The tolerance of newly emerged broadleaved tree seedlings to the herbicides clopyralid, cycloxydim and metazachlor

IAN WILLOUGHBY*, RICHARD L. JINKS AND VICTORIA STOKES

Forest Research, Forestry Commission, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, England
*Corresponding author. E-mail: ian.willoughby@forestry.gsi.gov.uk

Summary

In direct-seeded woodlands and nursery seed-beds, weeds can rapidly invade newly sown areas, leading to death or suppression of tree seedlings. Hand weeding is usually expensive, and the safe use of broad-spectrum contact herbicides is seldom possible. Hence in the work reported here, the tolerance of young tree seedlings to the potentially more selective, post-emergence herbicides clopyralid, cycloxydim and metazachlor, was tested. Seedlings of *Fraxinus excelsior* L. (ash) with two to four expanded true leaves (2–4 ETL), *Fagus sylvatica* L. (beech) (2–4 ETL), *Acer pseudoplatanus* L. (sycamore) (4–6 ETL), *Prunus avium* L. (cherry) (6–8 ETL) and *Quercus robur* L. (oak) (6–8 ETL) appeared to tolerate applications of cycloxydim at a rate of 0.45 kg a.i. ha^{-1}. Applications of 0.2 kg a.i. ha^{-1} clopyralid or 1.25 kg a.i. ha^{-1} metazachlor also appeared to be generally tolerated, but did cause some suppression of annual growth increment in *Q. robur*, *P. avium*, *F. sylvatica* and *A. pseudoplatanus*, particularly where repeat applications were made to the earliest growth stages. Seedling survival was unaffected by any herbicide treatment. Mixtures of all three herbicides were no more damaging than the same herbicides applied separately. Therefore, depending on tree species and growth stage, it appears that clopyralid could potentially be safely used to control a range of herbaceous weed species, metazachlor a variety of seedling or germinating weeds and cycloxydim a range of established grass weed species, in direct sown woodlands or nursery seed-beds, although further research is advisable to confirm crop safety.

Introduction

Where the use of natural regeneration is not possible, out planting of seedlings raised in tree nurseries is the principal method for establishing woodlands in the UK. Recent research has also suggested that direct seeding, where tree seed is sown in the actual location which is to be afforested, may also have potential for broadleaved woodland creation (Willoughby *et al.*, 2004a). Both nursery production and direct seeding face similar vegetation-management challenges. Weeds can compete with seedlings for scarce resources such as light, nutrients and moisture, which may result in the suppression or death of young, newly germinated tree seedlings (Davies, 1987;

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Williamson and Morgan, 1994; Willoughby *et al.*, 2004b). Hand weeding is feasible although costly within nurseries, but it is not generally a practical option on extensive direct-seeded sites (Willoughby *et al.*, 2004a). Hence to control weeds within forest nurseries, seed-beds are often sterilized before sowing, and herbicides applied after sowing of tree seed but before emergence of weeds (Williamson and Morgan, 1994). Direct seeding systems might potentially require fewer chemical inputs than planting, but herbicides usually remain the most cost-effective means of controlling weeds on fertile ex-agricultural sites in the first 1–2 years after sowing. Pre-emergence herbicides have been identified that can be safely used to control germinating weed seed, while allowing direct-seeded broadleaved trees to germinate unharmed (Willoughby *et al.*, 2003, 2004a). However, if nursery seed-beds are not sterilized effectively, or once residual herbicides start to degrade in the soil, weeds can rapidly establish and dominate a sown area. The safe use of contact herbicides in seed-beds containing dense and irregularly spaced seedlings is seldom considered possible. There is therefore a requirement to identify herbicides that could be used to control selectively established weed species among newly emerged tree seedlings.

Three herbicides appear to have particular promise. Clopyralid is a pyridine carboxylic acid, auxin-type herbicide, that is adsorbed primarily by foliage and also through roots; after translocation to meristematic regions, it interferes with auxin growth hormones, affecting cell elongation and respiration, leading to growth defects and death in susceptible species. It gives effective post-emergence control of some established herbaceous species, particularly members of Asteraceae (Compositae) and Polygonaceae, but does not affect grasses (Tomlin, 1997; Reade and Cobb, 2002). Metazachlor is a chloroacetamide herbicide that is adsorbed by hypocotyls and roots of young plants, and is thought to inhibit cell division hence causing death in susceptible species. It is primarily used to give pre-emergence control of germinating seedlings, but it will also give a degree of post-emergence control of some newly emerged grass and herbaceous species (Tomlin, 1997; Reade and Cobb, 2002). Cycloxydim is a cyclohexanedione herbicide that is adsorbed by foliage, and after translocation to meristematic regions inhibits lipid biosynthesis, causing disruption to cell membranes, chlorosis and ultimately death in susceptible species (Tomlin, 1997; Reade and Cobb, 2002). It can give effective post-emergence control of a range of established grass species, such as *Anthoxanthum oderatum* L. (sweet vernal grass), *Cynosurus cristatus* L. (crested dog's tail), *Dactylis glomerata* L. (cocksfoot), *Festuca arundinacea* Schreb. (tall fescue), *Lolium perenne* L. (perennial rye grass), *Molinia caerulea* L. (purple moor grass), *Phleum pratense* L. (timothy grass), *Poa trivialis* L. (rough meadow grass) and *Arrhenathrum elatius* (L.) J. and C. Presl (false oat grass) (Clay *et al.*, in press), *Deschampsia flexuosa* (Dixon *et al.*, 2005b), *Agrostis gigantea* Roth. (black bent), *Agrostis stolonifera* L. (creeping bent), *Alopecurus myosuroides* Huds. (black grass), *Elytrigia repens* (L.) Nevski (common couch grass) and volunteer cereals (BASF, 2002).

There is good evidence that established transplants of many tree species are tolerant of overall applications of clopyralid, cycloxydim or metazachlor (e.g. Hall and Burns, 1991; Mason and Williamson, 1992; Williamson *et al.*, 1992; Lawrie and Clay, 1994; Willoughby and Clay, 1996; Dixon *et al.*, 2005a; F.L. Dixon, D.V. Clay and I. Willoughby, in preparation). However, information on tolerance of young seedlings, particularly of broadleaved species, is much more limited. *Picea sitchensis* (Bong.) Carrière (Sitka spruce), *Larix kaempferi* (Lindl.) Carrière (Japanese larch), *Betula pendula* Roth. (silver birch) and *Alnus glutinosa* (L.) Gaertn. (common alder) seedlings with one or more expanded true leaves (ETL) have been shown to tolerate rates of up to 0.3 kg a.i. ha^{-1} clopyralid (Clay *et al.*, 1992), although transient epinasty sometimes occurs, particularly on pines (South, 2000). Metazachlor at 0.27 kg a.i. ha^{-1} applied post-sowing and thereafter at 4-week intervals proved to be very damaging to emerging conifer and broadleaved seedlings (Williamson *et al.*, 1990), although there are reports of it being successfully used at similar rates by commercial conifer nurseries in the UK (J. Morgan, personal communication). Our work was therefore designed to gather more evidence on the tolerance of young seedlings of five broadleaved species to the potentially selective post-emergence seed-bed herbicides, clopyralid, cycloxydim and metazachlor.

Materials and methods

The experiment was located at Headley Research Nursery, UK (51° 08′ N, 1° 51′ W, UK grid ref SU808379), a site which receives an annual average of 804 mm of rainfall and 1798 growing degree days above 4°C. Soil type according to Mackney *et al.* (1983) is a humic–ferric podzol, Shirrell Heath 1 series, and a pH of 5.5 was maintained by liming before planting. A randomized block design was used, with each species treated separately. For each species (see below for details), three 30 × 1 m seed-beds were prepared, with each seed-bed forming a block. Individual 2 × 1 m treatment plots were then laid out, with a 1-m buffer left between plots. Hence each block comprised 10 treatment plots of one species, and there were 30 plots in total per species. Seed-beds were sterilized using methyl bromide (Fumyl-o-gas; 99.7 per cent w/w methyl bromide with amyl acetate; Brian Jones Ltd, Caxton Hill, Hertford, Herts). A base dressing of 475 kg ha^{-1} 0 : 24 : 24 (N : P_2O_5 : K_2O) straight fertilizer was applied before sowing and three top dressings of 150 kg ha^{-1} 1 : 1 : 1 fertilizer applied in June, July and August, followed by 6 mm of irrigation applied over 2 h if no rainfall occurred within 24 h of the fertilizer dressing. Seeds of *Querus robur* L. (pedunculate oak), *Acer pseudoplatanus* L. (sycamore), *Prunus avium* L. (wild cherry), *Fagus sylvatica* L. (beech) and *Fraxinus excelsior* L. (ash), pretreated as necessary to break dormancy (Gordon and Rowe, 1982), were sown in drills at a depth of 3 cm and a rate of 50 viable seeds per plot, on 9 to 11 April 2002. Seeds were sown in five rows, 15 cm apart, with 10 cm between the seeds. Seed-beds were then tamped, gritted, netted against birds and subsequently kept weed free by hand weeding. Plots were subject to 6 mm of irrigation when soil moisture tension at 20 cm depth fell to 50 kPa, as indicated by tensiometers, to prevent moisture stress developing in seedlings.

The four herbicide treatments used were 0.2 kg a.i. ha^{-1} clopyralid (Dow Shield; 200 g l^{-1} clopyralid; DowAgroSciences, Hitchin, Herts), 0.45 kg a.i. ha^{-1} cycloxydim (Laser; 200 g l^{-1} cycloxydim; BASF plc, Cheadle Hulme, Cheshire.) with the addition of the adjuvant Actipron (97 per cent highly refined mineral oil; Joseph Batsons Co Ltd, Tipton, West Midlands) at 0.8 per cent of the spray solution, 1.25 kg a.i. ha^{-1} metazachlor (Butisan S; 500 g l^{-1} metazachlor; BASF plc.) and a mixture of 0.2 kg a.i. ha^{-1} clopyralid, 0.45 kg a.i. ha^{-1} cycloxydim and 1.25 kg a.i. ha^{-1} metazachlor. Treatments were applied using an Oxford precision sprayer with an Allman No. 00 nozzle, giving an output of 510 ml min^{-1} at a pressure of 150 kPa and a volume rate of 300 l ha^{-1}. For each species, applications were made at two seedling growth stages, S1 and S2 (Table 1). However, due to variable germination and prevailing weather conditions, there was some variation between species in the actual number of Extended True Leaves (ETL) present at the date of spraying – see Table 1 for details. Heavy rainfall occurred within 1 h of the initial S1 application to *A. pseudoplatanus*, *P. avium* and *F. sylvatica*, and due to the risk that this had caused the herbicides to be washed from the seedling leaves prior to adsorption, this treatment was subsequently repeated. There were three replicates (blocks) of the five treatments (four herbicides and one control) applied at two dates, giving 30 plots in total, for each of the five species. Operations were carried out according to Good Experimental Practice (PSD, 2000).

Seedling numbers were recorded for each plot immediately prior to spraying and at the end of the growing season. In addition, at the end of the growing season, height and root collar diameter were assessed for a maximum of 20 randomly selected seedlings per plot, effectively giving the first-year growth increment of the seedlings. The height and root collar diameter data were $\log_e$ transformed to satisfy the assumptions of analysis of variance, which was carried using Genstat (1993). The significance of treatment differences was evaluated for the herbicide effect, the plant growth stage effect and the interaction between the two. A least significant difference test was then carried out, again using Genstat. Fisher's exact test was used to analyse survival (Genstat, 1993), as few plots suffered mortality, and on those that did there was generally a low number of dead trees, which meant that analysis using a more elaborate parametric modelling approach, such as generalized linear modelling, was inappropriate.

Table 1: Treatment dates and approximate numbers of Extended True Leaves present on each species of seedling for S1 and S2 growth stages

	F. sylvatica		*A. pseudoplatanus*		*P. avium*		*Q. robur*		*F. excelsior*	
Growth stage	Spray date	ETL	Spray date	ETL	Spray date	ETL	Spray date	ETL	Spray date	ETL
S1	21 May (rain)	2	21 May (rain)	2	21 May (rain)	4	20 June	6	6 June	2
S1 repeat treatment due to rain	6 June	2	6 June	4	6 June	6	–	–	–	–
S2	20 June	4	20 June	6	20 June	8	4 July	8	20 June	4

Table 2: Mean height, root collar diameter and % survival values for *F. excelsior* seedlings treated at growth stages S1 and S2

		Mean height increment* (cm)		Mean root collar diameter increment* (mm)		Mean % survival†	
		S1	S2	S1	S2	S1	S2
	Rate (kg a.i. ha^{-1})	Two ETL	Four ETL	Two ETL	Four ETL	Two ETL	Four ETL
Metazachlor	1.25	21.2 (3.00)	22.1 (3.07)	8.28 (2.11)	8.43 (2.13)	100, a	100, a
Cycloxydim	0.45	22.7 (3.12)	29.8 (3.39)	8.73 (2.17)	9.83 (2.29)	100, a	100, a
Clopyralid	0.2	18.7 (2.88)	20.5 (3.00)	8.51 (2.14)	8.84 (2.18)	100, a	100, a
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	19.2 (2.95)	23.1 (3.13)	8.81 (2.18)	9.11 (2.21)	100, a	100, a
Untreated control		20.5 (3.01)	25.5 (3.20)	8.49 (2.14)	9.16 (2.21)	96.1, a	100, a
s.e.d.: interaction ($\log_e$)		0.185		0.068		–	
Residual d.f.		18		18		–	
l.s.d.: interaction ($\log_e$)		0.389		0.143		–	
P: herbicide effect		0.213		0.285		–	
P: growth stage effect		0.055		0.066		–	
P: herbicide × growth stage interaction		0.961		0.852		–	

l.s.d. = least significant difference, s.e.d = standard error of difference of means.
* Analysis carried out on natural log-transformed data (shown in brackets), s.e.d.s, l.s.d.s and *P*-values based on the log-transformed data.
† Survival analysis carried out using Fisher's exact test, where a = not significantly ($P > 0.05$) different from 100% survival, b = significantly different from 100% survival.

Results

F. excelsior

None of the treatments had any significant effect on survival or growth of *F. excelsior* (Table 2).

F. sylvatica

None of the treatments had any significant effect on the survival of *F. sylvatica* (Table 3). However, metazachlor significantly ($P \leq 0.05$) reduced both height and stem diameter increments of S1

Table 3: Mean height, root collar diameter and % survival values for *F. sylvatica* seedlings treated at growth stages S1 and S2

	Rate (kg a.i. ha^{-1})	Mean height increment* (cm)		Mean root collar diameter increment* (mm)		Mean % survival†	
		S1‡	S2	S1‡	S2	S1‡	S2
		Two ETL	Four ETL	Two ETL	Four ETL	Two ETL	Four ETL
Metazachlor	1.25	18.2 (2.90)	28.2 (3.34)	4.66 (1.54)	6.19 (1.82)	98.3, a	100, a
Cycloxydim	0.45	39.6 (3.67)	33.9 (3.52)	8.07 (2.09)	7.27 (1.98)	100, a	100, a
Clopyralid	0.2	11.7 (2.46)	16.6 (2.81)	3.88 (1.36)	4.59 (1.52)	100, a	100, a
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	17.7 (2.85)	26.8 (3.28)	4.40 (1.48)	5.72 (1.74)	97.9, a	100, a
Untreated control		29.9 (3.39)	32.7 (3.46)	6.72 (1.91)	6.92 (1.74)	100, a	100, a
s.e.d.: interaction ($\log_e$)		0.134		0.097		–	
Residual d.f.		18		18		–	
l.s.d.: interaction ($\log_e$)		0.282		0.203		–	
P: herbicide effect		<0.001		<0.001		–	
P: growth stage effect		0.001		0.010		–	
P: herbicide × growth stage interaction		0.021		0.046		–	

l.s.d. = least significant difference, s.e.d = standard error of difference of means.
* Analysis carried out on natural log-transformed data (shown in brackets), s.e.d.s, l.s.d.s and *P*-values based on the log-transformed data.
† Survival analysis carried out using Fisher's exact test, where a = not significantly ($P > 0.05$) different from 100% survival, b = significantly different from 100% survival.
‡ Repeat dose applied to S1 treatment due to rain.

stage seedlings by over 30 per cent; clopyralid significantly ($P \leq 0.05$) reduced height and diameter increment for both growth stages by 30–60 per cent and the mixture of metazachlor, clopyralid and cycloxydim significantly ($P \leq 0.05$) reduced S1 seedling height and diameter increment by ~40 per cent. There were significant ($P \leq 0.05$) herbicide × growth stage interactions for height and diameter increment, indicating that for treatments containing metazachlor or clopyralid, growth increment of the smaller S1 stage seedlings was suppressed more than in the larger S2 stage seedlings. There was also an indication ($P = 0.05$) that cycloxydim may have had a small positive effect on the height increment of S1 seedlings.

P. avium

Around 30 per cent of the S1 plants in the untreated control plots died, but there were few deaths in the herbicide treatment. When treatments are compared with the S2 control plants, which had 100 per cent survival, none of the herbicides had any significant negative effect on the survival of *P. avium* (Table 4). However, metazachlor significantly ($P \leq 0.05$) reduced height increment of S1 stage seedlings by ~40 per cent. There were significant ($P \leq 0.05$) herbicide × growth stage interactions for height and diameter increment, indicating that for treatments containing metazachlor, growth increment of the smaller S1 stage seedlings was suppressed more than in the larger S2 stage seedlings.

Q. robur

None of the treatments had any significant effect on the survival of *Q. robur* (Table 5). However, metazachlor significantly reduced height increment of S1 seedlings by ~20 per cent; and clopyralid, and the mixture of all three herbicides, significantly ($P \leq 0.05$) reduced both height and

Table 4: Mean height, root collar diameter and % survival values for *P. avium* seedlings treated at growth stages S1 and S2

	Rate	Mean height increment* (cm)		Mean root collar diameter increment* (mm)		Mean % survival†	
		S1‡	S2	S1‡	S2	S1‡	S2
	(kg a.i. ha^{-1})	Six ETL	Eight ETL	Six ETL	Eight ETL	Six ETL	Eight ETL
Metazachlor	1.25	47.0 (3.85)	80.7 (4.39)	11.29 (2.42)	13.56 (2.61)	94.8, a	100, a
Cycloxydim	0.45	92.7 (4.52)	95.2 (4.55)	14.35 (2.66)	14.51 (2.67)	100, a	100, a
Clopyralid	0.2	90.9 (4.50)	87.2 (4.47)	14.33 (2.66)	13.30 (2.59)	100, a	100, a
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	74.2 (4.30)	83.1 (4.41)	12.80 (2.55)	13.30 (2.59)	100, a	100, a
Untreated control		77.3 (4.34)	99.4 (4.60)	12.41 (2.52)	14.62 (2.68)	69.9 b	100, a
s.e.d.: interaction ($\log_e$)		0.099		0.056		–	
Residual d.f.		18		18		–	
l.s.d.: interaction ($\log_e$)		0.207		0.118		–	
P: herbicide effect		<0.001		0.014		–	
P: growth stage effect		<0.001		0.018		–	
P: herbicide × growth stage interaction		0.005		0.023		–	

l.s.d. = least significant difference.
* Analysis carried out on natural log-transformed data (shown in brackets), s.e.d.s, l.s.d.s and *P*-values based on the log-transformed data.
† Survival analysis carried out using Fisher's exact test, where a = not significantly ($P > 0.05$) different from 100% survival, b = significantly different from 100% survival.
‡ Repeat dose applied to S1 treatment due to rain.

stem diameter increments for both growth stages by 20–50 per cent.

A. pseudoplatanus

Similarly to *P. avium*, ~30 per cent of the S1 plants in the untreated control plots died, but when treatments are compared with the S2 control plants which had 100 per cent survival, none of the herbicides had any significant negative effect on the survival of *A. pseudoplatanus* (Table 6). However, metazachlor significantly ($P \leq 0.05$) reduced height and diameter increment of both growth stages by 10–70 per cent; clopyralid significantly ($P \leq 0.05$) reduced height and diameter increment of S1 stage seedlings by ~30 per cent and the mixture of all three herbicides significantly ($P \leq 0.05$) reduced S1 height increment by ~25 per cent. There were highly significant ($P \leq 0.001$) herbicide × growth stage interactions, because treatments containing metazachlor or clopyralid suppressed growth increment of the smaller S1 stage seedlings more than in the larger S2 stage seedlings.

Discussion

In general, the herbicides tested had only minor negative effects on trees, despite the fact that in our experiments, applications were made to very small seedlings. None of the herbicides had any effect on tree survival, and none of them caused any shoot die back, but several treatments did reduce overall seedling growth increment. Usually, both height and diameter increment was affected, although early applications of metazachlor only significantly reduced height increment. The three-way mix of metazachlor, cycloxydim and clopyralid appeared to be no more damaging than the three actives applied separately.

Table 5: Mean height, root collar diameter and % survival values for *Q. robur* seedlings treated at growth stages S1 and S2

	Rate ($kg\ a.i.\ ha^{-1}$)	Mean height increment* (cm)		Mean root collar diameter increment* (mm)		Mean % survival†	
		S1	S2	S1	S2	S1	S2
		Six ETL	Eight ETL	Six ETL	Eight ETL	Six ETL	Eight ETL
Metazachlor	1.25	19.7 (2.98)	22.1 (3.10)	6.99 (1.94)	6.58 (1.88)	100, a	100, a
Cycloxydim	0.45	25.5 (3.23)	26.6 (3.27)	7.03 (1.95)	7.16 (1.97)	95.2, a	100, a
Clopyralid	0.2	16.2 (2.78)	13.4 (2.58)	5.27 (1.66)	5.09 (1.63)	100, a	100, a
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	19.1 (2.94)	19.0 (2.94)	4.82 (1.57)	5.85 (1.77)	100, a	97.9, a
Untreated control		24.8 (3.21)	27.5 (3.31)	7.21 (1.98)	7.59 (2.03)	100, a	100, a
s.e.d.: interaction ($\log_e$)		0.104		0.092		–	
Residual d.f.		18		18		–	
l.s.d.: interaction ($\log_e$)		0.219		0.194		–	
P: herbicide effect		<0.001		<0.001		–	
P: growth stage effect		0.790		0.424		–	
P: herbicide × growth stage interaction		0.261		0.335		–	

l.s.d. = least significant difference, s.e.d = standard error of difference of means.
* Analysis carried out on natural log-transformed data (shown in brackets), s.e.d.s, l.s.d.s and *P*-values based on the log-transformed data.
† Survival analysis carried out using Fisher's exact test, where a = not significantly ($P > 0.05$) different from 100% survival, b = significantly different from 100% survival.

Smaller plants are often more susceptible to herbicide damage, at lower doses, than older, larger specimens (Zimdahl, 1999), although this is species and herbicide dependent (Kudsk, 2002). Generally, the smallest seedlings in our work appeared to be no more vulnerable to reductions in survival than those sprayed at a later growth stage. Although those treatments containing metazachlor and clopyralid caused more growth suppression with the younger seedlings of *F. sylvatica*, *A. pseudoplatanus* and *P. avium* (in the latter case for metazachlor only), this may not have been solely due to the size of seedlings at the time of treatment. The earliest growth stages of *F. sylvatica*, *A. pseudoplatanus* and *P. avium* had been subject to repeat treatment, due to heavy rainfall (9 mm in total) commencing within 1 h of the original spraying. Metazachlor is adsorbed primarily through roots. Clopyralid can also be adsorbed through roots, and post-spraying irrigation in closed drainage systems has been shown to cause severe damage to otherwise tolerant tree species (Clay *et al*., 1996). It is therefore possible that rather than reducing uptake of the herbicides, as would normally be expected for exclusively foliar-acting herbicides (Kudsk, 2002), the heavy rainfall after the initial application actually increased root uptake of metazachlor and clopyralid. Thus, rather than it being simply the result of inherent species or size differences, it is also possible that the combination of heavy rainfall washing root-acting herbicides into the soil along with a repeat application 2 weeks later, may account for the greater growth-increment suppression in the smallest seedlings of *F. sylvatica*, *A. pseudoplatanus* and *P. avium*.

The lower survival of the untreated *A. pseudoplatanus* and *P. avium* seedlings at the earliest growth stage may possibly reflect damage caused to seedling roots by hand-weeding operations. Less hand weeding was required in the herbicide-treated plots, and hence there is a reduced potential for damage this means.

Table 6: Mean height, root collar diameter and % survival values for *A. pseudoplatanus* seedlings treated at growth stages S1 and S2

	Rate (kg a.i. ha^{-1})	Mean height increment* (cm)		Mean root collar diameter increment* (mm)		Mean % survival†	
		S1‡	S2	S1‡	S2	S1‡	S2
		Four ETL	Six ETL	Four ETL	Six ETL	Four ETL	Six ETL
Metazachlor	1.25	9.8 (2.28)	33.7 (3.51)	4.22 (1.44)	8.09 (2.09)	95.0, a	100, a
Cycloxydim	0.45	43.2 (3.76)	44.9 (3.80)	9.19 (2.22)	9.68 (2.27)	98.3, a	100, a
Clopyralid	0.2	28.5 (3.35)	41.5 (3.72)	7.82 (2.06)	9.56 (2.26)	98.3, a	98.3, a
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	33.0 (3.48)	38.7 (3.65)	8.52 (2.14)	8.89 (2.18)	98.3, a	100, a
Untreated control		44.8 (3.80)	42.1 (3.74)	9.62 (2.26)	9.23 (2.22)	65.1, b	100, a
s.e.d.: interaction ($\log_e$)		0.069		0.045		–	
Residual d.f.		18		18		–	
l.s.d.: interaction ($\log_e$)		0.145		0.095		–	
P: herbicide effect		<0.001		<0.001		–	
P: growth stage effect		<0.001		<0.001		–	
P: herbicide × growth stage interaction		<0.001		<0.001		–	

l.s.d. = least significant difference.
* Analysis carried out on natural log-transformed data (shown in brackets), s.e.d.s, l.s.d.s and *P*-values based on the log-transformed data.
† Survival analysis carried out using Fisher's exact test, where a = not significantly ($P > 0.05$) different from 100% survival, b = significantly different from 100% survival.
‡ Repeat dose applied to S1 treatment due to rain.

Based on the results of our work, Table 7 gives an indication of potential seedling tolerance at different growth stages for the species and herbicides tested. This implies that for first-year nursery seed-beds or direct sown crops, once the effects of sterilization or post-sowing residual herbicides start to diminish, cycloxydim can be safely used to control competing susceptible grass species among young seedlings of *F. excelsior*, *F. sylvatica*, *A. pseudoplatanus*, *P. avium* and *Q. robur*. Clopyralid appears to be safe to use for controlling established herbaceous weeds, and metazachlor seedling or germinating weeds, among seedling *F. excelsior* and *P. avium*, but may suppress the growth of *Q. robur*, *A. pseudoplatanus and F. sylvatica*, particularly with younger seedlings and where heavy rainfall follows applications made to sandy soils or where double dose applications are made. Where the intention is to control mixed weed populations, mixtures of all three herbicides appear to be no more damaging than separate applications. Cycloxydim, clopyralid and metazachlor all have UK Department for Environment, Food and Rural Affairs (DEFRA) approval for use in forest nurseries or farm woodlands (Whitehead, 2005), and guidelines for their effective use in direct sown woodlands have recently been produced (Willoughby *et al.*, 2004a). Repeat experiments across a variety of sites, on heavier textured soils and in different years and climates would be required to be able to give unequivocal recommendations on crop safety. Therefore, the categories adopted in Table 7 are more conservative than those usually used for efficacy recommendations (PSD, 2003).

In practice, for herbicide treatment to be worthwhile, growers must make their own judgement as to whether the anticipated growth suppression from weed competition will outweigh

Table 7: Indicative potential tolerance of tree seedlings to metazachlor, cycloxydim and clopyralid

	Rate (kg a.i. ha^{-1})	Equivalent l ha^{-1} product†	*F. excelsior*		*F. sylvatica*		*P. avium*		*Q. robur*		*A. pseudo-platanus*	
			Two ETL	Four ETL	Two ETL	Four ETL	Six ETL	Eight ETL	Six ETL	Eight ETL	Four ETL	Six ETL
Metazachlor	1.25	2.5	R	MR	MS*,‡	MR	MS*,‡	MR	MR*	MR	MS*,‡	MR*
Cycloxydim	0.45	2.25	R	R	R‡	R	R‡	R	R	R	R‡	R
Clopyralid	0.2	1.0	MR	MR	MS*,‡	MS*	R‡	MR	MS*	MS*	MS*,‡	R
Metazachlor + cycloxydim + clopyralid	1.25 + 0.45 + 0.2	2.5 + 2.25 + 1.0	MR	MR	MS*,‡	MR	R‡	MR	MS*	MS*	MS*,‡	R

R = resistant: <5% reduction in survival, and <10% reduction in growth increment, compared with the untreated control. MR = moderately resistant: <10% reduction in survival, and 11–25% reduction in growth increment, compared with the untreated control. MS = moderately susceptible: <10% reduction in survival, and >26% reduction in growth increment, compared with the untreated control. S = susceptible: >10% reduction in survival, compared with the untreated control.
* Significantly different from the control treatment using least significant difference test at $P \leq 0.05$.
† Equivalent product rates per hectare are given for Dow Shield (200 g l^{-1} clopyralid), Laser (200 g l^{-1} cycloxydim) and Butisan S (500 g l^{-1} metazachlor).
‡ Based on a repeat dose applied 2 weeks after initial treatment.

the possible reduction in growth increment that may result from spraying. However, if the survival of tree seedlings is thought to be threatened by an invasion of susceptible weed species, then the herbicides tested clearly offer a potential means of reducing weed competition with relatively little risk of tree death. By contrast, this work has also shown that hand weeding may not always be a completely risk-free alternative.

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