Project title	Liverwort control using novel techniques
Project number:	HNS 175
Project leader:	Jill England, ADAS Boxworth
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Key words:	Liverwort, glucosinolate hydrolysis product, growing media amendment, suppression, growing media, *Marchantia polymorpha*

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Jill England
Horticultural Consultant
ADAS

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GROWER SUMMARY

Headline

- Mulch applications of *Sinapis alba* seed meal can significantly reduce liverwort establishment, although the actual mechanism of control and its true commercial potential on container grown plants has yet to be conclusively ascertained.

Background and expected deliverables

Liverwort growing on the surface of growing media is a major problem to the horticultural industry, affecting both protected and outdoor grown hardy nursery stock; the cost of hand removal of moss, liverwort and weeds at dispatch has been estimated at 4% of total annual production costs (Scott and Hutchinson, 2001), equating to over £1,700 per hectare based on 2008-9 figures (Crane and Vaughan, 2009). Zero tolerance of liverwort in certification schemes and a lack of approved chemical products make its control a technical priority for growers. There are currently no herbicides approved for use over plants under protection that will control liverwort and the use of loose fill materials (such as bark) and pot toppers over the surface of the growing media have practical limitations.

The aim of this project is to build on work completed in HDC projects HNS 126 and HNS 93c by investigating further any herbicidal effect of glucosinolate (GSL) hydrolysis products found in oil seeds on liverwort and the suppression of liverwort growth by unknown biological or physical factors within certain growing media components.

GSLs and their hydrolysis products (isothiocyanates, ITCs) are responsible for the distinctive pungent smell and hot taste of cabbages, mustards and other brassicas and are known to have toxic effects against plants, root knot nematodes and fungal species; brassicas are successfully used in bio-fumigation of soils against weeds and diseases. GSLs could potentially be used to control weeds in container grown crops; however each brassica variety has a distinctive profile of one or more glucosinolates, each of which could have a different effect on liverwort.

Year 1 of this project was comprised of two trials investigating the effect of five brassica oil seeds (*Brassica carinata, Sinapis alba, Camelina sativa* and two different *Brassica napus* - oilseed rape samples), and five growing media amendments (Melcourt Sylvafibre®, Melcourt Growbark®, Perlite, Vital Earth Green Compost and sterilised loam) on liverwort establishment and growth. The seed meal trial found that *Sinapis alba*, *Brassica napus* '00'

and *Camelina sativa* significantly reduced liverwort establishment, whilst the growing media amendment trial found that amendment with Sylvafibre® and sterilised loam significantly reduced liverwort establishment, although neither reduced liverwort levels to what would be commercially acceptable levels.

The expected deliverables from this work include the development of an effective novel control for liverwort infestation based on:

- Growing media amendment with seed meal or a combination of seed meals to reduce liverwort establishment (either through herbicidal effect and/or natural barrier effect).

- Growing media amendment with materials to provide natural microbial suppression of liverwort in addition to any physical effect.

Summary of the project and main conclusions

Two trials were carried out during 2010/11, the first investigated the effect of application method and dose rate of *Sinapis alba* seed meal on liverwort establishment and growth, and the second examined the fate of the glucosinolate content of the seed meal. Both trials were carried out under protection.

Seed meal suppressive effect

This trial focused on the use of *Sinapis alba* 'Braco' seed meal (containing glucosinolates) to control liverwort (Figure GS 1) when applied as pellets (mulch), ground seed meal (mulch), ground seed meal plus bark (mulch), ground seed meal incorporated into the growing media and a managed treatment (ground seed meal applied as a mulch on two separate occasions), identifying the most effective application method, determining effective application rates and any effects on crop growth and quality. For the managed treatment, two applications of ground seed meal were made, with the second being applied when liverwort infestation first started to appear in the trial (three weeks after the initial treatment). For the control, no seed meal was applied. Application rates used were 3, 6 and 9g per pot for each treatment. *Clematis* 'Ernest Markham' was used throughout this trial to test any beneficial or adverse effects on plant growth as it is known to be sensitive to herbicides (e.g. isoxaben the active ingredient in Flexidor 125). The growth habit of the plant also minimises shading of the growing media surface which increases the likelihood of liverwort establishment.

Seed meal treatments: a) ground seed meal b) seed meal reformed as pellets

The seed meal was processed to a fine meal and analysed for glucosinolate content. Plots consisted of 10 x 9cm pots, each planted with a plug of *Clematis* 'Ernest Markham'. A peat growing medium and overhead irrigation regime was adopted to provide conditions favourable to liverwort growth. Liverwort inoculum was provided by a 'spreader' pot (one pot per plot) containing liverwort.

The trial was set up on 19 August 2010 and the treatments applied on 2 September 2010. The dose rate of each of the seed meal treatments was easily distinguished once applied, with the 9g ground seed meal and all the seed meal with bark treatments completely covering the surface of the growing media. The pots were well watered after the treatments were applied, and this resulted in the seed meal swelling and covering a greater proportion of the growing media surface (Figure GS2).

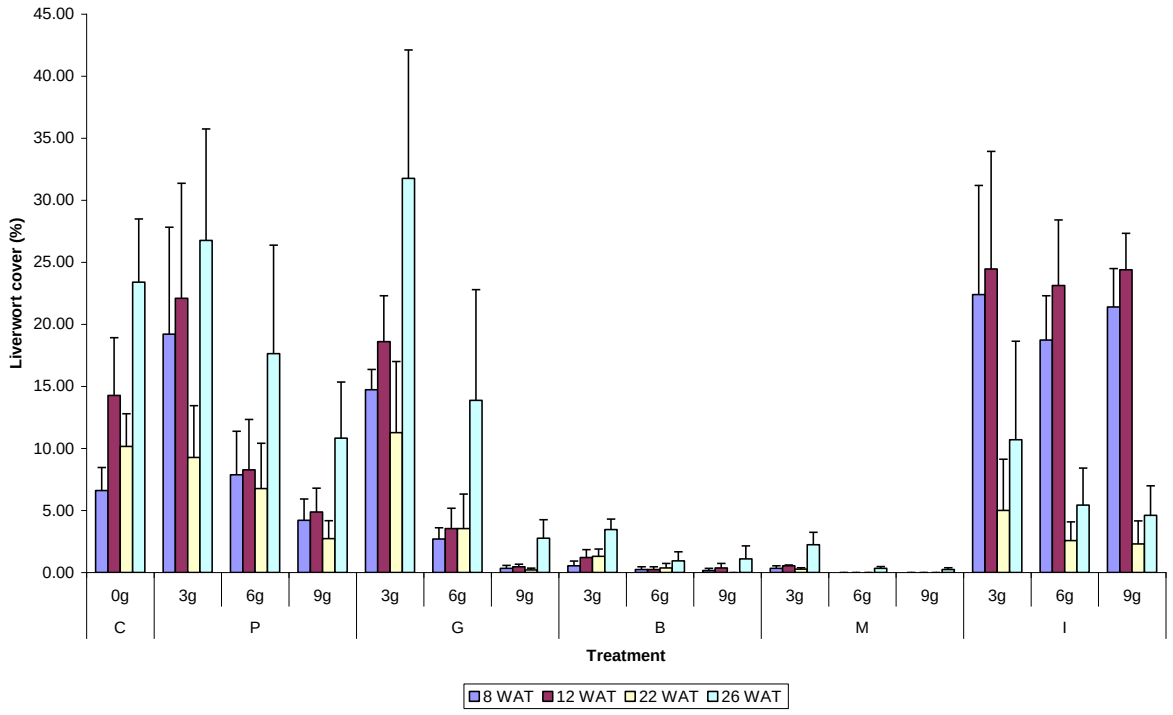
Ground **Pelleted**

Treatments: ground and pelleted seed meal (9g dose) post watering.

Liverwort cover

Throughout the trial the least amount of liverwort established in the managed and seed meal with bark treatments (Figure GS3). The managed application of seed meal and the seed meal with bark treatments covered most of the growing media surface and this physical capping may have helped to reduce liverwort establishment in these treatments (i.e. it was difficult to separate chemical control from physical control with these treatments). At 26 weeks after treatment (WAT) there was evidence of a small amount of liverwort establishment around the edge of some pots with these treatments.

In general, liverwort cover increased over time, except for the incorporated treatments. With these treatments the greatest amount of liverwort establishment was noted around 8 to 12 WAT. At later assessment dates the level of liverwort had substantially declined.



Liverwort cover (%) (WAT = weeks after treatment).

C = no seed meal applied. P = pelleted seed meal, G = ground seed meal, B = seed meal combined with bark, M = managed treatment and I = seed meal incorporated into growing media.

At 22 WAT it was observed that some of the liverwort had died back, relative to that recorded 12 and 26 WAT in the majority of treatments (Figure GS3). This was possibly either the result of the drier conditions experienced at this time, as the irrigation system was disconnected during the winter and plants were hand watered, or due to the extreme cold conditions.

Plant height

Plant height was recorded 8 WAT. The data collected suggested greater height of the *Clematis* at the 6g dose rate across all treatments except for the incorporated treatment where plant height was reduced. Least growth was recorded in the incorporated seed meal treatment (all dose rates) and the seed meal with bark treatment at the 9g dose rate. Plant height at the 6g dose rate in the pelleted seed meal and seed meal with bark treatments were both greater than in the control.

Phytotoxicity

By 15 September 2010 (2 WAT) phytotoxicity was noted on all plots where seed meal was incorporated. Most damage was found in the 9g treatments where the leaves were completely scorched on up to 50% of the plants, and marginal leaf scorch was observed on some of the remaining plants. Some scorching was also evident on the leaf margins of new growth. Within the 6g treatments leaf margin scorch was noted on 50-70% of plants, affecting older leaves. There was also reduced vigour and some marginal leaf scorch noted on new leaves. Marginal leaf scorch was noted on up to 25% of the plants in the 3g treatments.

Symptoms were typically greater at 8 WAT than 12 WAT suggesting that the phytotoxic effect of the treatments reduced over time.

Root development

Root development was assessed 26 WAT. Root development was compared with the control and graded on a scale of 1-5, ranging from no roots to root development comparable with those found in the control plots. There was a clear adverse effect of the seed meal on root development in the incorporated treatment, with root development decreasing with increased seed meal dose rate. The general trend in response to dose rate was for greater root development at the lower dose rates (ground seed meal, seed meal with bark and incorporated seed meal). However, root development was greater at the 6g dose rate for the managed treatment and at the 9g dose rate in the pelleted seed meal treatment.

Glucosinolate fate

A trial was designed to investigate the fate of the glucosinolate content of *Sinapis alba* 'Braco' seed meal via sample analysis (NIAB), determining the residual glucosinolate content of the seed meal. Fresh *Sinapis alba* seed was sourced for the 2010 trials due to the low residual glucosinolate found in seed meal used in the 2009 trials.

10 g of seed meal was placed on the surface of 100 x 9 cm pots containing growing media and arranged in a single block of 100 pots. *Sinapis alba* 'Braco' seed meal samples were

analysed for glucosinolate content before the trial, and then a total of 100 g was removed from the pots weekly until the threshold dropped below a measurable quantity.

Glucosinolate concentration in the seed meal decreased rapidly and was present in quantities too small to measure two weeks after treatment.

Overall, the *Sinapis alba* managed treatments and the seed meal with bark treatments were the most promising. Incorporating seed meal into growing media was the least successful in terms of phytotoxicity and reduced root development compared to the other treatments.

Financial benefits

There are currently no herbicides approved for use over plants under protection that will control liverwort. The cost of moss, liverwort and weed control at dispatch is estimated at 4% of the total annual production costs, equating to over £1,700 per hectare based on 2008-9 figures. Consequently, the development of a novel control method will prove particularly beneficial for use within protected environments.

A cost-benefit analysis for the novel control method will be carried out in the final project year.

Action points for growers

Further investigation of the effect of seed meal on liverwort and any associated phytotoxic effects on crop plants is required before any specific recommendations can be made for growers.

Growers could consider including a proportion of Sylvafibre® or sterilised loam in potting mixes as an aid to limiting liverwort development, particularly in short term crops (refer to the Year 1 Annual Report for further details).

SCIENCE SECTION

Introduction

Liverwort growing on the surface of growing media is a major problem to the horticultural industry, affecting both protected and outdoor grown hardy nursery stock. The cost of hand removal of moss, liverwort and weeds at dispatch has been estimated at 4% of total annual production costs (Scott and Hutchinson, 2001), equating to £1,763 per hectare based on 2008-9 figures (Crane and Vaughan, 2009). Zero tolerance of liverwort in certification schemes and a lack of approved chemical products make its control a technical priority for growers.

The aim of this project is to build on work completed in HDC projects HNS 126 and HNS 93c by investigating further the herbicidal effect of glucosinolate (GSL) hydrolysis products found in oil seeds on liverwort, and the suppression of liverwort growth by unknown biological or physical factors within certain growing media components.

GSLs and their hydrolysis products (isothiocyanates, ITCs) are responsible for the distinctive pungent smell and hot taste of cabbages, mustards and other brassicas and are known to have toxic effects against plants, root knot nematodes and fungal species; brassicas are successfully used in biofumigation of soils against weeds and diseases. GSLs could potentially be used to control weeds in containers; each brassica variety has a distinctive profile of one or more glucosinolates, each of which could have a different effect on liverwort.

Year 1 of this project was comprised of two trials investigating the effect of five brassica oil seeds (*Brassica carinata, Sinapis alba, Camelina sativa* and two different *Brassica napus* - oilseed rape samples), and five growing media amendments (Melcourt Sylvafibre®, Melcourt Growbark®, Perlite, Vital Earth Green Compost and sterilised loam) on liverwort establishment and growth. The seed meal trial found that *Sinapis alba*, *Brassica napus* '00' and *Camelina sativa* significantly reduced liverwort establishment, whilst the growing media amendment trial found that amendment with Sylvafibre® and sterilised loam significantly reduced liverwort establishment.

Year 2 of this project, following consultation with the project's Industry Representatives, focused on the effect of *Sinapis alba* on liverwort establishment and growth as this seed meal showed most promise in year one, aiming to investigate further the effects of different application rates and application method.

Year 2 Objectives

- *Seed meal suppressive effect:* to investigate a variety of application methods for *Sinapis alba* 'Braco' seed meal to identify the most effective application method, determine effective application rates and any effects on crop growth and quality.

- *Glucosinolate fate:* to investigate the fate of glucosinolate content of *Sinapis alba* 'Braco' seed meal via sample analysis (NIAB), determining the residual glucosinolate content of the seed meal.

Materials and methods

The trial was set up on 19 August 2010 and the treatments applied on 2 September 2010. The trials were sited under glass at John Richards Nurseries. Growing conditions were managed as normal for the site. Trays were placed on benches lined with polystyrene with drainage holes. Irrigation was provided by overhead sprinklers, and by hand watering during the winter when automatic irrigation was not used.

Sinapis alba 'Braco' seeds (Supplier: Farm Direct, Cumbria) were crushed and the oil extracted by Alan Brewer (Selby House Farm, cold extraction), then reformed into pellets prior to supply. The oil was extracted as it would otherwise become rancid.

The potting mix (pH 4.0) was comprised of:

- 100% Bulrush peat (standard)
- Osmocote Exact + trace elements (3-4 months, 4.0 kg/m^3)
- Lime (2.4 kg/m^3)
- Exemptor (vine weevil control, 0.3 kg/m^3)

The irrigation water (pH 7.32) used was collected rainwater, purified through iris beds:

- Alkalinity as HCO_3 83 mg/l
- Conductivity 198 µS/cm
- Nitrate-N 0.4 mg/l

Objective 1: Seed meal suppressive effect

Experimental design

Treatments were arranged in a randomised block design with 3-fold replication (Appendix 1). Liverwort inoculum was provided by a 'spreader' pot (one pot per plot) containing liverwort. Plots consisted of 10 x 9 cm pots, placed in trays each with one liverwort 'spreader' pot. Each pot was planted with a plug of *Clematis* 'Ernest Markham' (Supplier: Micropropagation Services Ltd). *Clematis* 'Ernest Markham' was used throughout this trial to minimise shading of the growing media surface and to test any beneficial or adverse effects on plant growth as it is known to be sensitive to herbicides (e.g. isoxaben).

Treatments

The seed meal was either incorporated or applied as a mulch; the mulch treatments were ground seed meal, seed meal pellets, and ground seed meal combined with bark (Melcourt propagation bark) (Figure 1 and Table 1). For the managed treatment a second application of ground seed meal only was applied once liverwort first appeared in the treatments, three weeks after treatment. For the control no seed meal was applied. Application rates used were 3, 6 and 9 g per pot.

Figure 1. Seed meal treatments: a) ground seed meal b) seed meal reformed as pellets

Table 1. Seed meal suppressive effect

Factor		**Treatment**
Application method	P	Mulch (pellets)
	G	Mulch (ground seed meal)
	B	Bark (ground seed meal with bark)
	I	Incorporated (ground seed meal)
	M	Managed treatment (ground seed meal)*
	C	No seed meal
Application rate	1	3 g
	2	6 g
	3	9 g

*For the managed treatment a second mulch of ground seed meal was applied once liverwort first appeared in the treatments (three weeks after treatment).

Assessments

Assessments were carried out as follows:

Date	WAT	Action	Data collection
15.09.10	2	Inspection	
21.09.10	3	Inspection	
29.09.10	4	Inspection	
27.10.10	8	Assessment	Liverwort cover Phytotoxicity Plant height
22.11.10	12	Assessment	Liverwort cover Phytotoxicity
01.02.11	22	Assessment	Liverwort cover
02.03.11	26	Assessment	Liverwort cover Root development

*WAT = weeks after treatment

Statistical analysis was carried out using GenStat Release 12.1 (PC/Windows XP).

Objective 2: Glucosinolate fate

Treatments and experimental design

10 g of ground seed meal was placed on the surface of 100 x 9 cm pots containing growing media and arranged in a single block of 100 pots. *Sinapis alba* 'Braco' seed meal samples were analysed for glucosinolate content before the trial, and then a total of 100 g was removed from the pots weekly until the threshold dropped below a measurable quantity. Glucosinolate content was measured by NIAB using test procedures based on British Standard BS 4289 Part 9: 1993 ISO 9167-1 1992.

Results and Discussion

Objective 1: Seed meal suppressive effect

The trial was set up on 19 August 2010 (Figure 2**) and the treatments applied on 2 September 2010. Liverwort started to appear three weeks after treatment, and a second application of ground seed meal was made to the managed treatment. The dose rate of each of the seed meal treatments was easily distinguished once applied, with the 9 g ground seed meal and all the seed meal with bark treatments covering the surface of the growing media (**

Figure 3). The pots were well watered after the treatments were applied, and this resulted in the seed meal swelling and covering a greater proportion of the growing media surface (Figure 4).

Figure 2. Trial set up prior to application of treatments

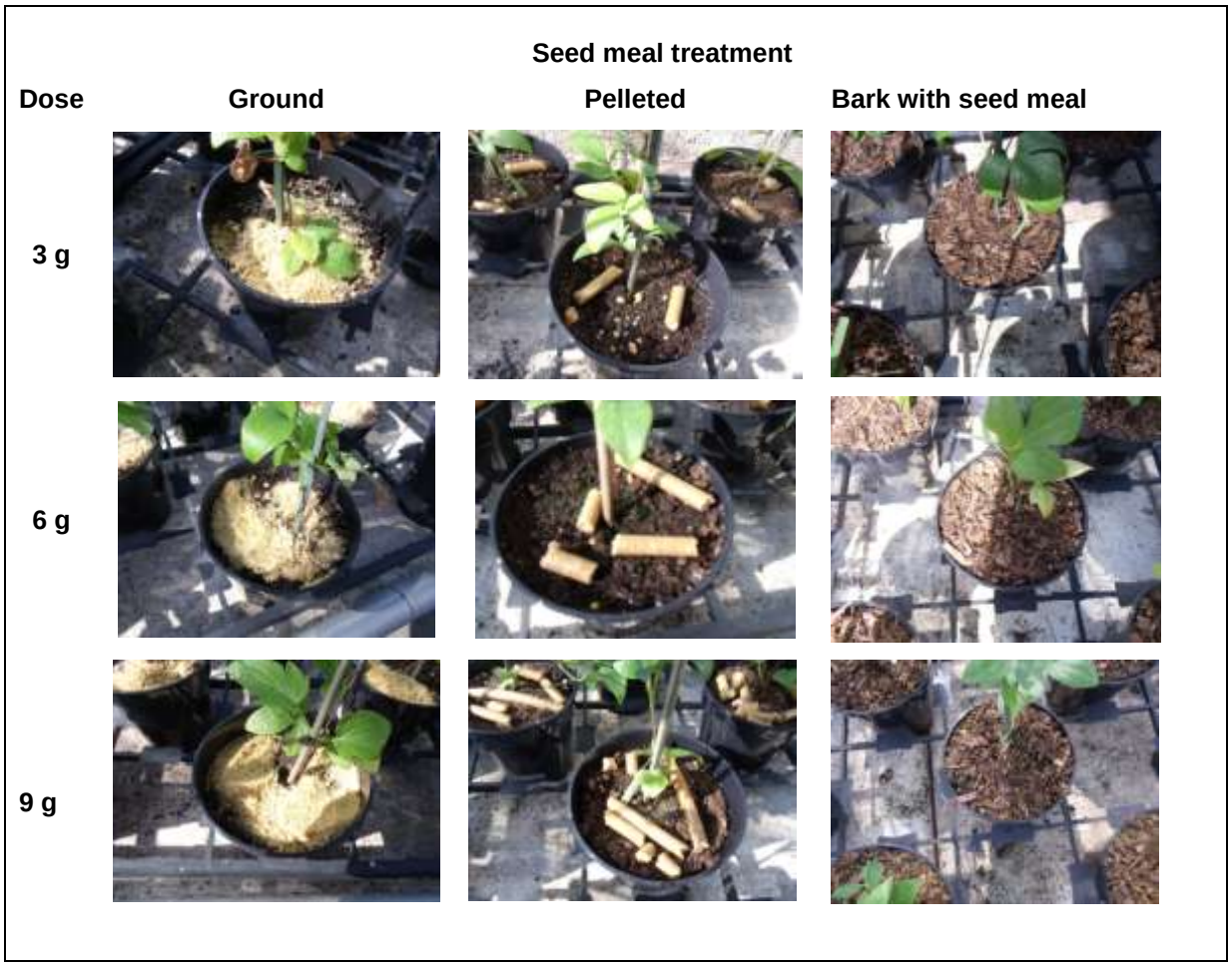


Figure 3. Treatments: ground seed meal, pelleted seed meal and bark mixed with ground seed meal, prior to watering.

Ground

Pelleted

Figure 4. Treatments: ground and pelleted seed meal (9 g dose) post watering.

Liverwort cover

Throughout this trial least liverwort established in the managed and seed meal with bark treatments. At 26 weeks after treatment (WAT), however, there was evidence of liverwort establishment around the edge of some pots (Figure 5). The seed meal in the managed treatment appeared to have formed a crust over the surface of the growing media, where liverwort did not seem to establish. The use of bark and a second application of seed meal provided greater cover of the growing media surface and this may have helped to reduce liverwort cover in these treatments. It was noted that by 12 WAT liverwort was growing over the edge of some pots containing liverwort inoculum and spreading into the bark treatments, rather than growing on the surface of the bark.

Managed treatment

Seed meal with bark

Figure 5. Liverwort infestation around the edge of pots, 26 WAT

In general, liverwort cover increased over time, except for the incorporated treatments. However, at 22 WAT it was observed that some of the liverwort had died back, possibly due to drier conditions as the irrigation system was disconnected during the winter and plants were hand watered, or due to the extreme cold conditions; it is evident from the data that liverwort establishment was reduced at 22 WAT compared to 12 and 26 WAT in the majority of treatments (Figure 6).

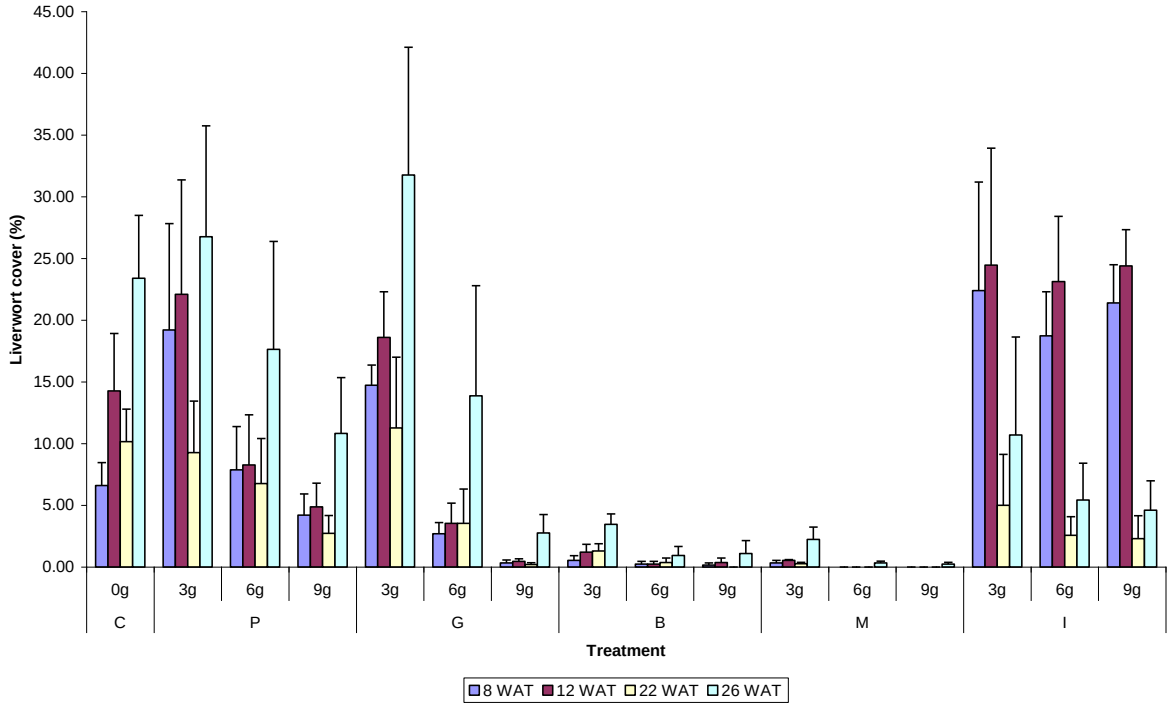


Figure 6. Liverwort cover (%) (WAT = weeks after treatment). P = pelleted seed meal, G = ground seed meal, B = seed meal combined with bark, M = managed treatment, I = seed meal incorporated into growing media, C = no seed meal applied

At 8 and 12 WAT the greatest amount of liverwort established in the incorporated treatment at all dose rates; liverwort cover was greater than in the control treatment in the pelleted seed meal (3 g) and ground seed meal (3 g) treatments and all incorporated treatments. However, by 26 WAT liverwort cover was only greater than the control in the pelleted seed meal (3 g) and ground seed meal (3 g) treatments.

Liverwort cover decreased with increased dose rate in all treatments except where seed meal was mixed with bark where there was a slight increase at 26 WAT.

Statistical analysis using ANOVA (**Table 2**) showed that there was a significant effect on liverwort cover due to treatment ($F_{4,30} = 7.95$, $P<0.001$) and dose rate ($F_{2,30} = 6.48$, $P=0.005$) at 26 WAT and throughout the trial, however there was no interaction between these factors. Closer inspection of the data indicates that liverwort cover in the bark with seed meal, incorporated and managed treatments were less (very highly significantly less) than the control due to treatment; liverwort cover at the 6 g and 9 g dose rates was very highly significantly less than the control.

Table 2. Analysis of variance (ANOVA) comparing liverwort cover, 26 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Block	2	633.02	316.51	4.30	0.023
Control	1	595.87	595.87	8.10	0.008
Treatment	4	2341.15	585.29	7.95	<.001
Dose	2	953.39	476.69	6.48	0.005
Interaction	8	800.07	100.01	1.36	0.254
Residual	30	2207.32	73.58		

Regression analysis (Table 3) indicated that by 26 WAT the seed meal with bark (6 g) and the managed treatments (6 and 9 g) had less than 5% liverwort cover in over 97% of pots. The control had less than 5% liverwort cover in 43% of pots, less than the pelleted seed meal, 3 g (33%) and ground seed meal, 3 g (27%).

Table 3. Liverwort cover: proportion of pots showing less than 5% liverwort cover, 26 WAT

Treatment	0g		3g		6g		9g	
	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.
Control	43	0.122						
Pellets			33	0.116	50	0.123	60	0.121
Ground			27	0.109	50	0.123	83	0.093
Bark			70	0.113	97	0.045	87	0.085
Managed			87	0.085	100	0.001	100	0.001
Incorporated			67	0.117	73	0.110	70	0.113

Plant height

Plant height was recorded 8 WAT (Figure 7). The data collected suggested greater height of the *Clematis* at the 6 g dose rate across all treatments except for the incorporated treatment where plant height was reduced. Least growth was recorded in the incorporated seed meal treatment (all dose rates) and the seed meal with bark treatment at the 9 g dose rate. Plant height at the 6 g dose rate in the pelleted seed meal and seed meal with bark treatments were both greater than in the control. Plant height was reduced at the 9 g dose rate in all mulch treatments (but not the incorporated treatment).

Statistical analysis of the data indicated a very highly significant difference (Table 4) between the treatments and the control ($F_{4,30}$ = 20.01, P<0.001) and further examination of the data indicated that plant height in the incorporated seed meal treatment was significantly less than the control.

Earlywine *et al.* (2008) reported that nitrogen available from the seed meal often resulted in increased plant biomass in tall fescue, perennial ryegrass and bermuda grass, with the effect increasing with higer dose rates of *Sinapis alba* seed meal. However, they observed mixed effects on turfgrass varieties where plant density and height were reduced in white clover (67%) in 2008, and plant density was reduced in large crabgrass (75%), buckhorn plantain (69%) and annual bluegrass (73%) although plant height was not reduced.

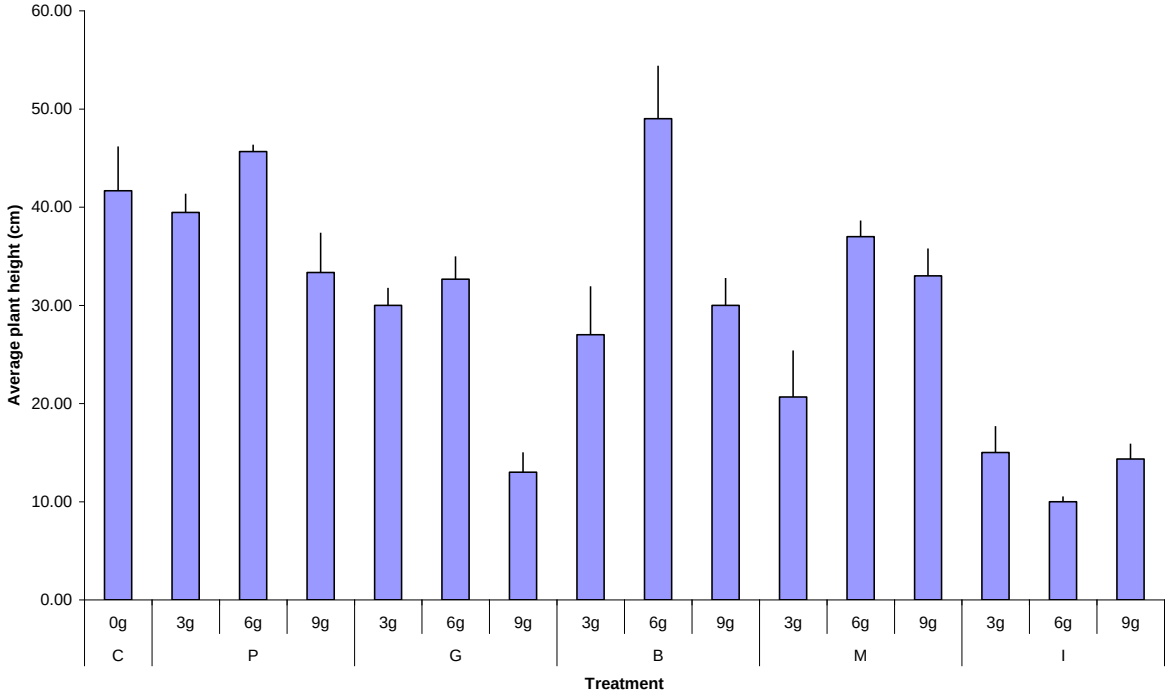


Figure 7. Plant height (cm), 8 weeks after treatment. P = pelleted seed meal, G = ground seed meal, B = seed meal combined with bark, M = managed treatment, I = seed meal incorporated into growing media, C = no seed meal applied.

Table 4. Analysis of variance (ANOVA) comparing average plant height, 8 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	2	5.67	2.84	0.09	0.914	
Control	1	61.25	61.25	1.96	0.172	
Treatment	4	2506.68	626.67	20.01	<.001	***
Dose	2	120.65	60.33	1.93	0.163	
Interaction	8	357.45	44.68	1.43	0.226	
Residual	30	939.76	31.33			

Phytotoxicity and plant quality

By 15 September 2010 (2 WAT) phytotoxicity was noted on all plots where seed meal was incorporated (

6 g

9 g

Figure 9) and in mulch treatments where higher rates were used. Most damage was found in the 9 g treatments where the leaves were completely scorched on up to 50% of the plants, and marginal leaf scorch was observed on some of the remaining plants. Some scorching was also evident on the leaf margins of new growth. Within the 6 g treatments leaf margin scorch was noted on 50-70% of plants, affecting older leaves. There was also reduced vigour and some marginal leaf scorch noted on new leaves. There was little new growth in the 3 g treatments and marginal leaf scorch was noted on up to 25% of the plants. Least phytotoxicity was noted in the seed meal with bark treatments, suggesting that the bark may ameliorate the phytotoxic effects of the seed meal

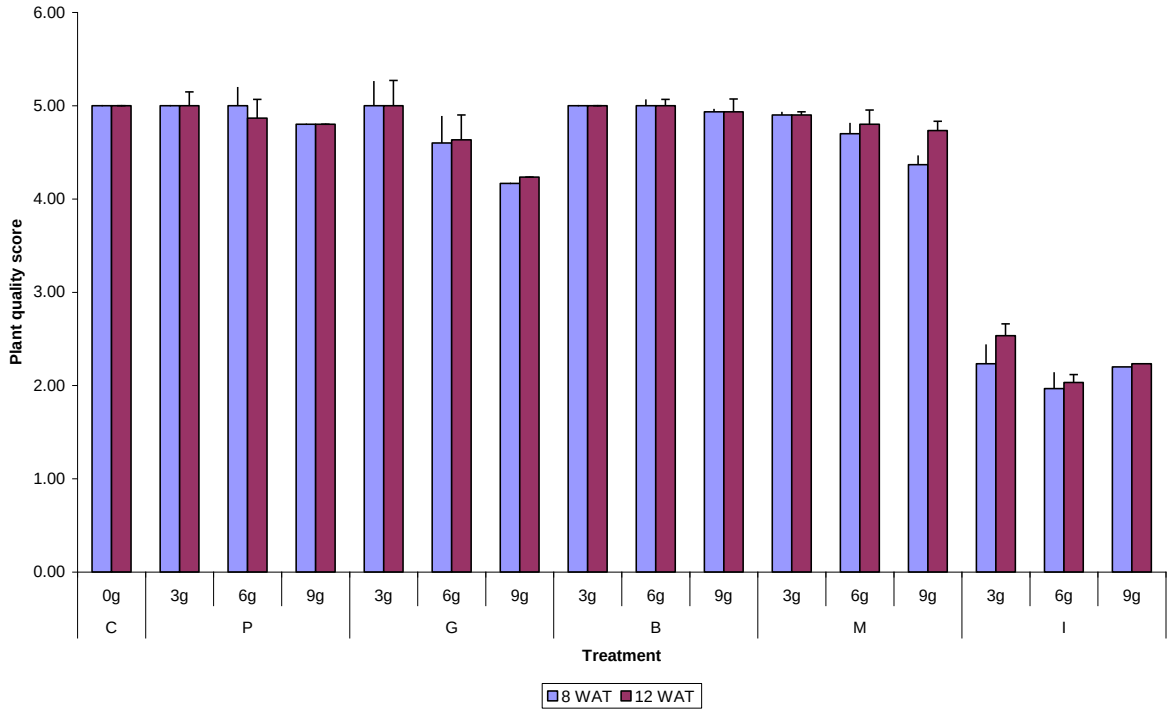


Figure 8. Plant quality scores: 1 (plant death) to 5 (no damage observed). P = pelleted seed meal, G = ground seed meal, B = seed meal combined with bark, M = managed treatment, I = seed meal incorporated into growing media, C = no seed meal applied. WAT = weeks after treatment

Phytotoxicity symptoms were recorded as quality scores on a scale of 1-5 (1 = plant death, 5 = no damage observed) and the data, collected 8 and 12 weeks after treatment, clearly showed an adverse effect on the plants due to incorporation of seed meal into the growing media (Figure 8). Statistical analysis using analysis of variance (ANOVA) determined the main effects of the treatment and dose to be very highly significant (Table 5 and Table 6) at both 8 WAT and 12 WAT however there was no significant interaction between treatment and dose.

6 g **9 g**

Figure 9. Phytotoxicity in incorporated seed meal treatment, 6 g and 9 g dose rates, 2 WAT

The main effect of treatment 8 WAT ($F_{4,30}$ = 235.05, P < 0.001) and 12 WAT ($F_{4,30}$ = 233.34, P < 0.001) was significantly greater phytotoxicity in the incorporated seed meal, ground seed meal and managed treatments than the control. In all treatments where there was a difference in phytotoxicity scores between 8 WAT and 12 WAT, greater phytotoxicity was observed at 8 WAT, with the exception of the pelleted seed meal treatment where phytotoxicity was greater 12 WAT.

Where phytotoxicity occurred it increased with dose rate other than in the incorporated treatment. Phytotoxicity was found in all treatments where seed meal was applied at the 9 g rate, but symptoms were typically greater at 8 WAT than 12 WAT suggesting that the phytotoxic effect of the treatments reduced over time. The exception to this was in the pelleted seed meal treatment where greater symptoms were observed 12 WAT than 8 WAT at the 6 g dose rate, and symptoms remained the same at 12 and 8 WAT at the 9 g dose rate.

When considering dose rate, phytotoxicity was significantly greater at all dose rates than in the control at 8 WAT ($F_{4,30}$ = 7.57, P < 0.05), however by 12 WAT ($F_{2,30}$ = 7.08, P < 0.05) only the 6 g and 9 g dose rates showed significantly greater phytotoxicity than the control (Table 5 and Table 6).

Table 5. Analysis of variance (ANOVA) comparing phytotoxic effects of seed meal, 8 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	2	0.14042	0.07021	1.27	0.294	
Control	1	1.54939	1.54939	28.12	<.001	***
Treatment	4	51.80311	12.95078	235.05	<.001	***
Dose	2	0.83378	0.41689	7.57	0.002	***
Interaction	8	0.85956	0.10744	1.95	0.089	
Residual	30	1.65292	0.05510			

Table 6. Analysis of variance (ANOVA) comparing phytotoxic effects of seed meal, 12 WAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	2	0.07875	0.03938	0.77	0.472	
Control	1	1.32612	1.32612	25.92	<.001	***
Treatment	4	47.74311	11.93578	233.34	<.001	***
Dose	2	0.72400	0.36200	7.08	0.003	***
Interaction	8	0.65156	0.08144	1.59	0.169	
Residual	30	1.53458	0.05115			

Regression analysis (Table 7) indicated that by 12 WAT for all treatments, except for incorporated seed meal for the 3 g dose rate over 97% of plants had plant quality scores greater than 4, and for the 6 g dose rate more than 80% of the plants had scores greater than 4, indicating low levels of symptoms and high quality plants in terms of any adverse effects of the seed meal treatments.

Table 7. Phytotoxicity: plants with quality scores of 4 to 5, 12 weeks after treatment. Scores: 1 (plant death) to 5 (no damage observed).

Treatment	0g		3g		6g		9g	
	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.
Control	100	0						
Pellets			100	0	97	0.039	87	0.074
Ground			100	0	87	0.074	50	0.108
Bark			100	0	100	0	93	0.055
Managed			97	0.039	80	0.087	77	0.092
Incorporated			3	0.039	0	0	0	0

Root development

Root development was assessed 26 WAT. Root development was compared with the control and graded on a scale of 1-5, ranging from no roots to root development comparable with those found in the control plots (Figure 10).

Figure 10. Root scores (left to right): 1 = no visible root, 2 = some visible root, 3 = visible root growing down, 4 = slightly reduced rooting (compared to control), 5 = root growth comparable with control.

The data collected showed a clear adverse effect of the seed meal on root development in the incorporated treatment, with root development decreasing with increased seed meal dose rate (Figure 11). The general trend due to dose rate was for greater root development at the lower dose rates (ground seed meal, seed meal with bark, incorporated seed meal). However, root development was greater at the 6 g dose rate for the managed treatment and at the 9 g dose rate in the pelleted seed meal treatment.

Statistical analysis confirmed that the main effect due to treatment was very highly significant different ($F_{4,30}$ = 122.97, $P < 0.001$) (Table 8), and close inspection suggested that all treatments were significantly different to the control.

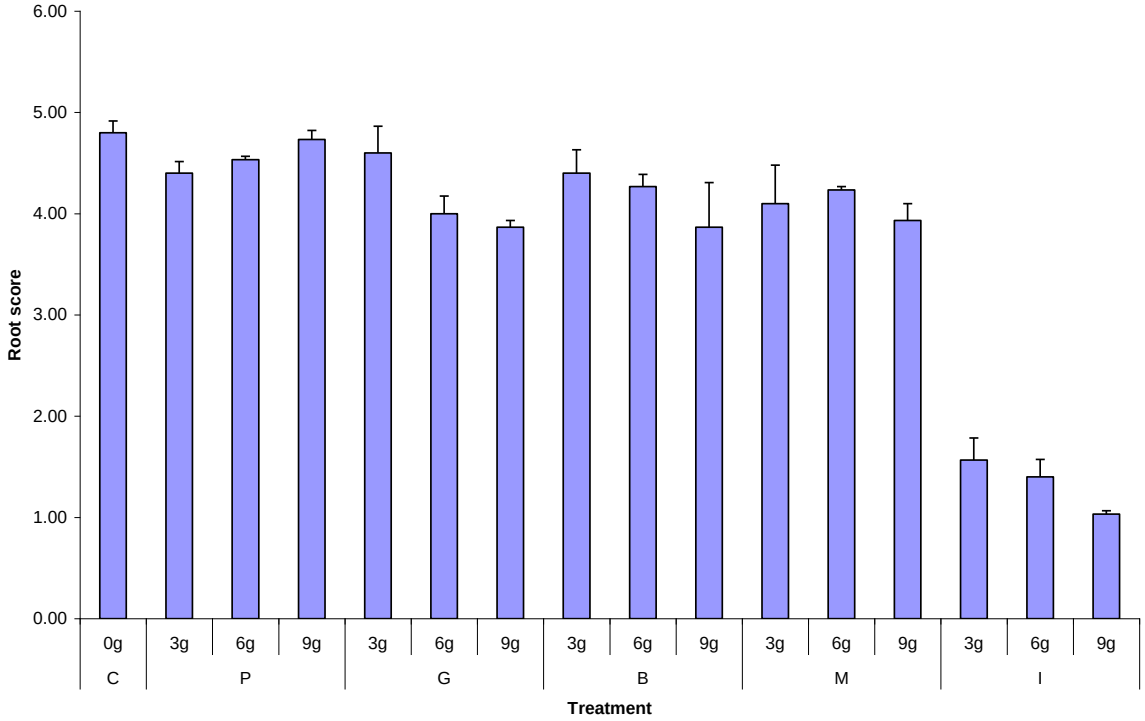


Figure 11. Root development scores, 26 WAT. Scores: 1 = no visible root, 2 = some visible root, 3 = visible root growing down, 4 = slightly reduced rooting (compared to control), 5 = root growth comparable with control. P = pelleted seed meal, G = ground seed meal, B = seed meal combined with bark, M = managed treatment I = seed meal incorporated into growing media, C = no seed meal applied.

Table 8. Analysis of variance (ANOVA) showing the effect of seed meal on root development, 26 weeks after treatment.

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.	
Block	2	0.1254	0.0627	0.50	0.614	
Control	1	3.6409	3.6409	28.78	<.001	***
Treatment	4	62.2169	15.5542	122.97	<.001	***S
Dose	2	0.8138	0.4069	3.22	0.054	
Interaction	8	1.3151	0.1644	1.30	0.281	
Residual	30	3.7946	0.1265			

Regression analysis (Table 9) indicated that by 26 WAT for all treatments except for the incorporated treatment, 57% of plants had a root development score of 3 or over; for the incorporated treatment only the 3 g dose rate had plants which fell within this range (3%). It is of interest that in a number of treatments over 90% of plants had root development scores of 3 and above: seed meal with bark (3 g), ground seed meal (3 g) and pelleted seed meal (6 g and 9 g).

Table 9. Root development: the proportion of plants with a root development score of 3 to 5, 26 WAT. Scores: 1 = no visible root, 2 = some visible root, 3 = visible root growing down, 4 = slightly reduced rooting (compared to control), 5 = root growth comparable with control.

Treatment	0g		3g		6g		9g	
	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.	Mean (%)	s.e.
Control	96	0.043						
Pellets			87	0.081	93	0.060	97	0.043
Ground			93	0.060	67	0.113	63	0.115
Bark			90	0.072	80	0.096	57	0.119
Managed			63	0.115	70	0.110	60	0.117
Incorporated			3	0.043	0	0.000	0	0.000

Objective2: Glucosinolate fate

Fresh *Sinapis alba* seed was sourced for the 2010 trials due to the low residual glucosinolate found in seed meal used in the 2009 trials (Table 10). Glucosinolate content decreases once milled, estimated at up to 50% over 6 months under refrigerated conditions (personal communication, H. Appleyard, NIAB). The analyses carried out indicated that pellets retained greater levels of glucosinolate than either flakes or ground seed meal.

Table 10. Initial glucosinolate content of *Sinapis alba* 'Albatross' seed meal from 2009

Seed meal sample	Glucosinolate content µm/g
Sinapis alba 'Albatross' pellets	62.3
Sinapis alba 'Albatross' flakes	3.6
Sinapis alba 'Albatross' (ground)	29.4

Glucosinolate concentration in the seed meal decreased rapidly and was too small to measure by two weeks after treatment (Table 11). Glucosinolate hydrolysis is catalysed by a myrosinase enzyme released following mechanical damage in the presence of water, and including moisture held in the air or applied as irrigation.

Table 11. Glucosinolate content of *Sinapis alba* 'Braco' seed meal

Sample date		Glucosinolate content µm/g
31.08.10	Pre-treatment	102.46
08.09.10	1 WAT	1.13
15.09.10	2 WAT	0.00
21.09.10	3 WAT	0.00

*WAT = weeks after treatment

Conclusions

Objective 1: Seed meal suppressive effect

The adverse effects on plant height and root development, and increased phytotoxicity combined with poor liverwort control due to the incorporated *Sinapis alba* seed meal treatments, indicate that this is not a viable treatment to pursue.

Greatest promise was shown by all dose rates of the seed meal with bark and the managed treatments, and the ground seed meal (9 g) treatments as less liverwort established throughout the trial under these conditions. The use of a second application of seed meal in the management treatment (without bark) and the use of bark will have increased the mulching effect of the treatments, and this may have had implications in reducing liverwort cover. In the seed meal with bark treatment, any effect of the seed meal (rather than the bark as a mulch) would have been apparent had there been a second control using bark only (not combined with seed meal). A bark only mulch will be used in future trials to identify if the addition of seed meal to the bark results in any further reduction in liverwort.

There did appear to be a growth inhibitory effect due to the mulch treatments 8 WAT compared to the control, with a slight adverse effect on root development 26 WAT and some phytotoxicity. However, over 94% of the seed meal with bark treatments, and 77% of the managed treatment showed phytotoxicity scores of 4 and over, indicating that the phytotoxicity caused little visible damage; even in the ground seed meal (9 g) treatment over 50% of the plants showed phytotoxicity scores of 4 and over.

Plant height at 8 WAT increased in the seed meal with bark (6 g) and the managed treatments (6 g), and this effect warrants further investigation as it could suggest some growth promotion properties of the seed meal when applied at specific dose rates.

Overall least phytotoxicity was noted in the seed meal with bark treatments, suggesting that the bark may ameliorate the phytotoxic effects of the seed meal. The 9 g dose rate of all mulch treatments had an adverse effect on plant height and also increased plant phytotoxicity, however a comparable effect was not observed in the root development.

Objective 2: Glucosinolate fate

The glucosinolate levels in the seed meal were found to decrease rapidly in this trial, and therefore must have a transient effect on liverwort establishment. Glucosinolates degrade to form volatile ITCs in the presence of myrosinase enzymes and moisture. ITCs are known to quickly degrade further to form SCN^- which is less biologically active than ITCs, and limiting any effects of glucosinolate degradation products to a short time frame. Reduced liverwort

establishment may also be due to or aided by an effect other than ITCs; for example a smothering effect of the bark or second seed meal application.

This trial also suggests a potential problem in preserving glucosinolate levels in any product developed. However, research published in 2010 investigated glucosinolate preservation in seed meal (*Brassica napus* 'Sunrise', *B. juncea* 'Pacific Gold' and *Sinapis. alba* 'Ida Gold') and stored post grinding and oil removal (Morra and Borek, 2010). This work compared storage in paper bags, sealed polythene bags and vacuum bottles (purged with a N_2 atmosphere) and kept under +4 and +25 ^{0}C over 30 months. The largest decrease in glucosinolate content in the *S. alba* samples content was recorded in seed meal stored in paper bags (57%) at +4 ^{0}C; from an average initial concentration of 131.4 µmol g^{-1} of 4-OH benzyl glucosinolate. No losses were recorded at +25 ^{0}C when stored in either paper bags or polythene. Morra and Borek concluded that seed meal storage up to 30 months was viable providing the relative humidity of the storage atmosphere is maintained low enough to avoid fungal growth. The primary requirement for preserving glucosinolate concentration was to limit water content, thus avoiding microbial growth and endogenous myrosinase activity.

Summary

The *Sinapis alba* managed treatments and the seed meal with bark treatments were the most promising, particularly where combined with increased plant height and reduced phytotoxicity. However, these treatments also acted as a physical barrier, and any improvement may have been due to a smothering effect, rather than control due to glucosinalotes. Incorporating seed meal into growing media was least successful in terms of phytotoxicity and reduced root development compared to other treatments.

These results suggest that future work could be based around the managed and seed meal with bark treatments, further investigating any effect on plant growth and development in addition to liverwort control and phytotoxicity across a range of plant species; a comparison of the effect of a bark only mulch with a seed meal with bark mulch would provide clarification the effect of the individual components of the treatments, and highlight any synergistic effects.

Technology transfer

- Article for HDC News

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References

Crane, R and Vaughan, R. (2009) *Horticulture Production in England. Farm Business Survey 2008/2009.* Rural Business Research, University of Reading.

Earlywine, D. T, Smeda, R. J, Teuton, T. C and Sams, C. E. (2008) Response of annual weeds and select turfgrasses to yellow mustard seed meal. North Central Weed Science Society Proceedings. 63:102.

Morra, M. J. and Borek, V (2010) Glucosinolate preservation in stored Brassicaceae seed meals. Journal of Stored Products Research. 46, 98-102.

Scott, M and Hutchinson, D. 2001. HNS 93. Nursery stock propagation: moss, liverwort and slime control. Horticulture Development Council, East Malling.

APPENDICES

Appendix 1.

Objective 1: Seed meal suppressive effect

Treatment	Application method	Treatment	Application Rate
P	Mulch (pellets)	1	3 g
G	Mulch (ground)	2	6 g
B	Mulch (seed meal with bark)	3	9 g
I	Incorporated		
M	Managed treatment (second seed meal application, mulch)		
C	Control (no seed meal)		

Block 1		Block 2		Block 3	
I3	F2	I3	B3	F3	G2
M1	G2	I2	G2	M1	B3
B2	F3	F3	G1	B1	M2
F1	M3	F2	B2	M3	G1
I3	G1	M1	G3	F1	C
M2	C	C	M3	I3	G3
B3	I2	B1	I1	I1	F2
B1	G3	M2	F1	I2	B2