The potential of safeners and protectants to increase tolerance of tree seeds to pre-emergence herbicides

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SUMMARY: The application of pre-emergence herbicides is often necessary to achieve efficient establishment of trees in nurseries and direct-sown woodlands, but due to environmental and commercial pressures, and the current European Union review of herbicide registration, the number of herbicides available in the future will be reduced. Alternative approaches for finding effective treatments are needed. The utilisation of safeners and protectants to permit the use of otherwise phytotoxic herbicides has been investigated in many crops over the past 50 years. Some safening chemicals are currently used as seed coating treatments or included in herbicide products to achieve crop tolerance. The future potential of this approach for tree seed protection is reviewed in this paper. Activated charcoal has been the most commonly used protectant in the past, and its potential as a seed-coating treatment for preventing damage from subsequent pre-emergence herbicide application was tested in a trial using container grown plants. It gave effective protection of ash (*Fraxinus excelsior* L.), cherry (*Prunus avium* (L.) L.) and field maple (*Acer campestre* L.) from simazine damage, but there appeared to be little evidence for a protective action with napropamide or pendimethalin. Further research is therefore required before the use of activated charcoal as a protectant against herbicide damage to tree seed in nurseries and direct sown woodlands can be recommended more widely.

Introduction

Pre-emergence herbicides are regularly used in forest nursery seedbeds to reduce the risk of weed competition on small tree seedlings (Figure 1), (Aldhous and Mason, 1994). There is also interest in their use in woodland establishment with direct sown seed (Willoughby et al., 2003). However, the number of herbicides available for these minor crop uses is declining due to commercial decisions and the withdrawal of many products under the European Union pesticide review (Whitehead, 2003). There is therefore a need to investigate alternative approaches to allow the optimum use of the reducing number of cost-effective herbicides that may be available.

Soon after the introduction of herbicides for vegetation management in the 1940s, consideration was given to means of manipulating crops so that effective but otherwise damaging herbicides could be used on them. Three main methods have been used to date:

1. Alteration of the genetic make-up of sensitive crops to make them tolerant or resistant to a cost-effective broad-spectrum herbicide. This has been done for glyphosate and glufosinate for a few major crops and been adopted widely, particularly

Figure 1. Post sowing, pre-emergence herbicides are often used in nursery seedbeds.

on soya (*Glycine max* (L.) Merr.) and oil seed rape (*Brassica napus* ssp. *oleifera* (DC.)) (Kirkwood, 2002). Poplar (*Populus* sp.) species have been modified for glufosinate tolerance (Paques et al., 1995) but given the public apprehension over the introduction of genetically modified organisms, this solution is unlikely to be acceptable for use in forestry in the foreseeable future, and so this option is not considered further here.

2. Treating seed, soil or crop foliage with a chemical that alters the metabolism of the crop so that it can tolerate a herbicide application in mixture with the chemical or applied subsequently. Such chemicals are termed safeners or antidotes.

3. Applying products to seeds or roots to prevent a post-planting herbicide being taken up by the crop seedling or transplant during early growth thus achieving selective use of the herbicide. Such products are termed protectants or adsorbents.

The historical use of safeners and protectants, and their potential for use on tree seeds, are considered in more detail below.

Historical use of safeners

The possibility of safening crops to herbicides was first tested in the 1940s (Davies & Caseley, 1999) but the first chemical to offer the possibility of consistent protection of crop seed to herbicides, naphthalic anhydride, was not patented until 1971. When mixed with seeds of a range of monocotyledonous crops it gave protection against thiocarbamate herbicides. This use was commercialised for a time but withdrawn when more cost-effective chemicals were introduced as seed or soil treatments. More recently, chemicals that give protection to crops when applied in mixture with post-emergence herbicides have been developed for use in cereals (Whitehead, 2003).

Tests with naphthalic anhydride and a variety of other potential safeners have been made on dicotyledonous crops, mainly treating seed before planting and assessing response to subsequent pre-emergence herbicides, but no commercial uses have resulted. Safening effects on broad-leaved crops of other types of chemicals such as fungicides, insecticides and growth regulators have been reported (Phatak & Vavrina, 1989). But it may be fair to assume that these effects were never large, otherwise more work would have been carried out to exploit them. There may also be problems with safening treatments increasing weed tolerance to herbicides.

Potential of safeners for tree seeds

The commercially developed safeners are only available in the UK as product mixtures with herbicides, none of which are pre-emergence herbicides. Naphthalic anhydride is available as an industrial chemical, so experimental work on its potential for safening as a seed treatment is feasible. Turner et al. (1981) found that naphthalic anhydride seed treatment reduced damage from napropamide in some unspecified conifer species and this treatment may merit further investigation.

Historical use of protectants

This topic has been comprehensively reviewed by Hoagland (1989). Since the introduction of soil-acting herbicides it has been known that their toxicity to plants is affected by soil properties; doses required for weed control are normally higher on soils with a large clay fraction or high organic matter content. The adsorbent properties of charcoal for removing soil toxins to aid plant growth was recognised in 1916.

The use of activated charcoal for protecting seeds from herbicide injury was patented in 1945. Subsequently it was shown that charcoal applied to the soil or foliage could prevent toxicity of herbicides such as 2,4-D. Activated charcoal was found to be effective as a protectant when applied as a seed dressing, as a band to the soil around or above seeds or as a root dip for transplants. Pelleted seed containing activated charcoal was tested successfully with some crops (Gupta, 1976). Activated charcoal was effective in protecting crops from damage from many classes of soil-acting herbicides. Efficacy varied according to herbicide properties, since non-ionic herbicides were better adsorbed than ionic ones, and adsorptivity decreased with increasing water solubility. The efficacy of activated charcoal as a protectant results from strong adsorption on the very large surface area of the material; total surface area of commercially available activated charcoals are given as 500 to 1500 m^2g^{-1} (Gupta, 1976). Adsorption capacity depends on surface area but rate of adsorption on particle size. Subsequent desorption of herbicides after mixture with activated charcoal is reported with some herbicides, for example 2,4-D (Hoagland, 1989).

The amount of activated charcoal required to give protection varies with soil type; heavier soils and those with a higher organic matter content are in themselves more adsorptive so the amount of activated charcoal required to protect crops from residual herbicide injury on such soils is less. Where charcoal slurry has been sprayed as a band above sown crops, rates of 200 to 400kg ha^{-1} have been used to achieve protection from subsequent herbicide treatment.

There was much experimental work with activated charcoal for protection of sown vegetable crops and transplanted crops in the 1960s and 1970s and some commercial use. Activated charcoal was widely used commercially in the UK in the 1970s as a root dip for strawberry (*Fragaria ananassa* (Duchesne) Duchesne) runners prior to simazine application (Lovelidge, 1978). Doses of up to 2kg a.i. (active ingredient) ha^{-1} simazine were tolerated when applied after planting to runners whose roots had been dipped in a slurry of 1kg activated charcoal in 9 litres water. There are a few reports of successful use of the root dipping technique for tree transplants (Fitzgerald & Fortson, 1977). The protectant effect of activated charcoal plus vermiculite when used as a ‘plug mix’ for establishing seedlings was also tested (Swain 1980; Rubin et al. 1982). However, Hoagland (1989) reported that the only wide-scale commercial use was for protecting turf grass and nursery stock against various herbicide treatments. He attributed the decline in commercial use in other crops to the need for a specialised application method and equipment, the expense of the material and application costs. In addition, efficacy and safety were not always certain; weed roots could penetrate the charcoal layer and escape control or crop roots could grow outside the layer and take up toxic levels of herbicide.

More recently, Ramsey et al. (2004) reported on work examining the use of charcoal combined with polymer water thickeners as a tree seedling root dip. The treatment gave initial protection against damage from soil applications of hexazinone, but as roots started to outgrow the protective coating of charcoal the plants once against became susceptible to higher doses of the herbicide. As far as can be ascertained, there has been no commercial use of such techniques in the UK.

The major focus for the agronomic use of activated charcoal to date has been for the detoxification of herbicide residues in soil. Because of its adsorbent properties it has been shown that incorporation of activated charcoal into soil containing residues of herbicides such as atrazine locks up the herbicide and enables otherwise sensitive crops to be grown. Rates of activated charcoal needed to achieve this depend on the texture and organic matter content of the soil, but in many studies ratios of 200 to 400:1 w/w charcoal: residue have been effective (Hoagland, 1989).

Potential of protectants for tree seeds

There appear to be no reports in the literature of successful experimental or commercial use of activated charcoal for protection of tree seeds from subsequent residual herbicide applications.

Pellet material for most agricultural seeds is applied to dry, non-dormant seeds. Its role is usually to make seeds larger and rounder to facilitate handling, mechanical singling and sowing - sometimes the pellet material provides a carrier for pesticides, or plant growth regulators, but ultimately the pellet material is usually intended to fall away from the seed as soon as it is sown and irrigated. However, most tree seeds are dormant and need a moist dormancy breakage pre-treatment before sowing. Pellet material for tree seeds therefore needs to remain intact throughout the moist pre-treatment phase and only fall away from the seed after sowing and subsequent seed-bed irrigation. Consequently tree seed pellet material needs to be much more durable so that it can act like an additional 'coat-imposed dormancy'; this may compound the germination problems of these species, or damage moist seed if applied after pre-treatment or if attempts are made to dry seed after pre-treatment (P. Gosling, pers comm.). Therefore, any utilisation of activated charcoal as a seed dressing in forest nurseries or for direct seeding will probably necessitate hand treatment with powder prior to sowing. With winged species, this may actually allow a greater amount of the powder to be taken up on each seed, compared to pelletised, regularly shaped seed. The cost of activated charcoal itself for this treatment may not be prohibitive.

	Expected percentage germination from suppliers viability test	Seeds sown per trough	Total charcoal per seed (mg)
Ash	50	40	23
Cherry	75	30	16
Field maple	40	50	24

Table 1. Seed and seed coating details.

Experimental study of charcoal as a protectant

In view of the likely protective effect of activated charcoal for tree seeds against pre-emergence herbicide damage, a small experiment was carried out to assess the potential of the technique. It investigated the effects of three common pre-emergence nursery seedbed herbicides in nursery seedbeds (napropamide, pendimethalin and simazine) applied at three doses on the emergence and growth of three tree species (ash (*Fraxinus excelsior* L.), cherry and field maple (*Acer campestre* L.)) on uncoated seed, and on seed previously treated with activated charcoal.

Methods

On 20 February 2002, seeds of ash (Forestry Commission identity number 00(304)F), cherry (01(439)F) and field maple (99(439)F) were obtained from Forestart Ltd, Church Farm, Hadnall, Shropshire, UK. All seed had been suitably pre-treated to break dormancy (Jinks et al., 1995; Gordon and Rowe, 1982). Half of the seed was then chosen at random, then evenly moistened and coated with activated charcoal (CAS No. 7440-44-0, Draco G-60,-100 mesh powder (Sigma-Aldrich Company Ltd., The Old Brickyard, New Road, Gillingham, Dorset, UK)). The few wings remaining on the ash and maple seeds were not removed before treatment. Wet seeds of each species sufficient for all protectant treatments were placed in polythene bags, charcoal was added and the bags shaken until the seeds would not take up any more charcoal. Seed numbers per trough and coating details are presented in Table 1.

Seeds were sown on to the soil surface of rigid plastic troughs approximately 60cm x 15cm and 15cm deep (1/3 of the trough for each species); these contained moist compost consisting of four parts sterilised loam, two parts peat and one part Cornish grit, with Osmocote fertilizer (5–6 month duration) at 4.5g litre^{-1} and magnesium limestone at 2.7g litre^{-1} added. Seeds were covered with their own depth of compost and watered lightly overhead; there were equal quantities of troughs sown with seeds coated with charcoal and not coated. At the time of sowing the ash seeds were dormant; the cherry were mostly chitting with radicles showing and approximately 10% of the maple seeds were chitting.

Herbicide treatments of napropamide (Devrinol; 450g litre^{-1} Suspension Concentrate (United Phosphorus Ltd, Birchwood Park, Warrington, Cheshire, UK)) at 1.0, 3.0 and 6.0kg a.i. ha^{-1}, pendimethalin (Stomp 400 SC; 400g litre^{-1} Suspension Concentrate (BASF plc., P.O. Box 4, Earl Road, Cheadle Hulme, Cheadle, Cheshire, UK)) at 0.6, 2.0 and 4.0kg a.i. ha^{-1} and simazine (Gesatop; 500g litre^{-1} Suspension Concentrate (Syngenta Crop Protection UK Ltd., Whittlesford, Cambridge, UK)) at 0.25, 0.75 and 2.25kg a.i. ha^{-1} were sprayed with a laboratory track sprayer at 287 kPa in 440 litre ha^{-1} on 22 February 2002. Rates were based on low normal, high normal, and double highest normal application rate, as recommended by Willoughby et al. (2004)

Figure 2. Photo of experiment taken 4 months after sowing.

and Williamson & Morgan (1994). There were three replicates of each herbicide / charcoal and control treatment, giving 72 troughs (plots) in total per species, of which 18 plots were control treatments (9 with, and 9 without charcoal), After spraying troughs were set out on sand beds outdoors, arranged in a fully randomised design (Figure 2).

Plant health was assessed visually 2 and 3 months after treatment using a score on a continuous scale from 0 – 7, where 0 = dead, 4 = 50% growth reduction compared with best untreated and 7 = as best untreated, and plant number and shoot fresh weights were recorded in June. Data were subject to analysis of variance using Genstat (Genstat 5 Committee, 1993), and s.e.d.'s generated. All results quoted as significant are at the p=0.05 level.

Results

The amounts of charcoal held by seeds did not differ widely being 16, 24 and 23mg per seed for cherry, field maple and ash respectively (Table 1). Emergence of all species with or without charcoal was very good on untreated troughs giving an even cover of seedlings.

Charcoal had the greatest protectant effect with simazine (Table 2, Figure 3). With uncoated seed, the highest dose of simazine caused death or severe damage to all species. However, when seed was coated with charcoal, only the highest dose of simazine on cherry had any damaging effects, where it significantly reduced plant health. Some protectant effects were also evident with the middle dose of simazine.

Differences between charcoal coated and uncoated seed were less evident with the other herbicides. Napropamide caused some plant health and growth reduction at the highest dose on ash, cherry and maple, but there was generally no significant difference in damage with charcoal treated seeds.

The highest dose of pendimethalin reduced plant health and growth of maple, the highest and middle doses reduced health and growth of cherry, and the highest dose reduced plant numbers in ash. However, coating seed with charcoal had no consistent significant effect on damage.

Discussion

The amounts of charcoal taken up by the seeds of the three species did not differ markedly (Table 1) and the results showed that there was sufficient to have a protectant effect. There was somewhat less on the cherry, an effect that was anticipated given its smooth seed surface compared with the rough surfaces of the other species. Since a ratio of 200 to 400 parts charcoal to 1 part herbicide is often required for inactivation in soil (Hoagland, 1989) the 20mg coating each seed received should have been sufficient to adsorb the amount of herbicide applied above it, say, 0.02mg cm^{-1} of active ingredient from a dose of 2kg ha^{-1}.

The response of the three tree species to pre-emergence herbicides is similar to that found in earlier experiments on container-grown plants (Willoughby et al. 2003), simazine being the most phytotoxic and napropamide the least damaging herbicide at the doses used (Table 2). However, where the seeds had been coated with charcoal there was considerably less damage in terms of plant health and growth from simazine, indicating that coating tree seeds with charcoal prior to planting has potential for protecting seedlings from damage. Where pendimethalin and napropamide had caused damage, especially to cherry and maple, there appeared to be little evidence for a protective effect from the charcoal

Figure 3. Troughs sown with cherry, maple and ash seed and sprayed with simazine at 2.25kg a.i./ha. Front, no charcoal; back, seed mixed with charcoal pre-sowing. Photograph taken 4 months after sowing.

treatment. However, this apparent lack of effect may be due to the failure to induce substantial amounts of damage from applications of the herbicides napropamide and pendimethalin. Further applications at higher doses to more seeds would be required to confirm that safeners had no effect with these herbicides.

The experiments reported here add to the body of evidence suggesting that charcoal can be effective as a protectant against simazine for susceptible species in a range of crop situations (Hoagland, 1989). The apparent difference in the protective properties of charcoal between herbicides may be due in part to the lack of substantial damaging effects from the herbicides themselves, and in part to a difference in the mode of action in soil. Generally simazine is taken up into the plant through the roots, whereas pendimethalin and napropamide are taken up by the hypocotyl. Consequently it is likely that sufficient simazine was adsorbed by the activated charcoal

		22 April 02			12 June 02					
	Dose (kg a.i. ha^{-1})	Plant health score (0-7)			Shoot fresh weight (g per trough)			Number of plants per trough		
Treatment		**Ash**	**Cherry**	**Maple**	**Ash**	**Cherry**	**Maple**	**Ash**	**Cherry**	**Maple**
Charcoal										
Napropamide	0.99	6.0	6.3	6.7	61	120	57	25	14	17
	2.97	7.0	6.7	7.0	47	177	73	24	20	23
	5.94	6.3	5.0	6.0	45	109	56	20	14	18
Pendimethalin	0.60	6.0	6.7	6.7	39	143	64	17	16	16
	2.00	6.0	6.0	6.3	46	135	73	21	19	21
	4.00	6.7	5.0	4.7	47	100	33	21	14	9
Simazine	0.25	6.3	7.0	7.0	38	142	73	24	17	21
	0.75	6.7	7.0	6.7	56	185	77	22	27	22
	2.25	5.3	4.7	5.7	42	200	71	18	17	18
No herbicide		6.0	6.9	6.3	55	204	64	27	24	19
No charcoal										
Napropamide	0.99	7.0	6.7	6.3	71	181	65	31	21	17
	2.97	6.7	6.3	6.3	49	181	72	26	21	21
	5.94	6.7	4.7	6.0	64	77	46	27	11	15
Pendimethalin	0.60	6.7	6.7	6.3	58	213	62	28	24	18
	2.00	6.7	6.0	7.0	72	148	65	30	20	18
	4.00	6.3	5.0	4.3	48	107	29	25	17	9
Simazine	0.25	6.3	7.0	7.0	56	204	78	26	26	24
	0.75	5.0	5.7	4.7	34	179	47	18	24	16
	2.25	3.0	2.3	1.7	3	13	3	2	2	1
No herbicide		7.0	7.0	6.5	58	201	71	26	24	19
S.E.D. $ (df = 50)		0.45	0.34	0.44*	7.22	22.04	8.06*	2.39	2.81	2.37*

$ For comparing no herbicide and herbicide treatments
*df = 49

Table 2. Effect of charcoal on response of ash, cherry and maple to pre-emergence herbicide application.

before it could be taken up by roots and hence damage was reduced. A possible increase in the protectant effect with simazine may also come from the adsorption by activated charcoal of herbicide exuded from roots in wet soil as it is being moved in the transpiration stream. With the other herbicides, the hypocotyl may have been outside the charcoal layer when exposed to them, with more damage resulting. The numerous reports of safening effects from charcoal on crops treated with herbicides of the same chemical groups as napropamide and pendimethalin, have been in situations where a slurry of charcoal has been sprayed above the seed thus probably affording more protection (Hoagland, 1989).

Further investigations on the protective abilities of charcoal applied as a seed coating would be desirable before any firm conclusions can be drawn, especially for field conditions. There may be other materials that could be used as protectants. Activated charcoal has also been widely used as a water treatment for removal of chemical residues but new compounds such as polymeric adsorbents have now been developed for this use (Kunin, 1977); currently, there is no information on whether such materials would be effective as herbicide protectants.

Conclusions

The potential for use of safeners for protecting tree seeds from post-planting injury for a wide range of herbicide injury does not look promising. A limited amount of work with naphthalic anhydride as a seed treatment might be worthwhile to assess the cost-effectiveness of any safening effect.

However, for herbicide protectants, further evaluation of the potential of activated charcoal on tree seed is worthwhile. If future results are also positive, attention needs to be given to application technique and economics, before the technique could be adopted on a large scale

The possible use as tree seed protectants of more modern adsorbents, developed originally for water purification, may also merit further investigation.

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