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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.

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## GROWER SUMMARY

### Headline

- Addition of 12.5% of bark fines or 6.3% each of bark fines and mature green waste compost (GWC), with water, to peat casing was either beneficial or neutral to mushroom yield
- Recycled cooked-out casing used at 25% had no overall effect on mushroom yield. A MushComb casing separator machine is a possible option in recycling spent casing in the shelf system. The recycled casing could be added to fresh casing in the conveyor to a head-end filler of shelves
- Positive Taqman PCR test results for *P. tolaasii* and large increases in *Pseudomonas* sp. populations in the casing from application to after the second flush generally corresponded with the occurrence of moderate or severe bacterial blotch

### Background and expected deliverables

Previous research has shown that the most promising peat substitutes in mushroom casing are composted bark fines, mature GWC, coir, recycled casing, recycled granulated waste rockwool slabs and filter cake clays. Coir was incorporated into some commercial blends for several years but it is no longer used due to the increased demand and cost of the raw material, particularly for uses such as strawberry substrate production. However, spent coir is a significant disposal problem for the soft fruit industry. In this project, the effect of using the above materials individually and in combinations of materials was investigated. The specific objectives of the project were:

1. To update and summarise any recent information on peat alternatives in casing published since HDC project M 53
2. To produce data that meets the requirements of the Environment Agency's low risk waste status and/or food safety regulations
3. To undertake commercial farm trials with the five most promising alternative materials identified from small-scale experiments in M 38 and M 53
4. To test how experimental physical, chemical and microbial standards for casing materials relate to mushroom yield, quality and blotch incidence on commercial farms
5. To electronically monitor crop water management and casing water status, and determine how these interact with the performance of casing materials and the occurrence of blotch
6. To communicate and disseminate results to industry
7. To monitor industry uptake of peat substitute casing materials.

## **Summary of the project and main conclusions**

Discussions with several European casing manufacturers suggest that decreasing availability of wet dug peat for mushroom casing is a problem not only in Britain but also in the Netherlands and Belgium. Other types of peat and peat production by-products are available in Britain in sufficient quantities to supply the mushroom industry. A review of potential alternatives to wet dug peat has shown that the most promising materials were composted bark fines, granulated recycled rockwool slabs, recycled casing, spent coir from strawberry grow bags, PAS 100 Green Waste Compost (GWC), and filter cake clays.

The following casing materials were used as peat substitute materials in the experiments: (a) pine bark fines (b) mature GWC (c) used granulated rockwool slabs (d) cooked-out separated spent mushroom casing (e) clay from sand quarries (f) spent coir from strawberry grow bags. The materials were used as individual peat substitutes and in two- and three- way mixes in some of the trials. Peat substitute materials were tested in four peat-based casing materials: three were commercial products containing wet dug peat and sugar beet lime (SBL) (Harte, Sterckx and Topterra) and a fourth casing (Everris) consisted of blocking peat, milled peat fines and SBL or ground chalk.

The main conclusions from the review and mushroom cropping trials conducted at five farms were:

1. The supply of wet dug peat has substantially reduced in Britain and dwindling supplies in Germany are also of concern to casing manufacturers in the Netherlands and Belgium.
2. The most commonly used casing in Britain is Harte (Ireland) with smaller quantities from Scotland, Belgium and the Netherlands.
3. Other types of peat and peat production by-products are available in Britain in sufficient quantities to supply the mushroom industry.
4. A review showed that the most promising alternatives to peat were composted bark fines, granulated recycled rockwool slabs, spent coir from grow bags, PAS 100 GWC, and filter cake clays.
5. Mushroom yields and quality from an Everris casing prepared from partially dried blocking peat and milled peat fines were similar to Harte and Topterra casings prepared from wet dug peats; however, the casing needed wetting during pre-mixing and the crop needed more frequent irrigation events of shorter duration than with wet dug peat casing.
6. The effects of adding 12.5 to 25% bark fines on mushroom yield were inconsistent between farms.
7. GWC was unsuitable at an inclusion rate of 25% but at 12.5% had no overall effect. It was best used at 6.3% in conjunction with a similar volume of bark when the effect was

either beneficial or neutral to mushroom yield; this blend would also be cheaper than using 12.5% bark.

8. The effect of addition of 25% recycled rockwool at all three farms where it was tested and in three types of casing was not significant compared with the respective peat control casings.
9. Recycling spent casing at 25% had no overall effect on mushroom yield. Casing with salt or disinfectant must be avoided for use in recycling in casing. A MushComb casing separator machine is an option for in recycling spent casing in shelves.
10. Filter cake clay at 20% reduced mushroom yield but the effect of 12.5% clay was not significant. However, the material was difficult to mix evenly through the casing.
11. Spent coir was unsuitable for casing because it encouraged green mould.
12. Casing materials with a volumetric water retention at saturation of at least 67% were more suitable than materials with a lower water retention when saturated
13. Maintaining a casing volumetric water content of at least 61% during cropping produced a better yield than maintaining a lower water volume in the casing.
14. Casing water tensions were consistently greater in the second flush than in the first across all the farms in spite of second flush yields being similar or lower than first flush yields; this indicates that more water needs to be applied after the first flush, without excessive draining into the compost.
15. The occurrence of bacterial blotch was not primarily related to the initial population of *Pseudomonas* sp. in casing materials; blotch was mainly associated with one farm which may have had environmental conditions conducive to the disease.
16. The occurrence of blotch generally corresponded with positive results obtained with a Taqman PCR test for *P. tolaasii* on casing samples taken after the second flush, although blotched mushrooms were obtained from casing treatments that tested negative and *vice versa*.
17. Large increases in *Pseudomonas* sp. populations in the casing from application to after the second flush generally corresponded with the occurrence of bacterial blotch or severe blotch.

### **Financial and environmental benefits**

Recycling of spent casing is a viable option if the casing is cooked out, not treated with disinfectant or large amounts of salt, and a method for removing the casing layer from the compost is available. This work has shown that the MushComb casing separator is a possible option in the shelf system. The recycled casing can be added with fresh casing in the hopper of the head-end filling machine. Table A1 shows the potential benefits and costs of recycling casing. This assumes that crops are cooked-out and there is no mushroom yield difference

between fresh casing and casing containing 25% recycled casing; this work has shown that there may be a yield benefit in recycling casing if it is rewetted before reuse. Alternatively, it may be possible to recycle a greater proportion of casing (30 - 50%) without a mushroom yield penalty, but this requires further investigation.

**Table A1.** Benefits and costs of recycling casing

Benefits	Costs
Saving in casing cost (25%)	Casing separating machine and trailer for collecting separated casing
Separated compost with increased fertiliser value (lower pH, higher plant nutrient content) or for reuse in Phase I compost	Removal of salt patches from casing after cook-out
Reduced cost of SMC disposal (12%)	Hopper and conveyor for feeding recycled casing into head-end casing hopper

HDC project M43 showed that 33% of spent compost (with casing layer removed) can be reused in Phase I compost with no effect on mushroom yield compared with non-amended Phase I compost. There is therefore a potential to save on straw and other compost ingredients, if composting is conducted in the vicinity of mushroom production.

Casing prepared from dried blocking peat and milled peat fines, and rewetted before use, can produce comparable mushroom yields and quality to casing prepared from wet dug peat. This could reduce dependency of the British mushroom industry on imports of wet dug peat. However, the casing needs to be wetted in a casing mixer and requires more frequent and smaller waterings. The cost of the casing materials would be similar to that of casing prepared from wet-dug peat and sugar beet lime, but there would additional costs in blending the casing ingredients.

The addition of bark and/or GWC at inclusion rates of 6.3-12.5% v/v, together with additional water, to peat casing may give yield benefits on some farms. These ingredients can be added to the casing hopper. The cost of the casing would therefore be similar to non-amended casing.

The Taqman PCR test for *P. tolaasii* and the measurement of *Pseudomonas* sp. in casing should help to identify conditions that are conducive to bacterial blotch.



### **Action points for growers and casing producers**

- Investigate removal and re-use of cooked out casing – disinfectants or large amounts of salt must not be applied to the casing before reuse, and salt patches must be removed from casing after cook-out.
- The cooked-out casing needs to be rewetted before reuse; the wetted material can be added to the hopper of the head-end filling machine in shelves.
- Addition of small (6.3 – 12.5%) amounts of bark and GWC, together with additional water, to peat casing may give yield benefits on some farms.
- Water tension in the casing is much greater in the second flush than in the first flush, indicating that more water needs to be applied after the first flush, without excessively draining into the compost. Volumetric water content of the casing should be kept at least 61% during cropping.
- In the event of a blotch problem, testing of casing during the cropping period using the Taqman PCR test for *P. tolaasii*, and for the total population of *Pseudomonas* sp. may identify where conditions are favourable for the disease.

## SCIENCE SECTION

### Introduction

#### ***Commercial and scientific developments that have occurred in peat substitution since M 55***

Previous HDC funded research in projects M 38 and M 53 and a subsequent review in project M 55 showed that the most promising peat substitutes in mushroom casing were coir, composted bark fines, green waste compost, recycled casing and recycled granulated waste rockwool slabs. Coir was incorporated into some commercial blends for several years but it is no longer used due to the increased demand and cost of the raw material, particularly for uses such as strawberry substrate production. However, about 20,000 cubic metres of spent coir are discarded annually from the soft fruit industry and this is a potential source of material for mushroom casing. Bark was not taken up commercially due to cost. However, composted bark fines (the most suitable grade for casing) are now more available (Melcourt) and large supplies of aged bark are available in Scandinavia (Lindum). Small-scale experiments in M 53 also showed that bark could be used beneficially in peat-based casing at 25% v/v. Increased cost of landfill disposal of used rockwool slabs means that granulating for re-use is now a cheaper option (Grodan, Cultilene).

Results presented in the review M 55 showed that separated spent casing performed better than mixed spent mushroom compost (SMC) as a casing ingredient. A machine for separating casing from the compost at emptying has been developed by MushComb in the Netherlands (see report of visit below). Royse et al. (2008) developed a method for removing the casing layer from mushroom beds after cropping by inserting a plastic mesh layer between the compost and casing layer during shelf filling.

Sturgeon (2011) reported on the use of composted green waste in casing. Cropping results with proportions of mature green waste compost (GWC) in casing in project M 53 were variable, although cropping results with one source of matured compost used at up to 25% by volume with Eversis casing were comparable with yields from the control Eversis casing. The variability in mushroom yield obtained from different sources of GWC was not explained by differences in physical or chemical properties such as electrical conductivity. As with sugar beet lime (SBL) used in casing (Visscher 1988), it is likely that maturity and stability of GWC are important characteristics. Due to the abundance (>4 million m<sup>3</sup>), local availability and relatively low cost of GWC, identifying suitable sources and desirable characteristics of the material, would be worthwhile, even for low inclusion rates in mushroom casing.

Following research conducted at HRI in the 1990s, multi-roll filter cake (MRF), a clay filter-cake by-product from the coal mining industry was developed as a commercial casing ingredient by Tunnel Tech-ECB and used by several mushroom farms for over six years. The development achieved a 'Science into Practice Grower of the Year Award' for Tunnel Tech in 2005. However, new Environment Agency (EA) waste legislation prevented the use of MRF in mushroom casing from 2008 until the material was granted a low risk waste exemption in 2012. The increase in sand and aggregate washing plants means that similar clay-like filter cakes are now more widely available than MRF. Pale colour means that these filter cakes are more attractive as a casing ingredient than MRF. Due to similar water holding characteristics, filter-cakes and mature composts also have the potential to replace SBL, a significant cost component in casing. The decline in the sugar industry in Britain and Ireland means that there is less SBL available and transportation distances to casing production has increased. Results from small-scale experiments in M 53 showed that a combination of a dense material (filter-cake) and a light material (used rockwool) performed better as a casing peat substitute than the individual materials.

Other materials that have been tested for use as casing materials for cultivation of *Agaricus bisporus* or *Agaricus blazei* in recent years include leached sugarcane bagasse (Booyens 2012; van Rooyen 2012), subsoil and charcoal (De Siqueira et al 2009; Coluato et al 2011), lime schist (Colauto et al 2010), composted vine shoots (De Juan et al 2003; Pardo et al 2003) and vermicompost (Garcia et al 2008; Zakaie et al 2013) (Table 1). However, cropping results were generally inferior to those obtained from peat casing.

In Britain, supplies of wet dug peat are no longer available and all wet dug peat casing is imported from Ireland, Belgium and the Netherlands. Casing in the latter two countries is produced using German peat, the supplies of which will be exhausted in the next 20 years. The Polish mushroom industry still has plentiful supplies of peat in Poland and the Baltic States.

In the UK, black humified peat for blocking composts, milled brown or blond peats and by-product peats are still available in sufficient quantity to supply the British mushroom industry. Unlike wet dug peat, these other sources of peat are partially dried during harvesting and processing, and therefore require rewetting before use as mushroom casing. Casing producers in the Netherlands are currently examining increasing the proportion of blond peat, which is still widely available, and reducing the quantity of black peat, which is becoming scarcer. Research in INAGRO, Belgium has examined the use of wood fibre and vermiculite as casing ingredients, although the trials were not very successful (Pyck, pers. comm. 2015).

Wood fibre performed poorly in M 53 due to insufficient water retention. Vermiculite was examined extensively as a casing ingredient at HRI (Noble & Gaze, 1995) but was too expensive and tended to stick to the mushrooms.

- *BVB Substrates Netherlands (Jos Amsing)*. BVB currently produces casing from mixtures of frozen black peat and unfrozen deep dug black peat together with sugar beet lime. They are investigating using more blond peat and less black peat in the casing. Natural clay is too variable to use in casing. They have investigated using sludge from a vegetable washing plant (mainly small particle soil) dewatered with polymers (particularly natural polymers). Research is also looking at relating properties (e.g. plasticity and water holding characteristics) of casing to performance.
- *CNC Netherlands (Caroline van der Horst and Wim Aarts)*. They have examined the use of granulated rockwool in casing but had problems with plastic pieces because the blocks were not de-sleeved before grinding.

Wageningen University (*Chris Blok*) They have examined the use of digestate in casing.

- *Topterra Netherlands (Ge Wijnands and Lam Janssen)*. They are conducting peat alternatives research in conjunction with Wageningen University.
- *McDon Peat Ireland (Martin McCourt)*. Currently they prepare casing mixes containing wet deep dug peat together with sugar beet lime, chalk and ground marble.
- *Harte Peat Ireland (Aidan O'Harte)*. They are not currently conducting research into peat alternatives but are interested in any usable developments.

**Table 1.** Peat alternative casing materials examined in the review and the maximum and minimum rates used.

Material	Rates, % v/v		References
	Min	Max	
Bark	25	100	7
Charcoal	25	30	1
Clay, MRF	25	90	8
Coir	25	100	8
Composted vine shots	25	25	2
Green waste compost	10	25	1
Lime schist	100	100	1
Rockwool used	100	100	3
Soil	50	100	3
Spent casing	50	100	3
Spent coir	Not tested in casing		
Sugar cane bagasse	?	?	2
Vermicompost	50	100	2

### ***Developments in the recycling of spent casing, particularly in the Netherlands***

There has been a large amount of research into using spent mushroom compost (SMC) for re-use in casing, most recently by Barry et al (2008) who demonstrated a clear negative effect on yield. This effect was alleviated by leaching but this is not a practical solution in the UK. More promising has been the re-use of separated spent casing (Nair & Bradley, 1981). Royse et al. (2008) developed a method for removing the casing layer from mushroom beds after cropping by inserting a plastic mesh layer between the compost and casing layer during shelf filling. Similarly, Farsi et al (2011) removed casing from compost using a perforated plastic mesh inserted between the compost and casing layers at filling. This had no effect on mushroom yield. Recycled casing was composted for three weeks and then leached. Mushroom yield from the recycled casing was not significantly different to that from fresh casing.

MushComb in the Netherlands have developed a machine for separating the casing from the compost on emptying shelves. Oei (2011) states that by using this technology, 50-75% of casing can be recycled with only a 5-10% reduction in yield compared with using fresh casing. Experiment results in project M 53 have shown that 25% v/v can be recycled without a mushroom yield penalty.

*Discussion with Bob Holtermans and Roel Drissen, MushComb at Sikes mushroom farm, Ysselsteijn, Venray, Netherlands*

MushComb have produced one large machine for separating casing from compost at emptying in the Netherlands and two smaller machines (for UK and Malaysia). The machine at Sikes is used at four different sites.

No crops are fully cooked-out in the Netherlands but nets are steamed and rooms may be given a short steaming treatment before emptying. The casing separator is placed between the emptying winch and the shelving. Casing is removed from compost during emptying by an Archimedean screw mechanism (Fig. 1). The separated casing is emptied into a separate trailer to that used for the underlying compost (Fig. 2). Emptying speed is similar to a conventional shelf emptying machine. The compost is sent to Germany because field disposal in the Netherlands is no longer allowed under EU Nitrate Vulnerable Zones (NVZ) rules. A small amount of compost goes to burning but no casing must be present in this material. Casing can be disposed of in the Netherlands on to the field because it has a much lower nitrogen content than the compost. Transport cost of disposal is therefore reduced compared with disposal of the entire SMC. There are no facilities on-site at Sikes for remixing casing. The separated casing is sent to another site and stored for three weeks to allow the mushroom residues to break down. More mushroom residues are present on the casing in machine harvested crops than in hand picked crops. A shorter period than three weeks for casing from hand-picked crops may be possible. The stored casing is then steamed at 65 °C before being sent to a casing producer for remixing with new casing. This was done by Topterra and BVB Euroveen but due to mould problems, this is now done at another site which is not producing conventional casing. An inoculum of *Bacillus subtilis* (Serenade) is added to the steamed casing to reduce the risk of *Trichoderma* growing on the chogs. The reused casing is mixed at 30% with fresh casing.



**Fig. 1.** MushComb casing separator in use at a shelf farm in the Netherlands



**Fig. 2.** Separated casing and compost filled into two trailers before being sent for recycling or land spreading respectively.

### **Changes in the legislation affecting the use of different raw materials in growing media**

PAS100 composts are marketed as soil amendments and are not classified by the EA as wastes. Multi-roll filter cake has a low risk waste exemption from the EA for use in mushroom casing. Spent compost or casing recycled on-site in new casing would be exempt from waste regulations, other than storage limits. Spent mushroom compost also has a low risk waste exemption for bagging, but there is a restriction in quantity of 50 tonnes on-site before bagging. Used rockwool is classified as a waste; it currently has a low risk status waste exemption for re-use in the production of new rockwool growing slabs. It does not yet have this exemption for use in a mushroom casing material. Spent coir from strawberry grow bags would also be classed as a waste. Analysis of these spent materials and mushrooms grown on them for pesticide residues and heavy metals would be needed to achieve a waste exemption from the EA for use in mushroom casing.

SMC now has an end-of waste exemption from the Northern Ireland Environment Agency, and is not regarded as a waste in France or Spain, nor is cooked-out SMC in Germany (Anon, 2010a,b, 2013). SMC can be used as a growing medium component, including mushroom casing, in these countries.

### **Defining the properties of the optimum casing material**

There are currently no parameters for defining the properties of the best peat or peat substitute casings. Increasing salt content or electrical conductivity of casing has generally been found to reduce mushroom yield; this was confirmed in HDC project M 53. Previous work has found conflicting evidence for the importance of water and air holding characteristics of casing materials for mushroom yield. Project M 53 found that the Air Filled Porosity (AFP) of a casing material explained 50% of its cropping potential, with an optimum AFP value of about 19% v/v. Coluato et al (2010) found that the yield of *Agaricus blazei* was optimum when the proportion of microporosity in a casing was 49 to 55% of total porosity (microporosity was defined as the volume of water retained divided by the drainage water from a sample). Zied et al (2011) found that the yield of *Agaricus blazei* correlated positively with the water holding capacity and negatively with bulk density of ten different soils used for casing but there was no effect of casing porosity on mushroom yield. Rangel et al (2008) measured the porosity and water holding capacity of different casing materials but no clear relationship with mushroom yield was found.

### **Improved control of water management and blotch using different casings**

In project M 35, the availability of water from peat casing was measured electronically using tensiometers which record the water tension (matric potential) on a data logger. Watering patterns on different farms and in different crops and casings were monitored with electronic tensiometers. Periods with over-wet or dry casing were detected from the graphical output of the tensiometer data and related to cropping problems and the occurrence of water stress symptoms such as watery flesh, distorted caps or leggy stems. Watering regimes on mushroom farms are often aimed at bacterial blotch prevention rather than maximising mushroom yield and quality. Data from electronic tensiometers should enable the water requirements of the mushroom crop grown with different casing materials to be controlled more precisely.

Previous work has shown that casing materials differ in the populations of pseudomonad bacteria present, and there is a large increase in the casing population during the life of a crop (Noble et al. 2003, 2009). However, it is not known what proportion of the



pseudomonads cause blotch. A real time PCR diagnostic test for mushroom blotch pathogenic *Pseudomonas tolaasii* has been developed in project M 54. The test can distinguish bacterial blotch causing strains from other pseudomonads in pot experiments using introduced inoculum. Although ginger blotch causing strains are not distinguished, this is not currently regarded as a significant cause of crop loss. The diagnostic test can potentially be used on commercial casing materials to determine the risk from blotch if conditions are also conducive. It is well established that inclusion of organic amendments such as bark and composts into peat based growing media can suppress soil-borne plant pathogens (Noble & Coventry, 2005), although the effect of casing amendments on the occurrence of bacterial and fungal mushroom pathogens is unknown.

### ***Survey of casing materials currently used in Britain***

A total of 20 farms and five casing suppliers were contacted to determine current casing usage in Britain. Two companies that produced casing at the start of the project no longer do so (Everris, McArdle) and two companies that supplied casing no longer supply significant quantities into Britain (CNC, McDon). All the blends currently used are mixtures of wet sphagnum peat and sugar beet lime (Table 2). One site also uses a small amount of additional chalk to increase the casing pH. The predominant type of casing is Harte from Ireland, with smaller quantities of casing from MacGregor, Sterx and Topterra. On nearly all farms, ready-mixed casing was applied through a filling hopper, with small volumes of water sprayed if necessary. Only two farms still had casing mixers, and only one of these was still in use. The majority of tray farms used Phase 3 compost for cacing instead of casing inoculum CI; shelf farms generally used ruffling to inoculate the casing. The same types of casing materials are used for white and brown strains.

**Table 2.** Number of farms in survey using various casing materials

Casing	Harte	MacGregor	Sterx	Topterra
Source	Ireland	Scotland	Belgium	Netherlands
No. farms	15	2	1	2

## Materials and methods

### ***General cropping procedure***

Replicated tray cropping experiments were conducted at Chelbury Farm, Glocs. (twice), Flixton Mushrooms, Norfolk (once), Little Hall Farm, Lancs. (twice) and Thakeham Farm, Sussex (twice). Larger-scale validation trials were also conducted at Little Hall Farm (twice) and Chelbury Farm in trays and at May Farm, Cambs. in shelves.

Compost spawn-run with the strain A15 was filled into wooden cropping trays or shelves (Table 3). Spawn-run compost (cacing) or CI was mixed into wetted casing materials, except at farm E where compost was 'ruffled' into the casing layer at filling of shelves (Table 3). Casings were applied to trays or shelves to a depth of 50 mm. The trays were stacked five high. The trays or shelves were watered after application, at two-day intervals until airing, after the first mushrooms were about 15 mm diameter, and after the first and second flushes of mushroom were picked. The water application in the initial mixing of the casings, and watering after application of the casings to the cropping trays was adjusted to maintain a moisture content of about 60-65% v/v. The air in the cropping rooms was recirculated and the relative humidity maintained at 95 -98% until mycelial growth in the casing layer had become established, about 6 days after application. Fresh air was then introduced into the growing room and the relative humidity reduced to 88 - 91%. Mushrooms were picked mainly with the veils closed (large buttons) at a diameter of 30 – 40 mm, over a 23 day period (three flushes) with the first flush being picked c. 17 days after the application of the casings. The fresh weights of mushrooms were recorded daily. The appearance of bacterial blotch, green mould and other diseases was recorded.

Representative samples of the casing materials used in the above farm trials were also used in small tray cropping tests at Moreton Mushrooms, Daventry, Northants. according to the methods described in HDC report M 53.

**Table 3.** Tray or shelf plot area, compost fill and application rates for cacking, casing inoculum (CI) and casing at different farms.

Farm	Area m <sup>2</sup>	Compost fill kg/m <sup>2</sup>	Cacking or CI per m <sup>3</sup> casing	Casing L/m <sup>2</sup>
A	0.93	97 (Phase II)	CI, 2 L	54
B	2.40	125 (Phase II)	CI, 0.97 kg	54
C	2.30	78 (Phase III)	cacking, 6.3 kg	54
D	2.20	78 (Phase III)	cacking, 9.2 kg	54
E	5.00	80 (Phase III)	ruffled	54

### ***Casing treatments***

The following casing materials were used as peat substitute materials:

- (a) Growbark Pine, composted bark fines, Melcourt Industries Ltd., Tetbury, Gloucs.
- (b) Green waste compost (GWC), PAS 100 standard, composted and matured for at least 6 months, White Moss Horticulture, Kirkby, Liverpool, Merseyside.
- (c) Used granulated rockwool slabs (obtained from cucumber and tomato growers), composted for 6 months, Materialchange, Helmsden, Northants.
- (d) Recycled, cooked-out separated spent mushroom casing (obtained on-farm)
- (e) Clay from sand quarries (Marshalls, Rawtenstall, Lancs. and Dewsbury, W. Yorks)
- (f) Spent coir from strawberry grow bags (Hugh Lowe Farms, Kent and Haygrove Ltd, Hereford).

The above materials were used as individual peat substitutes. Bark (a), GWC (b) and clay (e) were also used in two- and three-way mixes in some of the trials (Table 4). Peat substitute materials were tested in four peat-based casing materials (Table 4). Three of the peat-based casings were commercial products containing wet dug peat and SBL (Harte, Sterckx and Topterra) and when used alone or with 20% clay require little or no wetting before application. The Everris casing consisted of 50% v/v blocking peat, 30% v/v milled peat fines and 15% SBL (British Sugar) or ground chalk, <2 mm grade (Needhams Chalks) and required wetting before application (Table 4). All the other substitute materials used with Harte, Topterra, Everris or Sterckx casing required various quantities of water during mixing before application to achieve a volumetric water content of 67% (Table 5). Where substitute materials, except clay, were added to these casings, additional water needed to be added during mixing. Everris casing prepared from partially dried peats required wetting during mixing, irrespective of whether substitute materials were added.

**Table 4.** Casing treatments used at farms A, B, C and D in replicated tray experiments.

A1, A2, C1, C2, D1 and D2 refer to two crops grown at farms A, C and D.

Casing substitute, % v/v	Peat + Sugar beet lime or chalk casing		
	Harte	Everris	Topterra
Control, none	<b>A1, A2, B, C2, D1, D2</b>	<b>A1, A2, C1, C2, D2*</b>	<b>C1, C2</b>
Bark, 12.5	<b>A2, D1</b>	<b>A2, C2, D2*</b>	<b>C2</b>
Bark, 25	<b>A1, A2, B</b>	<b>A1, A2</b>	<b>C1</b>
GWC, 12.5	<b>A2, D1</b>	<b>A2, D2*</b>	-
GWC, 25	<b>A1</b>	<b>A1</b>	-
Spent casing, 25	<b>A1</b>	<b>A1</b>	-
Spent coir, 25	<b>A1, B</b>	<b>A1</b>	<b>C1</b>
Recycled rockwool, 25	<b>A1, B</b>	<b>A1, C1</b>	<b>C1</b>
Clay, 12.5	-	<b>C1</b>	<b>C2</b>
Clay, 20	<b>B</b>	-	-
Bark 12.5; Clay 12.5	-	<b>C2</b>	-
Bark 6.3; GWC 6.3	<b>D1</b>	<b>D2*</b>	-
Bark 6.3; GWC 6.3; Clay 6.3	<b>D1</b>	-	-

\* with ground chalk

- treatment combination was not examined

**Table 5.** Volume of water added at mixing in casing trials in Table 3 to achieve a target volumetric water content of 67%, litres water/m<sup>3</sup>.

Casing substitute, % v/v	Harte	Everris	Topterra
Control, none	0,9,0,0,0,0	129,156,129,129,228*	0, 0
Bark, 12.5	45, 46	182, 129, 228*	66
Bark, 25	16, 59, 60	118, 163	66
GWC, 12.5	45, 46	201, 228*	-
GWC, 25	21	118	-
Spent casing, 25	18	118	-
Spent coir, 25	41, 51	94	59
Recycled rockwool, 25	53, 133	129, 129	81
Clay, 12.5	-	133	20
Clay, 20	0	-	-
Bark 12.5; Clay 12.5	-	129	-
Bark 6.3; GWC 6.3	60	228*	-
Bark 6.3; GWC 6.3; Clay 6.3	65	-	-

\* with ground chalk

- treatment combination was not examined

**Table 6.** Casing treatments used at farms A, C and E in larger-scale validation trials.

Casing substitute, % v/v	Peat + Sugar beet lime or chalk casing		
	Harte	Everris	Sterckx
Control, none	<b>A3, A4, A5, C3</b>	<b>A5*</b>	<b>E</b>
Spent casing, 25	<b>A3, A4, C3</b>	-	<b>E</b>
Bark 6.3; GWC 6.3	-	<b>A3*</b>	-
With net to remove casing	<b>A3</b>	-	-
Spent casing, 25 + plastic mesh to remove casing	<b>A4</b>	-	-

\* with ground chalk

- treatment combination was not examined

In the initial replicated tray experiments, casings were mixed by shovel in half pallet boxes, and applied to trays by hand. In subsequent validation trials, casings were mixed and applied mechanically to trays or shelves. At farm E, blending of spent casing with fresh casing was achieved by loading a bulk bag of spent casing in the loading hopper (Figs. 3 and 4).

At farm A, the use of a plastic shelf net or plastic mesh (19 x 19 mm holes), placed on the Phase III compost in the trays before casing, on cropping was investigated. This enabled the spent casing to be removed from the spent compost at the end of cropping (Figs. 5 and 6). The shelf netting was supplied by Tencate Geosynthetics Europe, Alemelo, Netherlands. The plastic mesh was obtained from Fiberweb Ltd, Maldon, Essex and Mesh Direct, Burslam, Staffs.

In each replicated tray crop, three replicate trays of each treatment were prepared, with the three replicates positioned in different stacks and layers. In validation trials, ten trays or part shelves were cased with individual treatments (Table 6).



**Fig. 3.** Adding 25% spent casing with fresh casing in the delivery lorry at farm E.



**Fig. 4.** Blending of 25% spent casing with fresh casing before shelf filling at farm E.



**Fig. 5.** Plastic shelf net applied to compost surface before casing, to remove spent casing layer at the end of cropping at farm A.





**Fig. 6** Plastic mesh applied to compost surface before casing, to remove spent casing layer at farm A. Black material; Fiberweb Ltd; green material, Mesh Direct.

At farm C, spent casing was pre-wetted and then filled into a loading box which had a trap door at the bottom. This was held over the filling hopper containing casing, the recycled casing was then emptied on top (Fig. 7). Further casing was filled into the hopper to achieve a 25% blend. The recycled casing was mixed into the casing as it passed from the hopper, up the conveyor and into the filling hopper for the trays (Fig. 8).



**Fig. 7** Adding spent casing to casing hopper at farm C



**Fig. 8** Applying casing with recycled casing to trays at farm C

### ***Small scale cropping and mycelium growth bioassay tests***

Representative samples of the casing materials produced at commercial sites were tested in small-scale cropping tests against standard peat-based casings in replicated tray experiments at Moreton Mushrooms, Daventry, Northants. Mushroom pinning, yield, quality grades, cleanness and disease incidence from each material was assessed according to methods described in HDC Report M 55. Mycelial growth rate in casing materials was determined in glass cylinders according to the method in Noble et al, (1999).

### ***Measurement of casing water tension***

The availability of water from the casing was measured electronically using tensiometers which measure the water tension (matric potential) on a continuous basis using data loggers. Miniature tensiometers (type SWT5, Delta-T Devices Ltd, Cambridge) were positioned in the casing layers at the time of casing application. The matric potential of the casings was continuously recorded on a data logger (type DL2e Delta-T).

### ***Properties of casing materials***

Samples of casing (three 200 g samples from different trays of each treatment) in each test crop were analysed for gravimetric moisture content.

The following physical and chemical analyses were conducted on two replicate samples of the peat + SBL casing samples and alternative materials before and after mixing: AFP, compacted bulk density, pH and electrical conductivity (EC) (Noble & Dobrovin-Pennington, 2012). AFP was measured using two different methods, described in HDC report M 35. These are based on the volume of drainage water from a saturated sample, and on the fresh and dry weights of a saturated and drained sample, and a formula based on the density of organic matter in peat. The water holding capacity after free drainage in a 'Campot' test cylinder was determined according to HDC Report M 35.

### ***Compost stability test by measuring carbon dioxide evolution***

A method for measuring the stability of bark and GWC samples used in the casing experiments was based on a modified method of Llewellyn (2005). Two further GWC samples from J. Moody, Wolverhampton and Organic Recycling, Peterborough were tested for comparison. Compost or bark samples (200 g), wetted to 50% w/w moisture, were placed in a 2 L glass flask and incubated at 30°C. The CO<sub>2</sub> concentration in the flasks was measured and the air inside the flasks was then purged at daily intervals for five days. The respiration of the compost sample was determined from the total CO<sub>2</sub> evolved from the sample and the organic matter (volatile solids) content of the sample. Two replicates of each compost batch were analysed.

### ***Total pseudomonad populations in casing samples***

Pseudomonad populations in the casing materials at the start (fresh casing samples) and between the second and third flush of the experiments were determined by preparing suspensions of two replicate samples of 1 g casing in 9 mL sterile deionised water (Noble et al, 2009). Serial dilutions of the suspension were spread on pseudomonad isolation agar (PIA) (Difco Laboratories, Detroit, Michigan) and incubated at 25°C for 48 h to determine the total pseudomonad populations as colony forming units per fresh weight casing (cfu g<sup>-1</sup> F.W.).



### ***Evaluation of a real-time PCR test for detection of *Pseudomonas tolaasii****

The real-time PCR test results for detection of *P. tolaasii* are given as Critical Threshold (CT) values (critical threshold at which amplification starts). Samples of casing were taken at the start (fresh casing) and between the second and third flush of crops. DNA extracted from each sample was tested in duplicate. A CT of 40 indicated no amplification within the maximum number of 40 PCR cycles (i.e. a negative test). A CT value below 40 indicated amplification (a positive test) and the lower the CT the earlier amplification had started, indicating higher target DNA concentrations in the sample. As a general rule, a difference of 3 CTs represents a 10 fold difference in target DNA levels. In the test validation work in HDC project M 54, DNA extracted from a pure culture of *Pseudomonas tolaasii* containing around  $10^8$  cells per ml gave CT values of 19 - 22.

### **Residue and heavy metal analysis**

Samples of peat + SBL casing (two), recycled rockwool (five) and spent coir (two), 500 g of each, were analysed by Groen Agro, Netherlands for pesticide residues using gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS). Samples of first and second flush mushrooms (500 g) from two different crops were also analysed for pesticide residues.

Dried samples (100 g) of the above casing materials and mushrooms were also analysed for heavy metals by Groen Agro.

## **Results**

### ***Analysis of casing raw materials***

All the non-peat casing materials used had a pH value of 5.43 to 7.60. The EC values of green waste compost, spent casing and used rockwool were higher than those of peat and the other materials used (Table 7). The non-peat materials, except spent coir, had lower water volumes at saturation, and allowed more water to drain (Table 7). Bark and spent casing had higher air filled porosities (AFPs) than the other materials; clay and green waste compost (GWC) had the highest compacted bulk densities (CBDs).

**Table 7.** Properties of peat sources and raw materials; mean of two replicate samples.

Property	units	Bark	Clay	GWC	Spent casing	Used rockwool	Spent coir	Peat (Everris)
pH		5.43	6.04	7.06	7.39	7.60	5.87	4.19
EC	µS/cm	185	67	1056	1070	742	96	61
Moisture	% w/w	49.4	24.6	44.6	75.7	42.6	89.4	79.0
Water volume	% v/v	58.0	40.6	58.1	49.8	69.5	73.5	73.6
Drainage water	% v/v	16.7	19.8	12.9	36.5	13.1	12.4	10.8
AFP	% v/v	30.9	19.3	20.1	42.1	15.0	20.4	18.3
CBD	g/L	389	1127	701	474	322	583	538

**Compost stability test by measuring carbon dioxide evolution**

According to the standardised WRAP method for measuring compost stability (ORG0020) (Llewelyn, 2005) all the samples of GWC from White Moss Horticulture used in the experiments were mature and stable, i.e. produced less than 6 mg CO<sub>2</sub>/g VS/d (Table 8). Composted bark used in the experiments, and two other sources of GWC were also mature and stable according to the standardised method.

**Table 8.** Carbon dioxide evolved from compost samples, mg CO<sub>2</sub>/g Volatile Solids/d. Each value is the mean of two replicate samples.

Source	Sample			
	1	2	3	4
White Moss Horticulture	0.51	1.26	2.51	—
Organic Recycling	3.06	0.62	0.34	1.65
J. Moody	1.40	—	—	—
Melcourt (Bark)	0.21	—	—	—

**Analysis of casing materials**

Physical and chemical analyses of peat-based casing materials are shown in Table 9, and the effect of adding 6.3 to 25% of substitute materials to the casing on the analyses are shown in Tables 10 to 14. Values significantly greater or less ( $P \leq 0.05$ ) than the respective peat-based casing values are shaded in green and pink, respectively. All the casing materials had pH values of between 7.08 and 7.96 (Table 10). Addition of 25% spent casing, used rockwool or GWC generally increased the EC of the casing (Table 11).

Addition of 12.5% GWC to peat-based casing slightly increased the water retention and reduced the volume of water that drained, and the air filled porosity (AFP) (Tables 12, 13 and 14). Addition of GWC or spent casing at 25% had no overall effect on the water or air holding characteristics of casing, whereas addition of the other substitute materials to peat-based casing generally reduced the water volume retained after drainage of a saturated sample (Table 12), and increased the volume of water that drained (Table 13) and the AFP (Table 14). However, these effects were small and there were exceptions to these general statements. Adding 25% bark to Harte casing at farm A or to Topterra casing at farm C had no effect on the volume of water retained after saturation. Spent coir added at 25% increased the AFP in Harte and Topterra casings but not in Everris casing (Table 14). Clay added at 12.5% to Everris casing reduced the AFP whereas clay added to Harte casing at 20% increased the AFP. Addition of clay to peat-based casing increased compacted bulk density. Addition of 25% rockwool increased compacted bulk density of peat casing, except in Everris casing at farm A (Table 15). Addition of bark, spent coir, spent casing or GWC in one- or two-way mixes had only small and inconsistent effects on compacted bulk density of casing.

**Table 9.** Properties of peat casings containing SBL or ground chalk\* at four farms. Each value is the mean of two replicate samples.

(a) pH

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2
Everris	7.58	7.76	7.47*	-	7.39	7.32	-	7.70	7.71*
Harte	7.61	7.70	7.43	7.82	-	7.31	7.22	7.56	-
Topterra	-	-	-	-	7.45	7.08	-	-	-

(b) Electrical conductivity,  $\mu\text{S cm}^{-1}$

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2*
Everris	290	609	443*	-	458	750	-	538	232*
Harte	192	191	590	135	-	288	353	368	-
Topterra	-	-	-	-	256	352	-	-	-

(c) Water volume retained after drainage of a saturated sample, %v/v

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2*
Everris	68.4	71.5	74.3	-	68.1	72.2	-	66.0	67.0
Harte	72.0	72.6	71.0	74.5	-	71.5	77.2	78.9	-
Topterra	-	-	-	-	69.6	68.8	-	-	-

(d) Volume of drainage water after saturation, %v/v

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2*
Everris	18.2	11.1	8.4	-	13.9	11.1	-	14.4	12.4
Harte	17.3	16.5	16.8	14.4	-	19.1	10.0	9.9	-
Topterra	-	-	-	-	18.2	19.0	-	-	-

(e) Air-filled porosity, %v/v

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2*
Everris	22.9	16.3	8.4	-	20.9	16.1	-	22.4	19.5
Harte	20.4	16.5	16.8	17.8	-	21.1	13.6	12.9	-
Topterra	-	-	-	-	22.5	23.2	-	-	-

(f) Compacted bulk density, g/L

Farm/Trial	A1	A2	A5	B1	C1	C2	D1	D2	D2*
Everris	710	779	757	-	631	713	-	549	587
Harte	627	701	750	654	-	703	609	607	-
Topterra	-	-	-	-	629	628	-	-	-

**Table 10.** pH of different casing treatments used on four farms. Mean of two samples.

Material % v/v	Peat + Sugar beet lime casing												
	Harte						Everris					Topterra	
Farm/ Trial	A#	A1 A2	A4 A5	B1	C# C3	D1	A1 A2	A3* A5*	C1	C2	D2*	C1	C2
Control none	7.56	7.61 7.70	7.86 7.43	7.82	7.55 7.80	7.22	7.58 7.76	- 7.48	7.39	7.32	7.71	7.45	7.08
Bark 12.5	-	- 7.68	- -	-	- -	7.29	- 7.90	- -	-	7.38	7.73	-	7.09
Bark 25	-	7.47 -	- -	7.66	- -	-	7.54 7.81	- -	-	-	-	7.32	-
GWC 12.5	-	- 7.54	- -	-	- -	7.41	- 7.96	- -	-	-	7.79	-	-
GWC 25	-	7.65 -	- -	-	- -	-	7.68 -	- -	-	-	-	-	-
Spent casing 25	7.47	7.46 -	7.35 7.43	-	7.47 7.55	-	7.62 -	- -	-	-	-	-	-
Spent coir 25	-	7.45 -	- -	7.71	- -	-	7.53 -	- -	-	-	-	7.41	-
Rockwool 25	-	7.57 -	- -	7.95	- -	-	7.58 -	- -	-	-	-	7.55	-
Clay 12.5	-	- -	- -	-	- -	-	- -	- -	7.60	-	-	-	7.37
Clay 20	-	- -	- -	7.86	- -	-	- -	- -	-	-	-	-	-
Bark 12.5 Clay 12.5	-	- -	- -	-	- -	-	- -	- -	-	7.51	-	-	-
Bark 6.3 GWC 6.3	-	- -	- -	-	- -	7.35	- -	7.51 -	-	-	7.76	-	-
Bark 6.3 GWC 6.3 Clay 6.3	-	- -	- -	-	- -	7.40	- -	- -	-	-	-	-	-

# data from M 53

\* with ground chalk

- treatment combination was not examined

**Table 11.** Electrical conductivity (EC) of different casing treatments used on four farms,  $\mu\text{S}/\text{cm}$ . Green shading indicates casing samples that were significantly higher than the respective peat control casings ( $P \leq 0.05$ ). Each value is the mean of two samples.

Material % v/v	Peat + Sugar beet lime casing												
	Harte						Everris					Topterra	
Farm/ Trial	A#	A1 A2	A4 A5	B1	C# C3	D1	A1 A2	A3* A5*	C1	C2	D2*	C1	C2
Control none	229	192 191	496 598	135	247 475	353	290 609	- 443	458	750	232	256	352
Bark 12.5	-	- 331	- -	-	- -	337	- 514	- -	-	723	245	-	333
Bark 25	-	233 -	- -	146	- -	-	300 530	- -	-	-	-	286	-
GWC 12.5	-	- 244	- -	-	- -	406	- 621	- -	-	-	289	-	-
GWC 25	-	452 -	- -	-	- -	-	617 -	- -	-	-	-	-	-
Spent casing 25	393	422 -	660 590	-	470 698	-	430 -	- -	-	-	-	-	-
Spent coir 25	-	216 -	- -	169	- -	-	291 -	- -	-	-	-	347	-
Rockwool 25	-	247 -	- -	291	- -	-	402 -	- -	-	-	-	569	-
Clay 12.5	-	- -	- -	-	- -	-	- -	- -	378	-	-	-	361
Clay 20	-	- -	- -	155	- -	-	- -	- -	-	-	-	-	-
Bark 12.5 Clay 12.5	-	- -	- -	-	- -	-	- -	- -	-	689	-	-	-
Bark 6.3 GWC 6.3	-	- -	- -	-	- -	379	- -	250 -	-	-	275	-	-
Bark 6.3 GWC 6.3 Clay 6.3	-	- -	- -	-	- -	381	- -	- -	-	-	-	-	-

# data from M 53

\* with ground chalk

- treatment combination was not examined

**Table 12.** Water volume retained after drainage of a saturated sample of different casing treatments used on four farms, %v/v. Green and pink shading indicates the water volume was significantly higher or lower ( $P \leq 0.05$ ) than in the respective control treatment. Each value is the mean of two samples.

Material % v/v	Peat + Sugar beet lime casing											
	Harte					Everris					Topterra	
Farm/ Trial	A1 A2	A4 A5	B1	C2 C3	D1	A1 A2	A3* A5*	C1	C2	D2*	C1	C2
Control none	72.0 72.6	74.8 71.0	74.5	71.5 71.3	77.2	68.4 71.5	- 74.2	68.1	72.2	68.0	69.6	68.8
Bark 12.5	- 75.0	-	-	-	75.1	- 70.9	-	-	69.0	68.1	-	68.7
Bark 25	72.1 -	-	71.1	-	-	64.1 70.7	-	-	-	-	69.9	-
GWC 12.5	- 75.7	-	-	-	81.0	- 71.7	-	-	-	68.5	-	-
GWC 25	72.0 -	-	-	-	-	65.2 -	-	-	-	-	-	-
Spent casing 25	69.1 -	78.9 62.5	-	-	-	69.1 -	-	-	-	-	-	-
Spent coir 25	69.6 -	-	68.9	-	-	68.7 -	-	-	-	-	68.3	-
Rockwool 25	70.3 -	-	68.1	-	-	67.4 -	-	-	-	-	69.0	-
Clay 12.5	- -	-	-	-	-	-	-	65.1	-	-	-	65.8
Clay 20	- -	-	66.9	-	-	-	-	-	-	-	-	-
Bark 12.5 Clay 12.5	- -	-	-	-	-	-	-	-	64.7	-	-	-
Bark 6.3 GWC 6.3	- -	-	-	-	76.9	-	70.6	-	-	68.0	-	-
Bark 6.3 GWC 6.3 Clay 6.3	- - -	- - -	- - -	- - -	73.1	- - -	- - -	- - -	- - -	- - -	- - -	- - -

\* with ground chalk      - treatment combination was not examined

**Table 13.** Volume of drainage water after saturation of different casing treatments used on four farms, %v/v. Green and pink shading indicates the water volume was significantly higher or lower ( $P \leq 0.05$ ) than in the respective control treatment. Mean of two samples.

Material % v/v	Peat + Sugar beet lime casing											
	Harte					Everris					Topterra	
Farm/ Trial	A1 A2	A4 A5	B1	C2 C3	D1	A1 A2	A3 A5	C1	C2	D2*	C1	C2
Control none	17.3 16.5	12.9 16.8	14.4	19.1 17.0	10.0	18.2 11.1	- 8.4	13.9	11.1	14.4	18.2	19.0
Bark 12.5	- 12.8	-	-	-	11.8	- 13.6	-	-	14.3	12.6	-	17.8
Bark 25	15.3 -	-	16.7	-	-	20.6 11.6	-	-	-	-	18.4	-
GWC 12.5	- 10.9	-	-	-	5.9	- 10.4	-	-	-	12.0	-	-
GWC 25	14.9 -	-	-	-	-	17.0 -	-	-	-	-	-	-
Spent casing 25	18.1 -	8.9 15.8	-	-	-	16.0 -	-	-	-	-	-	-
Spent coir 25	20.5 -	-	19.3	-	-	18.4 -	-	-	-	-	20.8	-
Rockwool 25	17.4 -	-	20.3	-	-	17.0 -	-	-	-	-	18.6	-
Clay 12.5	- -	-	-	-	-	-	-	10.5	-	-	-	18.2
Clay 20	- -	-	15.5	-	-	-	-	-	-	-	-	-
Bark 12.5 Clay 12.5	- -	-	-	-	-	-	-	-	17.8	-	-	-
Bark 6.3 GWC 6.3	- -	-	-	-	10.1	-	12.3	-	-	12.5	-	-
Bark 6.3 GWC 6.3 Clay 6.3	- -	-	-	-	9.8	-	-	-	-	-	-	-

\* with ground chalk      - treatment combination was not examined



**Table 14.** Air filled porosity of different casing treatments used on four farms, %v/v. Green and pink shading indicates the air volume was significantly higher or lower ( $P \leq 0.05$ ) than in the respective control treatment. Mean of two samples.

Material % v/v	Peat + Sugar beet lime casing											
	Harte					Everris					Topterra	
Farm/ Trial	A1 A2	A4 A5	B1	C2 C3	D1	A1 A2	A3 A5	C1	C2	D2*	C1	C2
Control none	20.4 16.5	15.3 16.8	17.8	21.1 20.1	13.6	22.9 16.3	- 8.4	20.9	16.1	19.5	22.5	23.2
Bark 12.5	- 16.6	- -	-	- -	15.3	- 13.6	- -	-	19.1	19.3	-	22.9
Bark 25	20.0 -	- -	20.4	- -	-	23.5 14.6	- -	-	-	-	21.6	-
GWC 12.5	- 15.4	- -	-	- -	8.9	- 10.9	- -	-	-	18.7	-	-
GWC 25	19.0 -	- -	-	- -	-	23.2 -	- -	-	-	-	-	-
Spent casing 25	22.2 -	14.2 15.8	-	- 19.8	-	22.1 -	- -	-	-	-	-	-
Spent coir 25	23.1 -	- -	23.6	- -	-	22.8 -	- -	-	-	-	24.2	-
Rockwool 25	21.3 -	- -	22.8	- -	-	22.1 -	- -	-	-	-	21.5	-
Clay 12.5	- -	- -	-	- -	-	- -	- -	18.3	-	-	-	20.0
Clay 20	- -	- -	18.3	- -	-	- -	- -	-	-	-	-	-
Bark 12.5 Clay 12.5	- -	- -	-	- -	-	- -	- -	-	20.1	-	-	-
Bark 6.3 GWC 6.3	- -	- -	-	- -	13.8	- -	16.6 -	-	-	19.0	-	-
Bark 6.3 GWC 6.3 Clay 6.3	- - -	- - -	-	- -	11.5	- -	- -	-	-	-	-	-

\* with ground chalk      - treatment combination was not examined

**Table 15.** Compacted bulk density of different casing treatments used on four farms, g/L. Green shading indicates the density was significantly higher ( $P \leq 0.05$ ) than in the respective control treatment. Each value is the mean of two samples.

Material % v/v	Peat + Sugar beet lime casing											
	Harte					Everris					Topterra	
Farm/ Trial	A1 A2	A4 A5	B1	C2 C3	D1	A1 A2	A3 A5	C1	C2	D2*	C1	C2
Control none	627 701	749 750	654	703 751	608	710 779	- 757	631	713	587	629	628
Bark 12.5	- 802	-	-	-	608	- 808	-	-	696	588	-	679
Bark 25	631 -	-	684	-	-	725 823	-	-	-	-	650	-
GWC 12.5	- 779	-	-	-	655	- 789	-	-	-	601	-	-
GWC 25	697 -	-	-	-	-	710 -	-	-	-	-	-	-
Spent casing 25	621 -	791 722	-	- 776	-	666 -	-	-	-	-	-	-
Spent coir 25	648 -	-	674	-	-	664 -	-	-	-	-	631	-
Rockwool 25	698 -	-	727	-	-	689 -	-	-	-	-	709	-
Clay 12.5	- -	-	-	-	-	-	-	720	-	-	-	727
Clay 20	- -	-	758	-	-	-	-	-	-	-	-	-
Bark 12.5 Clay 12.5	- -	-	-	-	-	-	-	-	755	-	-	-
Bark 6.3 GWC 6.3	- -	-	-	-	632	-	786	-	-	599	-	-
Bark 6.3 GWC 6.3 Clay 6.3	- - -	-	-	-	676	-	-	-	-	-	-	-

\* with ground chalk      - treatment combination was not examined

### ***Mushroom yields, quality and disease incidence***

A first flush of mushrooms in replicated tray experiments on farms A, B, C and D is shown in Fig. 9. Mushroom yields from an Everris casing prepared from dried blocking peat and milled peat fines were similar to Harte and Topterra casings prepared from wet dug peats (Table 16). More than 95% of mushrooms picked from all casings were Class 1.

**Table 16.** Mushroom yields from different peat casings containing SBL or ground chalk\* at four farms, kg/m<sup>2</sup>. Each value is the mean of three replicate trays.

Farm/Trial	A1	A2	A5	B	C1	C2	D1	D2	D2*
Everris	30.1	29.7	26.2	-	30.6	23.5	-	21.2	23.5*
Harte	28.5	28.4	26.2	32.3	-	23.8	28.7	23.6	-
Topterra	-	-	-	-	30.6	23.1	-	-	-



**Fig. 9** First flush of mushrooms in replicated tray experiments at farms A, B (upper) and C, D (lower).

The addition of 25% GWC to Harte or Everris casing at farm A reduced mushroom yield (Table 17). GWC added at 12.5% had no overall effect. The effect of addition of 25% recycled rockwool at all three farms and in all three types of casing was not significant compared with the respective control casings. Addition of 25% bark slightly but significantly improved yield from Harte casing at farm A, but reduced yield in Harte casing at farm B and in Everris and Topterra casings at farms A and C. This may have been due to insufficient water being added during the initial blending of the latter casing materials, as discussed in the following sections on casing moisture. Bark added at 12.5% slightly improved yield in Harte casing at farm D and in Topterra casing at farm C but had no effect in Harte casing at farm A or in Everris casing. Bark and GWC, each added at 6.3% at farm D, improved yield in Harte casing but had no effect in Everris casing.

At farm A, spent casing at 25% reduced yield in Everris casing but not in Harte casing. Spent coir resulted in green mould (*Trichoderma harzianum*) in Harte casings at farm B and in Topterra casing in farm C (data not shown). Clay at 20% reduced yield from Harte casing at farm B but the effect of 12.5% clay in Everris or Topterra casings at farm C was not significant. The effect of adding clay and bark, each at 12.5%, or clay, bark and GWC, each at 6.3%, was also not significant compared with the peat-based control casings.

**Table 17.** Mushroom yields from different casing treatments at four farms, kg/m<sup>2</sup>. Green and pink shading indicates yields that were significantly higher or lower ( $P \leq 0.05$ ) than the respective control yields. Each value is the mean of three replicate trays.

Material % v/v	Peat + Sugar beet lime casing												
	Harte						Everris					Topterra	
Farm/ Trial	A#	A1 A2	A4 A5	B1	C# C3	D1	A1 A2	A3 A5	C1	C2	D2*	C1	C2
Control none	24.0	28.5 28.4	24.6 26.2	32.3	25.5 27.1	28.7	30.1 29.7	- 26.2	30.6	23.5	23.5	30.6	23.1
Bark 12.5	-	- 28.4	- -	-	- -	30.0	- 29.8	- -	-	23.7	22.9	-	24.6
Bark 25	-	29.7 -	- -	29.8	- -	-	25.6 31.8	- -	-	-	-	27.7	-
GWC 12.5	-	- 28.7	- -	-	- -	31.7	- 27.2	- -	-	-	23.0	-	-
GWC 25	-	25.3 -	- -	-	- -	-	28.6 -	- -	-	-	-	-	-
Spent casing 25	24.4	29.5 -	25.1 26.5	-	25.5 31.5	-	26.7 -	- -	-	-	-	-	-
Spent coir 25	-	29.5 -	- -	28.4	- -	-	27.5 -	- -	-	-	-	25.2	-
Rockwool 25	-	29.2 -	- -	32.1	- -	-	29.8 -	- -	-	-	-	29.6	-
Clay 12.5	-	- -	- -	-	- -	-	- -	-	29.8	-	-	-	23.1
Clay 25	-	- -	- -	28.0	- -	-	- -	- -	-	-	-	-	-
Bark 12.5 Clay 12.5	-	- -	- -	-	- -	-	- -	- -	-	23.1	-	-	-
Bark 6.3 GWC 6.3	-	- -	- -	-	- -	31.7	- -	22.8 -	-	-	23.7	-	-
Bark 6.3 GWC 6.3 Clay 6.3	-	- -	- -	-	- -	28.6	- -	- -	-	-	-	-	-

# data from M 53

\* with ground chalk - treatment combination was not examined

### ***Casing moisture content during cropping***

The average volumetric water content of Everris, Topterra and Harte (farm A trial 1 and farm B) casings was around 61% (Table 18). Harte casing in the remaining experiments had an average volumetric water content of 65-73%.

**Table 18.** Average water volume during cropping from different peat casings containing SBL or ground chalk\* at four farms, %v/v. Each value is the mean of two samples.

Farm/Trial	A1	A2	A5	B	C1	C2	D1	D2	D2*
Everris	62.6	62.5	62.7	-	59.5	60.0	-	58.4	58.6*
Harte	60.7	65.0	68.1	62.6	-	64.9	73.7	73.2	-
Topterra	-	-	-	-	59.5	59.4	-	-	-

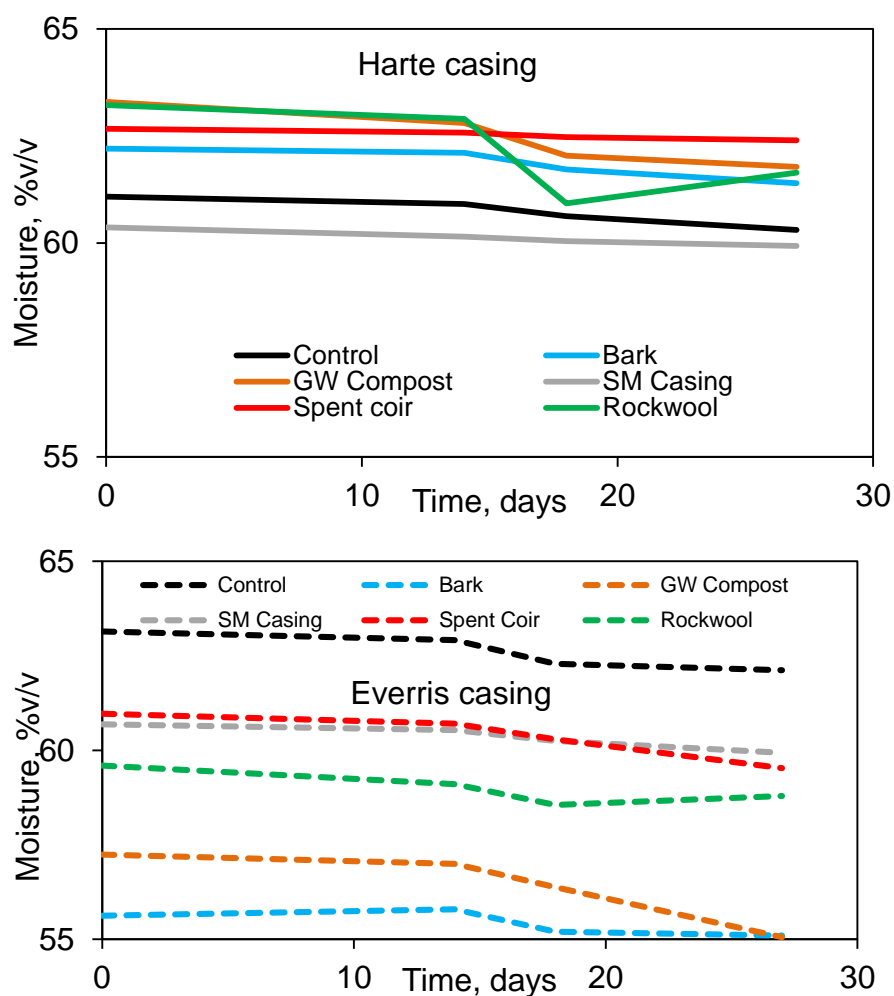
The volumetric water contents of peat-based casing and casing containing 25% spent coir or recycled spent casing or 12.5% bark or GWC during cropping were similar (Table 19; Figs. 10 to 15). The effects of adding 25% bark, GWC or rockwool on average volumetric moisture content of casing during cropping were inconsistent, with some crops showing slightly higher or lower values for the non-amended peat-based casings (Table 19, Figs. 10 to 15). Addition of 12.5 or 25% clay reduced the average volumetric moisture content of the casing.

**Table 19.** Average casing water volume in casing treatments during cropping at four farms. Green and pink shading indicates the water volume was significantly higher or lower ( $P \leq 0.05$ ) than in the respective control treatment. Each value is the mean of two samples.

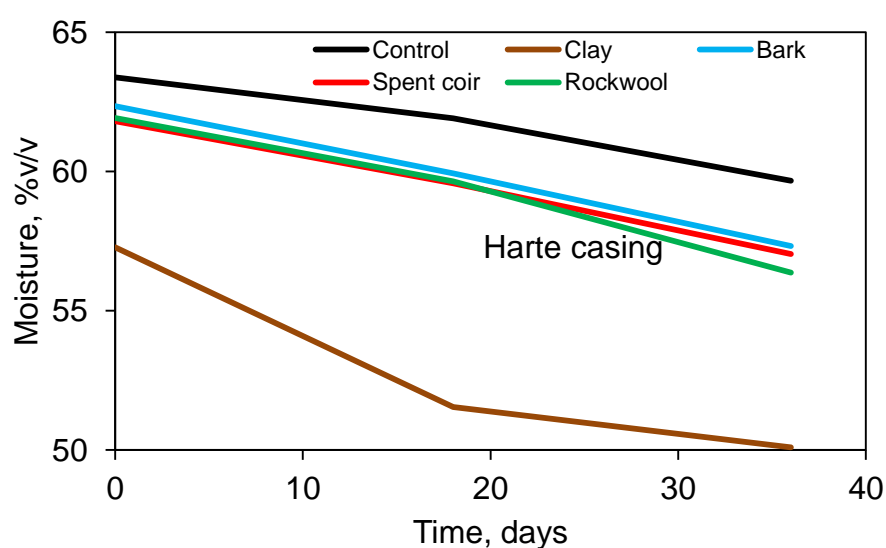
Material % v/v	Peat + Sugar beet lime casing												
	Harte						Everris					Topterra	
Farm/ Trial	A#	A1 A2	A4 A5	B1	C# C3	D1	A1 A2	A3 A5	C1	C2	D2*	C1	C2
Control none	62.2	60.7 65.0	68.0 68.1	62.6	62.6 66.9	71.7	62.6 62.5	- 62.7	56.6	60.0	58.4	59.5	59.4
Bark 12.5	-	- 69.1	-	-	-	70.3	- 62.8	-	-	57.3	58.6	-	61.4
Bark 25	-	61.9 -	-	59.9	-	-	55.2 63.7	-	-	-	-	60.5	-
GWC 12.5	-	- 69.2	-	-	-	71.9	- 61.5	-	-	-	59.5	-	-
GWC 25	-	62.4 -	-	-	-	-	56.4 -	-	-	-	-	-	-
Spent casing 25	62.1	60.1 -	68.9 68.1	-	62.6 64.8	-	60.4 -	-	-	-	-	-	-
Spent coir 25	-	62.5 -	-	59.5	-	-	60.4 -	-	-	-	-	60.1	-
Rockwool 25	-	62.2 -	-	59.3	-	-	59.0 -	-	-	-	-	61.2	-
Clay 12.5	-	-	-	-	-	-	-	-	53.5	-	-	-	57.1
Clay 25	-	-	-	52.5	-	-	-	-	-	-	-	-	-
Bark 12.5 Clay 12.5	-	-	-	-	-	-	-	-	-	54.9	-	-	-
Bark 6.3 GWC 6.3	-	-	-	-	-	69.1	-	-	-	-	59.1	-	-
Bark 6.3 GWC 6.3 Clay 6.3	-	-	-	-	-	66.9	-	-	-	-	-	-	-

# data from M 53

\* with ground chalk - treatment combination was not examined

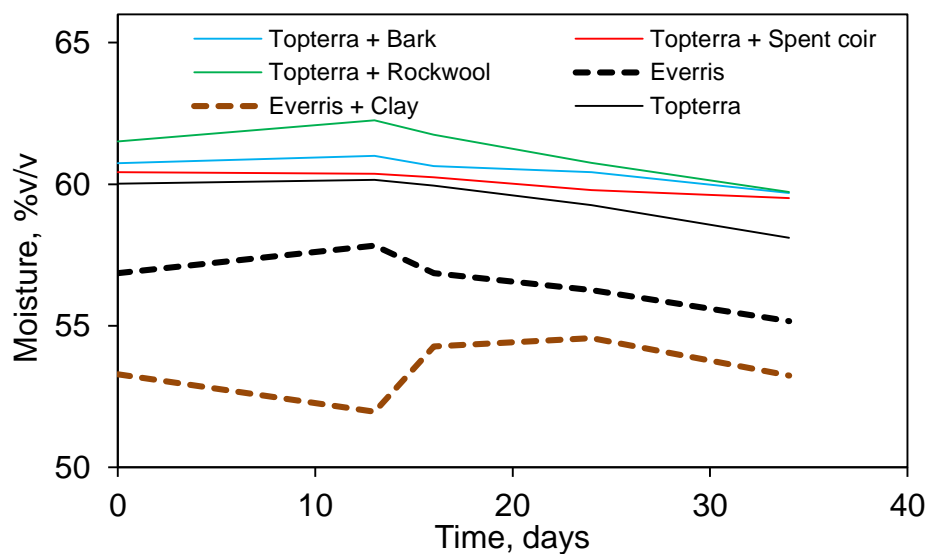


**Fig.10.** Moisture content of Harte and Everris casings containing 25% v/v of different substitute materials during Trial 1 at farm A.

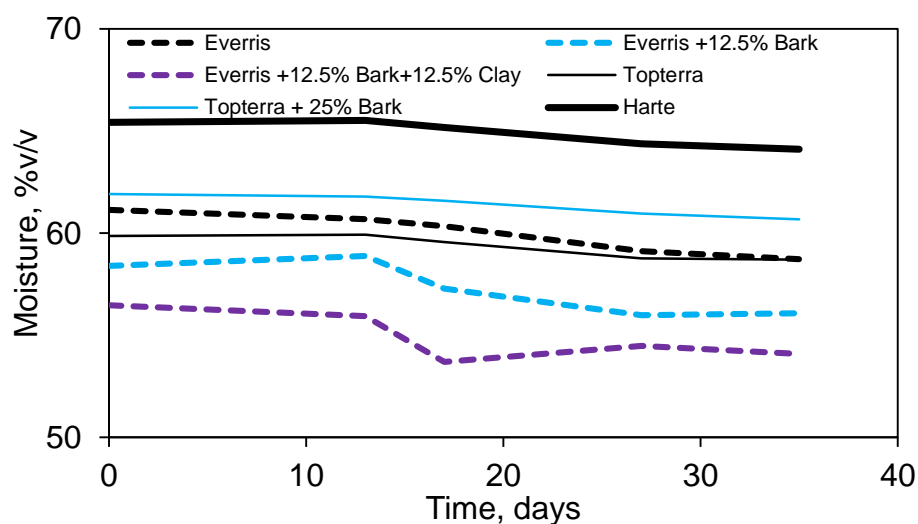


**Fig.11.** Moisture content of Harte casing containing 25% v/v of different substitute materials (or 20% clay) during cropping at farm B.

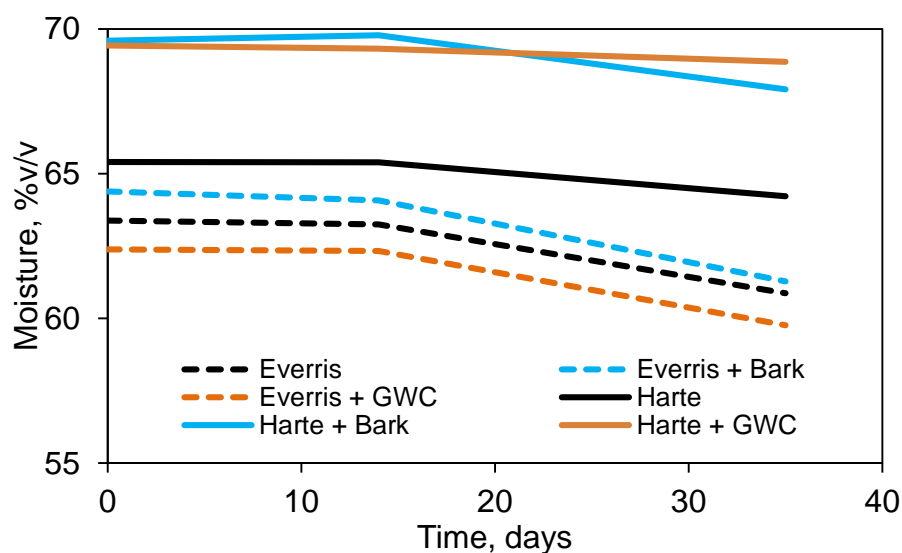




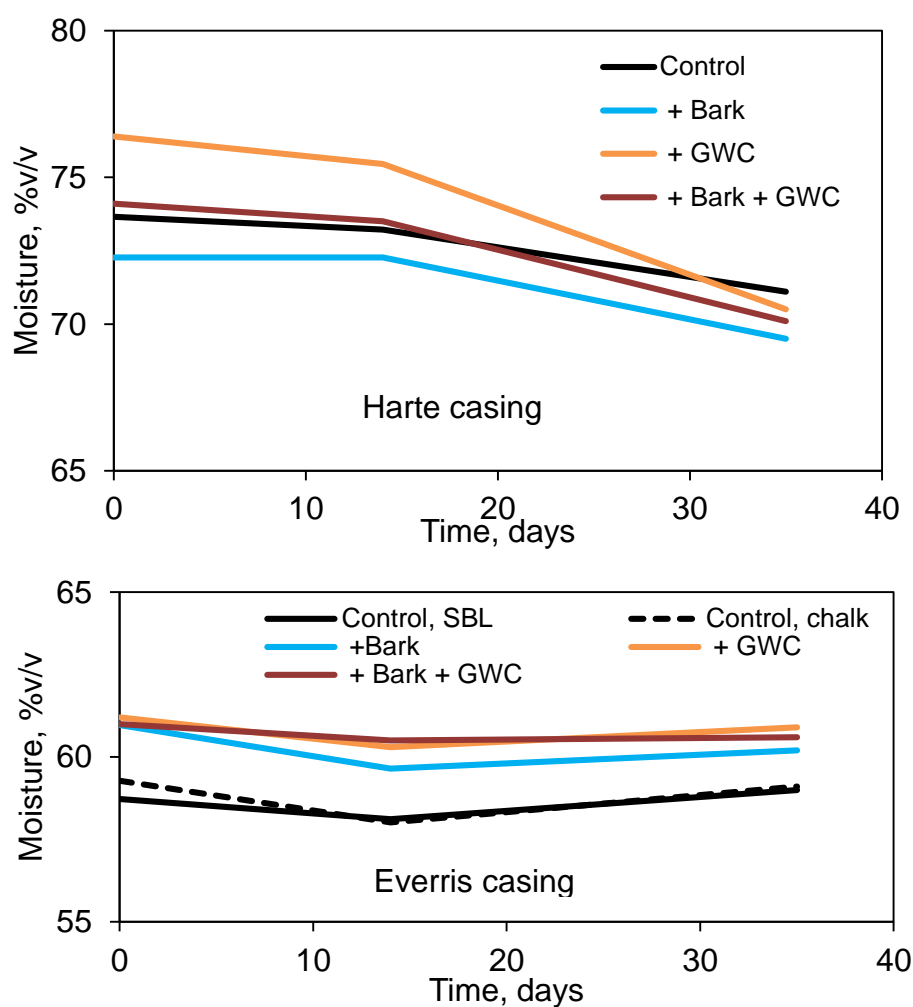
**Fig. 12.** Casing moisture of Topterra casing containing 25% of different substitute materials or Everris casing with and without 12.5% clay during Trial 1 at farm C.



**Fig. 13.** Casing moisture of Everris, Topterra and Harte casings with and without bark or bark+clay during Trial 2 at farm C.



**Fig. 14.** Casing moisture of Everris and Harte casings with and without bark or green waste compost at 12.5% v/v during Trial 2 at farm A.

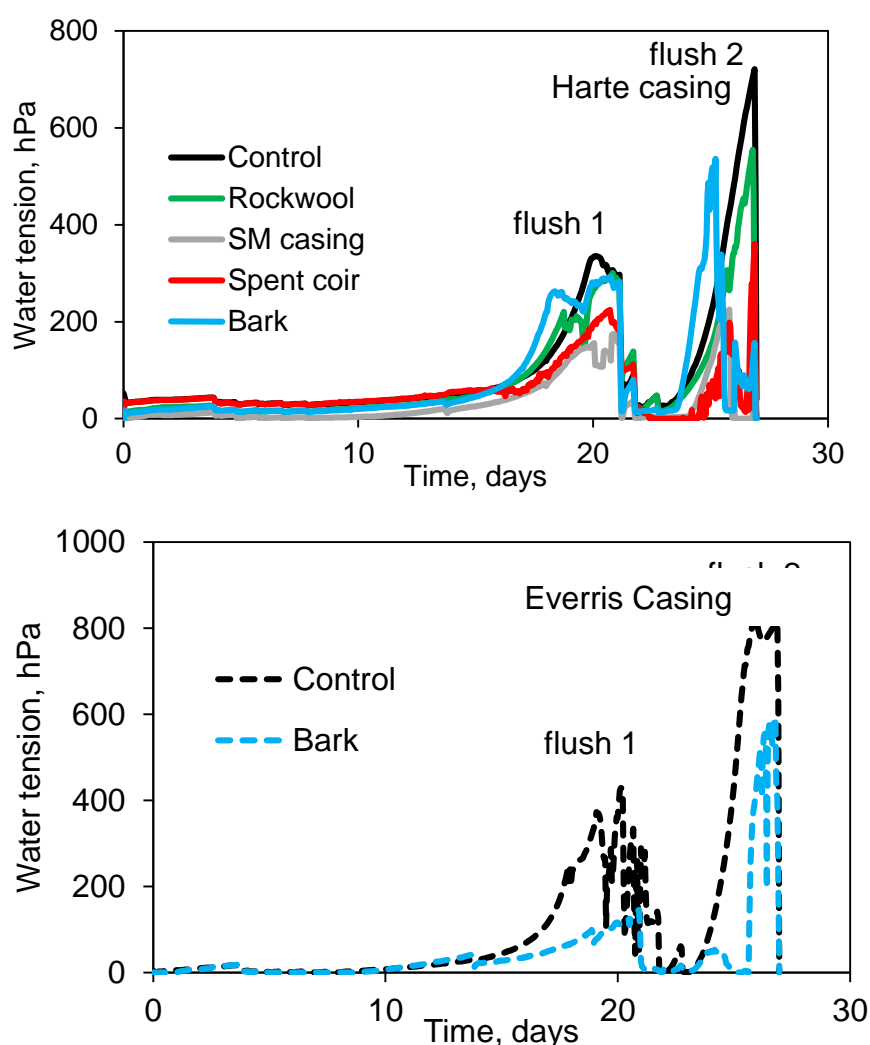


**Fig. 15.** Casing moisture of Everris and Harte casings with and without bark or green waste compost at 12.5% v/v during Trial 1 at farm D.

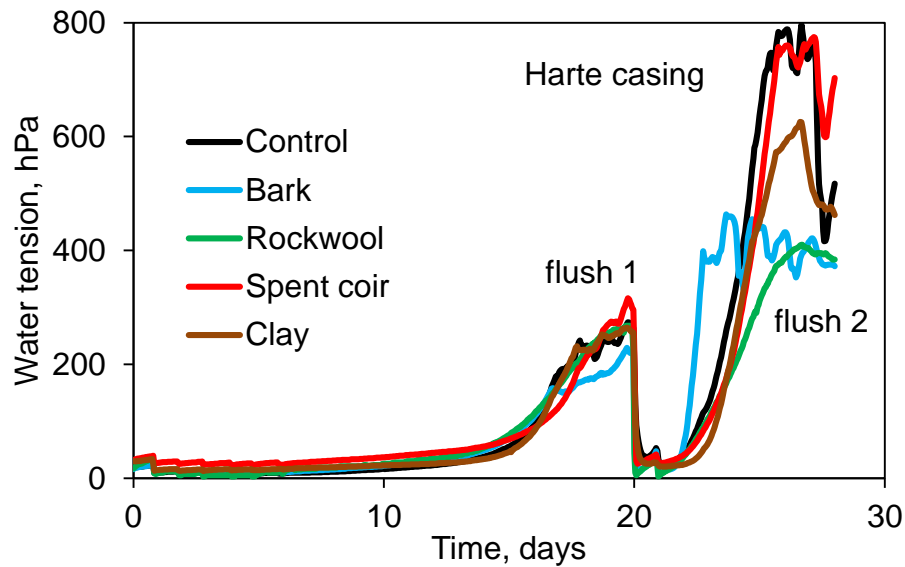
### ***Casing water tension during cropping***

Casing water tensions before the first flush were small in all four farms, and the casing water tension in the first flush was always smaller than in the second flush (Figs. 16 to 21). The casing water tensions in the first and second flushes at farms A, B and D were similar (Figs. 16, 17, 20 and 21) whereas the water tensions at farm C were much larger (Figs. 18 and 19).

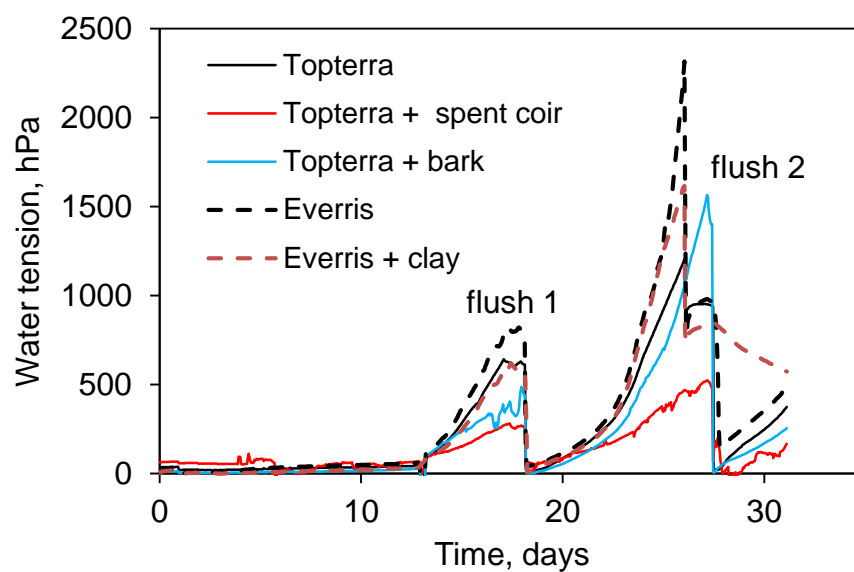
At farm A, casing water tension was slightly greater in the non-amended Everris casing than in Harte casing (Fig. 16). At farm C, casing water tension was greater in the non-amended Everris casing than in the Topterra casing (Fig. 18). Addition of substitute materials at 25% to casing generally reduced the water tension during the first and second flushes compared with the unamended peat-based control casings (Figs. 16 to 21). Exceptions were addition of 25% bark to casing in the second experiment at farms A and C (Figs. 19 and 20). Addition of bark or GWC at 12.5%, or both materials at 6.3% had no effect on the casing water tension (Fig. 21).



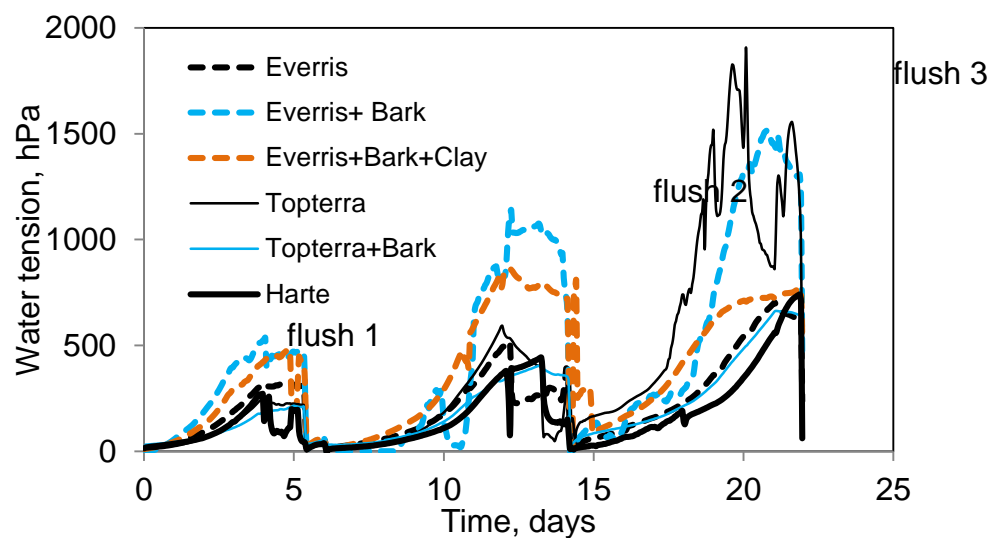
**Fig. 16.** Water tension in Harte and Everris casings containing 25% v/v of different materials during cropping in Trial 1 at farm A.



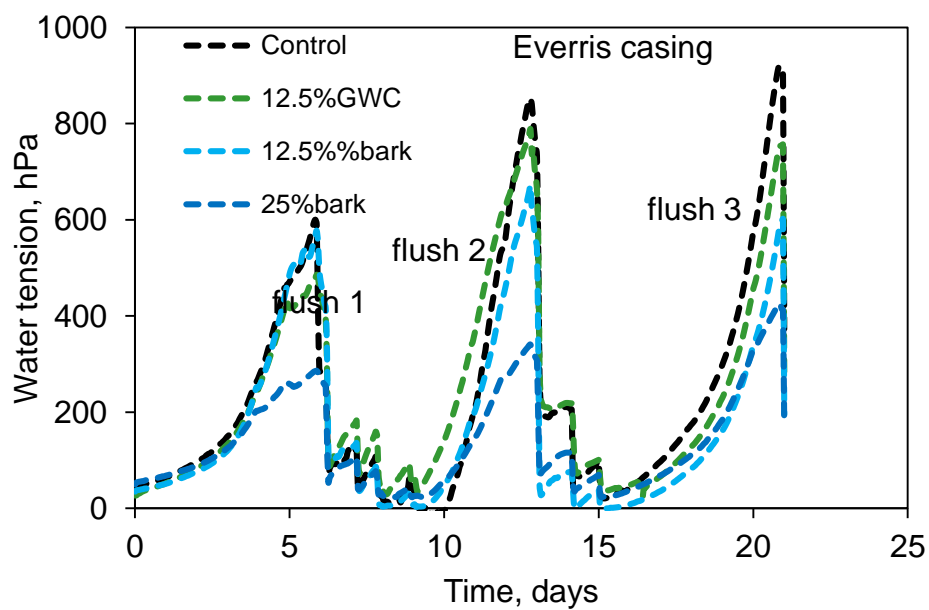
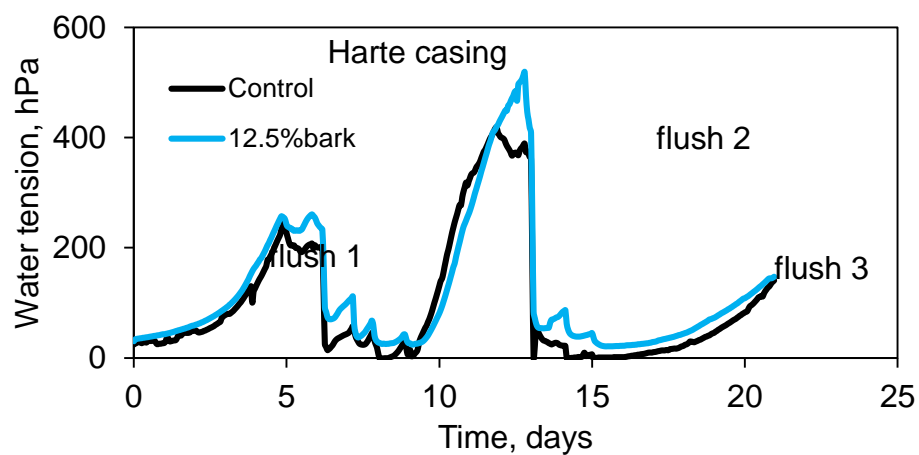
**Fig. 17.** Water tension in Harte casing containing 25% v/v of different substitute materials (or 20% v/v clay) during cropping at farm B.



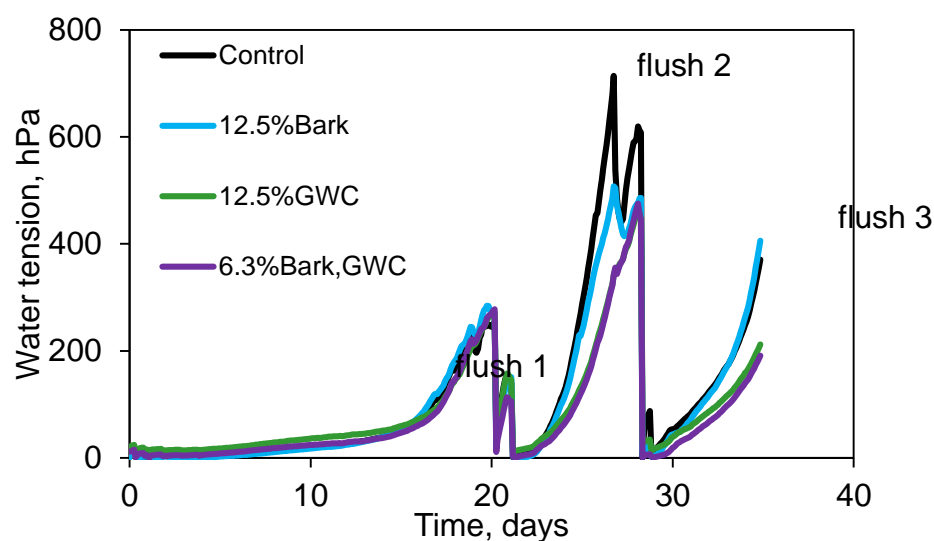
**Fig. 18.** Water tension in Topterra casing with and without 25% v/v spent coir or bark, or Everris casing with and without 12.5% v/v clay during cropping in Trial 1 at farm C.



**Fig. 19.** Water tension in Everris, Topterra and Harte casings with and without 25% bark or bark+clay during cropping in Trial 2 at farm C.



**Fig. 20.** Water tension in Everris and Harte casings containing different substitute materials during cropping in Trial 2 at farm A.



**Fig.21.** Water tension in Harte casings with and without 12.5% bark or GWC or 6.3%bark+6.3% GWC during cropping in Trial 1 at farm D.

### ***Large-scale validation trials***

First and third flushes of mushrooms in the larger scale casing trials at farms A, C and E are shown in Figs. 22 to 27. Total mushroom yields from the Everris + GWC (6.3%) + Bark (6.3%) casing and Harte casings at farm A were similar, although the Harte casing cropped better in the first flush and the Everris mix cropped better in the second and third flushes (Table 20). The use of a separating net or meshing between the compost and casing reduced yields by 9%. After cook-out, the casing separated easily from the compost layer.

At farms A and E, there was no difference in mushroom yield between shelves that were cased with 25% recycled casing, or with peat-based control casing (Tables 20 and 21). At Farm C, mushroom yield was higher from the 25% recycled casing treatment than from the Harte control casing (Table 21). The addition of 25% recycled casing to fresh casing did not have any effect on mushroom quality.



**Fig.22.** First flush in large-scale casing trial at farm A



**Fig. 23.** Third flush of mushrooms growing casing separated from the compost with netting.

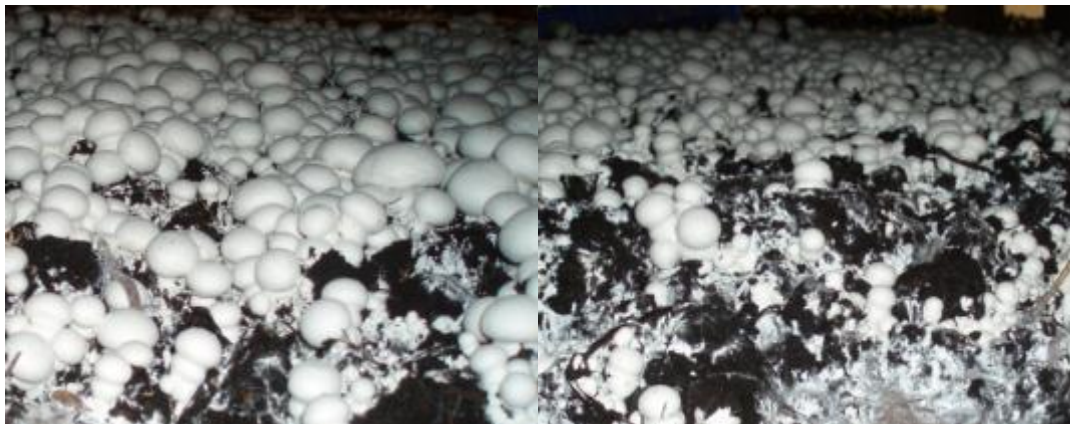


**Fig.24.** First flush in large-scale trial at farm E. Shelf with 25% recycled spent casing left; shelf with control casing right.





**Fig.25.** Large-scale trial at farm A. Tray with 25% recycled spent casing left; tray with control casing right.



**Fig.26.** First flush in large-scale trial at farm C. Tray with 25% recycled spent casing left; tray with control casing right.



**Fig.27.** Third flush in large-scale trial at farm C. Tray with 25% recycled spent casing lower left; tray with control casing upper right.



**Table 20.** Mushroom yields (kg/m<sup>2</sup>) in larger scale trials at farm A.

Casing treatment	Trial A3	Trial A4	Trial A5
Harte (control)	22.7	24.6	26.2
Everris	—	—	26.2
Everris + 6.3% bark + 6.3% GWC	22.8	—	—
Harte casing + separating netting	20.8	—	—
Harte + 25% recycled casing	—	25.1	26.5
Harte + 25% recycled casing + separating mesh	—	22.8	—

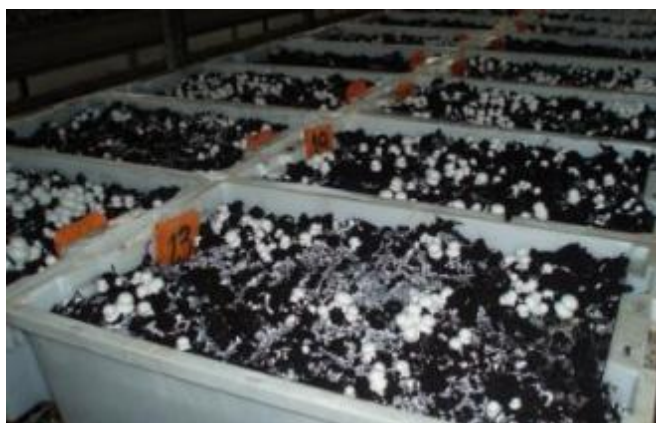
**Table 21.** Mushroom yields in larger scale trial at farms C and E, kg/m<sup>2</sup>.

Peat Substitute	farm C	farm E
None (control)	27.1	25.1
25% SM Casing	31.5	25.0

***Small scale cropping and mycelium growth bioassay tests***

Mushrooms growing in small-containers are shown in Fig. 28. Mushroom yields from the three types of peat-based casing (Harte, Everris and Topterra) in the small containers were similar. The addition of bark or GWC at 12.5% to Harte casing was beneficial to yield, whereas these substitutions had no effect on yield from Everris or Topterra casings (Table 22). Bark, recycled rockwool, spent coir or spent casing added at 25%, or bark + GWC each added at 6.3%, had no effect on mushroom yield compared with the respective non-amended peat control treatments, although GWC added at 25% reduced yield from Everris casing. Clay added at 12.5% reduced yield from Topterra casing.

Mycelium growth rate was slightly faster in Everris and Topterra casings than in Harte casing (Table 23). Bark or GWC added at 12.5 or 25% generally did not affect mycelium growth rate, although it was reduced by GWC added at 12.5% to Harte casing (Table 23). Spent casing, spent coir and rockwool all slightly increased mycelial growth rate in Harte casing but had no effect in Everris or Topterra casings.



**Fig. 28.** Mushroom cropping trial in small containers.

**Table 22.** Mushroom yields from casing treatments in small-scale cropping tests, kg/t Phase 3 compost. Green and pink shading indicates yields that were significantly higher or lower ( $P \leq 0.05$ ) than the respective control yields. Each value is the mean of three trays.

Material% v/v	Peat + Sugar beet lime casing		
	Harte	Everris	Topterra
Control (none)	277	275	268
Bark 12.5	292	283	270
Bark 25	271	279	265
GWC 12.5	293	275	-
GWC 25	253	261	-
Spent casing 25	271	269	-
Spent coir 25	285	278	262
Rockwool 25	284	281	276
Clay 12.5	-	270	250
Bark 6.3 + GWC 6.3	285	274	-

- treatment combination was not examined

**Table 23.** Mushroom mycelial growth rate in different casing treatments in glass cylinders, mm/d. Green and pink shading indicates growth rates that were significantly higher or lower ( $P \leq 0.05$ ) than the respective control growth rates. Each value is the mean of three replicates.

Material% v/v	Peat + Sugar beet lime casing		
	Harte	Everris	Topterra
Control (none)	4.21	4.46	4.46
Bark 12.5	4.12	4.49	4.49
Bark 25	4.31	4.37	4.42
GWC 12.5	3.93	4.46	-
GWC 25	4.30	4.62	-
Spent casing 25	4.46	4.46	
Spent coir 25	4.48	4.49	4.51
Rockwool 25	4.46	4.56	4.47
Clay 12.5	-	4.65	4.63
Bark 6.3 + GWC 6.3	4.05	4.64	-

- treatment combination was not examined

***Bacterial blotch in casing treatments and evaluation of a real-time PCR test for detection of *Pseudomonas tolaasii****

No bacterial blotch was observed in the trials at farms A, B and D, apart from one tray of Harte casing amended with 25% recycled rockwool (Table 24). Severe bacterial blotch was observed in both crops at farm C (Fig. 29): on all trays cased with unamended Topterra casing, or Topterra casing containing 25% recycled rockwool, 12.5% clay or 12.5% bark. Blotch was also observed on one out of three trays cased with Topterra casing and 25% spent coir. No blotch was observed in the unamended Everris casing or in Topterra casing containing 25% bark in the first crop at farm C. However, blotch was observed in two out of three trays in the unamended Harte and Everris casings, Everris casing with 12.5% bark or 12.% bark + 12.5% clay in the second crop at farm C.



**Fig. 29.** Severe bacterial blotch on mushrooms grown with Topterra casing at farm C

Using the Taqman PCR *Pseudomonas tolaasii* test, all the fresh samples of casing materials used at farms A, C and D had a CT value of 40 indicating that the level of *P. tolaasii* was below the detectable limit (samples from farm B were not tested). After the second flush, the lowest CT values (and therefore most positive for *P. tolaasii*) were found in Topterra casing samples from farm C, generally corresponding with the most severe bacterial blotch (Table 24). However, Topterra with 25% bark, which did not produce blotch symptoms had a CT value of 32.0, whereas Everris casing samples (with and without 12.5% bark) in the second

crop at farm C had CT values of 40 but did produce some blotch symptoms in mushrooms. This was probably due to the variability in *Pseudomonas* populations in casing across the beds.

**Table 24.** Taqman PCR *Pseudomonas tolaasii* CT results in casings between second and third flushes on different farms. All fresh casing samples had CT values of 40. Each value is the mean of two test samples. Brown cells: severe blotch; pale brown cells: some blotch observed; white cells (where treatment was examined): no blotch observed; grey cells: not tested.

Material% v/v	Peat + Sugar beet lime casing								
	Harte			Everris				Topterra	
Farm/Trial	A1	C2	D1	A1	C1	C2	D2*	C1	C2
Control none	40.0	37.7	40.0	40.0	37.2	40.0	40.0	29.7	30.8
Bark 12.5	-	-	n.t.	-	-	40.0	40.0	-	31.2
Bark 25	40.0	-	-	n.t.	-	-	-	32.0	-
Spent casing 25	40.0	-	-	n.t.	-	-	-	-	-
Spent coir 25	n.t.	-	-	n.t.	-	-	-	31.7	-
Rockwool 25	n.t.	-	-	n.t.	-	-	-	26.5	-
Clay 12.5	-	-	-	-	33.2	-	-	-	35.8
Bark 12.5 Clay 12.5	-	-	-	-	-	37.0	-	-	-
Bark 6.3 GWC 6.3	-	-	40.0	-	-	-	40.0	-	-

\* with ground chalk

- treatment combination was not examined

n.t. not tested with Taqman PCR

### **Total pseudomonad populations in casing samples**

The initial total populations of *Pseudomonas* sp. in the Harte, Everris, and Topterra peat-based casings were between  $3.1 \times 10^5$  and  $4.3 \times 10^6$  cfu/g (Table 25). The addition of bark, GWC, spent coir, spent casing, used rockwool or clay at up to 25% v/v did not affect the initial population of *Pseudomonas* sp. There was no difference in the initial *Pseudomonas* sp. population of casing materials that resulted in severe blotch (Topterra casings at farm C) and

casings that did not result in blotch (Table 26). In all of the casings at farms A and D, the total populations of *Pseudomonas* sp. increased by between x2 and x24 by time of the second sampling, after the 2<sup>nd</sup> flush (Table 26). The additions of bark, GWC, spent casing, spent coir, clay or rockwool to fresh casing had little or no effect on the final *Pseudomonas* sp. populations in the casings (Tables 26). At farm C, the increase in population of total *Pseudomonas* sp. in casing during cropping was more pronounced, increasing by between x17 and x137. The high counts of *Pseudomonas* sp. in the casing at farm C generally corresponded with the high levels of bacterial blotch at the farm (Table 26).

**Table 25.** Total *Pseudomonas* sp. in casing at start of crops on different farms. Each value is the mean of two test samples (cfu g<sup>-1</sup> casing).

Material %v/v	Peat + Sugar beet lime casing								
	Harte			Everris				Topterra	
Farm/Trial	A1	C2	D1	A1	C1	C2	D2*	C1	C2
Control none	3.1 x 10 <sup>5</sup>	3.5 x 10 <sup>6</sup>	3.8 x 10 <sup>6</sup>	4.3 x 10 <sup>6</sup>	1.2 x 10 <sup>6</sup>	2.8 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	3.1 x 10 <sup>6</sup>	3.9 x 10 <sup>6</sup>
Bark 12.5	-	-	3.7 x 10 <sup>6</sup>	-	-	2.9 x 10 <sup>6</sup>	4.2 x 10 <sup>6</sup>	-	3.8 x 10 <sup>6</sup>
Bark 25	1.7 x 10 <sup>6</sup>	-	-	n.t.	-	-	-	2.6 x 10 <sup>6</sup>	-
GWC 12.5	8.9 x 10 <sup>5</sup>	-	3.6 x 10 <sup>6</sup>	n.t.	-	-	1.5 x 10 <sup>6</sup>	-	-
Spent casing 25	1.4 x 10 <sup>6</sup>	-	-	1.9 x 10 <sup>6</sup>	-	-	-	-	-
Spent coir 25	n.t.	-	-	n.t.	-	-	-	4.0 x 10 <sup>6</sup>	-
Rockwool 25	n.t.	-	-	n.t.	-	-	-	9.9 x 10 <sup>5</sup>	-
Clay 12.5	-	-	-	-	9.5 x 10 <sup>5</sup>	-	-	-	3.0 x 10 <sup>6</sup>
Bark 12.5 Clay 12.5	-	-	-	-	-	2.7 x 10 <sup>6</sup>	-	-	-
Bark 6.3 GWC 6.3	-	-	4.1 x 10 <sup>6</sup>	-	-	-	2.4 x 10 <sup>6</sup>	-	-

\* with ground chalk

- treatment combination was not examined n.t. not tested for total pseudomonads

Table 26. Total *Pseudomonas* sp. in casing after second flush in crops on different farms. Each value is the mean of two test samples (cfu g<sup>-1</sup> casing). Brown cells: severe blotch; Pale brown cells: some blotch observed; white cells (where treatment examined): no blotch observed.

Material % v/v	Peat + Sugar beet lime casing								
	Harte			Everris				Topterra	
Farm/ Trial	A1	C2	D1	A1	C1	C2	D2*	C1	C2
Control none	1.1 x 10 <sup>6</sup>	6.7 x 10 <sup>7</sup>	5.9 x 10 <sup>7</sup>	2.3 x 10 <sup>7</sup>	7.4 x 10 <sup>7</sup>	3.8 x 10 <sup>8</sup>	2.4 x 10 <sup>7</sup>	1.1 x 10 <sup>8</sup>	3.7 x 10 <sup>8</sup>
Bark 12.5	-	-	5.6 x 10 <sup>7</sup>	-	-	3.7 x 10 <sup>8</sup>	8.8 x 10 <sup>7</sup>	-	6.4 x 10 <sup>7</sup>
Bark 25	2.1 x 10 <sup>6</sup>	-	-	n.t.	-	-	-	6.9 x 10 <sup>7</sup>	-
GWC 12.5	5.1 x10 <sup>6</sup>	-	5.8 x 10 <sup>6</sup>	n.t.	-	-	6.5 x 10 <sup>6</sup>	-	-
Spent casing 25	2.4 x 10 <sup>6</sup>	-	-	1.8 x 10 <sup>7</sup>	-	-	-	-	-
Spent coir 25	n.t.	-	-	n.t.	-	-	-	1.0 x 10 <sup>8</sup>	-
Rockwool 25	n.t.	-	-	n.t.	-	-	-	1.2 x 10 <sup>8</sup>	-
Clay 12.5	-	-	-	-	9.6 x 10 <sup>7</sup>	-	-	-	8.7 x 10 <sup>7</sup>
Bark 12.5 Clay 12.5	-	-	-	-	-	3.7 x 10 <sup>8</sup>	-	-	-
Bark 6.3 GWC 6.3	-	-	1.6 x 10 <sup>7</sup>	-	-	-	4.4 x 10 <sup>7</sup>	-	-

\* with ground chalk

- treatment combination was not examined

n.t. not tested for total pseudomonads

### ***Residue and heavy metal analysis***

Concentrations of heavy metals in filter cake clay were similar to those in peat + SBL casing, except chromium (Cr) which was higher in the peat-based casing, and copper (Cu) and (Zn) where higher values were obtained in the clay (Table 27). Values for arsenic (As), cadmium (Cd), and mercury (Hg) were lower in recycled rockwool than in peat + SBL casing, but values for cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni) and Zinc (Zn) were higher. However, all the heavy metal values in peat + SBL casing, filter cake clay and recycled rockwool were below the regulatory limit for land application for organic wastes, except nickel in one of the samples of recycled rockwool.

Heavy metal contents except copper and zinc were below detectable limits in all first and second flush mushrooms sampled (Table 28). Copper and zinc contents in mushrooms grown on peat + SBL, 25% recycled rockwool or 12.5% clay were similar, although the concentrations were consistently higher in the second flush than in the first (Table 28).

**Table 27.** Heavy metal analysis of casing ingredients; maximum and minimum values in three analyses, mg kg<sup>-1</sup> dry weight.

Heavy metal	Peat casing		Recycled rockwool		Filter cake clay		Regulatory limit*
	Min.	Max.	Min.	Max.	Min.	Max.	
As	<0.4	0.9	-	<0.4	<0.4	0.9	5
Cd	<0.1	0.2	-	<0.1	-	<0.1	0.75
Co	-	<0.05	1.3	4.0	-	<0.05	75
Cr	<0.1	3.1	10	67	<0.1	1.1	70
Cu	<0.05	5.2	10	30	<0.05	9.1	70
Hg	<0.01	0.02	-	<0.01	<0.01	0.02	0.4
Ni	<1.0	1.1	5	50	<1.0	0.9	25
Pb	<0.1	0.6	<0.1	13	<0.1	0.5	45
Zn	<0.05	8.6	100	120	<0.05	66.7	200

\* EU and UKROFS regulatory limits for composts applied to agricultural land (European Commission, 2004)



**Table 28.** Heavy metal analysis of mushrooms grown on peat/SBL casing and casings substituted with 25% recycled rockwool or 12.5% clay, and EU regulatory limit for mushrooms, mg kg<sup>-1</sup> dry weight.

Heavy metal	Peat casing		Recycled rockwool		Clay		EU limit*
	1st	2nd	1st	2nd	1st	2nd	
As	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	-
Cd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
Co	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-
Cr	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-
Cu	23.4 - 23.8	47.2 – 48.1	22.3	56.5	23.0	46.9	-
Hg	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.3
Ni	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Pb	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3
Zn	40.6 - 43.5	70.5 - 74.2	46.7	81.9	45.3	70.9	-

\* EU limit for Agaricus mushrooms (European Commission, 2001).

Trace levels of residues of several pesticides approved for tomatoes, cucumbers and strawberries were detected in the recycled rockwool and spent coir (Tables 29 and 30). Azoxystrobin, Bupirimate, Cyprodinil, Fludioxinil and Myclobutanil were detected in four out of five samples of recycled rockwool. All the other pesticides were only detected in one out of five samples. Boscalid, Dimethomorph, Myclobutanil and Penconazole were detected in both samples of spent coir tested. All of the pesticide residues detected were below the EU maximum residue limits (MRLs) for tomato fruit, except Fludioxinil and Myclobutanil in recycled rockwool and Quinoxifen in spent coir. However, all of the residue levels detected in the recycled rockwool and coir samples were below the MRLs for tomato fruit when used at 25% by volume in peat casing. No pesticide residues were detected in any of the first or second flush mushrooms grown on 25% recycled rockwool or 25% spent coir casings.

**Table 29.** Maximum and minimum pesticide residues detected by Groen Agro in five samples of granulated 6-month composted used rockwool slabs obtained from Grodan and Materialchange, and EU maximum residue levels in tomato fruit (mg kg<sup>-1</sup>). Detection threshold was 0.01 mg kg<sup>-1</sup>.

Pesticide	Detection method	Min	Max.	Positive samples	MRL tomato
Azoxystrobin	LC-MS	0.32	1.00	4	3.0
Boscalid	LC-MS	0.03	0.15	1	3.0
Bupirimate	LC-MS	0.12	0.42	4	2.0
Carbendazim	LC-MS	-	0.01	1	0.3
Cyprodinil	LC-MS	0.14	0.55	4	1.0
Fludioxonil	GC-MS	1.12	3.70	4	1.0
Imazalil	LC-MS	-	0.01	1	0.5
Imidacloprid	LC-MS	-	0.01	1	0.5
Iprodione	GC-MS	-	0.02	1	5.0
Metalaxyl	GC-MS	0.03	0.13	1	0.3
Myclobutanil	LC-MS	0.27	0.65	4	0.3

**Table 30.** Maximum and minimum pesticide residues detected by Groen Agro in two samples of used spent coir, and EU maximum residue levels in tomato fruit (mg kg<sup>-1</sup>). Detection threshold was 0.01 mg kg<sup>-1</sup>.

Pesticide	Detection method	Min	Max.	MRL tomato
Boscalid	LC-MS	0.04	0.23	3.0
Dimethomorph	LC-MS	0.09	0.30	1.0
Myclobutanil	LC-MS	0.14	0.15	0.3
Penconazole	GC-MS	0.01	0.04	0.1
Quinoxifen	LC-MS	-	0.06	0.02

## ***Discussion***

This work has shown that a suitable mushroom casing can be prepared from black blocking peat and milled peat fines. However, unlike casing prepared from wet dug peat, this casing requires rewetting before use, although blocking peat readily absorbs moisture and does not need a pre-soaking treatment that brown milled peats did when they were used for mushroom casing more than 20 years ago. The casing also required more precise irrigation scheduling (more frequent and smaller quantities) than is needed for wet dug peat casing. For farms that no longer have casing mixers, some reinvestment would be required for such a casing to be used. The cost of the casing is similar to that prepared from wet dug peat.

The effects of adding 25% bark fines or GWC on mushroom yield were inconsistent between farms. However, addition of 12.5% of bark fines or 6.3% each of bark fines and GWC to peat casing, with water, was either beneficial or neutral to mushroom yield. The latter mix would be cheaper than 12.5% bark because GWC is cheaper than bark. The composted bark fines and GWC were shown to be mature and stable according to a maturity test based on CO<sub>2</sub> evolution from samples.

Recycling spent casing at 25% worked better with Harte casing than with Everris casing although the latter mix may work better if more water is added. Recycled casing is only suitable if cooking out is used, and this is not practiced on all farms. Although machinery is available for separating casing on emptying in shelves, it is an expensive option and not suitable for tray farms. The trials have shown that a separating mesh or net inserted between the compost and casing layers is not a viable option for casing removal after cook-out due to mushroom yield loss and the time taken to insert and remove the mesh layer. The use of salt for disease control must be minimized and spraying of casing with disinfectants at the end of cropping must be avoided, otherwise the recycled casing will be contaminated. For tray farms, heavily salted trays can be avoided for recycling. A mixture of salt and fine sand could be used for disease control in order to minimize the amount of salt applied. The validation trials showed that recycled casing (and possibly bark or GWC) could be readily mixed into fresh casing using existing casing hoppers and shelf filling equipment. If recycled on the same farm, spent casing is not considered to be a waste. However, potential residues of Sporgon must be considered if the recycled casing is to be used in a further crop where Sporgon will again be applied. The results confirm large-scale trials in HDC project M 53 which showed that adding 25% spent casing did not affect mushroom yield compared with fresh casing. It is possible that inclusion rates higher than 25% could be used without yield loss, although small-scale trials in M 53 indicated that 50% recycled casing was too much.

There is some loss in casing volume during cropping so that 1 m<sup>3</sup> fresh casing results in 0.73 m<sup>3</sup> spent casing. This means that to produce a 25% recycled blend, about 35% of the casing on a farm would need to be recycled; the rest would be disposed of with the remaining SMC. Because there would be a constant in-flow of 75% fresh casing into a blend, and not all casing would be recycled, there would be no need to re-start the process at intervals during the year. SMC usually contains around 67% w/w substrate and 33% w/w casing. If 35% of the spent casing is recycled, this would be equivalent to about 12% w/w of the total SMC. Separated compost will have higher crop NPK fertilizer value than mixed SMC, which can also pose problems due to high pH caused by the lime in the casing. HDC project M43 showed that 33% of spent compost (with casing layer removed) can be reused in Phase I compost with no effect on mushroom yield compared with non-amended Phase I compost. There is therefore a potential to save on straw and other compost ingredients, if composting is conducted in the vicinity of mushroom production.

Casing including 25% granulated recycled rockwool slabs produced mushroom yields comparable with peat casings at all three farms where it was tested, and it did not stick to the mushrooms. Although trace levels of approved pesticide residues were detected in the raw material, they were below the MRL for tomato fruit when used at 25% in casing and no pesticide residues were detected in the mushrooms. However, the material would require a waste license from the EA if used commercially for producing mushroom casing (it currently has an EA low risk waste exemption for re-use in growing slabs). In view of the limited supply of this material in Britain (about 8,000 cubic metres per year) it is only likely to be viable if the much larger quantities in the Netherlands and Belgium (around 60,000 cubic metres per year) are also available for use as mushroom casing. Discussions with Dutch and Belgian casing producers indicated that they are not currently interested in preparing a waste exemption since the quantities available do not satisfy the requirements of their markets.

The effect of adding filter cake clay to casing at 12.5% or at 6.3% in a 3-way mix with bark and GWC on mushroom yield was not significant. However, addition of clay at 20% was detrimental to mushroom yield. The material was difficult to blend evenly in the casing and its high bulk density would increase transport costs.

The results have shown that casing materials with a volumetric water retention at saturation of at least 67% were more suitable than materials with a lower water retention when saturated. However, there was no relationship between casing water retention above 67% and mushroom yield obtained from different casings. Maintaining a casing water volume of at least 61% during cropping produced a better yield than maintaining a lower water volume.

Mushroom quality was unaffected by the casing treatments, with more than 95% of mushrooms picked as Class 1 from all treatments.

Within the range of values obtained in this work, there were no relationships between mushroom yield and the other physical characteristics of casing that were measured: air filled porosity, water drained out of a saturated sample, and compacted bulk density. EC (135 to 750  $\mu\text{S cm}^{-1}$ ) and pH (7.08 to 7.95) also did not influence mushroom yield.

Casing water tensions were consistently greater in the second flush than in the first flush across all the farms; this is in spite of second flush yields being similar or lower than first flush yields. This indicates that water stress is greater in the second flush than in the first, and that more water needs to be applied after the first flush, without draining into the compost.

The occurrence of bacterial blotch was not primarily related to the initial population of *Pseudomonas* sp. in casing materials. Blotch was mainly associated with farm C which may have had environmental conditions conducive to the disease. Watering at farm C, determined from tensiometer data, was similar to that at farms A, B and D. The occurrence of blotch generally corresponded with positive results obtained with a Taqman PCR test for *P. tolaasii* on casing samples taken after the second flush, although blotched mushrooms were obtained from casing treatments that tested negative and *vice versa*. This was probably due to the variability in *Pseudomonas* populations in casing on trays. Large populations of *Pseudomonas* sp. in the casing ( $>10^8$  cfu g $^{-1}$ ) after the second flush generally corresponded with the occurrence of moderate or severe bacterial blotch. However, smaller increases in *Pseudomonas* sp. population during the cropping period of less than x25 were more usual and were not indicative of the occurrence or absence of blotch. This increase in population and final counts *Pseudomonas* sp. of  $10^6$ - $10^7$  cfu g $^{-1}$  are similar to those found previously for peat-based casing (Noble et al. 2003; Noble et al. 2009). In Project M 54, mushroom culture in high humidity and conducive to blotch resulted in higher than usual casing populations of *Pseudomonas* sp. ( $10^8$  cfu g $^{-1}$ ) (Elphinstone et al, 2012).

## Conclusions

1. The supply of wet dug peat has substantially reduced in Britain and dwindling supplies in Germany are also of concern to casing manufacturers in the Netherlands and Belgium.
2. The most commonly used casing in Britain is Harte (Ireland) with smaller quantities from Scotland, Belgium and the Netherlands.

3. Other types of peat and peat production by-products are available in Britain in sufficient quantities to supply the mushroom industry.
4. A review showed that the most promising alternatives to peat were composted bark fines, granulated recycled rockwool slabs, spent coir from grow bags, PAS 100 green waste compost, and filter cake clays.
5. Mushroom yields and quality from an Everris casing prepared from partially dried blocking peat and milled peat fines were similar to Harte and Topterra casings prepared from wet dug peats; however, the casing needed wetting during pre-mixing and the crop needed more frequent irrigation events of shorter duration than with wet dug peat casing.
6. The effects of adding 12 - 25% bark fines on mushroom yield were inconsistent between farms.
7. GWC was unsuitable at an inclusion rate of 25% but at 12.5% had no overall effect. It was best used at 6.3% in conjunction with a similar volume of bark when it was either neutral or beneficial to mushroom yield; this blend would also be cheaper than using 12.5% bark.
8. The effect of addition of 25% recycled rockwool at all three farms where it was tested and in three types of casing was not significant compared with the respective peat control casings.
9. Recycling spent casing at 25% had no overall effect on mushroom yield. Casing with salt or disinfectant must be avoided for use in recycling in casing. A MushComb casing separator machine is an option for in recycling spent casing in shelves.
10. Filter cake clay at 20% reduced mushroom yield but the effect of 12.5% clay was not significant. However, the material was difficult to mix evenly through the casing.
11. Spent coir was unsuitable for casing because it encouraged green mould.
12. Casing materials with a volumetric water retention at saturation of at least 67% were more suitable than materials with a lower water retention when saturated
13. Maintaining a casing volumetric water content of at least 61% during cropping produced a better yield than maintaining a lower water volume in the casing.
14. Casing water tensions were consistently greater in the second flush than in the first across all the farms in spite of second flush yields being similar or lower than first flush yields; this indicates that more water needs to be applied after the first flush, without excessive draining into the compost.
15. The occurrence of bacterial blotch was not primarily related to the initial population of *Pseudomonas* sp. in casing materials; blotch was mainly associated with one farm which may have had environmental conditions conducive to the disease.
16. The occurrence of blotch generally corresponded with positive results obtained with a Taqman PCR test for *P. tolaasii* on casing samples taken after the second flush, although

blotched mushrooms were obtained from casing treatments that tested negative and *vice versa*.

17. Large increases in *Pseudomonas* sp. populations in the casing from application to after the second flush generally corresponded with the occurrence of bacterial blotch or severe blotch.

## Glossary

AFP Air filled porosity

CBD Compacted bulk density

CT Critical Threshold: lower values indicate higher DNA levels (e.g. for pseudomonads)

CI casing inoculum

EA Environment Agency (UK)

EC Electrical conductivity

GWC Mature green waste compost

HRI Horticulture Research International

MRL Maximum residue level

MRF Multi-roll filter cake, a coal mining industry clay by-product

NVZ Nitrate vulnerable zone

PCR Polymerase chain reaction

SBL Sugar beet lime

SMC Spent mushroom compost

## Knowledge transfer activities

Presentation and demonstration trays at mushroom growers' event, Stratford-upon-Avon, March 2015.

AHDB Horticulture Factsheet. Peat and alternative materials for mushroom casing

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