The effects of selected pre-emergence herbicides on the germination, survival, health and germinant morphology of Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

J. O'Carroll[1] and C. O'Reilly

Department of Crop Science, Horticulture and Forestry,
University College Dublin, Belfield, Dublin 4

[1]Current address: Medite of Europe Ltd., Redmonstown, Clonmel, Co. Tipperary

Summary

The effects of five pre-emergence herbicides, diphenamid, oxyfluorfen, napropamide, isoxaben and oxadiazon, on the germination of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seeds over a 6 week period when sown under grit or soil were investigated. The main objective was to find a herbicide that could be used as an alternative to diphenamid, the principal pre-emergence herbicide used in Irish nurseries. The number of surviving seedlings was determined at the end of the test, 2 weeks later, after all germination was complete. The results under soil and grit were consistent, although as expected, seeds germinated more slowly and final germination was lower under soil. Only the results from the grits are reported. Diphenamid and oxyfluorfen had little detrimental effect on the speed of germination and final (cumulative) germination of seeds, and the survival of germinants. Final germination (approximately 80%) and survival (approximately 90%) were almost the same as in the control. Morphological development and health scores, seedling height, root length and seedling dry weights also differed little from the control. Germination and survival were a little lower in those treated with napropamide (74% and 80% respectively), and the seedlings were healthy. Shoot growth, and to a greater extent, root growth, were however adversely affected by this treatment. Germinants in this treatment also had lower dry weights than in those from the control, diphenamid and oxyfluorfen treatments. Germination and all other variables were very poor in those treated with isoxaben and oxadiazon. In all cases, the herbicides tested had no effect on mycorrhizal associations in germinants.

Introduction

Weed competition in nursery seedbeds is a major problem, as it is in other phases of nursery production. Herbicides are widely used in their control. Post-seeding or pre-emergent treatment is generally more effective than post-germination application for full season weed control (Owston and Abrahamson, 1984). Diphenamid is the most widely used pre-emergence herbicide in Irish forest nurseries. Diphenamid can reduce hand weeding by up to 75%, with considerable savings in labour costs (McDonald *et al.*, 1974). This herbicide is highly effective, but there is concern about the over-reliance on it. Furthermore, diphenamid may be withdrawn from the market, posing a serious set-back to the nursery industry in Ireland. Alternative chemicals therefore need to be screened for use as pre-emergence herbicides, particularly for use on Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedbeds. This species accounted for approximately 80% of the afforestation and reforestation programme of about 21,000 ha in 1993. Although several herbicides other than diphenamid have been used successfully on conifers in other countries (such as napropamide, bifenox and DCPA), there is little information on herbicide suitability for use on Sitka spruce. However, some limited information of this kind is now available for this species (Williamson *et al.*, 1993).

The success of a pre-emergence herbicide treatment largely depends on the presence of a high concentration of the herbicide in the upper 3 cm of the soil, where most of the annual weeds germinate (Klingman and Ashton, 1975). Most conifers are however sown under a covering material such as grit (1 cm used in Ireland), where the seed is likely to be in direct contact with the chemical. In one study using red pine (*Pinus resinosa* Ait.), direct contact of herbicides with seeds had no effect on germination, but newly germinating seedlings were greatly affected (Kozlowski and Saskai, 1968). As the soil absorbs herbicides (Delorit and Ahlgren, 1973), soil covering may offer more protection from the herbicide than grit.

The objective of this study was to find a suitable alternative pre-emergence herbicide for use on Sitka spruce seedbeds, particularly as a substitute for diphenamid. To this end, the effect of five herbicides (diphenamid, oxyfluorfen, napropamide, isoxaben and oxadiazon) applied to both soil and grit covered seeds on the germination, early seedling morphology and mycorrhizal associations was investigated in a controlled greenhouse environment trial. No attempt was made to test the effectiveness of the herbicide in weed control.

Materials and methods

Soil from the Coillte Teo. Ballintemple Nursery, Co. Carlow, was placed to a depth of 5 cm in each of 72 plastic trays (35 x 22 x 7 cm). The soil was a sandy loam of pH 5.7, with an organic matter content of 0.78% and sand, silt and clay fractions of 66%, 19% and 15% respectively. The soil is considered ideal for germinating Sitka spruce.

Stratified Sitka spruce seeds (registration code 94R91) of Washington origin were sown in May, 1993, in five rows containing 10 seeds per row in each tray (50 seeds/tray), giving a density of 800 seeds/m^2. After sowing, 36 trays were covered with 1 cm grit (the same grit as that used operationally at Ballintemple), while the remaining trays were covered with a similar amount of soil. The trays were then placed in an experimental greenhouse unit at University College Dublin. After covering, all trays were well watered. After 24 hours, each herbicide was applied to six trays of each covering material (12 trays), using a hand-held bottle sprayer at recommended concentrations (Table 1). Twelve untreated trays served as controls. The trays were then randomly arranged within each of six blocks, each herbicide by covering material combination replicated once within each block. The trays were raised off the floor of the greenhouse using polystyrene laths, to avoid the possibility of cross contamination through runoff water.

As high temperatures (35°C) and low humidity (40% or lower) are common in greenhouses at the time of year during which the trial took place, three small polythene tunnels, each spanning two blocks, were erected for the duration of germination. A shade cloth was placed over the tunnels to lower the temperature. Temperatures were monitored daily using a maximum-minimum thermometer, while humidity was checked periodically with a digital hygrometer (Delta Ohm HD8501H, Padova, Italy). Temperatures were less than

Table 1. *Rates used for the application of herbicides to Sitka spruce seeds before germination. Herbicide abbreviations are given parenthetically.*

Herbicide	*Rate of application (g ai/ha)*
Diphenamid (DIPH)	4,000
Oxyfluorfen (OXYF)	72
Napropamide (NAPR)	3,150
Isoxaben (ISOX)	125
Oxadiazon (OXAD)	2,000

30°C and relative humidity was between 75-95% at all times during the trial.

All weeds were removed as they emerged, and no attempt was made to assess the effectiveness of the herbicides in weed control. No fertilisers were applied to the trays.

Data collection

Beginning 7 days after sowing, the trays were monitored daily until germination began. At this point, and thereafter at 2-day intervals, the number of new germinants were recorded until germination ceased 42 days later. After another 14 days, the health (severe, slight or no damage) and stage of development (seed cap present, cotyledons emerged, primary needles present, shoot elongation) of each germinant was subjectively scored.

Total germination alone is perhaps not the most meaningful way to quantify germination. The speed of germination is also important as it takes into account the vigour of the seeds. To account for differences in both speed of germination and total germination, Czabator's formula was used to calculate the germinative value (Czabator, 1962). This value is a product of mean daily germination over the whole test period and the mean daily germination over the most vigorous component of the test period (beginning at zero).

Seedling morphology, dry weight measurements and the number of surviving seedlings were recorded at the end of the experiment, 56 days later. Every fifth seedling was removed until a total of 10 seedlings was obtained from each tray for morphological assessments. As there were usually fewer than 50 seedlings per tray, each tray was scanned more than once. In a few cases, there were fewer than 10 germinants per tray. Shoot and root lengths were measured. The seedlings were then placed in an oven at 65°C for 24 hours, after which, dry weight determinations were made.

Data analyses and presentation

As the data were consistent across covering material, only the effects of herbicides on grit-covered seed trays were evaluated. For most variables, the data (mean value per tray) were subjected to an analysis of variance following a randomised block design using the SAS software package to test the effects of treatments and blocks (SAS Institute Inc., 1982). This test was followed by the least significant difference test to determine which herbicides were significantly different. In the case of the morphology data, two treatments were excluded as they contained too few seedlings in some replications. Nevertheless, mean values for all treatments are presented for comparison. Data on health and stage of development are presented for descriptive purposes only. These data were not subjected to statistical analysis as they were derived from observations of all surviving germinants, with sample sizes varying accordingly.

Mycorrhizae

In December, 1993, a further five seedlings were removed from each tray and stored in plastic bags at 4°C. Over the following few weeks, seedling roots were examined for the presence of mycorrhizae. The roots were washed in running tap water and rinsed in deionised water. Following washing, the seedlings were placed in petri dishes containing deionised water and examined under a stereo microscope (20x) to determine the presence of mycorrhizae. Notes were made of the colour and size of the hyphae. The number of infected root tips was recorded for each germinant as a percentage of the total number of root tips.

To identify the species of mycorrhizae, the root tip was mounted on a slide in cotton blue stain (10-15 seconds), and then squashed firmly under a cover glass. The squash was observed under a compound microscope (400x), and the mycorrhizae identified, using mantle and hyphal characteristics (Ingleby, 1990). In an attempt to speed up this procedure, a staining techniques using 0.1% Ponceau S (acid red 112) diluted in 10% acetic acid was tested, following the procedure outlined by Daughtridge *et al.* (1986). Although used successfully to detect ectomycorrhizal fungi in *Quercus rubra* L., this technique was not effective in detecting ectomycorrhizal fungi of Sitka spruce in this study.

Results

The effects of replication were significant for a number of variables, but this was largely attributable to the slightly smaller values recorded for two blocks closest to one edge of the experiment. These blocks may have received some additional 'drift' watering, due to their position adjacent to a separate trial. The effect of replication will not be addressed further in this paper.

Germination and survival

Herbicides had a highly significant effect on final or cumulative germination ($p=0.023$) and germination value ($p=0.013$). Seeds from the control had the highest final germination, but not significantly higher than in those from DIPH, OXYF and NAPR (Figure 1). Final germination was over 80% in the control, DIPH and OXYF, but was a little lower in NAPR (74%). Seeds from ISOX and OXAD treatments had the lowest final germination (≤55%). The pattern for germination values, which takes the speed of germination into consideration, showed an almost identical pattern to that of final germination (Figure 2).

The survival of the germinants showed a similar pattern among treatments. However, these data were not statistically analysed due to the low sample sizes available in some treatments. DIPH and the control had survival rates of over 90%, while survival was a little lower at 86% in OXYF. Approximately 73% of the germinants of ISOX survived, but only 21% of the OXAD germinants lived.

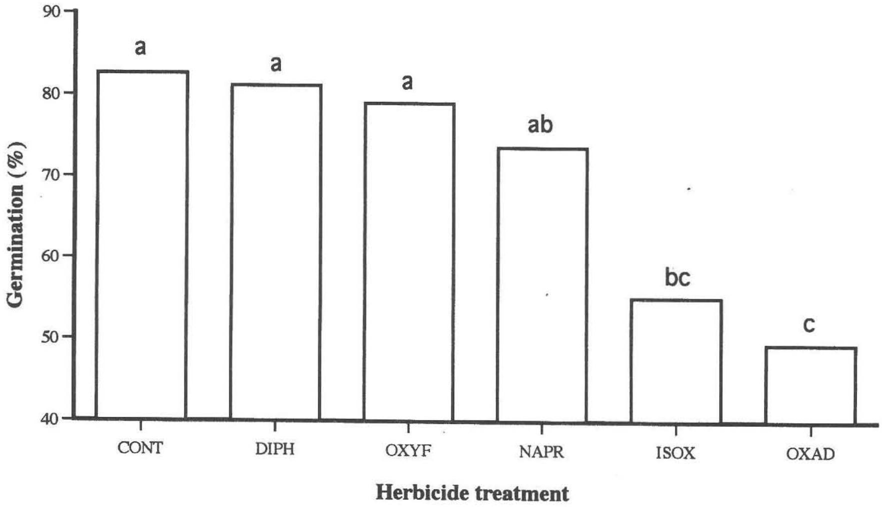

Figure 1. *Effect of pre-emergence herbicides on final germination of Sitka spruce seeds after 42 days in the greenhouse. The treatments are control (CONT), Diphenamid (DIPH), Oxyfluorfen (OXYF), Napropamide (NAPR), Isoxaben (ISOX) and Oxadiazon (OXAD). Bars having the same letter are not significantly different using the least significant means test.*

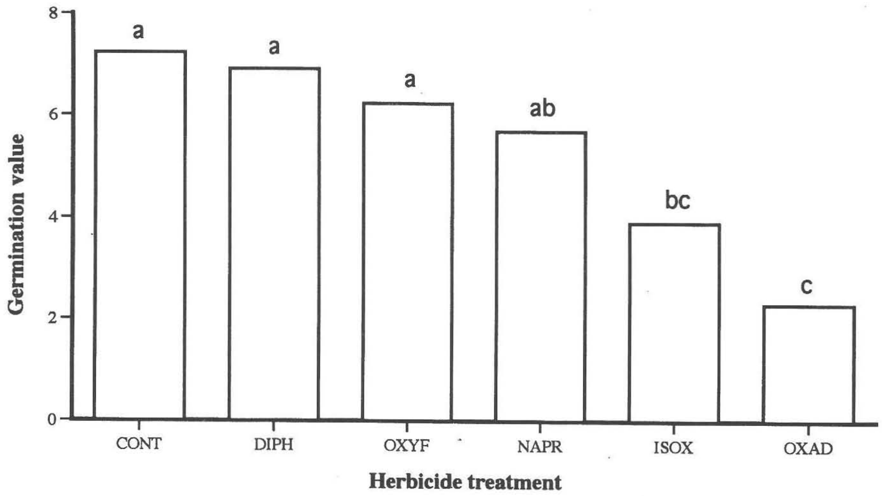

Figure 2. *Effect of pre-emergence herbicides on germination value of Sitka spruce seeds after 42 days in the greenhouse. Germination value is a composite index of both the speed and final germination values. The treatments are control (CONT), Diphenamid (DIPH), Oxyfluorfen (OXYF), Napropamide (NAPR), Isoxaben (ISOX) and Oxadiazon (OXAD). Bars having the same letter are not significantly different using the least significant means test.*

Seedling condition and morphology

Seedling morphology differed among treatments (Figures 3 and 4). Due to the small sample sizes available in ISOX and OXAD, however, these treatments were excluded from the statistical analyses. Values for all morphological variables in germinants from NAPR were significantly different from other treatments in the analysis (CONT, DIPH, OXYF) (p=0.003).

Shoot length differed little among the control, DIPH and OXYF (32-34 mm) (Figure 3). Shoots from germinants in the NAPR and ISOX treatments were a little shorter (25 mm and 27 mm respectively), while those from the OXAD treatment were shortest (21 mm). Roots of germinants from the control treatments (27 mm) were longer than those from other treatments. Root length (20-22 mm) was similar in germinants from DIPH, OXYF and ISOX. Root length was shortest in those from NAPR (14 mm).

Reflecting the combined differences in shoot and root morphology, there were differences among treatments in germinant dry weights (Figure 4). Mean dry weights were similar in the control, DIPH and OXYF (8.8-9.1 mg). Germinants from the NAPR and ISOX treatments were lighter (6.0 mg and 7.0 mg respectively), while those from the OXAD treatment were close to half these weights (3.5 mg).

With the exception of the OXAD treatment, few of the surviving germinants showed evidence of treatment damage after 2 months of growth in the greenhouse. Undamaged germinants accounted for 98% (control), 97% (DIPH and OXYF), 95% (NAPR) and 94% (ISOX) of the total, compared with 49% in the OXAD treatment.

In contrast, the stage of development of the germinants varied among treatments. While a large proportion of the germinants from the control and the OXYF treatment underwent primary shoot elongation (88% approximately), very few of those from the NAPR (30%) and OXAD (27%) produced primary needles. Germinants from the DIPH (71%) and ISOX (79%) treatments were slightly less advanced in stage of development than those from the control and OXYF.

Mycorrhizae

More than 90% of the root tips of germinants from all treatments had mycorrhizae, although only 10 germinants from each treatment were examined. No significant difference among treatments was detected. Three species of mycorrhizae were identified – *Humaria hemisphaerica*, *Thelephora terrestris* and *Helbeloma mesophaeum.*

Discussion

The final germination percentage and germination value (composite index reflecting rate and final germination) after 42 days, and survival after 56 days in the greenhouse were little affected by the use of DIPH and OXYF herbicides. Final germination was nearly 80% and survival was between 86-94% – levels not greatly different from those in the control. Furthermore, these herbicides had little effect on seedling dry weight, morphology or health. OXYF appears to be a very promising alternative herbicide to DIPH on Sitka spruce seedbeds. DIPH is the most widely used pre-emergence herbicide in Irish nurseries, and there is concern about the over-reliance on this herbicide. Repeated applications of the same or similar herbicide formulations may lead to weed resistant populations (Sandquist

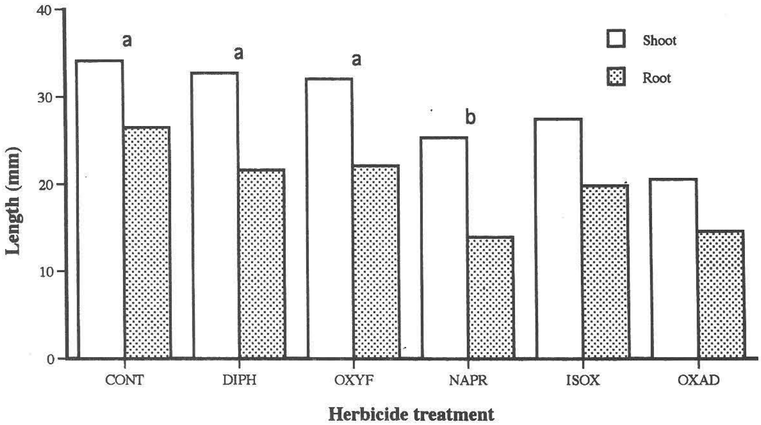

Figure 3. *Effect of pre-emergence herbicides on shoot and root length of Sitka spruce germinants after 56 days in the greenhouse. The treatments are control (CONT), Diphenamid (DIPH), Oxyfluorfen (OXYF), Napropamide (NAPR), Isoxaben (ISOX) and Oxadiazon (OXAD). Bars having the same letter are not significantly different using the least significant means test. Because of the low number of germinants in some replications in ISOX and OXAD, these treatments were excluded from the analysis.*

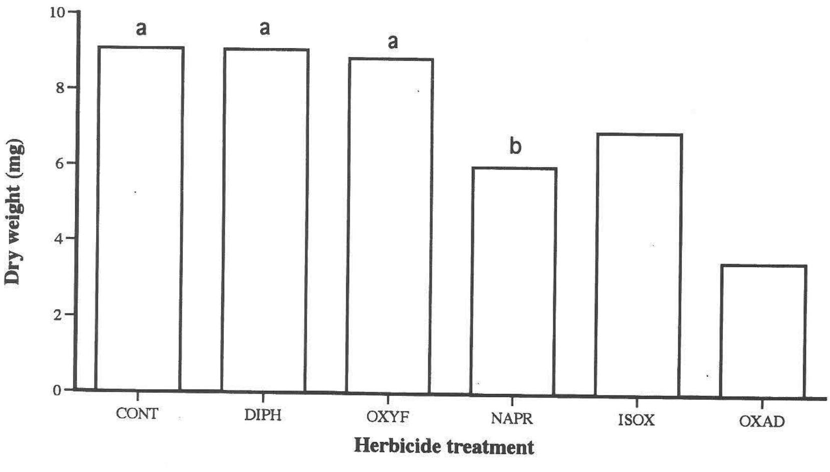

Figure 4. *Effect of pre-emergence herbicides on the dry weight of Sitka spruce germinants after 56 days in the greenhouse. The treatments are control (CONT), Diphenamid (DIPH), Oxyfluorfen (OXYF), Napropamide (NAPR), Isoxaben (ISOX) and Oxadiazon (OXAD). Bars having the same letter are not significantly different using the least significant means test. Due to the low number of germinants in some replications in ISOX and OXAD, these treatments were excluded from the analysis.*

et al., 1981). OXYF is very widely used as a pre- and post-emergence herbicide in North American nurseries to control a wide variety of broadleaved weeds and grasses (Owston and Abrahamson, 1984).

Although germination (74%) and survival (80%) were good with the use of NAPR, seedling growth was very poor, underlining the need for assessing variables in addition to germination percentage. In contrast, Sumaryono and Crabtree (1989) found that NAPR had little negative effect on the germination and growth of several coniferous species, including Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) and Japanese black pine (*P. thunbergii* Parl) when applied at higher concentrations (4 kg ai/ha) than used here (3.2 kg ai/ha). The application rates used in this trial were in line with those recommended for use in US nurseries for Douglas fir, lodgepole pine (*P. contorta* Dougl.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Owston and Abrahamson, 1984). However, the application rate used in this study was nearly 3.5 times that recommended by the British Forestry Commission guidelines (Mason and Williamson, 1988; Williamson *et al*., 1993). As root growth is perhaps more sensitive than shoot growth to herbicide toxicity (Kozlowski and Saskai, 1968), the very poor root development (14 mm vs. >20 mm; Figure 3) in germinants from this treatment is of concern. ISOX and OXAD show the least promise as pre-emergence herbicides on Sitka spruce seedbeds, with all variables being adversely affected. OXAD gave the worst results.

Despite the results presented here, further testing of ISOX, OXAD and NAPR herbicides at different concentrations than those used here may be needed. NAPR, for example, appears to be a promising pre-emergence herbicide for Sitka spruce and other conifers if used at approximately one-third of the label rates (Williamson *et al*., 1993). The same may also be true for the other herbicides.

Herbicide treatments had little effect on the frequency of mycorrhizal associations, although the number of samples examined was small. Similarly, Trappe (1983) found that three herbicides (bifenox, DCPA and napropamide) applied at two different rates did not reduce the proportion of feeder roots colonised by mycorrhizae fungi in Douglas fir and ponderosa pine seedlings, compared with the controls. In fact, Trappe noted that some treatments slightly enhanced mycorrhizal associations. Others have also found that herbicides had little effect or enhanced mycorrhizal associations in several tree species (Pope and Holt, 1978; South and Kelley, 1982; Palmer *et al*., 1980).

In conclusion, OXYF is recommended as a viable alternative pre-emergence herbicide to DIPH in Sitka spruce seedbeds. However, given that the results are based on a greenhouse trial, it is recommended that nursery managers conduct small-scale trials of their own before using the herbicide operationally. Factors such as weather conditions and soil type may influence the response to the herbicides (Williamson and Morgan, 1994). The efficacy of the herbicides in controlling weeds was not evaluated in this study.

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