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Results from six *Pinus taeda* nursery trials with the herbicide pendimethalin in the USA

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Pendimethalin is used by some nursery managers to control weeds in *Eucalyptu*s and *Pinus* seedbeds and cutting beds of *Pinus*. Six trials were implemented in open-rooted seedbeds to test the response of *Pinus taeda* to postemergence (to the crop) applications of 2.2 kg ha^{-1} active ingredient of pendimethalin (the formulation contained 455 g l^{-1}). No stunting was noticed when treating seedlings with 2.2 kg ha^{-1} four to 10 weeks after sowing. In one study, treated seedlings were larger than those not treated. Although the 2.2 kg ha^{-1} rate provided good control of the prostrate weed *Chamaesyce maculata*, pine seedlings developed herbicide galls on the stem near the groundline. The frequency of herbicide galls at 2.2 kg ha^{-1} varied by study and ranged from 0 to 28%. At present, it is not known if the frequency of gall formation depends on environmental or genetic differences.

Keywords: dinitroaniline, herbicide galls, loblolly pine, nursery, phytotoxicity

Introduction

To ensure effective weed control in open-root nurseries, it is important to have effective alternatives in case preferred herbicides or soil fumigants are withdrawn from the market. It may also be important not to rely too much on one family of herbicides, especially if weeding is lax and weeds are allowed to reproduce prior to removal. For example, after the introduction of effective diphenylether herbicides, weed populations in seedbeds declined and this allowed more sunlight to reach prostrate annual weeds such as *Chamaesyce maculata* (prostrate spurge). This weed is now considered troublesome at several nurseries that rely heavily on controlling annual weeds with diphenylether herbicides.

Pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) is a selective herbicide in the dinitroaniline family of herbicides with some activity on spurge (Gallitano and Skroch 1993). The chemical is absorbed through the roots and leaves of susceptible plants during germination and inhibits cell division and cell elongation. It has activity on several grasses and broadleaf weeds and is used in container nurseries in the USA (Riley et al. 1994) and in forest plantations (Woeste et al. 2005, Willoughby et al. 2009). An emulsifiable concentrate formulation of pendimethalin is labelled for use in forest tree nurseries in Australia, New Zealand and the United Kingdom. One label in New Zealand indicates that *Pinus radiata* may be treated 6–8 weeks after crop emergence with 0.99–1.3 kg ha^{-1} (n.b. all herbicide rates reported in this paper involve only the active ingredient). However, this label also indicates that seedbeds should be treated only when seed are covered with at least 0.3 cm of soil. Other species labelled for use in New Zealand include *Eucalyptus* (*E. botryoides*, *E. fastigata*, *E. globulus* and *E. saligna*), *Acacia* (*A. decurrens, A. mearnsii and A. dealbata) and Cupressus macrocarpa.*

Prostrate spurge (Euphorbiaceae) is a summer annual, native to North America (Krueger and Shaner 1982), but can now be found in a number of countries including Australia, Japan, Germany and Portugal. It can be a resilient weed in both container (Gallitano and Skroch 1993) and open-rooted nurseries and in some situations populations are high enough to stunt seedling growth. Oxyfluorfen (2-chloro-α,α,α-trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether) provides some level of preemergence control (Ruter and Glaze 1992) but once spurge germinates and becomes established it is difficult to control with postemergence applications of oxyfluorfen. Spurge not controlled by oxyfluorfen can produce copious amounts of seed. Some labels for metsulfuron methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) include prostrate spurge but stunting can result if too much of this herbicide is applied, especially when seedlings are less than eight weeks old. Nursery managers need a cost-effective method of controlling prostrate spurge without stunting seedlings. The objective of this research was to determine if pendimethalin could be used in pine nurseries without injuring seedlings.

Materials and methods

During 2007 and 2008, herbicide experiments were conducted at four forest nurseries in three states (Table 1). Herbicide plots (1.5 m × 3 m) were arranged in a randomised

Table 1: Selected soil properties at four nurseries (soil depth 15 cm), sowing, application and lifting dates, and rainfall from 1 April to 31 November

Site factor	Camden		Elberta	Jesup	Trenton
	2007	2008	2008	2008	2008
State	Alabama	Alabama	Alabama	Georgia	South Carolina
Latitude	32°04′ N	32°04′	30°27′ N	31°30′ N	33°44′ N
Longitude	87°20′ W	87°20′	87°31′ W	81°52′ W	81°49′ W
Sand (%)	78	66	72	77	80
Silt (%)	14	17	14	15	11
Clay (%)	8	17	14	8	9
Organic matter (%)	1.3	1.0	1.5	2.3	1.5
pH (H_2O)	5.1	4.9	5.2	5.2	5.1
Poylemer	Agrilock®	Agrilock®	Agrilock®	Agrilock®	Agrilock®
Mulch	None	None	None	Bark	None
Sowing date	9 April	24 April	30 April	19 April	16 April
Years since fumigation	3	3	0	0	0
First herbicide application	10 May	22 May	16 July	28 May	19 May
Second herbicide application	–	19 June	13 August	1 July	24 June
Lifting date	26 September	23 September	22 November	18 November	15 October
Rainfall (mm)	480	668	1 045	948	396

complete block design with five replications of seven treatments. An exception was at the Trenton Nursery in South Carolina where a supplemental rate study (Trenton–rate) involved eight treatments with two replications. In all the studies, stratified pine seed were sown in April and the soil was treated with a polymer to reduce soil erosion (Carlson et al. 1987). The genotype (i.e half-sib family) varied by nursery. Operationally, managers treated soil with an application of oxyfluorfen (typically at 0.56 kg ha^{-1}) and they irrigated beds to enhance germination. The herbicide treatments were applied at least four weeks after sowing (Table 1) after most of the pines had germinated. Herbicides tested varied but this document deals only with tests that involved pendimethalin. In four trials, a second application of 2.2 kg ha^{-1} was made to designated plots. The formulation involved microcapsules, suspended in an aqueous carrier, with 455 g l^{-1} of pendimethalin. An appropriate amount of the commercial product (Pendulum® AquaCap; BASF, Research Triangle Park, USA) was mixed in 3.8 l water and the solution was applied with a CO_2-powered sprayer. Seedlings were fertilised with nitrogen and were irrigated according to the nursery manager's preference and varied due to rainfall events and soil moisture.

Weed populations at three nurseries were low (i.e. almost weed free) due, in part, to sowing on recently fumigated soil (Table 1). However, at the Camden Nursery, it had been three years since the last soil fumigation and spurge was present in sufficient numbers to evaluate herbicide efficacy. Percentage weed cover was estimated using a score of zero to 100 (where 85 = 85% of the soil surface was covered with spurge). Cover estimates were made on 26 June 2007 and, the following year, number of spurge plants per sample plot (0.9 m^2) was recorded on 19 June 2008. On that date, weed cover was less than 5% on all plots.

Seedling tolerance to the herbicides was evaluated in the autumn. To determine seedbed density, seedlings from the plot-centre were sampled using a metal frame that encompassed 0.37 m^2 of seedbed. The number of seedlings, greater than 2 mm in diameter (at the root-collar), was recorded and seedlings were then lifted from soil using shovels. Seedlings were transported to Auburn University and 25 seedlings per plot were measured for height and diameter at the root-collar. In 2008, 25 seedlings per plot were examined for signs of swellings. Stems were cut at the root-collar and shoots and roots were placed in a drying oven at 70 °C. After 7 d of drying, samples were removed from the oven and the biomass recorded. A root mass ratio (RMR) was determined by dividing the root mass by seedling mass (i.e. g root/g plant).

Statistical analyses

Data were analysed with analysis of variance with replication as the blocking term and herbicide treatment as a fixed factor using the following linear model:

$$y_{ijk} = \mu + \text{rep}_i + \text{treatment}_j + \varepsilon_{ijk}$$

where y_{ijk} is the parameter of interest, μ = overall mean, rep_i = ith rep effect, i = 1,…5, treatment_j = jth fixed treatment effect, j = 1,…7 or 8, ε_{ijk} = random error associated with the ith rep, jth treatment and the kth tree, and where $\varepsilon_{ijk} \sim$ iid (0, σ^2).

Differences between treatments and controls were tested using contrasts (Mize and Schultz 1985, Saville 2003). The null hypothesis was rejected when p-values for contrast tests were significant ($\alpha = 0.05$). Least significant difference (LSD) values ($\alpha = 0.05$) were determined and provide the reader with an indication of the power of each statistical test.

Results

Treating emerged seedlings with pendimethalin did not reduce seedbed density (Table 2). Overall, average seedling density was the same (195 m^{-2}) for both control plots and for plots treated with 2.2 kg ha^{-1} (Table 3). For the sequential application (2.2 + 2.2 kg ha^{-1}), average stocking was 189 m^{-2} while stocking for control plots was 191 m^{-2}.

Table 2: Analysis of variance for *Pinus taeda* seedlings as affected by herbicides. RCD = root-collar diameter, RMR = root mass ratio

Site	Factor	*v*	Prob > *F*					
			Density	RCD	Height	Shoot	Root	RMR
Camden (2007)	Replication	4	0.0210	0.5356	0.2226	0.7333	0.1356	0.8964
	Treatment	6	0.2465	0.0001	0.0001	0.0215	0.0001	0.3827
	0 vs 1.1 kg	(1)	0.4749	0.0001	0.0001	0.0005	0.0001	0.1256
	0 vs 2.2 kg	(1)	0.5352	0.0119	0.0059	0.0333	0.0029	0.8569
	Error	28						
Camden (2008)	Replication	4	0.0452	0.8078	0.0352	0.2881	0.1127	0.1636
	Treatment	6	0.2468	0.0112	0.0003	0.0265	0.0917	0.1412
	0 vs 2.2 kg	(1)	0.8595	0.1526	0.1372	0.1408	0.9870	0.0086
	0 vs 2×	(1)	0.4288	0.0057	0.0512	0.0148	0.2579	0.0259
	Error	24						
Elberta	Replication	4	0.3857	0.3085	0.2072	0.6233	0.1794	0.0330
	Treatment	6	0.9340	0.2312	0.3417	0.4884	0.6325	0.2547
	0 vs 2.2 kg	(1)	0.7115	0.6402	0.0308	0.1004	0.5300	0.0333
	0 vs 2×	(1)	0.9018	0.1466	0.4287	0.8366	0.3422	0.0237
	Error	24						
Jesup	Replication	4	0.2827	0.0079	0.0427	0.0339	0.1549	0.3735
	Treatment	6	0.0018	0.2154	0.0617	0.8531	0.4486	0.0168
	0 vs 2.2 kg	(1)	0.3789	0.2771	0.0560	0.6097	0.6292	0.2373
	0 vs 2×	(1)	0.3300	0.5051	0.6983	0.8121	0.4828	0.4736
	Error	22						
Trenton	Replication	4	0.0143	0.0238	0.0095	0.2532	0.0852	0.7709
	Treatment	6	0.0603	0.0001	0.0315	0.1519	0.0104	0.6461
	0 vs 2.2 kg	(1)	0.5743	0.0001	0.0795	0.3028	0.0973	0.9443
	0 vs 2×	(1)	0.4143	0.0001	0.0833	0.0871	0.2063	0.2628
	Error	24						
Trenton (rate)	Replication	1	0.8101	0.5008	0.1555	0.7399	0.7399	0.4919
	Treatment	7	0.0442	0.0577	0.2457	0.1857	0.1857	0.0798
	0 vs 2.2 kg	(1)	0.6342	0.9607	0.9264	0.0460	0.0631	0.3858
	0 vs 4.5 kg	(1)	0.1347	0.4374	0.6322	0.3858	0.6211	0.5602
	0 vs 6.7 kg	(1)	0.4005	0.7793	0.5239	0.5602	0.7837	0.8035
	Error	7						

2× = two applications of pendimethalin at 2.2 kg ha^{-1} per application

At the Camden (2007) and Jesup Nurseries, seedling height growth increased after a single application of pendimethalin at a rate of 2.2 kg ha^{-1} (Table 3). In contrast, seedling height was decreased by the herbicide application at the Elberta Nursery.

Applying either a single application of 2.2 kg ha^{-1} or sequential treatments of pendimethalin did not reduce either shoot or root mass (Tables 2 and 3). The 2.2 kg ha^{-1} treatment increased shoot mass at the Camden Nursery (both years) and at the Trenton Nursery rate test in 2008. The root mass ratio was increased by both treatments at the Camden and Elberta nurseries in 2008.

Seedling diameter was not affected by pendimethalin at the Elberta and Jesup nurseries. However, the herbicide treatments increased root-collar diameter at the Camden Nursery (both years) and at the Trenton Nursery (Table 2).

For both years, pendimethalin at 2.2 kg ha^{-1} suppressed the germination of spurge at the Camden Nursery. In 2007, weed cover was 95% for the controls, and 87% and 46% for plots treated with 1.1 and 2.2 kg ha^{-1}, respectively (contrasts; $P>F$ = 0.0080 and 0.0001, respectively; $LSD_{0.05}$ = 8.9). In 2008, the number of spurge plants per sample plot were 21.4 and 0.1 for plots treated with 0 and 2.2 kg ha^{-1}, respectively (contrast $P>F$ = 0.0001; $LSD_{0.05}$ = 8.8).

Swellings on the stem were not noticed in the first study so no data on galls were collected in 2007. However, galls at or near the root-collar (Figure 1) occurred at all four nurseries in 2008 (Table 4). At two nurseries, the percentage of affected seedlings in treated plots treated with 2.2 kg ha^{-1} was less than 4% and not statistically different from zero ($P>F$ > 0.2). However, at two nurseries, treating with 2.2 kg ha^{-1} produced herbicide galls on more than 20% of the seedlings ($P>F$ < 0.01).

Discussion

Seedling tolerance

Three-needle pines appear tolerant of pendimethalin when applied either at sowing (South 1984, Lee et al. 1990, Vanner 1992, Peñuelas Rubira et al. 1995, Ortega et al. 2000) or soon after germination is complete. The height reduction observed at the Elberta Nursery might be a Type I statistical error (Snedecor and Cochran 1967) since average height for seedlings treated with the sequential treatment was similar to untreated seedlings. Even when treated with 6.7 kg ha^{-1}, seedlings at the Trenton Nursery were not significantly different from those in control plots (Table 2). However, herbicide galls can form at some nurseries if seedlings are treated soon after pines have germinated and have shed the seedcoat.

Seedlings treated with pendimethalin appeared greener than untreated seedlings. This is likely due to a physiological response from exposure to pendimethalin (Smith 2003). At the Jesup Nursery, there were few weeds present and seedlings were darker green about five weeks after treatment with pendimethalin.

Control of spurge

In the past, the population of spurge could be reduced by soil fumigation with methyl bromide. If nursery managers cease the use of methyl bromide fumigation, and if they allow spurge to produce seed (Ohnishi et al. 2008), the population of viable seed in the soil will increase. Spurge populations are typically low during the year after soil fumigation but can be relatively high during the third or fourth year after fumigation. Due to the growth habit and leaf structure, most selective herbicides provide ineffective control after spurge plants are 1 cm in diameter. Therefore, a few managers attempt to control emerged spurge plants by using directed applications of non-selective herbicides (Altland et al. 2002, Stallard 2005). Results from the Camden Nursery in 2008 indicate that emergence of spurge can be suppressed for several months if pendimethalin is applied before germination of spurge seed.

Covering spurge seed with only 2 cm of soil can reduce germination (Krueger and Shaner 1982). Mulching with either sawdust or pine bark is one way to reduce spurge germination. However, during rainfall events, these mulches may wash off seedbeds and expose soil to sunlight. With the adoption of soil stabilisers (Carlson et al. 1987) many managers no longer apply an organic mulch. Therefore, managers have been looking for an effective preemergence herbicide to control spurge.

Rainfall was well below average in 2007, with some areas of Alabama experiencing extreme drought. At the Camden Nursery, rainfall from 1 April to 31 November totalled 480 mm while the 30-year average (1951 to 1980) for these eight months was 886 mm. Spurge plants began to germinate in early May and some were present at time of treatment. These spurge plants were not greatly affected by the 10 May application, which may explain why weed cover in the 2.2 kg ha^{-1} treatment was 46% by June. A key to controlling spurge with pendimethalin is to apply the herbicide prior to germination of spurge seed. Even though spurge plants grew in plots treated with 1.1 kg ha^{-1}, the reduction in early weed competition was enough to improve both shoot and root growth of pine.

Rate

Crop injury depends on both the species and rate of pendimethalin applied. With *Taxus media*, stock treated with 2.2 kg ha^{-1} exhibited less foliar injury than seedlings treated with 9 kg ha^{-1} (McNiel et al. 1998). Injury to some hardwood species can occur from using the emulsifiable concentration formulation, even at rates as low as 0.6 kg ha^{-1} (Willoughby et al. 2007). Other species are relatively tolerant to pendimethalin, which explains why some managers use it to control weeds in hardwood seedbeds (Harmer 1999, South

Table 3: The effect of herbicide rate on seedling stocking (density), root-collar diameter (RCD), shoot height (height), shoot dry weight (shoot), root dry weight (root) and root mass ratio (RMR). Least significance difference (LSD; $\alpha = 0.05$) data are italicised

Site	Rate (kg ha^{-1})	Density (m^{-2})	RCD (mm)	Height (cm)	Shoot (g)	Root (g)	RMR
Camden (2007)	0	260	2.3	16.9	1.0	0.10	0.098
	1.1	266	2.8	20.0	1.6	0.17	0.100
	2.2	266	3.5	23.2	2.0	0.26	0.116
	LSD	*(13.8)*	*(0.36)*	*(2.14)*	*(0.54)*	*(0.04)*	*(0.023)*
Camden (2008)	0	218	2.8	19.9	1.4	0.16	0.103
	2.2	216	3.0	21.9	1.6	0.16	0.188
	2.2 + 2.2	206	3.2	22.6	1.8	0.18	0.190
	LSD	*(33.1)*	*(0.28)*	*(2.73)*	*(0.30)*	*(0.04)*	*(0.011)*
Elberta	0	181	4.6	28.8	3.9	0.37	0.087
	2.2	186	4.5	27.3	3.4	0.35	0.093
	2.2 + 2.2	182	4.8	28.3	3.8	0.39	0.094
	LSD	*(26.7)*	*(0.29)*	*(1.37)*	*(0.56)*	*(0.05)*	*(0.006)*
Jesup	0	198	4.9	29.1	4.0	0.75	0.158
	2.2	190	5.1	32.8	4.1	0.72	0.147
	2.2 + 2.2	190	5.1	30.2	4.0	0.72	0.152
	LSD	*(19.6)*	*(0.39)*	*(3.92)*	*(0.63)*	*(0.14)*	*(0.018)*
Trenton	0	168	4.1	20.5	3.1	0.51	0.144
	2.2	161	4.8	22.3	3.3	0.56	0.145
	2.2 + 2.2	178	4.9	22.3	3.5	0.55	0.135
	LSD	*(25.3)*	*(0.25)*	*(2.04)*	*(0.53)*	*(0.06)*	*(0.0166)*
Trenton (rate)	0	144	4.6	26.0	3.7	0.58	0.136
	2.2	151	4.6	25.9	4.1	0.62	0.133
	4.5	167	4.8	25.1	3.9	0.56	0.126
	6.7	132	4.5	24.8	3.6	0.49	0.120
	LSD	*(31.9)*	*(0.55)*	*(4.44)*	*(0.87)*	*(0.17)*	*(0.021)*

and Carey 2005). In the *Pinus taeda* studies, the 2.2 kg ha^{-1} rate was selected due to limited weed control observed at 1 kg ha^{-1} (South 1984). In New Zealand, pendimethalin (330 g l^{-1}; EC) is labelled for use on young *Pinus radiata* seedings (six to eight weeks after crop emergence) at 1.3 kg ha^{-1}. The rate tested for *Pinus taeda* (2.2 kg ha^{-1}) was about 70% higher. Although the New Zealand label states the herbicide should not be applied as the crop is emerging, the warning is likely due to the formulation containing 612 g l^{-1} chlorobenzene. The microencapsulated formulation does not contain this solvent.

Seedlings treated with sequential applications (a total of 4.5 kg ha^{-1}) exhibited no visual signs of stunting. When compared to a single treatment, the sequential applications increased the frequency of herbicide galls at the Jesup Nursery. At the Trenton Nursery, applying 4.5 kg ha^{-1} in a single treatment also increased frequency of herbicide galls (Table 4).

Figure 1: A herbicide gall produced by pendimethalin at the Jesup nursery

Herbicide galls

Certain dinitroaniline herbicides may cause swellings on the stem (Figure 1). 'Even in some established woody plants, certain preemergence herbicides can cause stems to enlarge and become brittle; examples include oryzalin in Monterey pine (*Pinus radiata*), and prodiamine in true firs (*Abies* spp.)' (Costello et al. 2003: 190). These trials are the first to demonstrate that pendimethalin can cause galls on *Pinus taeda* seedlings (when treatment is made four to 10 weeks after sowing). Galls near the root-collar have also been observed from using dinitroaniline herbicides on *Abies fraseri* (prodiamine), *Pseudotsuga menziesii* (oryzalin and prodiamine), *Pinus taeda* (prodiamine) and *Pinus radiata* (oryzalin and pendimethalin). Galls on hardwoods have occurred on *Acer rubrum* (pendimethalin) (Altland 2005) and *Celtis laevigata* (prodiamine plus pendimethalin).

Genotype

Tolerance to herbicides is under genetic control. Differences in genotype explain why some weeds develop herbicide resistance when selection pressure is provided by frequent use of triazine herbicides (Holliday and Putwain 1980). Within the genus *Pinus*, some species demonstrate a greater tolerance to herbicides than others (Kosinski and Holt 1985, Wood et al. 1993). Likewise, crop tolerance to pendimethalin is also demonstrated by differences in genus (Skroch et al. 1991, Haase and Rose 1998, Clay et al. 2006, Willoughby et al. 2007). For *Glycine max*, genetic differences can explain the amount of stem injury caused by pendimethalin (Glover and Schapaugh 2002). Reports from operational use of prodiamine and pendimethalin in hardwood seedbeds indicate *Celtis laevigata* responds by producing herbicide galls while no galls were observed on stems of *Quercus* spp. Within *Pinus taeda*, we know of no published reports of herbicide tolerance by genotype. We hypothesise that some genotypes of *Pinus taeda* are more prone to forming herbicide galls than others. Managers who intend to apply pendimethalin operationally to *Pinus taeda* should consider treating small lots first to determine if the genotypes they sow will form galls when exposed to this herbicide.

Table 4: The effect of herbicide rate on the frequency (%) of stem swellings on pine seedlings. Least significant difference (LSD; $\alpha = 0.05$) data are italicised

Pendimethalin (kg ha^{-1})	Camden[1]	Elberta	Jesup	Trenton	Trenton – rate
0	0	4	0	0	0
2.2 + 2.2	3	24	100	0	–
2.2	2	23	28	0	2
4.5	–	–	–	–	18
6.7	–	–	–	–	30
LSD	(*5*)	(*14*)	(*12*)	–	(*8*)
Prob > *F*	0.2193	0.0080	0.0001	–	0.0002

[1] Evaluation of stem swellings at Camden was made on 6 February 2009

Conclusions

When applied before weed germination, pendimethalin at 2.2 kg ha^{-1} can control a number of annual grasses and broadleaves. The ability to control grasses and prostrate spurge has resulted in the operational use in both pine and hardwood seedbeds. Pines appear relatively tolerant to preemergence applications and at four nurseries, no stunting was observed when 2.2 kg ha^{-1} was applied to newly germinated pines. In two trials, the root mass ratio was increased, which might improve seedling performance after transplanting. However, when treating with 2.2 kg ha^{-1} or more, *Pinus taeda* seedlings may develop herbicide galls near the root-collar. Future investigations are required to determine if differences among nurseries are due to a combination of genetics and environmental factors, or simply due to differences in the environment. Since the presence of herbicide galls on seedlings will likely affect customer satisfaction, managers should first treat a small number of plants at the recommended rate and should evaluate stems for the occurrence of galls about six months following treatment.

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