Final report

Detecting persistent herbicides in compost

Summary of investigations into the development of a robust plant response test that is sensitive to low levels of synthetic auxin herbicides, the sensitivity of different plant species to synthetic auxin herbicides, and the presence of these herbicides in green composts and growing media

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Front cover photography: Glasshouse experiments at Stockbridge Technology Centre

Executive summary

Background

Growing media manufacturers are actively looking for alternatives to peat to use in their products for the amateur garden market and for professional growers. The physical characteristics of green compost make it a potentially suitable alternative, and the material is used at low levels by some manufacturers for the domestic garden market – but there is concern over the suitability of this material for use in products for the professional sector. Testing in 2011 by Stockbridge Technology Centre indicated that a high proportion of green compost samples submitted for routine testing caused leaf twisting and distortion in field bean. The symptoms observed were typical of those associated with low level damage caused by synthetic auxin herbicides. The theoretical pathway for compost contamination is that grass clippings entering the green waste stream may contain residues of these persistent herbicides used by gardeners on their lawns or by turf care companies, and that proportions of these herbicides survive the composting process.

To begin to understand the potential extent and impact of persistent herbicide contamination in green composts, the project was split into four parts:

1. Developing and testing a bean bioassay to potentially replace or complement the current BSI PAS 100 weed and plant response test and provide an effective test for herbicide contamination. The sensitivity of three bean types to a range of parameters was tested, including electrical conductivity (which can be high in some green compost samples), herbicide residue levels and the glasshouse environment (to determine if the current BSI PAS 100 stipulated growing conditions were appropriate for a different plant species). An evaluation was also carried out to test whether or not the assay could be reduced to less than five weeks using module grown transplants instead of direct sowing.
2. Screening a range of plant species for their sensitivity to herbicide residues in green composts. The sensitivity of a wide range of bedding and vegetable plant species to clopyralid was tested by using green compost spiked with known concentrations of this herbicide.
3. Testing samples of green composts (GC) and green-food composts (GFC) for possible herbicide presence. Samples submitted for bioassay by laboratories appointed under the Compost Certification Scheme were tested for the presence of herbicide symptoms using field bean in a standardised test.
4. Testing a wide range of growing media available on the amateur market for herbicide residues. Samples were purchased from three different geographical regions and tested for the presence of herbicide symptoms using field bean in a standardised test.

Responding to suggestions from stakeholders given at a meeting held at STC in October 2012, the project was extended to carry out a series of experiments to confirm that the symptoms observed were due to herbicide contamination, and not any other cause, such as bacteria or heavy metal contamination.

A further extension to the project covered an experiment to test whether including powdered activated charcoal, which is known to absorb herbicide residues and render them unavailable to plants, could reduce the severity of symptom expression in field bean.

Conclusions and recommendations

1. Field bean showed both a wider range of visible symptoms to clopyralid than dwarf French bean, and clearer symptom expression than broad bean. Field bean was also tolerant of high electrical conductivity and grew well at lower temperatures and shorter day length.
2. Transplanted module raised field bean were sensitive to herbicide residues and could offer a quicker test than from direct sown seed.
3. Plant sensitivity screening using green compost treated with clopyralid confirmed that field bean, tomato and dahlia are all very sensitive. Lobelia, carrot, courgette, chilli peppers, cucumber, potato and pea were far less sensitive as were the other species tested.
4. Herbicide symptoms in field bean were observed for the majority of compost samples submitted for routine bioassay testing under the Compost Certification Scheme. These symptoms ranged in severity from slight leaf curling which was observed at three weeks after sowing to severe distortion of the growing point plus severe leaf curling.
5. There was no effect of the date of sample submission on the severity of herbicide symptoms confirming the results of similar research carried out by STC in 2011.
6. There was no apparent correlation between BSI PAS 100 certification status and the incidence of herbicide damage in field beans.
7. Of the 94 combinations of retail growing media and geographical location (of sample purchase) a total of 44 (47%) showed symptoms of herbicide damage in field bean. No herbicide symptoms were observed in growing media products known to exclude green compost.
8. Where BSI PAS 100 green compost samples were mixed with professional peat-based growing media at 1:2 by volume, herbicide symptoms were only observed on field beans. Considering that this incorporation rate is generally higher than that currently used in most branded peat-based growing media then most multi-purpose composts should be safe to use on all species except field beans and other related bean types.
9. The plant bioassay developed for field beans should be used to provide reassurance that BSI PAS 100 compost is potentially safe for including in growing media. Where a rapid result is required, using module raised transplants would reduce the duration of the test to four weeks.
10. The treatments applied to green compost and peat treated with clopyralid confirmed that the symptoms observed were not caused by anything else, such as bacteria or heavy metal contamination.
11. The current BSI PAS 100 plant response test using tomato should continue with the field bean bioassay used alongside for green compost samples that may be destined for use in growing media for the amateur market.
12. Consideration should be given to extending the current BSI PAS 100 plant response test to six weeks, or using transplanted tomato for four weeks as the current four week duration using seeded tomato is too short to detect herbicide effects.

Suggestions for further work

1. Continued testing of retail products known to contain green compost so that the extent of possible impacts can be determined and evaluated.
2. Testing the potential of using transplanted tomato alongside seeded tomato to determine if symptoms are observed within four or five weeks. This test would be less sensitive than field beans but may offer a more realistic assessment of green compost materials likely to cause problems if used in growing media products for the garden market.
3. The economic feasibility of adding powdered activated charcoal to green compost to reduce plant exposure to herbicide residues should be studied.

Contents

1.0 Introduction

Growing media manufacturers are actively looking for alternatives to peat to use in their products for the amateur garden market and for professional growers. The physical characteristics of green compost make it a potentially suitable alternative, and the material is used at low levels by some manufacturers for the domestic garden market – but there is concern over the suitability of this material for use in products for the professional sector. Testing in 2011 by Stockbridge Technology Centre indicated that a high proportion of green compost samples submitted for routine testing caused leaf twisting and distortion in field bean. The symptoms observed were typical of those associated with low level damage caused by synthetic auxin herbicides. The theoretical pathway for compost contamination is that grass clippings entering the green waste stream may contain residues of these persistent herbicides used by gardeners on their lawns or by turf care companies, and that proportions of these herbicides survive the composting process.

To begin to understand the potential extent and impact of persistent herbicide contamination in green composts, the project was split into four parts:

Part 1: Developing and testing a bean bioassay test to potentially replace or complement the existing BSI PAS 100 weed and plant response test
Part 2: Screening a range of plant species for their sensitivity to herbicide residues in green composts
Part 3: Testing samples of green composts (GC) and green-food composts (GFC) for possible herbicide presence
Part 4: Testing a wide range of growing media available on the amateur market for herbicide residues

An additional set of trials were commissioned in autumn 2012 following a stakeholder meeting. The aim of the additional work was to confirm that the symptoms observed on field bean were beyond reasonable doubt due to synthetic auxin herbicides and also to determine if the pH of the growing media affected symptom expression.

This final report summarises the key findings of the research programme conducted at Stockbridge Technology Centre and concludes with recommendations and suggestions for follow-up work.

2.0 Developing a robust plant response test to detect persistent herbicides

2.1 To determine the response of 10 cultivars of field, broad and dwarf French bean to electrical conductivity (EC) in a peat-based growing medium augmented with potassium chloride

Field beans have already been shown to be very sensitive to low levels of herbicides found in green compost. Dwarf French bean has been included as it may offer an alternative and, due to its more compact growth habit, may be more suitable – providing that it is equally sensitive to herbicides of concern. However, the type of bean must be tolerant to a range of EC in the peat + green compost mixes used in the BSI PAS 100 growth test due to the potentially high EC levels in some green compost materials.

Seven batches of Peat-Based Growing Medium (PBGM) were prepared in accordance with the BSI PAS 100 Annex D growth test using 0-25mm sphagnum peat (incorporating lime at 4g/l and soluble fertiliser (18:16:18) at 1g/l of peat as standard) to which 0-3g/l of laboratory grade potassium chloride was added. This provided mixes with a range of electrical conductivities (EC) ranging from 300-1100 µS/cm, as listed in Table 2-1.

Seeds of three cultivars of field bean, two cultivars of broad bean and five cultivars of dwarf French bean were therefore sown in each to determine their sensitivity to EC levels in respect of their speed of emergence and growth.

Table 2-1 Growing media analysis

PBGM Treatment (g/l KCl added)	EC (µS/cm)	
	Mean	Range
Nil - Control	312	296 – 326
0.5g/l	411	393 – 426
1g/l	603	550 – 683
1.5g/l	672	622 – 702
2g/l	880	818 – 932
2.5g/l	971	878 – 1071
3g/l	1084	1063 – 1109
Peat only	53	

The emergence of the bean seeds was slightly delayed where potassium chloride was incorporated into the peat at above 0.5g/litre of peat, but by five days later it was similar for field bean and broad bean. For dwarf French bean potassium chloride added at above 2.5g/l delayed final emergence and for some cultivars reduced final plant stand.

Plant size was generally similar for the field bean and broad bean for all except the highest rate of potassium chloride. However, for dwarf French bean plant vigour was reduced at the two highest rates at all assessment dates even at five weeks after sowing. Cultivars Cantares and Scuba appeared to be more sensitive to EC than the other cultivars of dwarf French bean. The effect on plant vigour was mainly a reduction in the bushiness of the plant.

At 5 weeks after sowing the plant heights were similar for most treatments, but dwarf French beans cultivars Laguna and Scuba were shorter at the highest two rates of potassium chloride.

At 5 weeks after sowing the plant weights were generally lower for all bean types at the higher rates of potassium chloride. There was a greater effect for the dwarf French beans, with lower mean plant weights at rates above 1.5g/l KCl.

Of the three bean types tested, dwarf French beans would be the least suitable for use in a compost bioassay as they appear to be more sensitive to high EC levels, which have a greater effect on plant weight at harvest. Foliage colour across all treatments was paler at three weeks after sowing compared to the field and broad bean plants which remained dark green. The preferred bean type would be field bean due to its tolerance of high EC levels with all three cultivars performing similarly.

2.2 Assessing ten cultivars of three bean types for their sensitivity to clopyralid herbicide

Field beans are known to be very sensitive to low levels of herbicide residues. Dwarf French bean may offer an alternative (notwithstanding their apparent sensitivity to EC, as outlined above), and due to its more compact growth habit may be a more suitable bioassay species provided it is also equally sensitive to clopyralid.

This study compared three cultivars of field bean (Fuego, Sultan and Tattoo), two cultivars of broad bean (Bunyards Exhibition and Monica) and five cultivars of dwarf French bean (Cantares, Faraday, Laguna, Parker and Scuba) covering both early and main season types for their sensitivity to clopyralid-treated compost.

These cultivars were sown in six growing media. Three media utilised commercially-sourced green compost product that had been treated ('spiked') with clopyralid at 0.000333, 0.00333 and 0.0333mg of active ingredient (ai) per litre of compost in 1:3 blends with sphagnum peat. The other three media were controls: a standard peat-based growing medium (PBGM) and two media incorporating un-spiked green composts in 1:3 blends with sphagnum peat. These two un-spiked green composts comprised:

- A commercially-sourced material (without herbicide addition); and
- A composite blend of green compost created from spare material submitted to STC for routine BSI PAS 100 testing.

Seedling emergence was three days earlier for dwarf French bean than field beans; broad beans were the slowest to emerge. Overall there did not appear to be an effect of herbicide rate on the speed of emergence or final plant stand. Plant size at two weeks after sowing was generally similar in all growing media treatments but the dwarf French beans were taller due to their earlier emergence.

Herbicide symptoms started to appear at two weeks after sowing for all bean types. The severity of symptoms were slight in the dwarf French bean at two weeks but by three weeks after sowing it had significantly increased at the highest rate of clopyralid with plants becoming severely stunted and the centre of the plant no longer growing. The plants turned very dark green. At the middle rate there was slight leaf curling in the centre of the plants only. Cultivars Cantares and Laguna showed more symptoms than the other three cultivars at the middle rate with only very slight symptoms at the lowest rate.

Broad bean and field bean cultivars showed a range of symptoms ranging from severe stunting of the growing point to slight leaf curling in compost treatments even where no obvious effects were visible in dwarf French bean. This damage increased from two to five weeks after sowing. Field bean showed symptoms for all compost treatments except the untreated PBGM control which contained no green compost. Overall, cultivars Fuego and Tattoo were more sensitive than Sultan.

The trial confirmed that field bean is very sensitive to clopyralid even at trace levels, and is a more reliable indicator than broad bean. Although dwarf French bean is very sensitive at higher rates of clopyralid they do not give as good a range of symptom expression at lower rates as field bean therefore would be less suitable for a bioassay test.

2.3 Comparing the effect on the growth and herbicide symptom expression of four glasshouse environmental regimes for field bean and dwarf bean

Field bean and dwarf (French) bean were grown under different glasshouse environmental regimes to determine the effect of temperature and supplementary lighting on plant growth and herbicide symptom expression. Plants were grown in peat-based growing medium (PBGM) and a mix of peat-based growing medium (PBGM) + green compost known to be contaminated with herbicide residues. The glasshouse temperature regimes compared the current recommendation in the BSI PAS 100 plant response test of 16°C minimum night temperature and minimum day temperature of 21°C (16/21°C) with a cooler regime of 12°C minimum night and 16°C minimum day (12/16°C). Half of the plants in each glasshouse were given supplementary lighting using high pressure sodium lights to create a 16 hour day length period with the other half under ambient light and natural day length.

Seeds of field bean cv. Fuego and dwarf (French) bean cv. Cantares were sown into two litre pots containing the two growing media. Seeds were sown on 8 March and 29 August with plants assessed over a five week period. The speed of emergence, plant size, plant height and herbicide symptom expression were assessed together with plant fresh weight at five weeks.

For the first sowing (8 March), seedling emergence was faster for dwarf French bean than field bean. Emergence was advanced where the warmer 16/21°C regime had been used, but final plant stand was similar to the cooler temperature regime of 12/16°C. Emergence was delayed for field bean grown in the contaminated compost mix with dwarf French bean less affected. Plant size at 3, 4 and 5 weeks and plant heights at 3 and 5 weeks were higher for 16/21°C regime. Plant size did not appear to be affected by lighting regime. Smaller plants were produced where the contaminated compost had been used for both bean types. This effect was similar for both temperature regimes. Plant weight at five weeks was higher for the 16/21°C regime, lower for the contaminated compost but similar for both lighting regimes. Herbicide symptom expression was higher for the 16/21°C regime with no increase in visible symptom expression from using supplementary lighting.

For the second sowing (29 August) a similar set of results were obtained showing a benefit on plant growth from using the 16/21°C regime. There was again no clear additional benefit from using supplementary lighting.

Overall, whilst plant emergence was slightly later at the lower temperature regime, plant growth was still good at the 12/16^{o}C regime. The supplementary lighting did not have a consistent effect on plant growth or herbicide symptom expression. To ensure adequate growth and herbicide symptom expression the higher temperature regime of 16/21°C should be used but there is probably no need to use supplementary lighting. However, there might potentially be a benefit from using supplementary lighting in December and January when ambient light levels are lower.

2.4 Investigation into the sensitivity to clopyralid and speed of symptom expression in direct sown and transplanted field bean and dwarf French bean

Module raised field bean and dwarf French bean transplanted into pots were compared with direct sown plants for their speed of symptom expression to auxin herbicide. Plants were grown in: peat-based growing medium (PBGM); a 2:1 mix of PBGM + green compost known to be contaminated with herbicide residues; a 2:1 mix of PBGM + a green compost product (GC) spiked with clopyralid at 0.0, 0.000333, 0.00333 and 0.0333mg ai/litre of GC. The trial was set up in a glasshouse with a minimum night temperature of 16°C and a minimum daytime temperature of 21°C. Supplementary lighting was provided to both extend the day length and increase light intensity to 6000 lux. Plants were assessed for growth and the severity of herbicide damage symptoms at 2, 3, 4 and 5 weeks.

Figure 2-1 Size of field bean (left) and dwarf French bean (right) at transplanting

Herbicide symptom expression was observed in the transplanted beans at two weeks after transplanting for both bean types. At three weeks after sowing a range of symptoms were observed in the field bean for all growing media treatments except the PBGM which remained free from any signs of damage. Symptoms were only observed in the dwarf bean at the two highest rates of clopyralid.

The damage in the field bean sown directly into the pots was generally delayed by a week and was less severe, with smaller plants compared to the transplanted plants.

Overall the trial has confirmed that field bean is far more sensitive to clopyralid residues than dwarf French bean. The use of module raised field bean transplants could reduce the length of the bioassay test to just 3 weeks without compromising the quality and robustness of the test. This would allow the test to be completed earlier although transplants would need to be grown and available for use in the test.

3.0 Screening a range of common flower, bedding plant and vegetable species

3.1 Flower and bedding plant species

3.1.1 Objectives

To screen a range of popular flower and bedding plant species grown by gardeners for their sensitivity to herbicide residues that may be present in growing media that contain green compost.

3.1.2 Experimental details

A. Plant species

Species and cultivar	Plant family
1. *Alyssum* cv. Bonnet Violet (F1)	Brassicaceae**
2. *Antirrhinum majus* cv. Appeal Pink (F1)	Plantaginaceae
3. *Begonia semperflorens* cv. Devil Pink (F1)	Begoniaceae (related to Cucurbitaceae)
4. *Dahlia* cv. Figaro Orange (F1)	Asteraceae*
5. *Dianthus* cv. Festival Violet Deep (F1)	Caryophyllaceae
6. *Fuchsia* (rooted cuttings) cv. Blackie	Onagraceae
7. *Geranium* cv. Palladium Scarlet (F1)	Geramiaceae
8. *Impatiens* (New Guinea) cv. Divine Violet (F1)	Balsaminaceae
9. *Lobelia erinus* cv. Cambridge Blue (F1)	Campanulaceae
10. Marigold cv. Orange Boy (F1)	Asteraceae*
11. Pansy cv. Matrix Blue Blotch (F1)	Violaceae
12. *Petunia* (seed raised) cv. Express Rose (F1)	Solanaceae*
13. *Polyanthus* cv. Piano Rose (F1)	Primulaceae
14. *Primula acaulis* cv. Bonneli Pink (f1)	Primulaceae
15. *Salvia* cv. Blaze of Fire (F1)	Laminaceae
16. *Verbena* cv. Quartz XP Carmine Rose (F1)	Verbenaceae
17. *Viola cornuta* cv. Sorbet XP Purple (F1)	Violaceae
Control indicator species	
1. Tomato cv. Shirley	Solanaceae*
2. Field bean cv. Fuego	Fabaceae*

*Families known to be sensitive to aminopyralid and clopyralid
**Families insensitive to aminopyralid and clopyralid

B. Growing media and herbicide levels

1. Peat-based growing media control (Levington M2)
2. Peat-based growing media (M2) + BSI PAS 100 compost test samples known to have levels of herbicide residue detectable by field bean bioassay (mix 2:1)
3. Peat-based growing media (M2) + green compost (GC) (mix 2:1) nil clopyralid incorporated
4. Peat-based growing media (M2) + green compost spiked with clopyralid at 0.000333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)
5. Peat-based growing media (M2) + green compost spiked with clopyralid at 0.00333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)
6. Peat-based growing media (M2) + green compost spiked with clopyralid at 0.0333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)

Note – the rates of clopyralid selected are those which showed a range of severity symptoms of herbicide damage to field beans in a previous WRAP project (OAV035-003). The highest treatment rate (treatment 6) showed a damage level similar to that observed in the worst case of contamination of compost samples observed in informal testing of BSI PAS 100 samples at STC in 2011.

A commercially available green compost soil conditioner product was obtained for treating with clopyralid at 0.0 – 0.0333mg ai/litre of GC. The clopyralid treatments were applied using a gas pressurised Oxford Precision Sprayer fitted with a single nozzle lance. The clopyralid treatment was applied as three separate applications with thorough incorporation between each to ensure uniform distribution within the green compost. The treated compost was then placed into a polythene bag and was allowed to rest for 11 days before mixing with Levington M2 at a rate of 2:1 (M2:green compost). Standard NPK fertiliser (18:16:18) was added at 1g/litre of green compost.

Plug plants of 15 species were produced in plastic module trays with rooted fuschia cuttings and *Begonia semperflorens* sourced from Ball Colegrave Ltd. The plug plants were grown in Levington F2 compost (which contains no green compost). Three plants of two species were transplanted into each of three six-pack trays containing the growing media treatments on 30 April. Species with similar growing habits were paired together to minimise vigour and competition issues. Tomato and field bean were also grown as plug plants and transplanted into trays of each media as indicator species. The plants were grown for 8 weeks with three replicates of each species and with two species per six-pack tray (9 plants per treatment).

Due to the need to avoid contamination of adjacent plots the six-pack trays were stood on upturned empty plastic seed trays. These empty trays were stood on capillary matting laid on a solid polythene sheet to contain any run off.

The trial was carried out in a glasshouse with a minimum temperature of 14°C at night and a minimum daytime temperature of 16°C with ventilation at 18°C.

3.1.3 Results

The first herbicide damage symptoms were observed at two weeks after transplanting in dahlia and field bean and after three weeks for tomato. These three species are known to be sensitive to clopyralid from previous work.

The damage increased over the following five weeks but no symptoms were observed on any of the other plant species except for Lobelia where there was very slight inward leaf curling.

The results are presented in Table 3-1 to Table 3-4 for each of the two affected bedding/flower plant species and the two indicator species.

Table 3-1 Herbicide damage symptoms (0=nil and 10=severe) on dahlia 2-8 weeks after transplanting

Treatment	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks
M2 (Control)	0	0	0	0	0	0
M2 + PAS 100 compost samples	0	0	0	0	0	0
M2 + Spiked GC product						
0.0mg ai clopyralid/l	0	0	0	0	0	0
0.000333mg ai clopyralid/l	0	0	0.3	0.3	0.7	0.7
0.00333mg ai clopyralid/l	0	0	0.7	0.7	1.0	1.0
0.033mg ai clopyralid/l	2.0	4.3	5.7	6.3	6.6	6.6

At two weeks after transplanting there was visible damage on the dahlia plants grown in the medium containing GC treated at the highest rate of clopyralid. The new leaves had become serrated and were thicker and duller. By four weeks the damage had increased and was also present at lower levels of the two other clopyralid treatments. There was no damage on the plants grown in M2 + PAS 100 samples.

Table 3-2 Herbicide damage symptoms (0=nil and 10=severe) on lobelia 3-8 weeks after transplanting

Treatment	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks
M2 (Control)	0	0	0	0	0
M2 + PAS 100 compost samples	0.3	0	0	0	0
M2 + Spiked GC product					
0.0mg ai clopyralid/l	0	0.3	0.3	0.3	0.3
0.000333mg ai clopyralid/l	0	0.7	0.3	0.3	0.3
0.00333mg ai clopyralid/l	0.3	0.3	0.3	0.3	0.3
0.033mg ai clopyralid/l	0.3	1.0	1.3	1.3	1.3

At three weeks after transplanting there was slight inward leaf curling on some of the plants. This was observed at a slightly higher level where clopyralid had been applied to the GC at the highest rate. Plants were also slightly smaller for this treatment, but flowering was unaffected.

Table 3-3 Herbicide damage symptoms (0=nil and 10=severe) on field bean 3-8 weeks after transplanting

Treatment	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks
M2 (Control)	0	0	0	0	0
M2 + PAS 100 compost samples	0	0.7	1.3	3.3	3.7
M2 + Spiked GC product					
0.0mg ai clopyralid/l	0	0	0	0.7	0.7
0.000333mg ai clopyralid/l	0	0	0	0.3	0.3
0.00333mg ai clopyralid/l	0.7	1.3	2.7	5.0	5.0
0.033mg ai clopyralid/l	4.7	5.7	6.0	7.3	7.3

At three weeks after transplanting there was visible damage on the field bean plants grown in the medium containing GC treated at the highest rate of clopyralid and a very slight effect at the middle rate of clopyralid. By four weeks the damage had increased and was also present at a lower level on the M2 + PAS 100 samples. By six weeks there was also damage on the 0.0mg ai/l clopyralid treatment and this suggests that there may have been some prior contamination of this material.

Table 3-4 Herbicide damage symptoms (0=nil and 10=severe) on tomato 3-8 weeks after transplanting

Treatment	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks
M2 (Control)	0	0	0	0	0
M2 + PAS 100 compost samples	0	0	0	0	0
M2 + Spiked GC product					
0.0mg ai clopyralid/l	0	0	0	0	0
0.000333mg ai clopyralid/l	0	0	0	0	0
0.00333mg ai clopyralid/l	0	0	0	0	1.7
0.033mg ai clopyralid/l	0	1.3	2.7	4.3	5.0

At four weeks after transplanting there was visible damage on the Tomato plants grown in the medium containing GC treated at the highest rate of clopyralid. By six weeks the damage had increased. By eight weeks there was also damage at the second highest rate of clopyralid treatment. These damage levels are lower than observed in field bean and again confirm that tomato is less sensitive to herbicide damage than field bean.

3.1.4 Conclusions

1. Herbicide damage symptoms were observed in only two of the flower and bedding species tested, indicating that the remaining 15 species are not sensitive to clopyralid at the rates used.
2. Dahlia was the most sensitive ornamental species and this confirms previous findings.
3. Lobelia also appeared sensitive but the effects were only very slight and did not appear to have an effect on plant size or flowering.
4. Fifteen of the seventeen flower and bedding species representing 14 plant families exhibited no herbicide damage symptoms at all. Surprisingly these included marigold (Asteraceae) and petunia (Solanaceae) from clopyralid-sensitive families.
5. Field bean and tomato transplants both showed damage which increased at the higher rates of clopyralid dosage. Damage levels were lower in the tomato.
6. Field bean showed herbicide damage symptoms in the M2 + PAS 100 compost samples but no effects were observed in the dahlia for this treatment. This is a surprising result due to the known sensitivity of dahlia to synthetic auxin herbicides.

3.2 Vegetable species

3.2.1 Objectives

To screen a range of popular vegetable plant species grown by gardeners for their sensitivity to herbicide residues which may be present in growing media available to amateur gardeners that contain green compost.

3.2.2 Experimental details

A. Plant species

Species and cultivar	Plant family
1. Tomato cv. Moneymaker	Solanaceae *
2. Runner bean cv. Scarlet Emperor	Fabaceae *
3. Beetroot cv. Bolthardy	Chenopodiaceae ***
4. Carrots cv. Early Nantes 2	Apiaceae *
5. Lettuce cv. Little Gem	Asteraceae *
6. Parsnip cv. Gladiator	Apiaceae *
7. Leek cv. Musselburgh Improved	Amaryllidaceae (was Alliaceae)***
8. Courgette cv. All Green Bush	Cucurbitae
9. Pea cv. Hurst Green Shaft (Main Crop)	Fabaceae *
10. Mustard cv. Salad Leaves Oriental Mustard	Brassicaceae **
11. Purple sprouting broccoli cv. Choice Selection Mixed	Brassicaceae **
12. Chilli cv. Prairie Fire	Solanaceae *
13. Cucumber cv. Marketer	Cucurbitae
14. Basil cv. Sweet Green	Laminaceae
15. Parsley cv. Champion Moss Curled	Apiaceae *
16. Potato cv Maris Piper	Solanaceae *
Control indicator species	
1. Field bean cv. Fuego	Fabaceae *
2. Tomato cv. Shirley	Solanaceae *

*Families known to be sensitive to aminopyralid and clopyralid
**Families insensitive to aminopyralid and clopyralid
***Families insensitive to clopyralid

B. Growing media and herbicide levels

1. Peat-based control (Levington F2)
2. Peat-based (F2) + PAS 100 compost test samples green compost known to have moderate levels of herbicide residue (mix 2:1)
3. Peat-based (F2) + green compost (mix 2:1) nil clopyralid incorporated
4. Peat-based (F2) + green compost spiked with clopyralid at 0.000333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)
5. Peat-based (F2) + green compost spiked with clopyralid at 0.00333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)
6. Peat-based (F2) + green compost spiked with clopyralid at 0.0333 mg ai/litre of green compost and allowed to rest for 1 week before use (mix 2:1)

Note – the rates of clopyralid which have been selected are those which showed a range of severity symptoms of herbicide damage to field beans in a previous WRAP project (OAV035-035). The highest treatment rate (treatment 6) showed a damage level similar to that observed in the worst case of contamination of compost samples observed in informal testing of BSI PAS 100 samples at STC in 2011.

A commercially available green compost soil conditioner product was obtained for treating with clopyralid at 0.0 – 0.0333mg ai/litre of GC. The clopyralid treatments were applied using a gas pressurised Oxford Precision Sprayer fitted with a single nozzle lance. The clopyralid treatment was applied as three separate applications with thorough incorporation between each to ensure uniform distribution within the green compost. The treated compost was then placed into a polythene bag and was allowed to rest for 11 days before mixing with Levington F2 at a rate of 2:1 (F2:green compost). Standard NPK fertiliser (18:16:18) was added at 1g/litre of green compost.

Seeds were sown into plastic module trays (104 cells/tray (4 trays per square metre) with a cell volume of 45ml) containing the growing media treatments on 1 May. Three potato tubers were planted into three litre pots containing the compost treatments. Field bean and tomato were also sown and included as indicator species. The plants were grown for 8 weeks after sowing with three replicates of each species and growing media and with 22 seeds sown per replicate (66 seeds per treatment).

Due to the need to avoid contamination of adjacent plots the module trays and pots were stood on upturned empty plastic seed trays. These empty trays were stood on capillary matting laid on a solid polythene sheet to contain any run off.

The trial was carried out in a glasshouse with a minimum temperature of 14°C at night and a minimum daytime temperature of 16°C with ventilation at 18°C.

3.2.3 Results

Seeding emergence was generally uniform across all species with no visible differences between the growing media plus herbicide treatments. However, emergence was slightly delayed for the F2 + PAS 100 compost samples – probably due to higher Electrical Conductivity levels.

The first herbicide damage symptoms were observed at three weeks after sowing in field bean but not until after four weeks for the test species. Damage increased over the following five weeks.

The results are presented in Table 3-6 for each of the seven affected plant species and in Table 3-5 for the two indicator species.

Table 3-5 Herbicide damage symptoms (0=nil and 10=severe) for the two indicator species three to eight weeks after sowing

Treatment	Field bean			Tomato cv. Shirley	
	3 weeks	4 weeks	6 weeks	4 weeks	6 weeks
F2 (Control)	0	0	0	0	0
F2 + PAS 100 compost samples	0	0	0	0	0
<u>F2 + Spiked GC product</u>					
0.0mg ai clopyralid/l	0	0	0	0	0
0.000333mg ai clopyralid/l	0	0	2.0	0	0
0.00333mg ai clopyralid/l	0	2.0	4.0	0	0
0.033mg ai clopyralid/l	4.0	7.0	6.0	2.0	5.0

Table 3-6 Herbicide damage symptoms (0=nil and 10=severe) for seven test species three to eight weeks after sowing or planting

Treatments	Chilli	Carrot		Tomato cv. Moneymaker		
	8 weeks	6 weeks	8 weeks	4 weeks	6 weeks	8 weeks
F2 (Control)	0	0	0	0	0	0
F2 + PAS 100 compost samples	0	0	0	0	0	0
F2 + Spiked GC product						
0.0mg ai clopyralid/l	0	0	0	0	0	0
0.000333mg ai clopyralid/l	0	0	0	0	0	0
0.00333mg ai clopyralid/l	0	0	0	0.3	0.3	0.7
0.033mg ai clopyralid/l	1.3	2.3	2.3	1.7	7.0	8.0

Treatments	Courgette	Pea			Cucumber	Potato		
	4 weeks	3 weeks	4 weeks	6 weeks	4 weeks	4 weeks	6 weeks	8 weeks
F2 (Control)	0	0	0	0	0	0	0	0
F2 + PAS 100 compost samples	0	0	0	0	0	0	0	0
F2 + Spiked GC product								
0.0mg ai clopyralid/l	0	0	0	0	0	0	0	0
0.000333mg ai clopyralid/l	0	0	0	0	0	0	0	0
0.00333mg ai clopyralid/l	0.3	0	0	0	0	0	0	0
0.033mg ai clopyralid/l	1.0	1.3	3.3	4.0	2.0	2.0	1.0	1.0

The species which were most severely affected were tomato and peas, with distortion of the growing point. For chilli, carrot, courgette, cucumber and potato the symptoms were slight – even at the highest rate of clopyralid.

The field bean and tomato used as indicator species both showed herbicide symptoms with field bean showing moderate severity at the highest rate. In the field bean only damage was also present at the two other rates of clopyralid but was less severe and slower to develop.

The species unaffected by the incorporation of clopyralid at the rates used in this test were parsley, basil, leek, mustard, beetroot, parsnip, purple sprouting broccoli, lettuce and runner beans.

3.2.4 Conclusions

1. Herbicide damage symptoms were observed in six of the vegetable species raised from seed and also potato grown from tubers, while nine species showed tolerance to clopyralid at the rates used. Five of these (tolerant) species were representative of three families known to be sensitive to clopyralid and two were Cucurbiteae which is a family that is widely recognised as being sensitive to hormone weedkillers.
2. Tomato cv. Moneymaker and pea were the most sensitive species, with distortion of the growing point at the highest rate of clopyralid. Tomato cv. Moneymaker was more sensitive than cv. Shirley used as the indicator species.
3. Chilli, carrot, courgette, cucumber and potato were less sensitive, showing only slight leaf curling.
4. Field bean (used as an indicator species) showed herbicide damage which increased over time, with damage also present at the lower rates of clopyralid. Damage levels were lower in tomato cv. Shirley (used as the second indicator species).
5. Nine species exhibited no herbicide damage symptoms in any growing media treatment. These included representatives not only from families said to be insensitive to clopyralid (beetroot, broccoli, leek and mustard; and basil – which has not been classified), but representatives of three families said to be sensitive to clopyralid (lettuce, parsnip, parsley and runner bean). This shows that even within families there can be a variety of responses to clopyralid and other herbicides.
6. Field bean has been confirmed as extremely sensitive to herbicide residues. Indeed, it demonstrates symptoms at (spiked) concentrations that do not produce symptoms in other species tested. Determining the highest (spiked) herbicide concentration at which no tested species exhibited symptoms other than field bean (Table 3-1 to Table 3-6), it is possible to derive a threshold symptom score below which symptoms in field bean indicate that the compost under test is likely to be suitable for growing media use, and above which symptoms indicate that the compost under test is unlikely to be suitable for use in growing media. This threshold score is 2 (equating to moderate symptoms). Should users of compost have species-specific concerns, then they should test the material using those species of interest.

4.0 Assessing for the presence of herbicides in compost and growing media

4.1 Composts

4.1.1 Objectives

To test samples of compost (including those derived from inputs that include a proportion of food waste) submitted in 2012 to NRM Ltd and other Compost Certification Scheme-appointed laboratories for BSI PAS 100 plant response testing, for herbicide symptoms using the field bean bioassay test.

4.1.2 Experimental details

Samples were provided and tested over a seven month period commencing March 2012. Green (and green/food) compost (GC) samples sent to NRM Ltd for BSI PAS 100 tomato plant response testing were all tested, as were samples supplied by other appointed laboratories through a request sent out by the Association for Organics Recycling (AfOR[1]).

The compost samples were tested within three to six weeks of arrival and were diluted with peat-based growing medium (PBGM) on a standard 1:2 (GC:PBGM) basis. This represents the current maximum dilution rate used in the BSI PAS 100 plant response test. A PBGM control was made using sphagnum peat.

The plants were monitored from emergence until five weeks after sowing. The plants were assessed for herbicide symptoms on a weekly basis using the scoring scale developed in a previous WRAP study (OAV035-003, Appendix 11).

4.1.3 Results

The results for herbicide symptom expression for all samples and sowing dates are presented in Table 4-1.

Table 4-1 Number of samples in each category for each field bean sowing date. Composts with symptoms scoring 2 and above may be unsuitable for use in growing media intended for general use

Sowing date	No. of samples	No symptoms (0)	Slight symptoms (1)	% with no to slight symptoms	Moderate Symptoms (2 & 3)	Severe Symptoms (4 & 5)	% with moderate to severe symptoms
8 March	52	1	35	69%	16	0	31%
22 March	15	1	9	67%	5	0	33%
11 April	18	2	9	61%	6	1	49%
1 May	23	3	7	44%	11	2	66%
21 May	15	5	5	67%	5	0	33%
13 June	31	7	5	39%	19	0	61%
6 August	35	7	18	71%	10	0	29%
13 August	37	2	16	49%	15	4	51%
29 August	13	2	11	100%	0	0	0%
10 Sept	27	7	7	52%	12	1	48%
17 Sept	5	1	4	100%	0	0	0%
27 Sept	11	2	3	46%	6	0	54%
Total	**282**	**40**	**129**	**60%**	**105**	**8**	**40%**

Overall, 282 samples were tested. Of these, 40% may be unsuitable for use in growing media destined for the open market (where there would be no control over the plant species grown), but could still be used to grow herbicide-tolerant species.

[1] *At the time of publication, AfOR has become the Organics Recycling Group (ORG) within the Renewable Energy Association (REA)*

There did not appear to be a consistent effect of sowing date on herbicide symptom expression. An assumption has been made that the sample submission date would be approximately 10-16 weeks after the start of the composting process. Thus no seasonal effect was identified.

The results presented in Table 4-2 are for samples submitted through NRM Ltd where the certification status of the composting company is known.

Table 4-2 Number of samples in each damage category for each status of certification

BSI PAS 100 status	Number of samples	Number in each damage category				% with symptoms
		Nil symptoms (0)	Slight symptoms (1)	Moderate symptoms (2 & 3)	Severe symptoms (4 & 5)	
Certified	216	31	100	73	12	86
Expired	16	1	4	11	0	94
Applying	8	2	3	2	1	75
Not in scheme	22	5	9	8	0	77
Total	**262**	**39**	**116**	**94**	**13**	**85**

A high proportion of the samples came from BSI PAS 100 certified sites. There did not appear to be any correlation between the incidence of symptoms with the certification status of the composted material.

4.1.4 Conclusions

1. Of the composts tested, 40% may not be suitable for use as a constituent in growing media that are used to grow herbicide-sensitive species. They would be suitable for herbicide-tolerant species.
2. There was no obvious effect from sampling/sowing date on symptom expression indicating that samples provided over the seven month period contained herbicides with no apparent increase during July and August. This period would normally be expected to potentially have a higher proportion of grass clippings that may have been treated with home and garden lawn weed killers – this assumes that samples were sent soon after the composting process had been completed.
3. There did not appear to be any correlation between the incidence of symptoms with the certification status of the composted material.

4.2 Amateur growing media

4.2.1 Objectives

To test a range of amateur peat-free and peat-based growing media for the presence of herbicide using a field bean bio-assay; and to compare the sensitivity of transplanted and seeded tomatoes with field beans in samples already known to be contaminated with these residues.

4.2.2 Experimental details

A total of 41 retail bagged products that are commonly available including multi-purpose (MPC) and specific use media and growing bags covering both peat-free and peat-based formulations were purchased from retail outlets around York (North), Coventry (Central) and Lincoln (East) or via the Internet. Where it was not possible to source 'key' products from all regions an additional bag of each was sourced from an alternative Yorkshire location. Eighteen brands were represented.

Part 1

Pots were filled on 14 March 2012 with the different growing media and sown with 10 seeds of field bean (cv. Fuego) with two pots filled from each available bag. They were grown in a glasshouse on benches and monitored for five weeks. Saucers were placed under the 2 litre pots to collect any run-off and to avoid cross contamination.

Part 2
Using those products showing herbicide symptoms in Part 1 a second test was started on 15 May 2012. This compared transplanted tomato (cv. Shirley) with three replicate pots (300 ml volume) and seeded tomato with three replicate half-sized seed trays (10 seeds sown per tray) plus three replicate two litre pots of field bean. These were monitored for six weeks so that field bean symptoms could be correlated with any symptoms observed on transplanted and direct seeded tomato. The test was longer than the standard duration of four weeks to allow symptom expression to develop in the tomato plants.

All tests were carried out in a glasshouse with a minimum temperature of 16°C at night and a minimum daytime temperature of 20°C with ventilation at 22°C.

4.2.3 Results

Part 1
The results for the herbicide symptoms observed in field bean at five weeks after sowing are presented in Table 4-3.

Table 4-3 Mean level of herbicide symptoms exhibited by field bean at 5 weeks after sowing (0=nil damage, 5=severe)

No.	Product	North	Central	East
1	Peat-based MPC	1.5*	2.5	2.0
2	Peat-based MPC + JI	0.5	0.5	0
4	Peat-based MPC	0	0	0
5	Peat-based MPC + JI	0.75	-	0.5
7	Peat-based MPC	-	0	0
8	Peat-based MPC	0	0	0
10	Peat-based MPC	0	0	-
11	Peat-based MPC	0	-	-
15	Peat-based MPC	0	0	0
27	Peat MPC	0	0	-
33	Peat-based All Purpose	0	0	0
35	Peat-based MPC	0	0	0
38	Peat-based MPC	0	0	0
39	Peat-based MPC	0	0	0
40	Peat-based MPC	-	-	0
13	Peat-based Seed and Cutting	0	-	-
41	Peat-based Seed and cutting	0	-	-
18	Peat-based Tub and Basket	0.25	0	0
26	Peat-based Tub and Basket	0.75	0	1.0
36	Peat-based Pot and Container	0	0	0*
37	Peat-based Container and Basket	0	0	0
32	Peat-based grow bag	0	0.75	1.0
24	Peat-based Planter	2.0*	0	1.0*
25	Peat-based grow bag	1.0	1.5	1.5
6	Peat-free MPC	2.0*	0*	2.0**
9	Peat-free MPC	1.5	1.0	1.0
12	Peat-free MPC	1.0*	3.0*	-
16	Peat-free Compost	1.0	-	-

No.	Product	North	Central	East
17	Peat-free Seed and Cutting	0.25	1.0	0.25**
28	Peat-free compost	3.5	-	-
29	Peat-free compost	1.5	-	-
34	Peat-free All Purpose	0	0	0
20	Peat-free garden compost	2.0*	2.5	1.75*
23	Peat-free potato planter	0.75	-	-
30	Peat-free Veg compost	-	1.0	-
31	Peat-free garden compost	3.0*	-	-
3	Organic and Peat free MPC	0	0.5	1.0
14	Organic MPC	0	-	-
21	Organic and Peat free Seed and Cutting	0	-	-
19	Organic and Peat free vegetable Compost	1.0	1.0*	1.5
22	Organic and Peat free Growbag	2.0*	0.75	1.25*

*second sowing due to poor emergence for first sowing
**additional bag from second Yorkshire location

Emergence was good overall but for some products it was poor so a second sowing was made and these results are reported.

Herbicide damage symptoms were observed within 4 weeks of sowing and for many products they increased slightly between 28 and 35 days after sowing. The symptoms ranged in severity from slight leaf curling in the head of the plant to moderate distortion of the growing point.

The damage was observed in both peat-free and peat-based MPC and the specific growing media products, with a higher number of organic and peat–free materials showing herbicide damage symptoms. Damage was observed in all four ‘growing bags’.

Part 2
The results for the second bio-assay for those products showing herbicide symptoms for Part 1 are presented in Table 4-4.

Table 4-4 Mean level of herbicide symptoms exhibited on field bean, transplanted and seeded tomato in selected products at 4, 5 and 6 weeks after sowing (0=nil damage, 5=severe)

No.	Growing media type	Source region	Field beans			Transplanted tomato			Seeded tomato		
			4wks	5wks	6wks	4wks	5wks	6wks	4wks	5wks	6wks
1	Peat-based MPC	C	2.0	2.7	2.7	0	0	0	0	0	0
1	Peat-based MPC	E	1.0	2.0	2.7	0	0	0	0	0	0
26	Peat-based Tub and basket	E	1.0	1.0	1.0	0	0	0	0	0	0
32	Peat-based grow bag	C	1.0	1.0	1.0	0	0	0	0	0	0
32	Peat-based grow bag	E	0	0	0.3	0	0	0	0	0	0
24	Peat-based Planter	N	1.0	2.0	2.0	0	0.5	0.5	0	0	0
24	Peat-based Planter	E	1.0	1.3	1.3	0	0	0	0	0	0
25	Peat-based grow bag	N	1.0	1.7	1.7	0	0	0	0	0	0
25	Peat-based grow bag	C	1.0	1.0	1.0	0	0	0	0	0	0
25	Peat-based grow bag	E	0	1.0	2.0	0	0	0	0	0	0
6	Peat-free MPC	N	0	1.0	2.0	0	0	0	0	0	0
6	Peat-free MPC	E#	1.0	2.0	2.0	0	0.25	0.5	0	0	0
9	Peat-free	N	1.0	1.3	1.3	0	0	0	0	0	0
9	Peat-free	C	1.0	1.3	1.3	0	0	0.5	0	0	0
9	Peat-free	E	0	0.7	1.0	0	0	0	0	0	0
12	Peat-free MPC	N	0.7	1.7	2.0	0	0	0	0	0	0
12	Peat-free MPC	C	0.7	2.0	2.3	0	0	0.5	0	0	0
16	Peat-free compost	N	1.0	1.3	1.3	0	0	0	0	0	0
17	Peat-free seed and cutting	E#	0.7	1.0	1.0	0	0	0	0	0	0
28	Peat-free compost	N	2.0	3.0	3.0	2.5	2.5	3.5	0	0	0.5
29	Peat-free compost	N	1.0	1.3	2.0	1.5	2.0	2.0	0	0	0.7
20	Peat-free garden compost	N	1.3	3.0	3.7	2.2	2.5	3.5	0	0	0.5
20	Peat-free garden compost	C	1.0	1.0	1.3	0	0	0	0	0	0
20	Peat-free garden compost	E	1.7	1.7	2.3	0.5	0.5	0.75	0	0	0
30	Peat-free Veg compost	C	0	0.7	1.3	0	0	0	0	0	0
31	Peat-free garden compost	N	1.0	1.7	2.3	2.0	2.0	2.5	0	0	0.5

No.	Growing media type	Source region	Field beans			Transplanted tomato			Seeded tomato		
			4wks	5wks	6wks	4wks	5wks	6wks	4wks	5wks	6wks
3	Organic and Peat-free MPC	E	0.7	1.3	1.7	0	0	0	0	0	0
19	Organic and Peat-free veg compost	N	1.0	1.0	1.0	0	0	0	0	0	0
19	Organic and Peat-free veg compost	C	0	1.0	1.3	0	0	0	0	0	0
19	Organic and Peat-free veg compost	E	0.7	1.0	1.0	0	0	0	0	0	0
22	Organic & Peat-free Growbag	N	2.0	2.7	2.7	0.5	1.0	1.5	0	0	0
22	Organic & Peat-free Growbag	E	1.0	1.0	1.0	0	0	0	0	0	0

additional bag from second Yorkshire location

Herbicide damage symptoms were observed on field beans in all of these 18 products – although as suggested above, the extreme sensitivity of field bean to herbicides may mean that some or all of these products were still suitable for their intended uses. Herbicide symptoms were also observed in transplanted tomatoes grown in nine growing media products. The damage was mainly confined to the new leaves at the top of the plant with slight distortion of the leaves commonly referred to as 'nettling' as the leaves become more serrated and start to look like nettle leaves. Very slight herbicide symptom expression on the seeded tomato was observed on four products, even after six weeks.

4.2.4 Conclusions

1. Herbicide symptoms were observed on field beans grown in 22 of the 41 bagged products from garden centres, DIY stores and other retailers. They included branded and own-brand products.
2. Damage symptoms on field bean ranged from very slight leaf curling to severe twisting and head distortion.
3. Tomato again proved to be less sensitive than field bean although using transplants, rather than seed, and growing for six weeks increased the expression of symptoms.
4. Compared with field bean, damage symptoms on transplanted tomato were far less severe – consisting of the characteristic 'nettling' in the head of the plants often not observed until four or five weeks after transplanting. The damage was observed in far fewer products, presumably because tomato requires higher levels of herbicide residue to express symptoms.
5. Damage levels usually increased over time for both crops with symptoms in tomato taking longer to develop.
6. These results clearly show that some products could affect the performance of tomato and other sensitive crops. This could be a potentially serious issue if gardeners used these products for filling raised beds, tubs and other containers for growing tomato and other vegetable crops which might accumulate more herbicide residues from the compost over time as the plants grew larger. This reinforces the need to establish a clear threshold for field bean bioassay symptoms, at (or above) which composts should not be used in growing media (and equivalent products) destined for amateur or professional use.

4.3 Evaluating a range of treatments to confirm that the symptoms observed in field beans are due to herbicide residues in green compost

This element of the project looked at a range of treatments which were intended to prove beyond reasonable doubt that herbicide residues in the various tested growing media were the cause of the symptoms found in field bean, which is known to be very sensitive to auxin based herbicides.

Unfortunately due to the very low levels of herbicide levels in the materials (estimated at less than 3ppb) laboratory determination is potentially impossible, or at best unreliable and expensive – so a series of field bean bioassays were used, with various treatments intended to rule out other possible causes of the symptoms seen. These treatments included sterilisation to kill the bacteria present, addition of powdered activated charcoal (PAC) to absorb any herbicide residues present, addition of EDTA to absorb any heavy metals present and use of Ethysorb™ to absorb ethylene in the vicinity of the plant.

These treatments were applied to peat-based growing media (PBGM), composite green compost samples retained from BSI PAS 100 tests, commercially available growing media known to cause low symptom expression (presumed to be due to low levels of herbicide contamination), commercially available growing media known to cause moderate symptom expression (presumed to be due to moderate levels of herbicide contamination), PBGM spiked with clopyralid @0.0011mg ai/l of peat and PBGM spiked with clopyralid @0.011mg ai/l of peat. Field beans, known to be very sensitive to herbicide residues, were sown on 24 January 2013 with the trial carried out in warm glasshouse.

There was no reduction in symptom expression following sterilisation of the growing media, so it can be assumed that the symptoms were not caused by bacteria present in the material as autoclaving for 25 minutes should have killed the bacteria. Likewise, there was no reduction in symptom expression following the application of EDTA or the use of Ethysorb™, indicating that the symptoms were not caused by the presence of any heavy metal or ethylene produced by bacteria in the growing media (unless the rates applied were too low).

4.3.1 Assessing the effect of pH on symptom expression in field beans

This element of the project looked at the effect of pH on the expression of symptoms considered to be caused by the presence of herbicide residues in growing media and green compost. Lime was added at three rates (0, 4 and 8g/litre of peat) to create three pH levels using peat-based growing media (PBGM) (sphagnum peat (50% fine (0-10mm) and 50% medium (0-20mm)) which had been treated with four rates of clopyralid (0, 0.00011, 0.0011 and 0.011mg ai/l of peat). These treatments were compared to PBGM which had been treated with ethylamine at 0.011mg product/l of peat to simulate the use of a blank (inactive) formulation of herbicide. Field beans were sown on 31 January 2013 with the trial carried out in a warm glasshouse.

Leaf curling and twisting of the growing point was observed within two weeks of sowing but no classic symptoms were observed throughout the assessment period on the PBGM control or on the ethylamine treatment after two weeks. The highest symptom expression was recorded on the field beans grown in the PBGM which had been treated (spiked) at the highest rate of clopyralid. These symptoms of leaf curling and severe twisting of the plants were most severe in plants grown at the highest rate of clopyralid in PBGM at pH4. Symptoms also developed on plants grown in the medium rate of clopyralid but at much lower levels with only slight leaf curling.

Table 4-5 Herbicide symptoms (0-5) at 14, 21, 28 and 35 days after sowing

Treatment	14 days after sowing	21 days after sowing	28 days after sowing	35 days after sowing
pH 7-8				
PBGM	0.15	0	0	0
Clopyralid low	0	0	0	0
Clopyralid medium	0	0.38	0.44	0.44
Clopyralid high	0.4	1.87	2.62	3.25
pH 5.5				
PBGM	0	0	0	0
Clopyralid low	0.15	0	0.07	0.07
Clopyralid medium	0	0	0.07	0.13
Clopyralid high	0.65	1.87	2.5	2.87
pH 4				
PBGM	0.5	0	0	0
Clopyralid low	0.9	0.63	0.5	0.44
Clopyralid medium	1.25	1.0	1.25	1.0
Clopyralid high	1.5	2.87	3.63	4.12
Mean of pH				
pH 7-8	0.1	0.45	0.62	0.74
pH 5.5	0.15	0.38	0.53	0.62

Treatment	14 days after sowing	21 days after sowing	28 days after sowing	35 days after sowing
pH 4	0.9	0.93	1.08	1.12
LSD (5%)	0.2 (***)	0.2 (***)	0.28 (**)	0.21 (**)

Reductions in the pH of the growing media were found to increase symptom expression, although most green composts sent for BSI PAS 100 testing typically have a pH value of 6-7, so symptom expression would not be maximised.

4.3.2 Comparing the effect of different rates of powdered activated charcoal on symptom expression in field beans

This element of the project looked at the potential for powdered activated charcoal to mitigate the presence of synthetic auxin herbicide residues in growing media. Rates of powdered activated charcoal ranged from 3 to 15g/2.27kg of growing media, which were from 2 to 10 times higher than those used in a previous study where no effects from incorporation were observed.

The powdered activated charcoal treatments were applied to peat-based growing media (PBGM), composite green compost samples retained from BSI PAS 100 tests, commercially available growing media known to cause low symptom expression (presumed to be due to low levels of herbicide contamination), PBGM spiked with clopyralid @0.0055mg ai/l of peat and PBGM spiked with clopyralid @0.011mg ai/l of peat. Field beans were sown on 12 March 2013 with the trial carried out in warm glasshouse and assessed for six weeks.

Leaf curling and twisting of the growing point was observed during the trial but with no symptoms observed on the PBGM control (not shown in Table 4-6). The highest symptom expression was recorded on the field beans grown in the PBGM which had been treated (spiked) at the highest rate of clopyralid, but symptoms were observed on plants grown in all other growing media.

The incorporation of powdered activated charcoal delayed the onset of symptom expression. There was a good dose response with the two higher rates (12 and 15g/2.27kg of growing media) significantly reduced the severity of the symptoms.

Overall, the addition of powdered activated charcoal reduced symptom expression for both spiked and non-spiked growing media indicating that the symptoms are almost certainly caused by herbicide contamination.

Table 4-6 Herbicide symptoms (0-5) at 42 days after sowing

Treatment PAC/2.27kg of growing media	BSI PAS 100 mix	Green compost low	PBGM + clopyralid 0.0055mg ai/l	PBGM + clopyralid 0.011mg ai/l
Untreated	1.4	2.0	2.0	3.6
3g	0.9	1.5	2.3	4.0
6g	0.9	1.0	0.8	4.0
9g	0.5	0.8	1.1	2.5
12g	0.5	0.6	1.0	1.5
15g	0.1	0.5	0.5	0.5
LSD (5%)	0.36 (***)	0.60 (***)	0.56 (***)	0.56 (***)

4.3.3 Discussion

None of the treatments applied to the growing media/composts had a significant effect on symptom expression except the powdered activated charcoal applied to the PBGM which had been treated (spiked) at the lower rate of clopyralid. This lack of any significant reduction in symptom expression would suggest that the cause of the symptoms is due to the presence of herbicide residues rather than bacteria or heavy metal contamination. The lack of any significant effect of the powdered activated charcoal is considered probably due to the low rate, which was determined based on scientific literature[2]. However, when powdered activated charcoal was added at rates up to ten times those in the initial test, the symptoms were substantially reduced. A small observational study was subsequently undertaken using single pots treated with powdered activated charcoal at rates up to six times those used in this project, and this did reduce symptom expression in green compost known to be contaminated with grass clippings treated with Verdone Extra (which contains clopyralid).

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[2] Powdered activated charcoal added at 1 level teaspoon (1.5g)/2.27 kg (5lbs) of soil (as described in "a quick test for herbicide carry-over in the soil", by Klein, Bernards and Shea, University of Nebraska Extension Publications, September 2008, www.ianrpubs.unl.edu/pages/publicationD.jsp?publicationId=1052)

5.0 Overall conclusions and recommendations

5.1 A protocol for a potential new bioassay test using field or dwarf bean

1. The most reliable bean type which can be expected to tolerate high Electrical Conductivity levels in growing media in BSI PAS 100 Annex D tests are field beans followed by broad beans.
2. Slight herbicide damage symptoms were more clearly visible in field bean than in dwarf French bean as leaf curling was only present on the leaves as they unfolded and the growing point was often obscured by the newly expanded leaves. Newly expanded leaves did not show the symptoms as clearly as the field bean.
3. Field bean was more sensitive to lower rates of contamination with cultivars Fuego and Tattoo exhibiting more symptoms than Sultan. This would make these two cultivars more suited for use in a bioassay.
4. Seedling emergence was quicker for the 16/21°C temperature regime for both sowings and for both bean types. Plant growth was better for the 16/21°C regime. Herbicide symptoms were more pronounced at the 16/21°C regime probably as the plants were slightly more advanced compared to those grown at the lower temperature regime.
5. Supplementary lighting had no obvious effect on plant growth and did not increase herbicide symptom expression, but both sowings were carried out when ambient levels are higher than in the middle of winter.
6. Auxin herbicide damage symptoms were visible in transplanted field bean within just two weeks of transplanting. This is faster than with direct sown beans and the symptoms are more obvious. The use of transplanted field bean could allow the current bioassay used to test for the presence of herbicides in green compost to be reduced in length to just three weeks after transplanting without compromising the reliability or accuracy of the test.

The current BSI PAS 100 plant response test using tomato should continue. For green composts that are to be used in growing media then it would be advisable to ensure that detectable residues of persistent herbicides were either absent or present below an acceptable threshold, as determined by use of the field bean bioassay.

The glasshouse environmental regime for the current tomato test should be used for the field bean bioassay test, with the potential to reduce the length of the test to three weeks by using module raised field bean transplants.

5.2 Identification of the sensitivity of a range of plant species to clopyralid residues in spiked green compost added to peat

1. Herbicide damage symptoms were observed in only two of the flower and bedding species tested indicating that the remaining 15 species are not sensitive to clopyralid at the rates used.
2. Dahlia was the most sensitive ornamental species and this confirms previous findings. Lobelia also appeared sensitive but the effects were only very slight and did not appear to have an effect on plant size or flowering.
3. Field bean showed herbicide damage symptoms in the M2 + BSI PAS 100 compost samples but no effects were observed in the dahlia for this treatment. This is a surprising result due to the sensitivity of dahlia to synthetic auxin herbicides.
4. Herbicide damage symptoms were observed in six of the vegetable species raised from seed and also potato grown from tubers with nine species showing tolerance to clopyralid at the rates used.
5. Tomato cv. Moneymaker and pea were the most sensitive species with distortion of the growing point at the highest rate of clopyralid. Tomato cv. Moneymaker was more sensitive than cv. Shirley used as the indicator species. Chilli, carrot, courgette, cucumber and potato were less sensitive showing only slight leaf curling.

The results provide reassurance that most species are insensitive to the low levels of persistent herbicide(s) that are likely to be present in most green composts. The main risk is for growing media that contain a high proportion of green compost, but these risks can be mitigated through testing with field bean.

5.3 Quantification of the extent of herbicide contamination in composts that could potentially be used in growing media

1. Of the composts tested, 40% may not be suitable for use as a constituent in growing media that are used to grow herbicide-sensitive species. It is likely that they would be suitable for herbicide-tolerant species.
2. There was no obvious effect from sampling/sowing date on symptom expression indicating that samples provided over the seven month period contained herbicides with no apparent increase during July and August. This period would normally be expected to potentially have a higher proportion of grass clippings that may have been treated with home and garden lawn weed killers - this assumes that samples were sent soon after the composting process had been completed.
3. The rate of green compost incorporation in this bioassay test was 33% which may be more than that used by most growing media manufacturers in their growing media products sold on the amateur market. These findings therefore generally represent a worst case scenario, although the green compost proportion has been rising and some mixes already contain in excess of 50%.
4. Based on the screening tests used to examine herbicide impacts on common flower, bedding plant and vegetable species, if these BSI PAS 100 green compost samples had been used in growing media the growth of most plant species would have been unaffected. However, sensitive species including dahlia, peas and beans could still be affected – depending on the proportion of the material used in the growing media.

On-going monitoring of composts for herbicide residues should continue and consideration should be given to using transplanted tomato as an alternative to the highly sensitive field bean. This could potentially provide a better indication of the 'safety' of the green compost for use in garden growing media, allowing the adoption of a +/- approach to herbicide scoring, rather than a 0 – 5 scoring approach.

5.4 Quantification of the extent of herbicide contamination in peat reduced and peat-free bagged growing media.

1. Herbicide symptoms were observed on field beans grown in 22 of the 41 bagged products from garden centres, DIY stores and other retailers. They included branded and own-brand products.
2. Herbicide symptoms were observed on field beans in 27 of the 34 (79%) of the peat-free and/or organic product samples and in 17 of the 60 (28%) of the peat-based product samples.
3. In the follow up test, all 22 peat-free product samples produced symptoms in field bean, nine showed symptoms in transplanted tomato (45%) and four showed very slight symptoms in seeded tomato (18%). Damage symptoms on field bean ranged from very slight leaf curling to severe twisting and head distortion.
4. In the follow up test, all 10 peat-based product samples produced symptoms in field bean, but only one sample showed symptoms in transplanted tomato (10%) and none exhibited symptoms in seeded tomato.
5. Tomato again proved to be less sensitive than field bean although using transplants, rather than seed, and growing for six weeks increased the expression of symptoms.
6. Compared with field bean, damage symptoms on transplanted tomato were far less severe – consisting of the characteristic 'nettling' in the head of the plants often not observed until four or five weeks after transplanting. The damage was observed in far

fewer products, presumably because tomato requires higher levels of herbicide residue to express symptoms.

7. Damage levels usually increased over time for both crops with symptoms in tomato taking longer to develop.
8. These results clearly show that some products could affect the performance of tomato and other sensitive crops in actual (domestic) use. This could be a potentially serious issue if gardeners used these products for filling raised beds, tubs and other containers for growing tomato and other vegetable crops which might accumulate more herbicide residues from the compost over time as the plants grew larger.

On-going independent testing of growing media (multi-purpose, specialist uses and growing bags) should continue so as to provide reassurance to growing media users. Those growing media which do show symptoms on field bean should then be tested with dahlia, tomato and peas. These tests could be part of a range of growing tests to ensure that products are 'fit for purpose', as the 2020 peat-free deadline approaches.

5.5 Proving beyond reasonable doubt that the symptoms observed in field bean are due to herbicide contamination

1. The growing media used in the trial provided a known range of contamination levels and/or exhibited a range of severity symptoms in field bean bioassay. There was no symptom expression in the peat-based growing media (PBGM) showing that there was no contamination of the peat, pots, saucers or seed. Any leaf curling or twisting of the growing point was caused by a residue within the growing media.
2. As there was no reduction in symptom expression following sterilisation of the growing media it can be assumed that the symptoms were not caused by bacteria present in the material as autoclaving for 25 minutes should have killed the bacteria.
3. As there was no reduction in symptom expression following the application of EDTA then it can be assumed that the symptoms were not caused by the presence of any heavy metal in the material unless the rate applied was too low.
4. As there was no reduction in symptom expression following the use of Ethysorb™ applied to the surface of the growing media after seedling emergence then it can be assumed that the symptoms were not caused by ethylene being produced by bacteria in the growing media. Any ethylene produced should have been absorbed by the coated granules prior to reaching the ethylene receptors in the aerial parts of the plants.
5. The reduction in symptom expression where powdered activated charcoal was applied to the PBGM spiked with a low level of clopyralid suggests that the symptoms are due to herbicide contamination. The lack of any reduction in symptoms for the other growing media would indicate that the rate recommended was insufficient for the levels of contamination present.
6. The significant reduction in the severity of symptom expression when powdered activated charcoal was incorporated at 12 and 15g/2.27kg of growing media provides sufficient proof that the symptoms observed in field bean and other plants are due to herbicide residues in the green compost.

Powdered activated charcoal could be taken forward as a treatment for compost that is to be used in growing media. However a cost benefit analysis at selected rates is required.

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