made in August or September(Figures 1 through 6).

Due to space and time limitations, it was not possible to compare the tolerance of these three conifer species to these three herbicides on a statistical basis in one combined experiment. However, assessments of either first or second year injury resulting from the slightly toxic rates of 0.5 or 1.0 kg/ha clearly indicated that white spruce was the most tolerant, followed by black spruce and jack pine, respectively. This relationship appeared to be true for all three herbicides(Figures 1 through 6).

3.2.2 Growth room studies of conifer tolerance to bialaphos and glufosinate-ammonium at different stages of growth

On the basis of per cent needle necrosis, glufosinate-ammonium(0.5 kg/ha) was clearly less toxic to white spruce, jack pine, red pine and white pine when applied during the second half of the growing season between 10 and 16 weeks after planting(Figure 7). For all four of the conifer species, there was a significant increase in tolerance to glufosinate-ammonium between 8 and 10 weeks after transplanting($P < 0.05$, Figure 7). A similar pattern, of greater tolerence to late-July through early September treatments, was observed for both bialaphos and glufosinate ammonium on jack pine and white spruce(Figures 8a and 8b). On the basis of needle necrosis, neither bialaphos or glufosinate-ammonium at 0.5 kg/ha were measurably toxic at the later four treatment times. However, at the four earlier treatment times, glufosinate-ammonium was significantly more toxic than bialaphos on both jack pine and white spruce($P < 0.05$, Figure 8a). This latter experiment was repeated with white spruce and red pine seedlings. Glufosinate-ammonium sprays were applied 4, 8, 12 and 16 weeks after transplanting. The seedlings were again most tolerant when treatments were delayed until 12 or 16 weeks. Epicuticular waxes were removed and quantified (according to the methods of Willis et.al., 1989) from companion seedlings at all of these time intervals. However, under these growth room conditions, epicuticular waxes did not increase significantly with time and thus could not be correlated with the increase in tolerance to foliar applications of glufosinate-ammonium.

3.2.3 Physiological action of bialaphos and glufosinate-ammonium in conifers

Similar to observations for other plants(Shelp and DaSilva, 1990), foliarly applied glufosinate-ammonium was poorly translocated in black spruce seedlings(Figure 9). At all harvest times from 12 to 48hr after treatment, 90% or more of the radioactivity was recovered from the needles or stems which had been directly exposed to the spray. There was some evidence of movement from needles to stems but no more than 10% of the radioactivity was ever translocated to the roots(Figure 9).

Several investigators have shown that the growth regulator, paclobutrazol can protect transplanted conifer seedlings from water stress by either increasing wax deposition on the needles or causing stomatal closure(Gao et.al., 1987; Marshall et.al., 1991). If, in fact, paclobutrazol does increase epicuticular wax deposition on conifer needles, we hypothesized that pretreating conifer seedlings may reduce the uptake of glufosinate-ammonium. At 48 hrs after treatment, only highest rate of paclobutrazol had a significant effect on glufosinate ammonium uptake, and the effect was to increase rather than to decrease the amount recovered in the needles after rinsing(Figure 10). The seedlings that were treated with the highest rate of paclobutrazol were shorter and the needles were much broader than control seedlings. Their greater uptake may have been due to increased interception and retention of the spray.

However there was no indication that any rate of paclobutrazol actually reduced uptake of glufosinate-ammonium.

White spruce stems and needles were able to metabolize glufosinate-ammonium-^{14}C, but the rate of metabolism, 40% in 16hr(Table 2), was not as rapid as is usually observed in plants with high metabolic tolerance to a herbicide(Hatzios and Penner, 1982). In parallel studies there was no evidence of glufosinate-ammonium-^{14}C metabolism in the aquatic plant, northern water milfoil(Table 2). The effect of glufosinate-ammonium on ammonia accumulation supported the conclusion this herbicide acts as a poorly translocated, poorly metabolized herbicide in white spruce as has been observed in other plant species(Haas and Muller, 1987; Shelp and DaSilva, 1990; Tachabana et.al., 1986; and Sauer et.al., 1987). Glufosinate-ammonium had their greatest effects on ammonia accumulation in the needles of white spruce, slight but significant effects in the stems and no effect on ammonia accumulation in the roots(Figure 11). Furthermore, between 24 and 48 hrs after spraying, it continued to cause further increases in ammonia accumulation in both the needles and the stems. In contrast, bialophos was inconsistent in its effects on ammonia levels in white spruce(Figure 11).

3.3 SUMMARY AND CONCLUSIONS

Both outdoor studies and indoor controlled environment studies indicated that most conifer species are likely to be less tolerant to either glufosinate-ammonium or bialaphos than to glyphosate. A major exception could be, white spruce, which in outdoor studies displayed equal tolerance to bialaphos or glyphosate at application rates as high as 2 kg/ha during either July or August. In our studies, white spruce was the most tolerant to either bialaphos or glufosinate-ammonium, followed closely by black spruce and then by the various pine species included in the study.

Glufosinate-ammonium appears to have the same mode of action in conifers as has been reported in other plant species. Foliar sprays caused rapid increases in ammonia levels in white spruce seedlings which is consistent with its mode of action as an inhibitor of the enzyme, glutamine synthetase, in plants. This toxic effect was more evident in the needles than in the stems and was not measurable in the roots. These effects, combined with studies that indicated little translocation or metab-olism of radiolabelled glufosinate-ammonium indicate that this herbicides act primarily as contact herbicide in white spruce as has been reported in other plant species. Bialophos appears to be slower acting than glufosinate-ammonium, perhaps because it needs to be metabolically activated to phosphinothricin acid in conifers as in other plant species.

White spruce, black spruce and jack pine tolerated higher rates of bialaphos or glufosinate-ammonium in outdoor studies than in controlled environment, indoor studies. In the indoor studies, regardless of confer species or herbicide, tolerance generally increased with increasing maturity of the new growth. In the outdoor studies, jack pine and black spruce appeared to be most susceptible to the September treatments. However, white spruce was equally tolerant at all application times.

3.4 ACKNOWLEDGEMENTS

Funding for this project was provided by the Northern Ontario Development Agreement's Northern Forestry Program and by the Green Plan Pest Management Network. Marvin Faber, University of Guelph is acknowledged for his collaboration in the metabolism studies. Appreciation is also extended to Garth Mitchell and John Studens, Canadian Forest Service, Sault Ste. Marie and to Margaret Carter, University of Guelph, for their technical assistance.

3.5 LITERATURE CITED

Beard, J. 1993. Crops stand up to killer chemicals. New Scientist, September 11, p. 16.

Bayer, E., Gugel,K.K., Haegele,K., Hagenmaier,H., Jessipov, S., Konig,W.A. and Zahner, H. 1972. Stoffwechselproduket von mikroorganismen. Phosphinothricin and phosphinothricyl-alanyl-alanin. Helv. Chim. Acta 55:224-239.

Campbell, R.A., Wood, J.E., Mitchell, E.G., Studens, J., and Wagner, R.G. 1994. A simple low volume laboratory spray system Weed Tech. 8:87-92.

Campbell, R.A. 1991. Silvicultural herbicides in Canada: Registration status and research trends. For. Chron. 66:520-527.

DeGreef, W., Delon, R., DeBlock, M., Leemans, J. and Botterman, J. 1989. Evaluation of herbicide reisitance in transgenic crops under field conditions. Biotechnology 7:61-64.

Deloitte & Touche Management Consultants. 1991. Economic benefit assessment of triclopyr in forest management. Prepared for the Forest Management Caucus. Deloitte & Touche Management consultants, Guelph, ON. 111p.

Devillard, C. 1992. Transformation in vitro du tremble(Populus tremula X Populus alba) par Agrobacterium rhizogenes et regeneration de plantes tolerantes au basta. C.R. Acad. Paris, 314(III):291-298.

Duke, S.O. 1988. Glyphosate. Chapter 1, pp. 1-70, In, Herbicides: Chemistry, Degradation and Mode of Action. V. 3, P.C. Kearney and D.D. Kaufman, Eds., Marcel Dekker, N.Y.,403pp.

Forestry Research Advisory Council of Canada. 1992. Forest research priorities in Canada, 1991. For. Chron. 68: 121-125.

Frans, R., Talbert, T., Marx, D., and Crowley, H. 1986. Experimental Design and Techniques for Measuring and Analyzing Plant Responses to Weed Control Practices, Chapter 2, p 29-46, In: Research Methods in Weed Science, 3rd Edition, N. D. Camper, Editor, S. Weed Sci. Soc., Champaign, Ill., 406pp.

Gallina, M.A. and Stephenson, G.R. 1992. Dissipation of glufosinate ammonium in two Ontario soils. J. Agric. Food Chem. 67:165-168.

Gao, J., Hofstra, G. and Fletcher, R.A. 1987. Anatomical changes induced by triazoles in wheat seedlings. Can. J. Bot. 66: 1178-1185

Haas, P. and Muller, F. 1987. Behaviour of glufosinate-ammonium in weeds. Brit. Crop Protec. Conf.-Weeds. 10 B-1:1075-1082.

Hatzios, K.K. and Penner, D. 1982. Metabolism of herbicides in higher plants. Burgess, Minneapolis, MN, 142pp.

Hintze, J. L. 1995. NCSS 6.0, User Guide I and II, Number Cruncher Statistical Systems, Kaysville, Utah, 1558pp.

Hoechst. 1994. Glufosinate, pp.147-149, In WSSA Herbicide Handbook, 7th Edition, Weed Sci. Soc. Amer., Champaign, Illinois.

Jobidon, R. 1991a. Potential use of bialaphos, a microbially produced phytotoxin, to control red raspberry in forest plantations and its effect on black spruce. Can. J. For. Res. 21: 489-497.

Jobidon, R. 1991b. Control of Kalmia with bialaphos, a microbially produced phytotoxin. Northern J. Appl. For. 8:147-149.

Kuehl, R. 1994. Statistical Principles of Research Design and Analysis. Wadsworth Pub. Co., Belmont, CA, 686pp.

Marshall, J.G., Scarratt, J.B. and Dumbroff, E.B. 1991. Induction of drought resistance by abscisic acid and paclobutrazol in jack pine. Tree Physiol. 8: 415-421.

Meiji Sieka Kaisha/ 1984. Meiji Seika Kaiasha: Fermentation Specialist. Farm Chem. 147:52.

Mersey, B.G., Hall, J.C., Anderson, D.M., and Swanton, C.J. 1990. Factors affecting the herbicidal activity of glufosinate-ammonium: Absorption, translocation, and metabolism in barley and green foxtail. Pestic. Biochem. and Physiol. 37:90-99.

Misato, T. and Yamaguchi, I. 1984. Pesticides of microbial origin. Outlook on Agric. 13(3):136-139.

Newmark. 1987. Plant genetic systems gets basta resistance. 1987. Biotechnology 5:321-322.

Omura, S., Hinotozawa, K., Inamura, M., and Murata, M. 1984. The structure of phosalacine, a new antibiotic containing phosphinothricin. J. Antibiot. 37:939-940.

Radosevich, S.R. and Bayer, D. E. 1979. Effect of temperature and photoperiod on triclopyr, picloram and 2, 4, 5-T translocation. Weed Sci. 27: 22-27.

Russelo, D., Edey, S. and Godfrey. 1974. Selected TAbles and Con versions Used in Meteorolgy and Related Fields. Canada Dep't. of Agric. Pub. No 1522., p 146.

Sauer, H., Wild, A., and Ruehle, W. 1987. The effect of phosphinothricin(glufosinate) on photosynthesis 2. The causes of the inhibition of photosynthesis. Z. Naturforsch 42:270-278.

Sekizawa, Y., and Takematsu, T. 1983. How to discover new antibiotics for herbicidal use. pp. 261-268,In, Pesticide Chemistry, Human welfare and the Environment. V.2, Natural Products. Takahashi,H., Yoshioka, H., Misato, T. and Matsunaka, S. (Eds), Pergamon Press, Oxford.

Shelp, B.J. and Da Silva, M.C. 1990. Distribution and metabolism of xylem-bourne ureido and amino compounds in developing soybean shoots. Plant Physiol. 94: 1505-1511.

Shelp, B.J.,Swanton, C.J. and Hall, J.C.1992. Glufosinate(phosphinothricin) mobility in young soybean shoots. J. Plant Physiol. 139:626-628.

Sprankle, P., Meggitt, W.F. and Penner, D. 1975. Rapid inactivation of glyphosate in the soil. Weed Sci. 23:224-228.

Stephenson, G.R., Baker, L.R., and Ries, S.K. 1971. Metabolism of pyrazon in sussceptible species and inbred lines of tolerant red beet(Beta vulgaris L.). J. Amer. Soc. Hort. Sci. 96: 145-147

Sy, M., Jobidon, R. and Margolis, H. 1994. Differential tolerance of coniferous species to the microbially produced herbicide, bialaphos. I. Morphological and growth effects. Can. J. Forest Research 24: 2191-2198.

Tachibana, K., Watanabe, T., Seikizawa, Y. and Takematsu, T. 1986. Inhibition of glutamine synthetase and quantitative changes of free amino acids in shoots of bialaphos-treated Japanese barnyard millet. J. Pestic. Sci. 11:27-31.

Vasil, I.K., Vasil, S., Castillo, A.M. and Fromm, M. 1994. Genetic transformation of wheat. 1994., pp. 11-14, In, Improvement of Cereal Quality by Genetic Engineering, R.J. Henry and J.A.Ronalds, Eds.,Plenum Press, New York, 174pp.

Wakabayashi, K., Matsuya, K., Ohta, H., and T. jikihara. 1978. Advances in Pesticide Science. 2nd Edition. Geissbuhlere, H., Brooks, G.T. and Kearney, P.C.(Eds). Pergammon Press, Oxford, pp. 256-260.

Willis, R. G., Stephenson, G. R., Fletcher, R. A., and Prasad, R. 1989. Seasonal variations in jack pine(Pinus banksiana) and white spruce(Picea glauca) tolerance to glyphosate and triclopyr. Weed Tech. 3: 33-38.

Wilms, L. 1989. Glufosinate, a new amino acid with unexpected properties. Pestic. Sci. 27: 219-221.

Table 1. Comparative properties and uses for the herbicides, glyphosate, glufosinate-ammonium and bialophos.

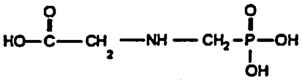
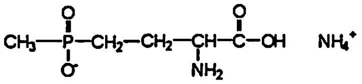
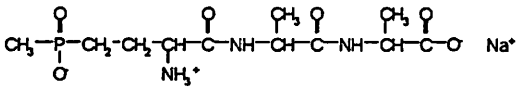
glyphosate	**glufosinate-ammonium**	**bialaphos**
 $HO-\overset{O}{C}-CH_2-NH-CH_2-\underset{OH}{\overset{O}{P}}-OH$	 $CH_3-\underset{O^-}{\overset{O}{P}}-CH_2-CH_2-\underset{NH_2}{CH}-\overset{O}{C}-OH \quad NH_4^+$	 $CH_3-\underset{O^-}{\overset{O}{P}}-CH_2-CH_2-\underset{NH_3^+}{CH}-\overset{O}{C}-NH-\overset{CH_3}{CH}-\overset{O}{C}-NH-\overset{CH_3}{CH}-\overset{O}{C}-O^- \quad Na^+$
ROUNDUP®, VISION®, RODEO® and others	BASTA, IGNITE®, and others	MEIJI HERBIACE
non-selective, post-emergent systemic herbicide for crop and non-crop areas	non-selective, post-emergent, contact herbicide for crop and non-crop areas	non-selective, post emergent and contact herbicide for use as a directed spray in fruit and vegetable crops
controls most annual and perennial plants	controls a broad spectrum of broadleaved weeds and grasses	-controls a broad spectrum of broadleaved weeds and grasses
-inhibits aromatic amino acid synthesis in plants -minimal metabolism in plants	-inhibits glutamine synthesis in plants -elevates ammonia levels in plants -minimal metabolism in plants	-inhibits glutamine synthesis in plants -elevates ammonia levels in plants -minimal metabolism in plants
-tightly bound to soil -negligible mobility, volatility -microbially degraded in soil -field half-life of 47 days -inactive in soil	-weakly adsorbed to soil -potentially mobile -microbially degraded in soil -field half-life of 7 days -inactive in soil	-microbially degraded in soil -field half-life of 20-30 days -inactive in soil
-AOLD$_{50}$ (Rat): 5600 mg/kg -AOLD$_{50}$ (Bobwhite Quail): >4640 mg/kg -96-h LC$_{50}$ (Rainbow Trout): 86 mg/L	- AOLD$_{50}$ (Rat): 1910-2170 mg/kg - AOLD$_{50}$ (Rat): (Japanese Quial): >2000 mg/kg -96-h LC$_{50}$(Rainbow Trout): 320 mg/L	-AOLD$_{50}$ (Rat): 268-404 mg/kg (technical) -AOLD$_{50}$ (White Leghorn): >5000 mg/kg (technical and formulation) -48-h LC$_{50}$ (Carp): 1000 mg/L
-very soluble in water -low solubility in acetone, chlorobenzene, ethanol, kerosene, xylene -pK$_a$ = 2.6, 5.6, 10.3	-soluble in water and organic solvents -pK < 2, 2.9, 9.8	-very soluble in water, soluble in methanol -insoluble in ethanol, n-butanol, acetone, ethyl ether, chloroform, benzene, benzane
-very soluble in water	-very soluble in water	-very soluble in water

Table 2. Metabolism of ^{14}C-glufosinate ammonium in white spruce and northern water milfoil (*Myriophyllum sibericum*, Komorov) shoots after 16 hours incubation in continuous light [1]

species	percent of recovered radioactivity at 16 hrs		
	glufosinate-ammonium	MPPA-3[2]	MPAA-2[3]
white spruce	60	16 ± 3	6 ± 6
N. water milofoil	97 ± 3	0	0

1. Intact shhots were vacuum infiltrated with ^{14}C-glufosinate-ammonium in 0.35M mannitol. Extractable (20% EtOH) radiolabelled compounds were separated by HPLC.
2. MPAA-3 is deaminated glufosinate acid.
3. MPAA-2 is deaminated and decarboxylated glufosinate acid.

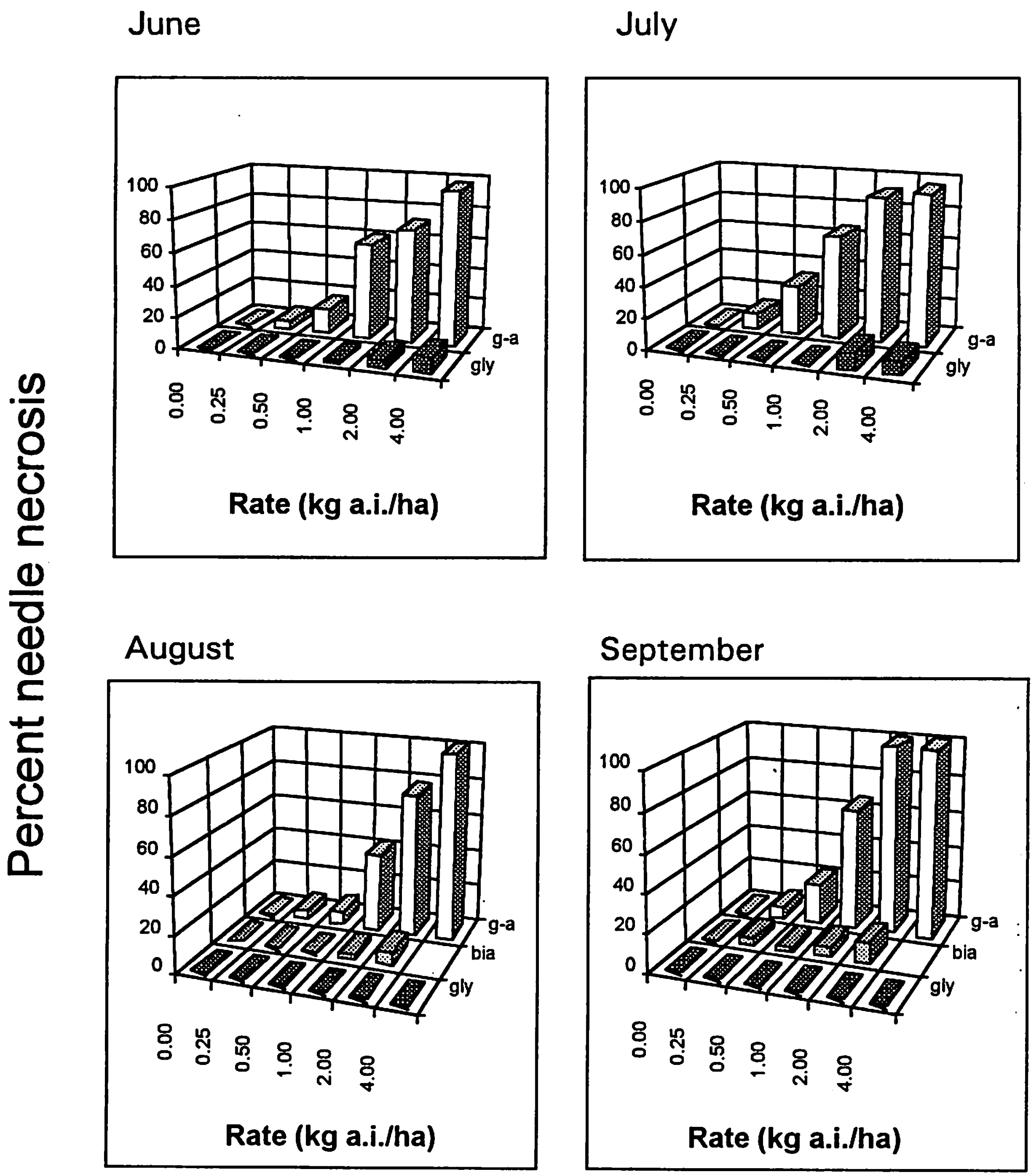


Figure 1. Foliar injury to white spruce (visual estimate of percent needle necrosis) in October following treatment with bialaphos (bia) glufosinate-ammonium (g-a) or glyphosate (gly) in June, July, August or September.

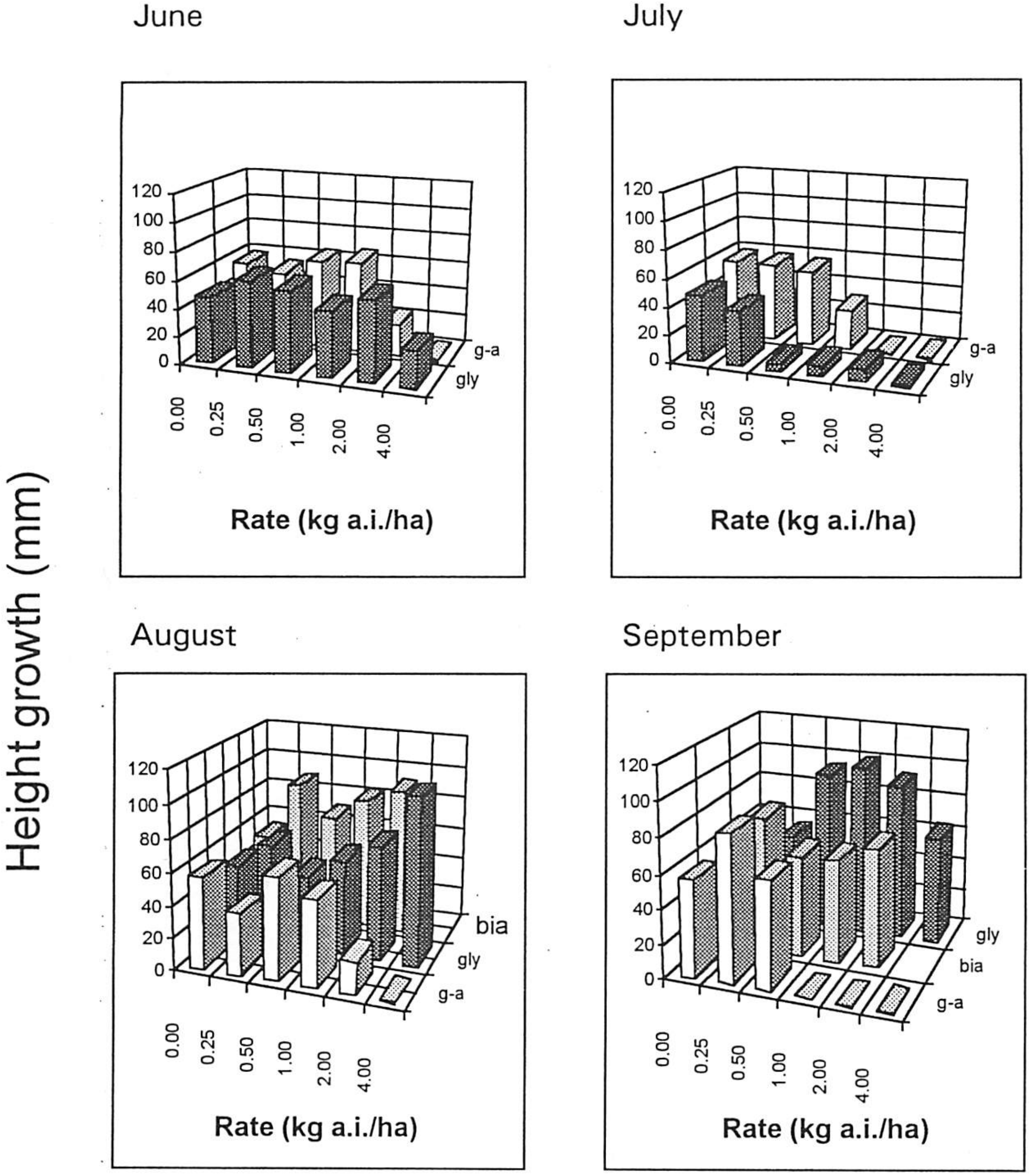


Figure 2. Height growth of white spruce during the season following treatment with bialaphos (bia), glufosinate-ammonium (g-a) or glyphosate (gly) during June, July, August or September of the previous year.

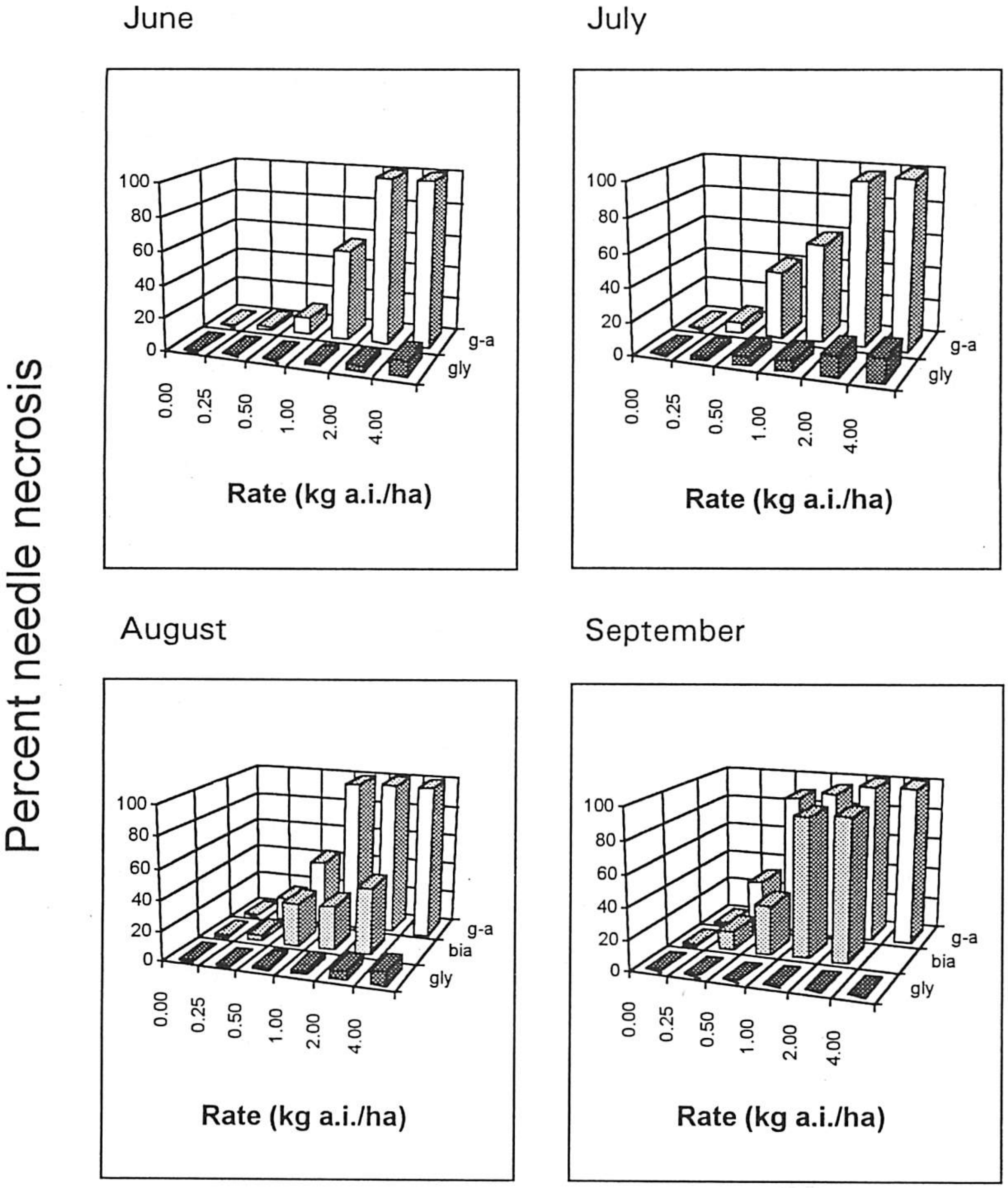


Figure 3. Foliar injury to black spruce (visual estimate of percent needle necrosis) in October following treatment with bialaphos (bia) glufosinate-ammonium (g-a) or glyphosate (gly) in June, July, August or September.

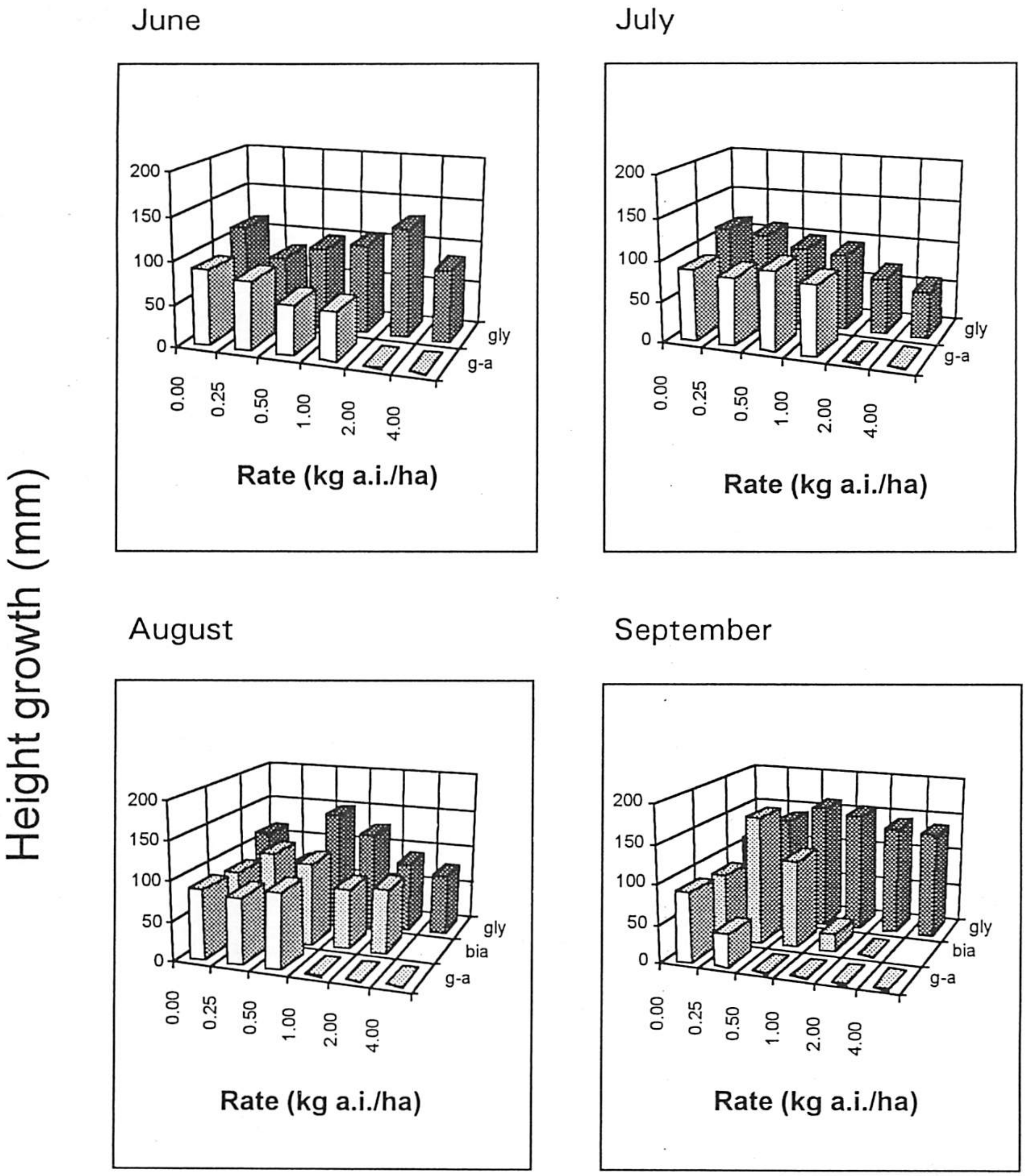


Figure 4. Height growth of black spruce during the season following treatment with bialaphos (bia), glufosinate-ammonium (g-a) or glyphosate (gly) in June, July, August or September of the previous year.

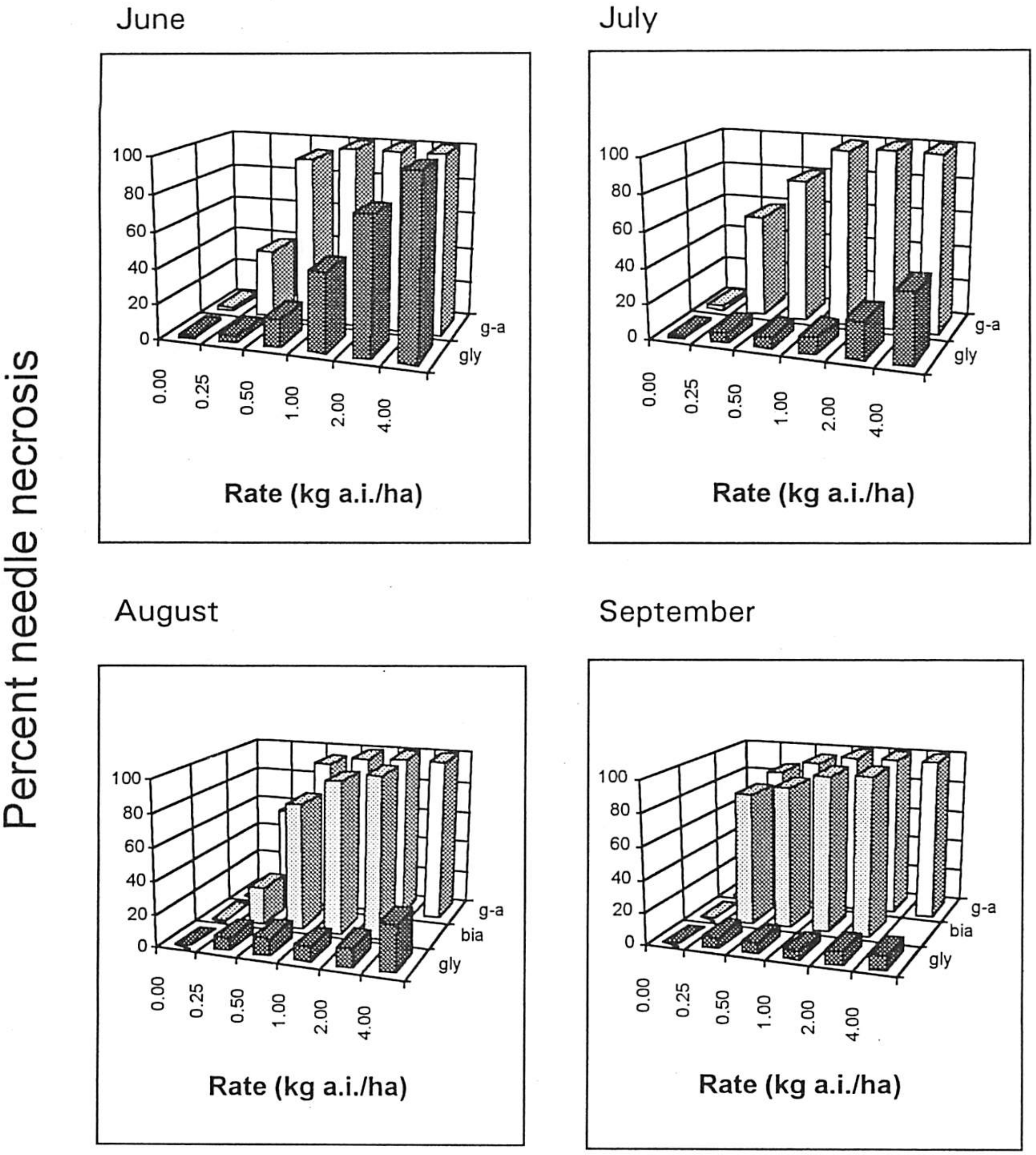


Figure 5. Foliar injury to jack pine (visual estimate of percent needle necrosis) in October following treatment with bialaphos (bia) glufosinate-ammonium (g-a) or glyphosate (gly) in June, July, August or September.

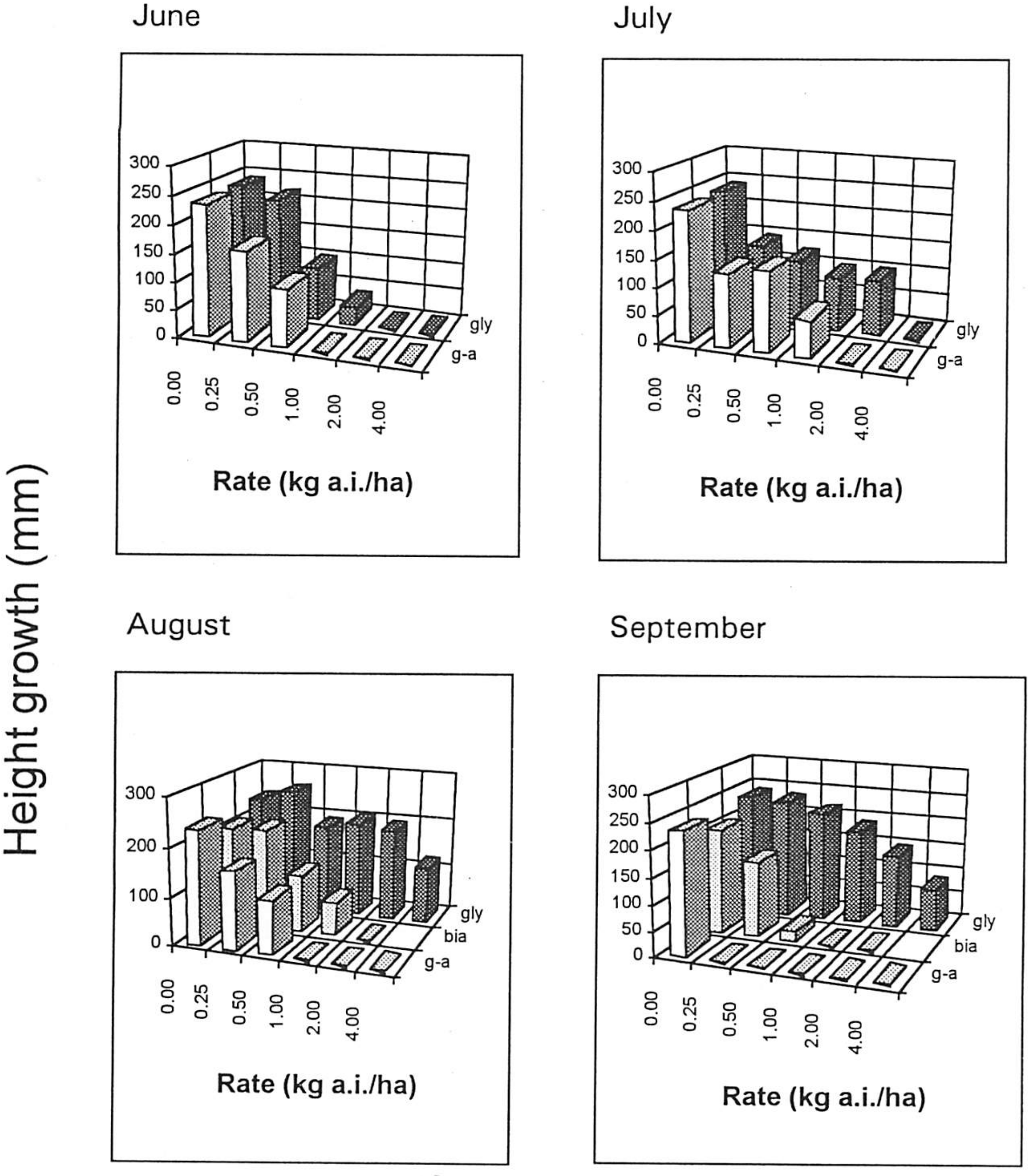


Figure 6. Height growth of jack pine during the season following treatment with bialaphos (bia), glufosinate-ammonium (g-a) or glyphosate (gly) during June, July, August or September of the previous year.

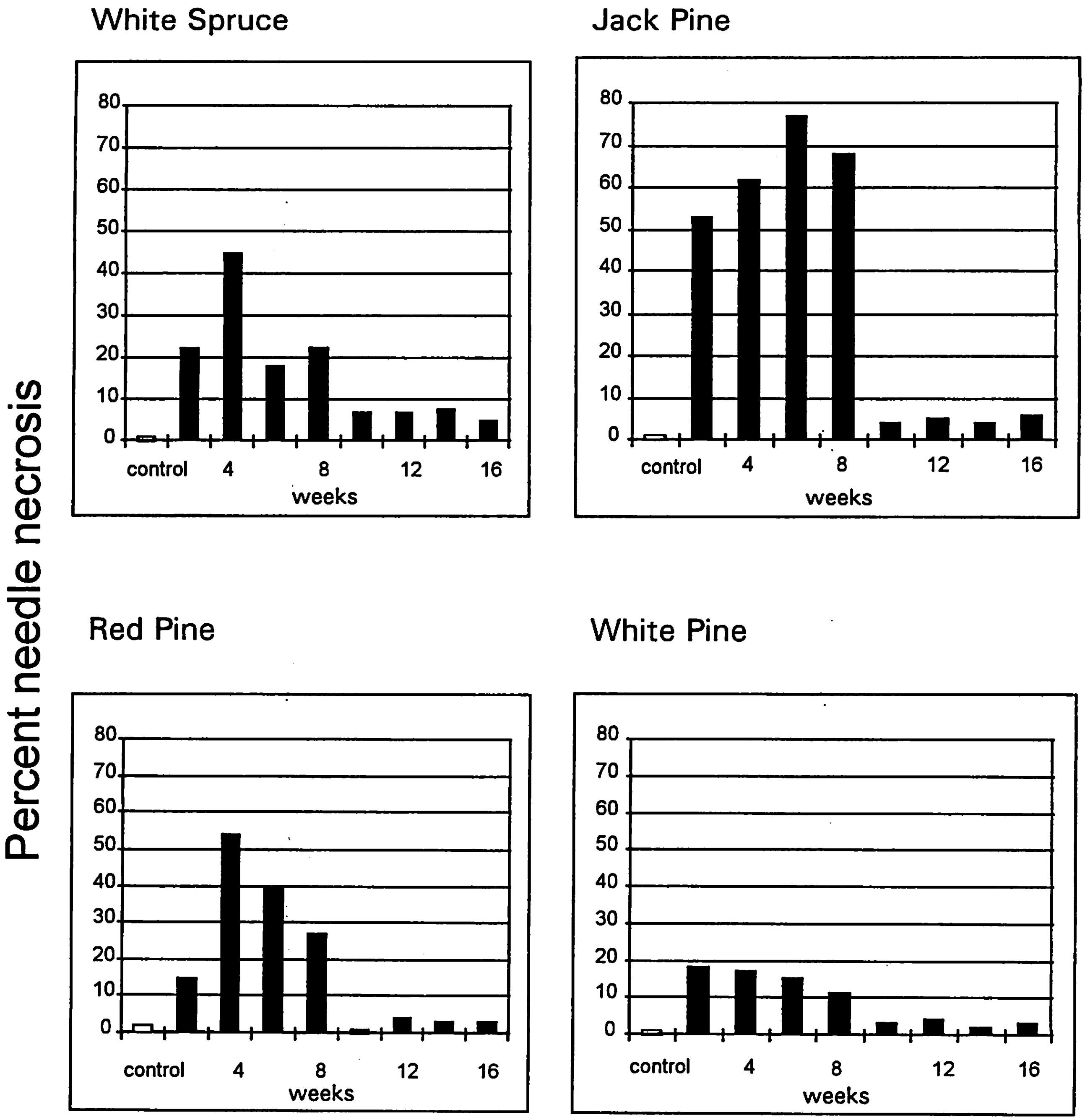
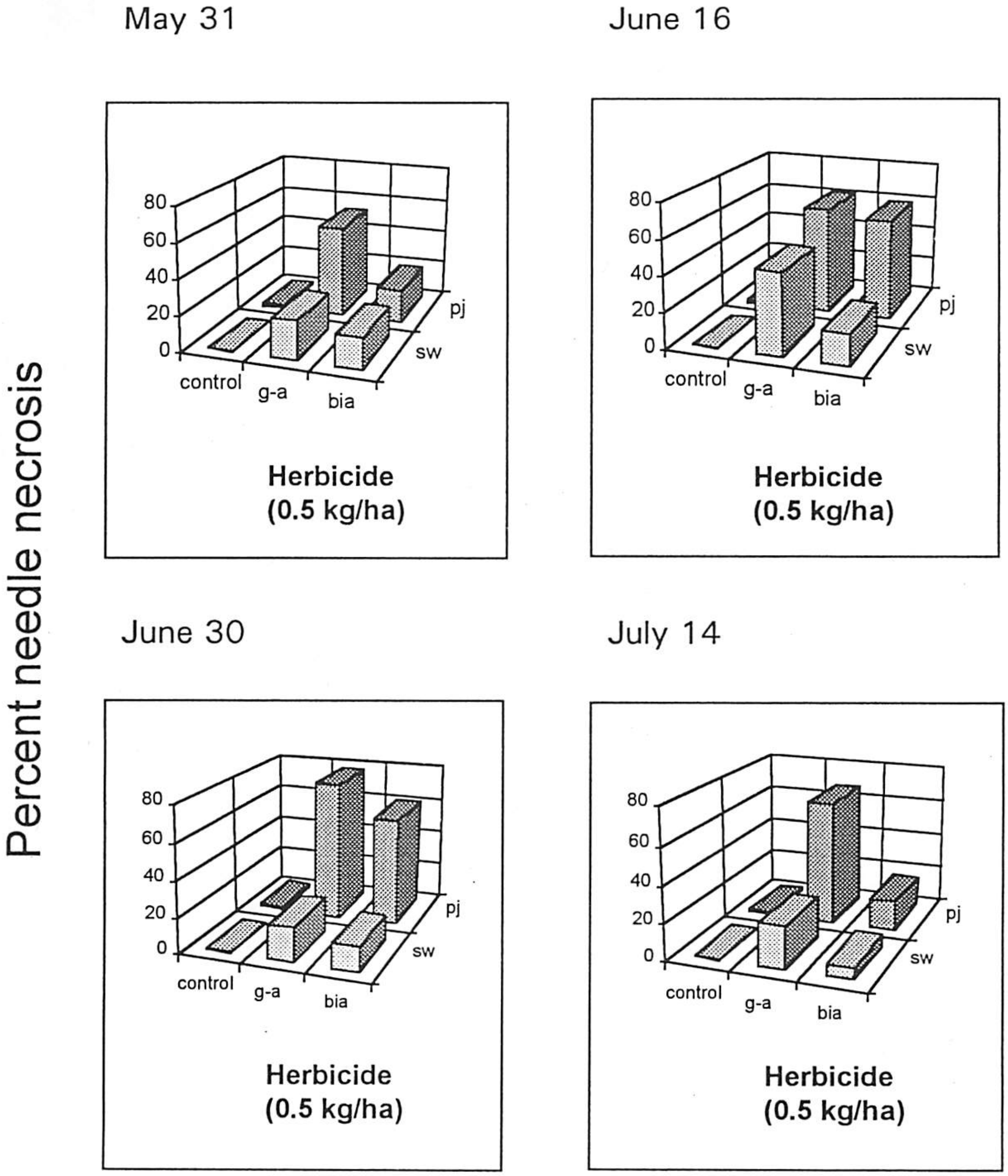


Figure 7. Foliar injury to transplants of four conifer species (visual estimate of percent needle necrosis) in October following foliar treatment with glufosinate-ammonium (0.5 kg/ha) at 2 to 16 weeks after planting (May 13).



May 31

June 16

June 30

July 14

Figure 8a. Foliar injury (visual estimate of percent needle necrosis) to white spruce (sw) and jack pine (pj) in October following treatment with 0.5 kg/ha glufosinate-ammonium (g-a) or bialaphos (bia) on May 31, June 16, June 30 or July 14.

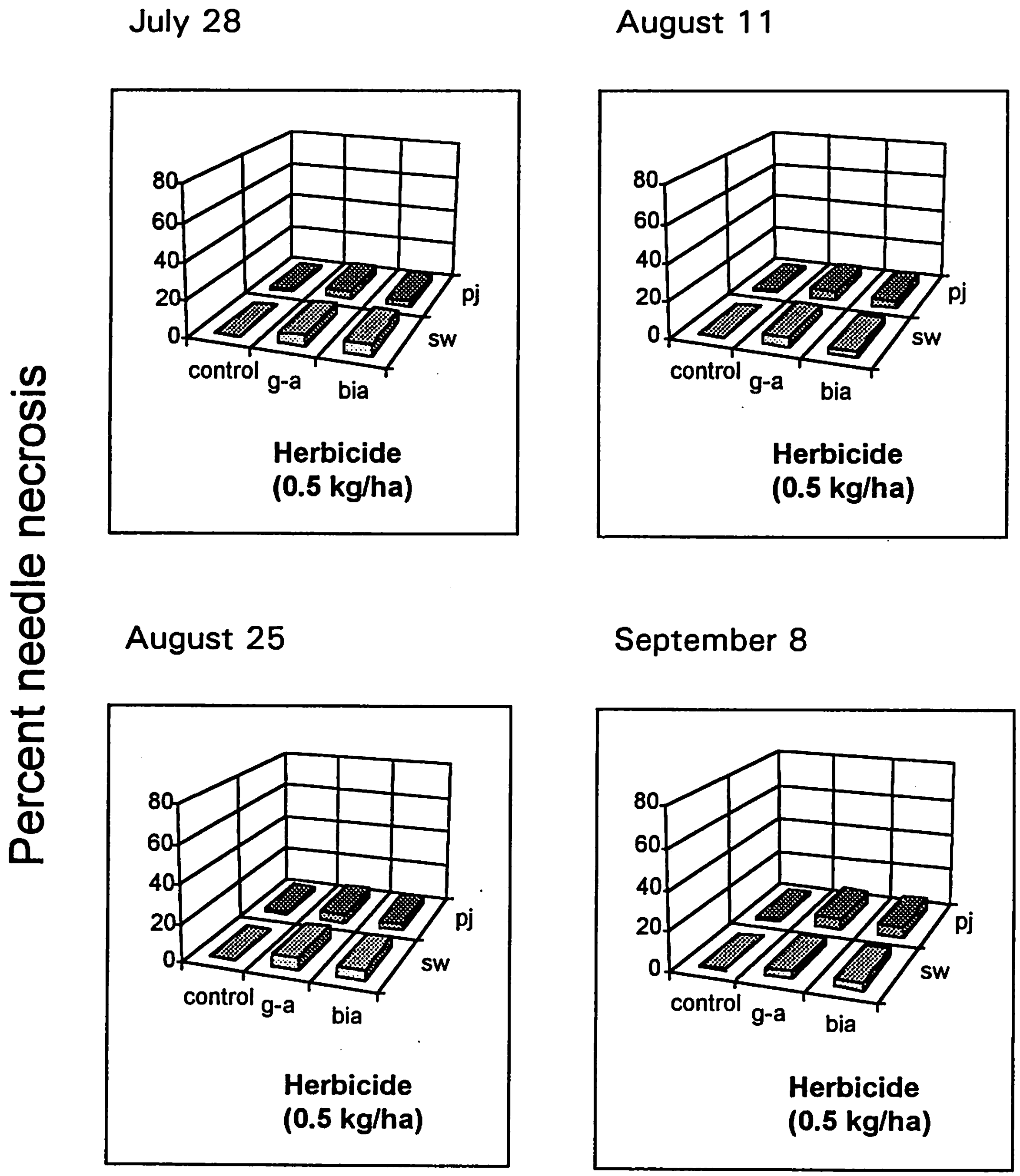


Figure 8b. Foliar injury (visual estimate of percent needle necrosis) to white spruce (sw) and jack pine (pj) in October following treatment with 0.5 kg/ha glufosinate-ammonium (g-a) or bialaphos (bia) on July 28, August 11, August 25, or September 8.

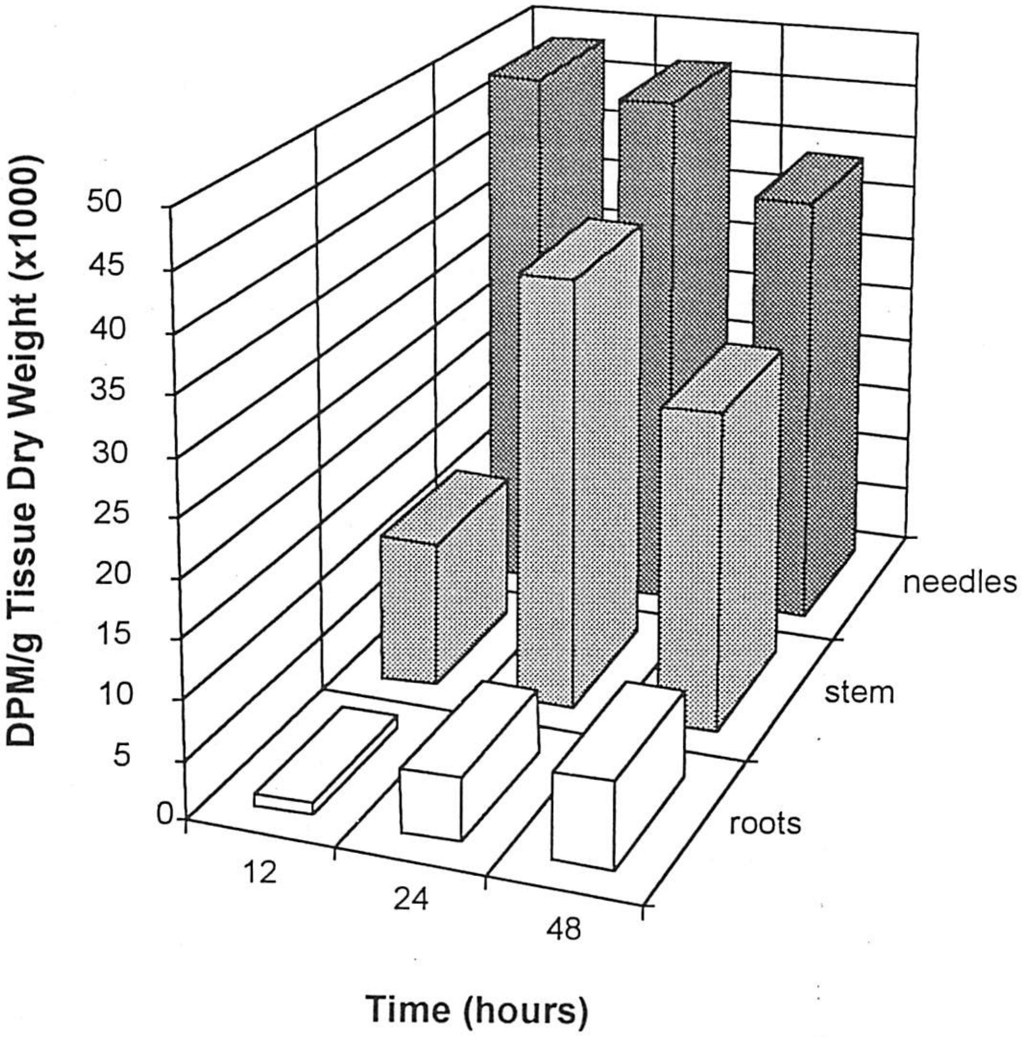


Figure 9. Uptake of glufosinate-ammonium-^{14}C applied as a spray to the shoots of black spruce seedlings.

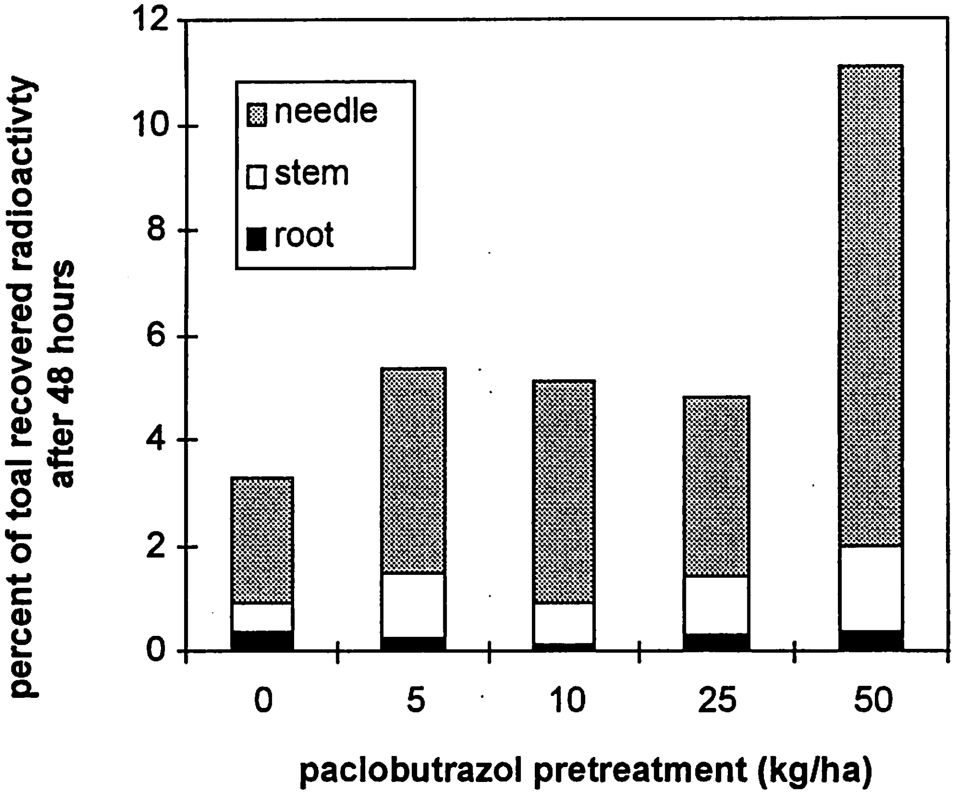


Figure 10. Influence of paclobutrazol pretreatment on the uptake of glufosinate-ammonium-^{14}C applied as a spray to black spruce seedlings.

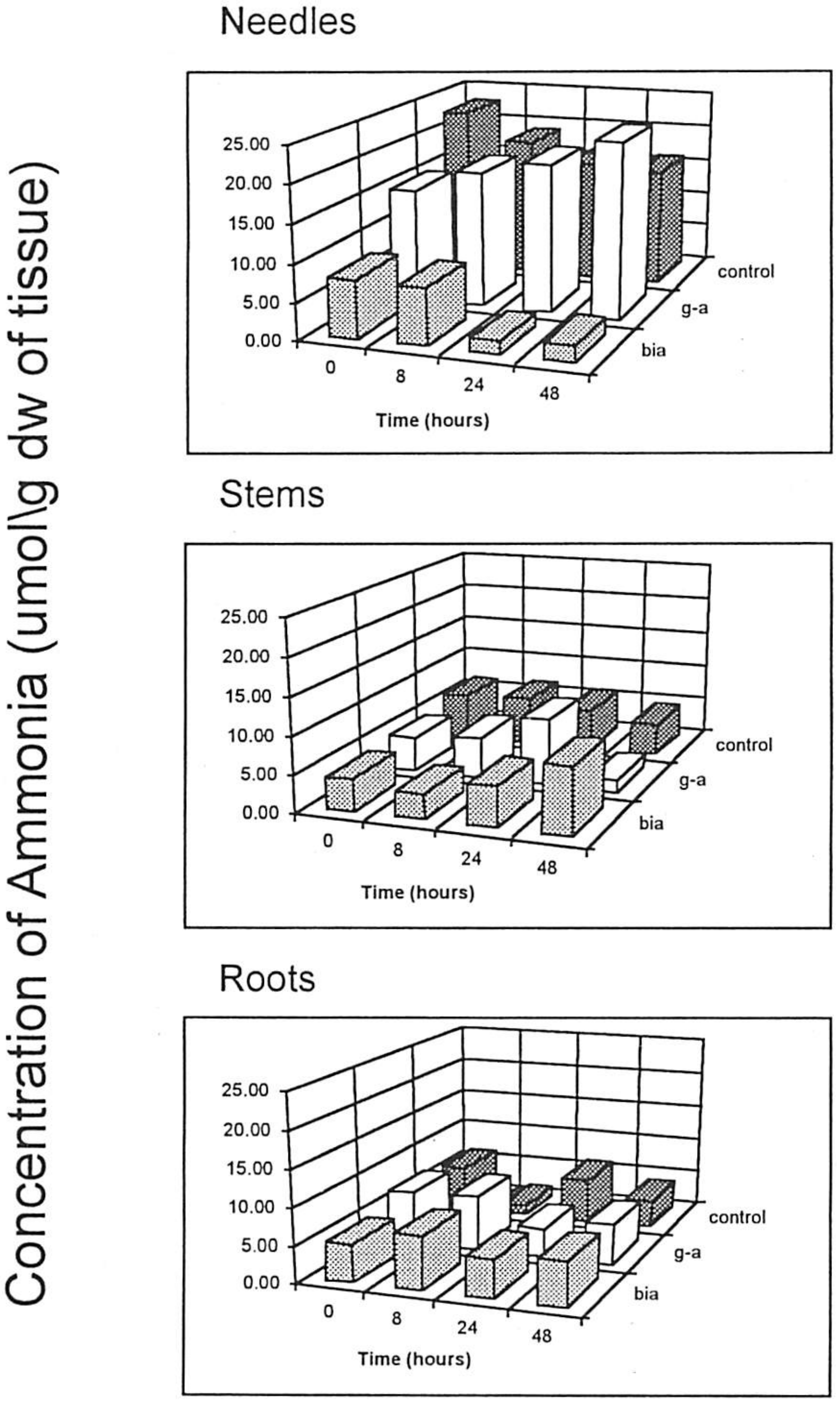


Figure 11. Influence of bialaphos (bia), glufosinate-ammonium (ga) or glyphosate (gly) applied as a foliar spray (1.0 kg (a.I.)/ha) on the accumulation of ammonia in white spruce seedlings.