# FINAL REPORT

To:
Horticultural Development Council
Bradbourne House
Stable Block
East Malling
Kent, ME19 6DZ

## IMPROVING THE EFFICIENCY AND ENVIRONMENTAL IMPACT OF MUSHROOM COMPOSTING

**M 3e HortLINK Project**
**CSA6365/HL0163LMU**

**Dr Ralph Noble**
**Warwick HRI**
**Wellesbourne, Warwick, CV35 9EF**

November 2006
Commercial - In Confidence

# Grower Summary

## M 3e Horticulture
## LINK Project
## CSA6365/HL0163LMU

### IMPROVING THE EFFICIENCY AND ENVIRONMENTAL IMPACT OF MUSHROOM COMPOSTING

**Final report 2006**

| | |
|---|---|
| Project Title: | Improving the efficiency and environmental impact of mushroom composting |
| Project Number: | M 3e Horticulture LINK Project CSA6365/HL0163LMU |
| Project Leader: | Ralph Noble |
| Report: | Final Report, November 2006 |
| Previous Reports: | Year 1, September 2004; Year 2, September 2005 |
| Key Workers: | Ralph Noble, Mairead Kilpatrick, Phil Hobbs, Shekar Sharma Andreja Dobrovin-Pennington, Gary Lyons |
| Locations of Project: | Warwick HRI, University of Warwick<br>Wellesbourne, Warwick, CV35 9EF<br><br>Agri-Food and Biosciences Institute,<br>Loughall, Co. Armagh, BT60 8JB<br><br>Institute of Grassland and Environmental Research,<br>North Wyke, Okehampton, Devon, EX20 2SB |
| Project Co-ordinator: | Mr Martyn Dewhurst, Tunnel Tech Ltd,<br>The Old Airfield, Winchester Street, Leckford, Hants., SO20 6JF |
| Date Project Commenced: | 1 October 2003 |
| Keywords: | Mushrooms, Compost, Odour, Smell, Straw, Spawn-running, Mycelial growth |

**Signed on behalf of:** **Warwick HRI**

***Signature***:…… Date: ……
***Name:*** Professor Simon Bright
Director and Head of Department

Intellectual property rights are invested in the Consortium of Horticulture LINK Project No. CSA6365/HL0163LMU.

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

**CONTENTS** Page

**Consortium members:**

Warwick HRI, University of Warwick

Agri-Food and Biosciences Institute

Institute of Grassland and Environmental Research

Horticultural Development Council

Mushroom Growers' Association

Blue Prince Mushrooms Ltd

Carbury Mushrooms Ltd

Custom Compost Ltd

Drinkwaters' Mushrooms Ltd

Greenhill Compost Ltd

J. Rothwell & Son Ltd

James A. Gooding Ltd

McGeary's Mushroom Compost

Monaghan Mushrooms Ltd

New Zealand Mushrooms Ltd

Shackleford Mushrooms Ltd

Tandragee Compost

Tunnel Tech Ltd

JEOL (UK) Ltd

Casella eti

Triton Technology Ltd

**Government Sponsors**

Department for Environment, Food and Rural Affairs

Department of Agriculture and Rural Development for Northern Ireland

## Grower Summary

### Headlines

Growing site and nitrogen fertiliser application had greater effects on the chemical analysis of wheat straw and its subsequent mushroom cropping performance than cultivar or applications of fungicide or growth regulator. High values for dry matter digestibility and/or hemicellulose and cellulose content (indicated by derivative thermogravimetric analysis) in wheat straw appear to be better for producing higher yield mushroom compost.

The rate of mushroom mycelial growth in compost and subsequent mushroom yield were related to the fall in compost pH during spawn-run. However, the amount of laccase enzyme at the end of spawn-run was a better indicator of subsequent mushroom yield. Increasing the rate of spawn to 0.8 % w/w, from the commercial standard rate of 0.5% w/w, reduced spawn-running time and increased mushroom yield. Supplementation at spawning with several proprietary supplements had no effect on spawn-running time or on subsequent mushroom yield. Vis-NIR analysis of compost samples may give an indication as to the subsequent mushroom mycelial growth rate (spawn–run); derivative thermogravimetric analysis of composts gave an indication of subsequent mushroom yield.

The odour intensity of goody water was closely related to the concentration of volatile sulphides. These were produced from leached poultry manure and could easily be detected with gas detection tubes. Low concentrations could also be detected using an electronic analyser. Aeration by submerged pipes or aerators and screening out solid material resulted in a significant reduction in goody water odour. Measuring the redox potential and electrical conductivity of goody water gave a good indication of the odour intensity – both measurements can easily be taken on-site.

### Background and Objectives of the Work

Processing time is a major factor influencing the cost of mushroom compost production. In particular, the rate of degradation to produce a suitable compost (in aerated or windrow Phase I), and the subsequent colonisation of the compost with mushroom mycelium in expensive spawn-running facilities (Phase III). This work was aimed at identifying the factors that influence the mushroom mycelial colonisation of compost, in order to reduce spawn-running time and associated capital and operating costs.

Wheat straw used in preparing mushroom compost is highly variable in terms of degradability and the properties change during storage. This causes a significant problem in producing a consistent compost. This work aimed to identify key physical and chemical properties of straw that relate to suitability for mushroom composting and subsequent cropping.

The mushroom industry is under environmetal pressure due to its odour emissions. If environmentally unacceptable levels of smell are to be eliminated, the use of recycled run-off or 'goody' water and watering management needs to be improved. Recycled water quality and management may also have effects on the efficiency of the composting process and subsequent mushroom growth. This work was aimed at determining the relevant chemical and microbial properties of recycled water, and the influences of water treatment on the odours from mushroom composting and water storage faclities.

Objectives

01 Determine the chemical and physical composition of straw that influence compost degradation and subsequent mushroom mycelium growth (spawn-running) and cropping.
02 Identify compost factors that can be manipulated to reduce spawn-running times from those currently found commercially by at least 10% (current average is 16 days).
03 Determine the effects of chemical and microbiological properties of recycled water on composting odours, sulphide emissions and compost quality, and identify improved methods of recycled water treatment and application.
04 On-site tests to adapt new methods for analysing straw, reducing spawn-running time, measuring odours and sulphide emissions, and managing water recycling and application in commercial-scale composting systems.

## Summary of results and conclusions

Effects of straw analysis on compost

- Growing site and nitrogen fertiliser application had greater effects on the chemical analysis of wheat straw and its subsequent mushroom cropping performance than cultivar or applications of plant growth regulator and fungicide. However, mushroom yields from the spring wheat cultivar Axona were better than from several winter wheat cultivars.
- Straw produced at the Limavady site in NI produced compost with a higher yield potential than straw from two other sites in NI and from GB. This straw had a higher dry matter digestibility than straw from the same treatments grown on the other sites.
- Straw produced from higher nitrogen fertiliser plots produced higher mushroom yield than straw from low nitrogen fertiliser plots. This difference corresponded with a higher hemicellulose and cellulose fractions in the straw and a lower lignin fraction.
- Samples of straw that had either a high dry matter digestibility and/or larger relative amounts of cellulose and hemicellulose to lignin (determined by thermogravimetry) produced better mushroom yields than straw samples with low values of these measurements.

- Large-scale experiments showed that the difference in mushroom yield between the 'best' and 'worst' straw sources were about 50 kg mushrooms/ tonne compost (20% yield difference)
- The tensile properties of straw did not relate to the subsequent mushroom yield potential or mycelial growth rate of the compost produced.
- Plant growth regulator (chlomequat) and fungicide applications to straw resulted in significant changes in the chemical composition of the straw, but they did not significantly affect either mushroom yield or mycelial growth rate from the compost produced.
- Straw that had been stored dry for 1 year produced better mushroom yields than fresh straw of the same type (wheat cultivar, nitrogen fertiliser, fungicide, and plant growth regulator applications), whereas two-year old straw produced significantly lower mushroom yields.

Reducing spawn-running time

- Fall in compost pH and increase in laccase enzyme were good indicators of the rate of spawn-run in compost and both measurements related to the final mushroom yield from the compost.
- Leaving compost unpressed until casing resulted in a more rapid spawn-run in terms of temperature increase and higher laccase enzyme, but not in terms of drop in compost pH.
- Increased compaction of compost by increasing the time of pressing in trays from 4 to 12 seconds retarded spawn-running and reduced mushroom yield
- A higher spawn rate (0.8% w/w) resulted in greater laccase content, greater compost pH drop and higher mushroom yield than a lower rate (0.5% w/w).
- None of supplements used at spawning (ProMycel, Natural Gold and Lambert T6) had a significant effect on spawn-running (except for an early increase in compost temperature) or mushroom yield.
- There was fairly close correlation between the vis-NIR spectral analysis of the compost samples prepared from different straw batches and subsequent mycelial growth rate and with fall in compost pH during spawn-run. This could be due to the availability of compost nutrients to mushroom mycelium, particularly amorphous hemicellulose and cellulose.
- There were no relationships between the vis-NIR spectral analysis of the composts and subsequent mushroom yield; the results from DTG analysis of compost samples did correlate with their subsequent mushroom yield

Effects of recycled water analysis on odours

- Aeration of goody water by submerged pipes, propeller type aerators, or continuous recirculation reduced odour and sulphides. However, this effect was confounded by aerated goody water pits having a lower dry matter content.
- Surface aeration of goody water pits was ineffective in reducing odour.
- Redox potential was found to be a better indication of the oxygen demand of goody water samples since a dissolved oxygen meter could not discriminate between several samples which had zero values, but differing redox potentials and odours.
- Goody water odour was correlated with redox potential and dry matter content (or electrical conductivity). Dry matter content and electrical conductivity of goody water were highly correlated.
- A large number of chemical compounds were detected, both in freeze-dried goody water and in air samples taken from above goody water pits. Sulphur-containing compounds explained most but not all the odour associated with goody water air. Indoles, amines, ketones, aldehydes and alcohols were also detected in variable amounts.
- Sulphides (hydrogen sulphide and dimethyl sulphide) from goody water could easily be detected with gas detector tubes. Low concentrations of these sulphides could also be detected with a pulsed fluorescence $SO_2$ analyser fitted with a sulphide to $SO_2$ converter.
- There was a wide range in the pH of goody water but this did not affect odour.
- Microbial profiles of goody water samples were determined using phospholipid fatty acid (PLFA) analysis and measurement of *Clostridia* spp. to estimate populations of sulphate reducing bacteria. Aerated goody water had a lower population of sulphate reducing bacteria and overall population of Gram +ve bacteria than non-aerated goody water.
- The addition of gypsum to goody water samples did not result in an increase in odour or volatile sulphides. However, the prevention of gypsum being washed into the goody water storage tank and forming a source of sulphur for sulphate reducing bacteria, must be regarded as good practice.
- Goody water samples with wide ranges in pH, EC, redox potential and dry matter content resulted in composts that had similar rates of mushroom mycelial colonisation and mushroom yield.
- The sulphide emissions and odours produced from flask composts were not related to the odour intensities and sulphide emissions of the goody water samples that were used to prepare them. The direct effect of goody water odour on composting sites is therefore likely to be more important than the effect of goody water on subsequent composting odours.

**Action points for growers**

- Background information should be obtained on the source of straw supplies for composting (particularly growing site, nitrogen fertiliser application and wheat cultivar) and the relative performance of different straw supplies compared. This work has shown that mushroom yield differences of 20% can be due to different straw sources.
- The pH of compost should be measured at spawning and after spawn-run. The compost pH drop gives an indication of the quality of the spawn-run and the potential yield from the compost.
- The effect of using a higher spawning rate should be examined. This work has shown that a higher yield and shorter spawn-run can be obtained if the rate is increased above the commercial standard rate of 0.5% w/w. Top-spawning did not produce any benefits in this work.
- If supplements are used at spawning, the benefits of using them should be re-examined, since no benefits were identified in this work.
- The effect of reducing the compression of spawned compost should be examined. Excessive pressure delayed spawn-run and reduced mushroom yield in this work.
- The quality of goody water should be regularly tested by measuring the electrical conductivity and redox potential. Values greater than 2 mS/cm or less than -300 mV respectively indicate that the screening of solids and/or the aeration system are not adequate.

**Anticipated practical and financial benefits**

Effect of straw selection

The work has shown that the difference in mushroom yield between 'good' and 'poor' sources of wheat straw from the same season is at least 50 kg mushrooms / tonne of Phase II compost produced (around 20% yield difference). At 20p/kg mushrooms (net of picking and packing), this has a potential value of £5 / tonne Phase II compost. One tonne of straw produces about 4 tonnes of Phase II compost. Assuming a transport cost of £1.80 per mile for a 25 tonne capacity lorry, it costs around £0.018 per tonne of Phase II compost produced, for each additional mile that the straw must be transported to the composting site. This means that it would be worth transporting the straw up to an additional 278 miles (£5/£0.018 per mile) to the site, if suitable growing sites were identified in place of local but 'poor' sources of wheat straw. If the value of mushrooms was higher than 20p/kg (net of picking and packing), it would be worth transporting suitable straw proportionately further.

Effect of spawn rate

Cost of additional spawn (0.8% w/w compared with 0.5% w/w) per tonne of compost is 3 kg x £1.66/ kg spawn = £5 per tonne of Phase II compost. Increased yield = 15 kg/tonne compost.

This would require a mushroom price (net of picking and packing) of 34p/kg to cover the cost of the extra spawn. A mushroom of 20p/kg mushrooms (net of picking and packing) (or £3.00 for 15 kg of increased yield) leaves a cost of £2 per tonne Phase II compost (£2.70 per tonne of Phase III compost) for the additional spawn. A one day (6%) shortening in spawn-run (reduced energy and capital running costs) would be worth more than a cost of £2.70 per tonne of compost.

Environmental benefits – reduced goody water odour

An electrical conductivity meter can be purchased for about £400 (e.g Fischer) and a Redox meter can be purchased for about £800 (e.g. ArrowDox). Regular monitoring with these instruments should lead to improved goody water quality and reduced pressure from environmental authorities.

Costs were obtained from The Agricultural Budgeting & Costing Book (Anon, 2004), Nix et al (2005) and transport costs from Organic Recycling Ltd.

**PROJECT MILESTONES**

| Mile-stone | Target Date | Primary Milestone |
|---|---|---|
| 1.1 | 9 months | Methods developed for the physical and chemical analysis of wheat straw obtained from experimental field sites, and of defined age, variety, N fertiliser regime, soil type, PGR application (HRI, IGER, DARD, Triton Technology) |
| 2.1 | 9 months | Laboratory methods developed for mycelial growth rate and extracellular laccase on compost samples (HRI) |
| 1.2 | 12 months | Straw samples from the experimental sites covering a range of cultivars, ages, and growing localities (soil types, N fertiliser regimes, PGR application) analysed using the above methods and related to biodegradability, mushroom mycelial growth and cropping (laboratory scale (HRI, DARD, IGER, Triton) |
| 3.1 | 12 months | Techniques developed for determining the microbial community structure, chemical composition and biological oxygen demand of recycled water samples, on their own and in laboratory-scale composting tests (HRI, IGER, Jeol) |
| 2.2 | 15 months | Mushroom mycelial colonisation of compost related to physical and chemical analyses of compost (laboratory scale) (HRI, IGER, DARD, Commercial composters) |
| 3.2 | 18 months | Odour panel and sulphide measurements on air surrounding recycled water samples related to the analyses in 3.1 (HRI, IGER, Jeol, Casella, Commercial composters) |
| 1.3 | 24 months | Biodegradation rate , mushroom spawn-running and cropping using straw batches and types obtained from the experimental field sites related to key properties identified in 1.2 tested in intermediate-scale facilities (HRI, DARD, Triton, Commercial composters) |
| 3.3 | 24 months | Effects of methods of water treatment on odour and sulphides determined on commercial sites (HRI, DARD, Casella, Commercial composters) |
| 2.3 | 27 months | Spawn-running time and mushroom cropping related to key factors identified in 2.2, intermediate-scale facilities (HRI, DARD, Commercial composters) |
| 4.1 | 34 months | Effects of wheat straw batches from well-defined sources (cultivar, soil type, fertiliser and pesticide/ PGR applications determined in large-scale experiments (HRI, DARD, Triton, Commercial composters) |
| 4.2 | 34 months | The effects of new composting formulations and composting methods on spawn-running time will be determined in large-scale facilities (HRI, DARD, IGER, Comm. composters) |
| 4.3 | 34 months | Methods for measuring and controlling application of recycled water and effects on odours and sulphides tested on commercial sites (HRI, DARD, IGER, Jeol, Casella, Commercial composters) |

4.4 36 months Drafting of user guidelines on improved composting procedures (all partners).

| Mile-stone | Target Date | Secondary Milestone |
|---|---|---|
| 1.4 | 3 months | Arrange supply of straw samples from experimental field sites (HRI, DARD, IGER) |
| 3.4 | 6 months | Arrange commercial sites for sampling recycled water and air odour samples (HRI, IGER, Commercial composters) |
| 3.5 | 9 months | Test electronic sulphide analysers on laboratory air samples (HRI, Casella, Commercial composters) |
| 1.5 | 12 months | Barley and rape straw samples tested for physical, chemical and biodegradability analyses developed in 1.1 and 1.2 (HRI, DARD). |
| 2.4 | 18 months | Recycled water samples used in bench-scale composting experiments to determine relationships between recycled water analysis and mycelial growth and mushroom cropping (HRI, IGER) |
| 2.5 | 24 months | Test effect of different mushroom strains and substrate supplement treatments, in combination with new substrates, on mushroom spawn-running time (HRI, DARD). |
| 4.5 | 24 months | Effects of recycled water analysis on mushroom spawn-running and cropping determined in intermediate-scale tests (HRI, IGER, Commercial composters) |
| 3.6 | 24 months | Arrange industry sites for large-scale experiments (HRI, DARD, Commercial composters) |
| 4.6 | 27 months | Techniques for measuring and controlled application of recycled water tested in large-scale systems (HRI, DARD, Commercial composters) |
| 4.7 | 34 months | Consortium to agree on draft guidelines (all partners) |

All of the primary project milestones and all of the secondary project milestones (except 1.5 concerning rape and barley straw) were completed.

# SCIENCE SECTION

### Abbreviations used in the report

DM dry matter
DMD dry matter digestibility
DTG derivative thermogravimetry
EC electrical conductivity
GC-MS gas chromatography – mass spectrometry
HDMD hemicellulose dry matter digestibility
HPLC high performance liquid chromatography
NIR vis-near infra red spectroscopy
PGR plant growth regulator
PLFA phospholipids fatty acid
TSC total soluble carbohydrates
TSP total soluble polyphenol

## Part 1: Effects of straw analysis on compost degradation and mushroom cropping.

### Introduction

Wheat straw used in preparing mushroom compost is highly variable in terms of degradability and the properties change during storage. This causes a significant problem in producing a consistent compost. The biodegradability of straw in composting has been estimated from its chemical composition using determinations of hot ethanol dry matter, ash, neutral detergent fibre, cellulose, hemicellulose, lignin, and derivative thermogravimetry and near-infra red spectroscopy (Chaleaux et al 1991, Savoie et al 1992, Lyons et al 2000). Previous work at DARD indicated that the biodegradability of wheat straw samples in composting was related to the enzymatic digestion of their dry matter in a hemicellulase-cellulase bioassay (Sharma et al 2000). Wheat cultivars are known to differ with respect to the physical stiffness of their straw (Anon 2002) and this information was used to pre-select wheat cultivars for use in mushroom composting experiments. However, no previous attempts have been made to relate the physical and chemical properties of straw to its suitability for mushroom composting and subsequent cropping.

The aim of this work was to identify the best sustainable sources of wheat straw for mushroom composting, and to adapt the composting process to better exploit the diversity and unpredictability of wheat straw supplies.

### Objectives

To test the following hypotheses:

- Is wheat straw biodegradability in composting related to its physical and/or chemical properties?
- Is the biodegradability of wheat straw in composting related to its subsequent suitability for mushroom mycelial growth and cropping?
- Can the biodegradability and suitability of wheat straw be related to its cultural source?

**Materials and Methods**

During the initial year, analytical methods were developed and adapted for measuring chemical and physical properties of wheat straw. In total, over 50 parameters were assessed and significant differences in the properties of wheat straw cultivars quantified using these methods. Consequently, over a number of successive seasons, selected cultivars of wheat straw grown under known crop husbandry conditions on experimental sites in Britain and Northern Ireland were sampled. These were analysed, selected samples composted in small and intermediate scale facilities and then used for mushroom growth and cropping tests.

Chemical analyses of straw samples

Straw samples were analysed for pH, electrical conductivity, dry matter content, nitrogen dry matter, total soluble carbohydrates (TSC), total soluble polyphenol (TSP), and Ash (Sharma & Kilpatrick 2000). The presence of plant growth regulator (chlormequat) and fungicide residues was determined using GC-MS. Straw samples were also analysed using derivative thermogravimetry (DTG) and vis-Near Infrared Spectroscopy (NIR) according to the methods outlined below. From year 2, wheat straw samples were further analysed by a procedure of *in-vitro* analysis of grass dry matter digestibility (DMD) using the ruminant enzymes pepsin and cellulase. A modified protocol was subsequently adopted for hemi-cellulose dry matter digestibility (HDMD) using the enzyme Pentopan 500BC.

*Derivative thermogravimetry (DTG)*

Wheat samples were ground in a Cyclotec mill (Foss) to pass through a 0.5mm screen. Material for analysis (3 - 3.2 mg) was taken by transferring 10-15 small sub-samples into an alumina crucible. All samples were weighed accurately on a Mettler MT5 microbalance. Thermogravimetric analysis was undertaken on a Mettler Toledo TGA/SDTA851 Thermal Analysis System, equipped with an autosampler and TSO801RO sample robot. Three replicates were analysed for each sample. A PC running STAR software (Mettler Toledo) controlled the system. Analysis was by a standard dynamic method. Samples were heated in a furnace from 32 – 600ºC at a heating rate of 20ºC $min^{-1}$. Sample weight loss was

measured against increase in temperature (or time). Compressed air was passed through the furnace at a rate of 20ml $min^{-1}$ to aid combustion and to flush out combustion products to stop secondary reactions. Thermogravimetric curves (thermograms) for each sample were evaluated using macros created in the STAR software. Data collected for each sample consisted of measurement of peak weight loss, height, width, temperature, and peak area for the major combustion products present on the thermograms. Data was measured using the thermogravimetric curve and also its first derivative (*dw/dt*). The latter technique is referred to as Derivative Thermogravimetry (DTG).

*Vis-near infrared spectroscopy (NIR)*

Eight sub-samples were taken from each wheat straw sample. The pieces of straw were cut into lengths of between 3-4 cm and conditioned overnight in a laboratory maintained at 20 $^{O}$C and 85% RH. Each was in turn packed into a Natural Product Cup and scanned on a Vis-NIR spectrometer (Foss NIRS 6500). Spectral reference data was recorded 64 times at 2 nm intervals for each scan, over the range 400-2498 nm and the 8 sub sample scans were averaged to produce 1050 data points. A band path of 10 nm was used with a wavelength accuracy of 0.5 nm. Reflectance readings were converted to absorbance and the data was collected and spectral results were converted from WINISI (Foss) to UNSCRAMBLER format for multivariate data analysis using principal component and partial least squares regression methods to determine relationships between samples and chemical data.

*Mineral analysis*

Dried (105$^{O}$C to constant weight), milled (1mm on Cyclotec mill) straw samples were ashed at 500$^{O}$C for a minimum of 16 hours and minerals extracted in 50% Nitric acid solution before analysing a broad range of minerals using ICP-OES (Inductively coupled plasma optical emission spectrometry).

*Fungicide and plant growth regulator residue analysis (GC-MS)*

An acetone (100 ml) extraction of the straw sub-samples (10 g) was accompanied by further maceration, which facilitated a more complete extraction. Anhydrous sodium sulfate was added to remove any water from the sample. The sample was concentrated by rotary evaporation prior to clean-up of the sample. Interfering material was removed from the extract by gel permeation chromatography (GPC). Post GPC the extract was again concentrated to 1 ml in order to achieve the reporting levels required. Samples were analysed for the presence of plant growth regulator (chlormequat) and fungicide residues using gas chromatography-mass spectrometry (GC-MS). Calibration curves were generated

for each of the pesticides sought using standards and a best-fit line was calculated by linear regression analysis.

*Dry matter digestibility (DMD)*

Dried wheat straw samples were ground in a Cyclotec mill (Foss) to pass through a 1.0 mm screen. Material for analysis (0.2 g) was taken by transferring a number of small sub-samples with all samples weighed accurately on a Mettler AT250 balance. Three replicates were analysed for each sample. Pepsin solution (25 ml) was added and the straw suspension placed in an incubator set at 50$^{O}$C for 24 hours. Sodium carbonate solution was used to adjust the pH to 4.7 and cellulase added before the samples were placed in the incubator (50$^{O}$C) for a further 24 hours. The straw samples were subsequently filtered using a vacuum pump and Buchner flask and the filtrate oven dried (105 $^{O}$C) for 5 hours. Crucibles were removed to the dessicator to cool; undigested residue was weighed and the percentage dry matter digestibility calculated.

*Hemi-cellulose dry matter digestibility (HDMD)*

The procedure was the same as above, up to the point of Pepsin solution addition. Instead, Pentopan 500 BC pre-conditioned (40$^{o}$C) buffer solution was added to each straw sample, with 50 ml cellulase solution. Samples were swirled gently to mix in the enzyme solution, and then replaced into the incubator (40$^{o}$C) for 48 hours. The straw samples were then filtered, oven dried, cooled and undigested residue weighed and percentage dry matter digestibility calculated as before.

<u>Mechanical properties of straw samples</u>

*Tensile strength tests*

Tensile tests were conducted with an Instron universal testing machine (model 4301, Instron Ltd, High Wycombe, UK) at 20$^{o}$C and 100% relative humidity. Strips of internodal straw were cut to 40 x 2 mm. Each individual strip was mounted using double-sided adhesive tape on to acetate templates. The strips were then placed across the pneumatic jaws on the Instron testing machine. The strips were then stretched at a rate of 1 mm/min until tissue failure. Measurements of increasing load (N) and displacement (mm) were used to calculate the stress (force or load per cross sectional area) and strain (displacement divided by original length).

*Dynamic mechanical analysis of straw*

The dynamic modulus (stiffness) was also determined using a dynamic testing machine at Triton Technology. Straw strip samples were prepared in the same way as for the Instron

testing machine. The tests were conducted both in air at 100% relative humidity and immersed in water, at temperatures from 20 to 80°C.

Composting tests on straw types

*Bench-scale composting*

Wheat straw samples (600 g) were mixed with 200 g of sieved (5 mm screen) poultry manure, 25 g gypsum and 2 litres of water. The ingredients were mixed at daily intervals in bins for 3 days. The mixed substrate ingredients were composted in 'Quickfit' multiadapter flasks immersed in thermostatically controlled water baths, each holding two 10-litre flasks (Noble *et al*, 1997). The prepared ingredients (about 2 kg samples) were placed on a perforated stainless steel platform within each flask and the flasks immersed in the waterbaths such that the water level was above the level of the enclosed substrate. Each flask was connected to ancillary equipment providing independent aeration of the compost. The oxygen concentration in the substrate was controlled regularly by adjusting the airflow through the compost in each flask within the range 0 - 16 litres $kg^{-1}$ substrate $h^{-1}$ by means of flow meters. The temperature of the substrate in the flasks was monitored with Squirrel multipoint temperature loggers (Grant Instruments Ltd, Cambridge, UK).

For the first 48 hours of the composting process, the thermostat of the waterbath was set at 48°C to allow a natural succession and gradual build-up of microorganisms. The substrate temperature was then increased to 72°C for 5 days, after which the substrate was re-mixed and the temperature reduced to 47°C for the remainder of the composting period, which was seven days, or prolonged until the air in the flask was clear of ammonia. An oxygen concentration of 11(± 1.5) % v/v was maintained in the substrate. The average analysis of the initial compost ingredients was: moisture content 75.9%, N, ammonium and ash contents 1.31, 0.495 and 11.3 % of DM respectively, and pH 7.88.

At the end of the composting period, the material in each flask was weighed. After samples were taken for analysis, 1.2 kg of the residual material was inoculated with mushroom spawn (spawned) at two percent of the fresh weight of compost with *Agaricus bisporus* spawn (Hauser A15) and filled into plastic pots, 230 mm diameter x 220 mm depth. The pots were placed in polythene bags in an incubator at 25°C and when the substrate was fully colonised with mushroom mycelium, about 15 days after spawning, the containers were cased with a moist mixture of peat and sugar beet lime (900 g). When mushroom mycelium was visible on the surface of the casing, the containers were transferred to a controlled environment chamber with an air temperature of 18°C, relative humidity of 90% and a $CO_2$ concentration of 0.1% to induce fruiting. Mushrooms were harvested daily over a 30 day period (cap diameter 25-30 mm). Two replicate composts were made from each batch of straw.

*Large-scale composting*

Straw from different years (stored dry under cover), wheat cultivars, growing sites, with and without PGR application were composted in windrows with standard amounts of poultry manure (475 kg/tonne straw) and gypsum (25 kg/tonne compost). Straw bales were formed into windrows and wetted on day 0. Poultry manure was added on days 2 and 10, gypsum on day 10; water applications and windrow turns were made on alternate days during a 16 day period. At the end of Phase I, the composts had an average moisture content of 76.5%. The average N, ammonium N and ash contents were 2.14, 0.41 and 26.1% respectively. Average compost pH was 7.9.

Composts were pasteurised at 58ºC for 6 hours and conditioned at 45-48ºC. For the Phase II pasteurisation regime, the tunnels were filled with 2.5 t of material from the Phase I. Following a 20 hour equalisation of compost temperature at 45 - 48°C, the composts were pasteurised at 58 - 60°C for 6 hours. Compost temperatures were then reduced to 46 - 49°C (conditioning). A minimum oxygen concentration of 13% was maintained during Phase II. Composting was completed when the compost temperature had fallen to within 1°C of the air temperature and ammonia could no longer be detected in the compost.

The mushroom cropping procedure is outlined in Noble & Gaze (1998). Composts were spawned with the strain Sylvan A15 at 0.5% w/w, unless otherwise stated. Three flushes of mushrooms were picked.

The rate of mushroom mycelial growth rate in compost prepared in bench- and large-scale facilities was determined using the growth tube method described in the spawn-running section (Part 2) of this report.

Where sufficient straw was available, two replicate composts were made from each type of straw.

*Compost analysis*

Analyses were conducted on freeze-dried, finely milled samples of the compost ingredients and of the substrates before and after composting in bench-scale or large-scale experiments. Dry matter (DM), N, ammonium ($NH^{+}_{4}$) and ash contents and pH were determined as described previously. Visible and NIR spectral analysis and derivative thermogravimetric analyses (DTG) were conducted on the compost samples, similar to those conducted on the wheat straw samples (previously described). Theses results are presented in Part 2.

Wheat straw samples

Wheat straw samples were grown at three sites in Northern Ireland: Crossnacreevy, Downpatrick and Limavady from experimental trials of the AFBI Plant Testing Station. Two sites were used in England: Kirton and Wellesbourne of Warwick HRI. Wheat cultivars used for analysis and in mushroom composting experiments are summarised in Fig. 1. The cultivars were selected to cover a range in straw types based on NIAB descriptions (Anon, 2002) : Malacca, Hereward and Tanker were short straw cultivars and Claire and Richmond were longer straw cultivars.

*Year 1 experiments*

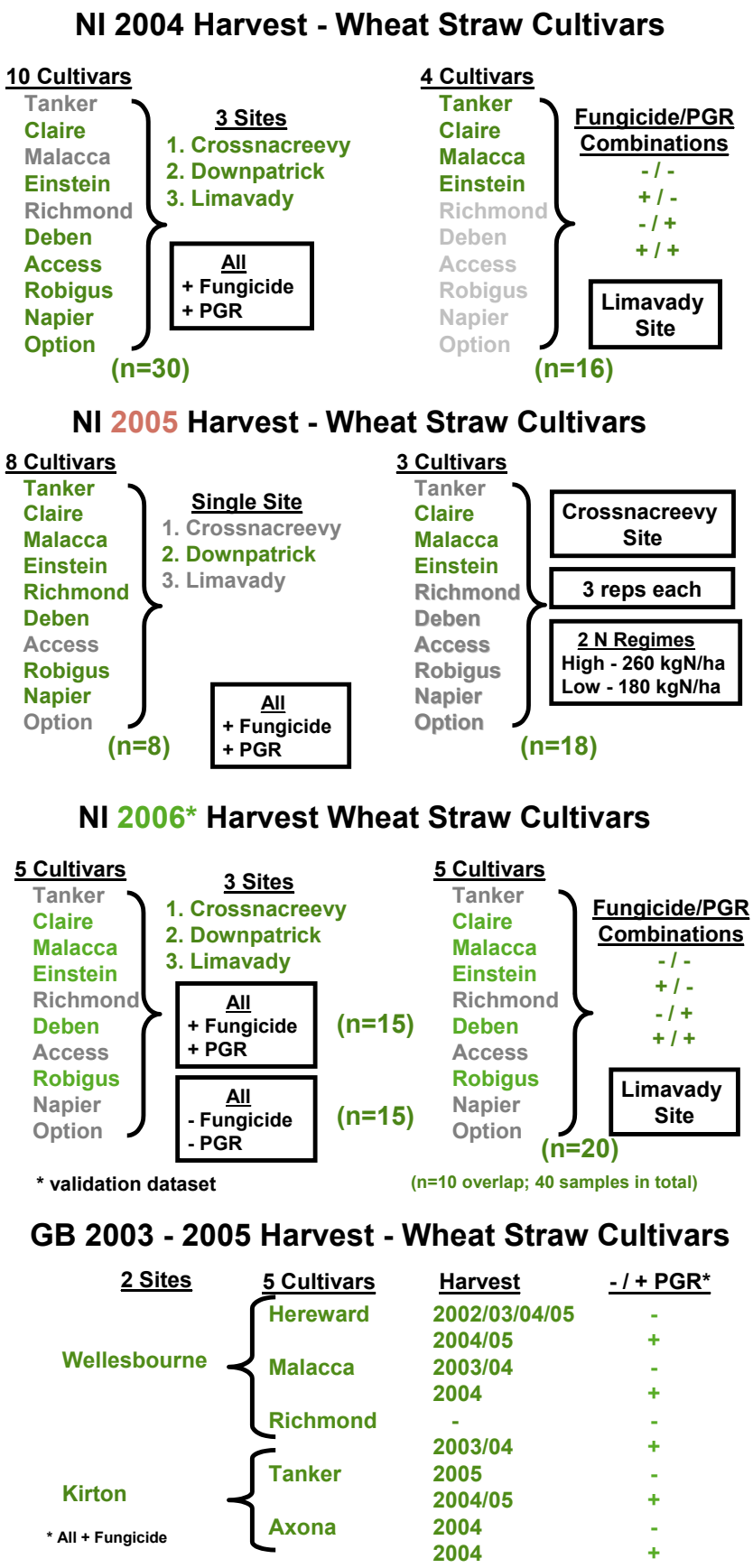
In Year 1 of the project, straw from 5 cultivars and two growing seasons was used (Table 1). Cultivar Claire was grown at two rates of nitrogen fertiliser and cultivar Hereward was grown with and without plant growth regulator, PGR (chlormequat). These samples were used only for analysis and bench-scale composting experiments.

Table 1. Wheat straw samples used in Year 1 experiments

| Cultivar | Growing site | Year | Age, months | Fungicide | PGR | N fertiliser kg / ha |
|---|---|---|---|---|---|---|
| Claire | Crossnacreevy, NI | 2003 | 6 | + | + | 100 |
| Claire | Crossnacreevy, NI | 2003 | 6 | + | + | 200 |
| Hereward | Wellesbourne, GB | 2002 | 18 | + | - | N/A |
| Hereward | Wellesbourne, GB | 2003 | 6 | + | - | N/A |
| Malacca | Wellesbourne, GB | 2002 | 18 | + | - | N/A |
| Richmond | Wellesbourne, GB | 2003 | 6 | - | - | N/A |
| Weston | Wellesbourne, GB | 2002 | 18 | + | + | N/A |

N/A not available

Fig. 1 Wheat straw cultivar factorial treatment experiments



*Year 2 experiments*

GB wheat straw cultivars, PGR (chlormequat) application and growing locality and year of straw for project Year 2 composting and analytical tests are shown in Table 2. These show a total of five winter wheat cultivars from the Wellesbourne and Kirton sites. The spring wheat cultivar Axona was also grown at Kirton. Two cultivars were grown with and without PGR. New season and one-year stored straw was obtained for three cultivars. All the samples were grown with fungicide (tebuconazole). These samples were used for analysis and both the bench-scale flask and large-scale composting experiments.

Table 2 Wheat straw samples from GB sites used in Year 2 composting experiments

| Cultivar | Growing site | Year | Age, months | Fungicide | PGR |
|---|---|---|---|---|---|
| Axona | Kirton | 2004 | 6 | - | + |
| Hereward | Wellesbourne | 2002 | 30 | + | - |
| Hereward | Wellesbourne | 2003 | 18 | + | - |
| Hereward | Wellesbourne | 2004 | 6 | + | + |
| Hereward | Wellesbourne | 2004 | 6 | + | - |
| Malacca | Wellesbourne | 2003 | 18 | - | + |
| Malacca | Wellesbourne | 2004 | 6 | - | + |
| Richmond | Wellesbourne | 2003 | 18 | - | + |
| Richmond | Wellesbourne | 2004 | 6 | - | + |
| Tanker | Kirton | 2004 | 6 | - | + |

Wheat straw samples, plant growth regulator and fungicide treatments for Northern Ireland straw samples used in the Year 2 composting experiments are shown in Table 3. The Northern Ireland straw came from two multi-site field experiments. Ten winter wheat cultivars were grown on three sites, all with fungicide (fenpropimorph) and plant growth regulator (chlormequat). Four of these cultivars were also grown at the Limavady site, with and without fungicide and plant growth regulator, in all combinations. Two of the cultivars (Richmond and Tanker) were common to those grown in England. The wheat cultivars were selected to cover a range in straw length and strength characteristics. All the NI samples were used for chemical analysis. The Northern Ireland samples of cultivars Claire, Malacca, Richmond and Tanker were used for bench-scale flask composting experiments.

Table 3. Wheat straw samples grown in 2004 in Northern Ireland for Year 2 composting experiments

| Cultivar | Growing site | Fungicide | PGR |
|---|---|---|---|
| Claire | DownP | + | + |
| Malacca | DownP | + | + |
| Option | DownP | + | + |
| Tanker | DownP | + | + |
| Napier | DownP | + | + |
| Access | DownP | + | + |
| Deben | DownP | + | + |
| Richmond | DownP | + | + |
| Einstein | DownP | + | + |
| Robigus | DownP | + | + |
| Claire | Cross | + | + |
| Malacca | Cross | + | + |
| Option | Cross | + | + |
| Tanker | Cross | + | + |
| Napier | Cross | + | + |
| Access | Cross | + | + |
| Deben | Cross | + | + |
| Richmond | Cross | + | + |
| Einstein | Cross | + | + |
| Robigus | Cross | + | + |
| Claire | LIM | + | + |
| Malacca | LIM | + | + |
| Option | LIM | + | + |
| Tanker | LIM | + | + |
| Napier | LIM | + | + |
| Access | LIM | + | + |
| Deben | LIM | + | + |
| Richmond | LIM | + | + |
| Einstein | LIM | + | + |
| Robigus | LIM | + | + |
| Claire | LIM | - | - |
| Malacca | LIM | - | - |
| Tanker | LIM | - | - |
| Einstein | LIM | - | - |
| Claire | LIM | - | + |
| Malacca | LIM | - | + |
| Tanker | LIM | - | + |
| Einstein | LIM | - | + |
| Claire | LIM | + | - |
| Malacca | LIM | + | - |
| Tanker | LIM | + | - |
| Einstein | LIM | + | - |

*Year 3 experiments*

Two cultivars were grown at the GB sites in 2005, with and without plant growth regulator (Table 4). The fungicide tebuconazole was used on all these samples. The straw was used for analysis and bench and large-scale composting experiments. In 2005, eight winter wheat cultivars were grown at the Downpatrick site in Northern Ireland (Table 4). Three of these cultivars (Claire, Malacca and Tanker) were also grown at the Crossnacreevy site at two rates of nitrogen fertiliser (180 and 260 kg N/ha). Straw from these cultivars were used for bench-scale composting experiments. Straw samples from all the treatments were used for analysis.

Table 4. Wheat straw samples grown in 2005 for the Year 3 composting experiments

| Cultivar | Growing Site | Fungicide | PGR | kg N/ha |
|---|---|---|---|---|
| Claire | DownPatrick, NI | + | + | N\A |
| Malacca | DownPatrick, NI | + | + | N\A |
| Tanker | DownPatrick, NI | + | + | N\A |
| Napier | DownPatrick, NI | + | + | N\A |
| Debden | DownPatrick, NI | + | + | N\A |
| Richmond | DownPatrick, NI | + | + | N\A |
| Einstein | DownPatrick, NI | + | + | N\A |
| Robigus | DownPatrick, NI | + | + | N\A |
| Claire | Crossnacreevy, NI | + | + | 180 |
| Malacca | Crossnacreevy, NI | + | + | 180 |
| Tanker | Crossnacreevy, NI | + | + | 180 |
| Claire | Crossnacreevy, NI | + | + | 260 |
| Malacca | Crossnacreevy, NI | + | + | 260 |
| Tanker | Crossnacreevy, NI | + | + | 260 |
| Hereward | Wellesbourne, GB | + | - | N\A |
| Hereward | Wellesbourne, GB | + | + | N\A |
| Tanker | Kirton, GB | + | - | N\A |
| Tanker | Kirton, GB | + | + | N\A |

Table 5. Chemical analysis of wheat straw samples used in Year 1 experiments

| Cultivar, N rate, Site (NI or GB) | pH | EC uS/cm | Dry matter, % | TSC g/kg DM | TSP g/kg DM | Ash % of DM |
|---|---|---|---|---|---|---|
| Claire, low N, NI | 7.87 | 598 | 95.3 | 12.4 | 3.5 | 4.4 |
| Claire, high N, NI | 8.97 | 627 | 95.6 | 11.8 | 3.6 | 4.6 |
| Hereward, GB | 7.50 | 314 | 94.4 | 9.3 | 2.6 | 5.3 |
| Hereward, GB | 7.87 | 318 | 93.2 | 9.5 | 2.4 | 5.4 |
| Weston, GB | 8.96 | 469 | 94.0 | 7.9 | 2.7 | 7.6 |
| Malacca, GB | 8.45 | 237 | 94.7 | 6.2 | 2.4 | 7.2 |
| Richmond, GB | 8.11 | 337 | 94.3 | 7.8 | 2.4 | 6.3 |

**Results**

Chemical analysis

*Year 1 experiments*

Analyses are shown in Table 5. Straw from cultivars Claire (high N application) and Weston were the most alkaline. Cultivar Claire also produced straw with the highest electrical conductivity, dry matter content and total soluble carbohydrates. Cultivars Weston and Malacca had the highest ash contents. The cultivar Claire grown in Northern Ireland had the highest fibre fraction as measured by derivative thermogravimetry (DTG). Near infra-red spectroscopy (NIR) analysis indicated significant chemical differences between GB samples and those of Northern Ireland origin.

*Year 2 experiments*

Differences between wheat cultivars were relatively small compared with site differences. Analytical ash content, % dry matter digestibility, total soluble carbohydrate and total soluble polyphenol for cultivars grown on three sites in Northern Ireland are shown in Fig. 2. Straw produced at Crossnacreevy had lower ash content, whereas straw produced at Downpatrick had a lower dry matter content. Narrowing the cultivar comparison but extending the site comparisions outside Northern Ireland to include data from GB (Kirton and Wellesbourne) indicated similar trends (Fig.3). Straw from the Limavady site had a higher dry matter content and higher dry matter digestibility than straw from the other sites.

The statistical comparison of the 10 wheat cultivars grown in Northern Ireland is shown in Table 6. Significant differences were quantified for % dry matter, electrical conductivity, pH, total soluble carbohydrate, total soluble polyphenols, elements of DTG analysis, ash and several minerals.

The combined effects of fungicide and PGR treated straw was evident when treated and untreated cultivars (Claire, Einstein, Malacca and Tanker) grown on the Limavady site were compared (Fig. 4). The nitrogen content and weight loss in the secondary peak (WL 2

– structural hemicellulose and lignin) of the treated samples was lower, whereas the dry matter, ash, %ash residue (DTG) and dry matter digestibility was significantly higher for treated samples. Significant differences were quantified for the analytical parameters of the straw for both the individual and combined effects of the fungicide and plant growth regulator treatment applications (Tables 7 and 8). There were no significant differences between the four cultivars grown at Limavady used in this comparison. The secondary peak (WL 2 – structural hemicellulose and lignin) in the DGT analysis of straw samples from the different sites were similar.

*Hemi-cellulose dry matter digestibility (HDMD).* Significant differences in HDMD were detected between the four cultivars (Claire, Einstein, Malacca and Tanker) ($P<0.05$) and between fungicide ($P<0.001$) and PGR application treatments ($P<0.05$) (Fig. 5). While similar patterns were detected for %HDMD for fungicide ($P<0.001$) and PGR ($P<0.05$), there were no cultivar differences.

*Year 3 Experiments*

In 2005, the effect of increasing the nitrogen application rate from 180 kg N /ha to 260 kg N/ha on the Crossnacreevy site was consistently evident on all three cultivars – Claire, Malacca and Tanker. Significant differences were observed for some of the analytical parameters, most notable being %NDM, %residue ash and minerals – Fe, Mg P and K (Table 9). Increasing nitrogen rates resulted in decreases in the % dry matter of the straw (cultivar specific) and changes in the DTG data (Peak Ht 1, Peak Area 2, Peak height 2) (Fig. 6). The DTG data indicates that the higher nitrogen rate resulted in greater hemicellulose and cellulose fractions but less lignin formation due to the faster stem growth reflected in increased internodal stem length.

The range of analytical parameters for the full database of 84 straw samples is shown in Table 10 and clearly quantifies the variability of the raw material across a large number of both chemical and physical parameters. Most notably, dry matter, conductivity, nitrogen dry matter, Ash, C:N, digestibility, mineral analysis, internodal lengths and diameters and tensile strength as determined by Young's Elasticity modulus.

Fig. 2 Dry matter, dry matter digestibility, Ash, TSC and TSP results for 10 wheat straw cultivars (+ fungicide, + PGR) on the three Northern Ireland sites (Year 2 experiment)

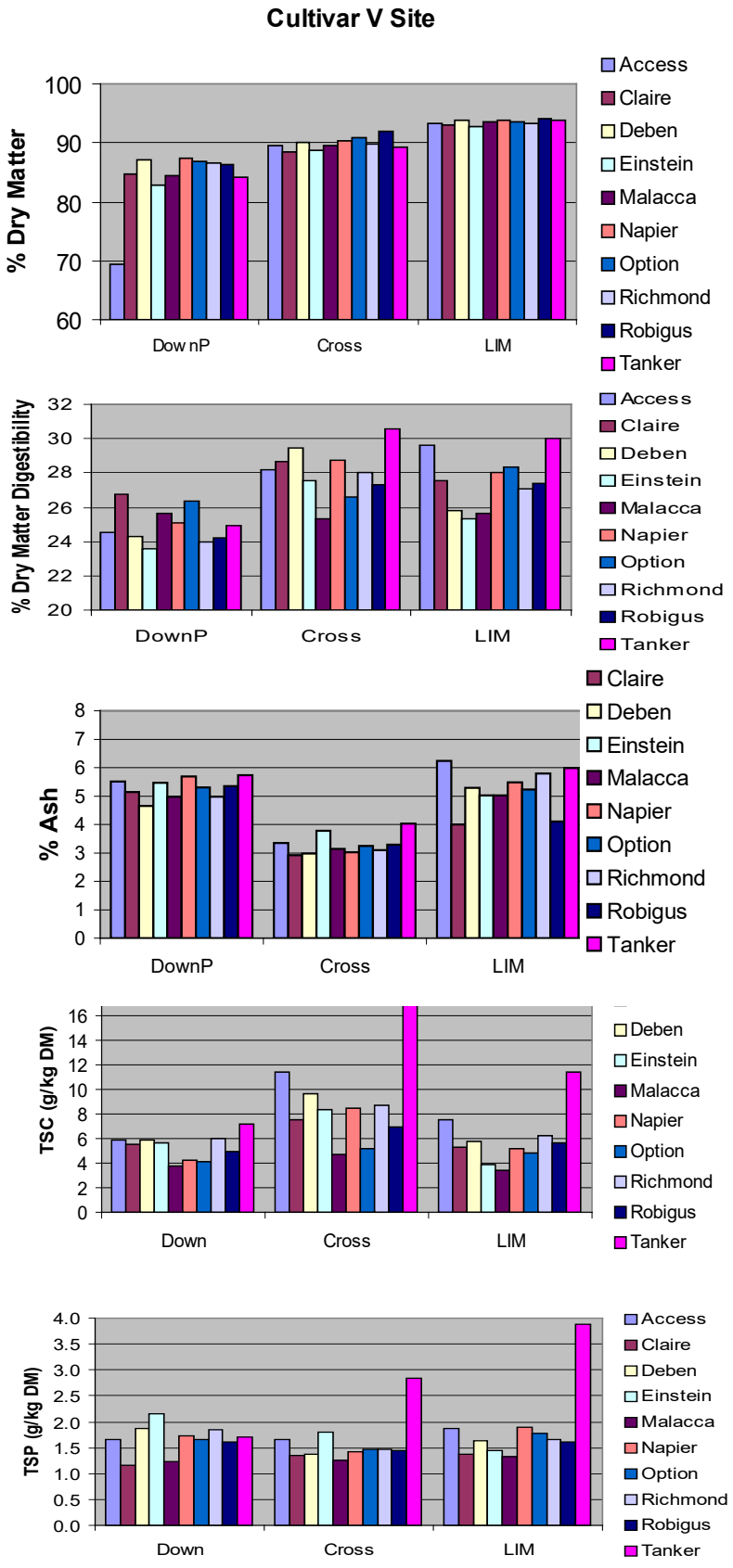
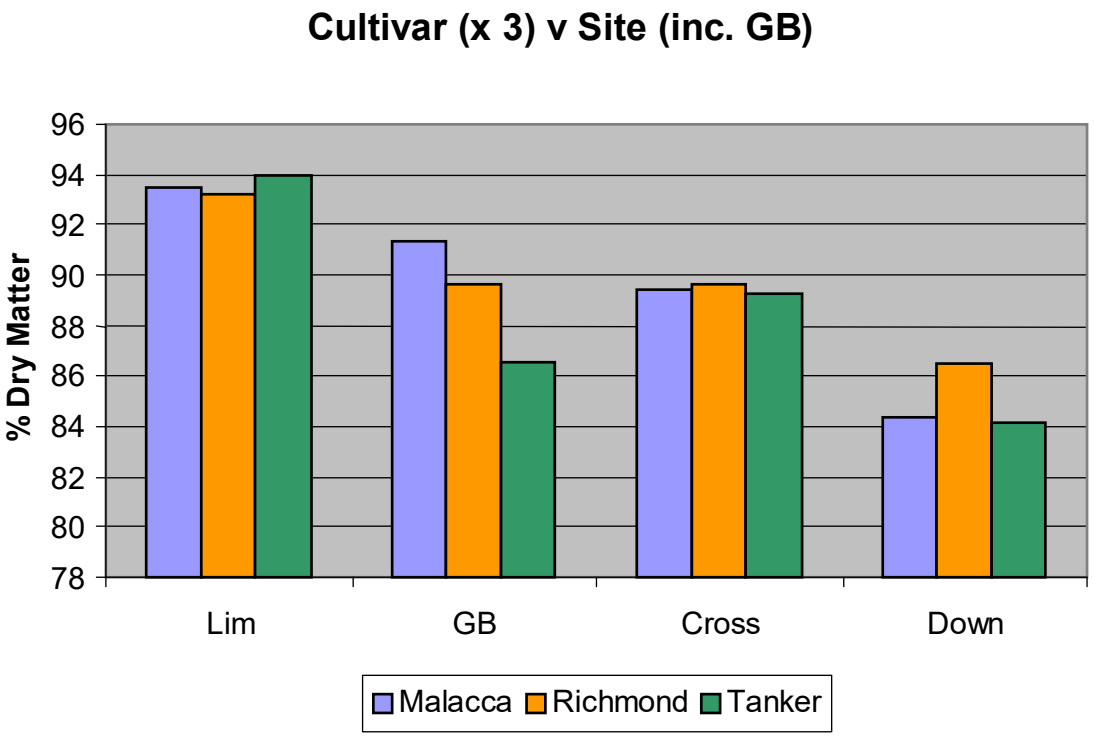
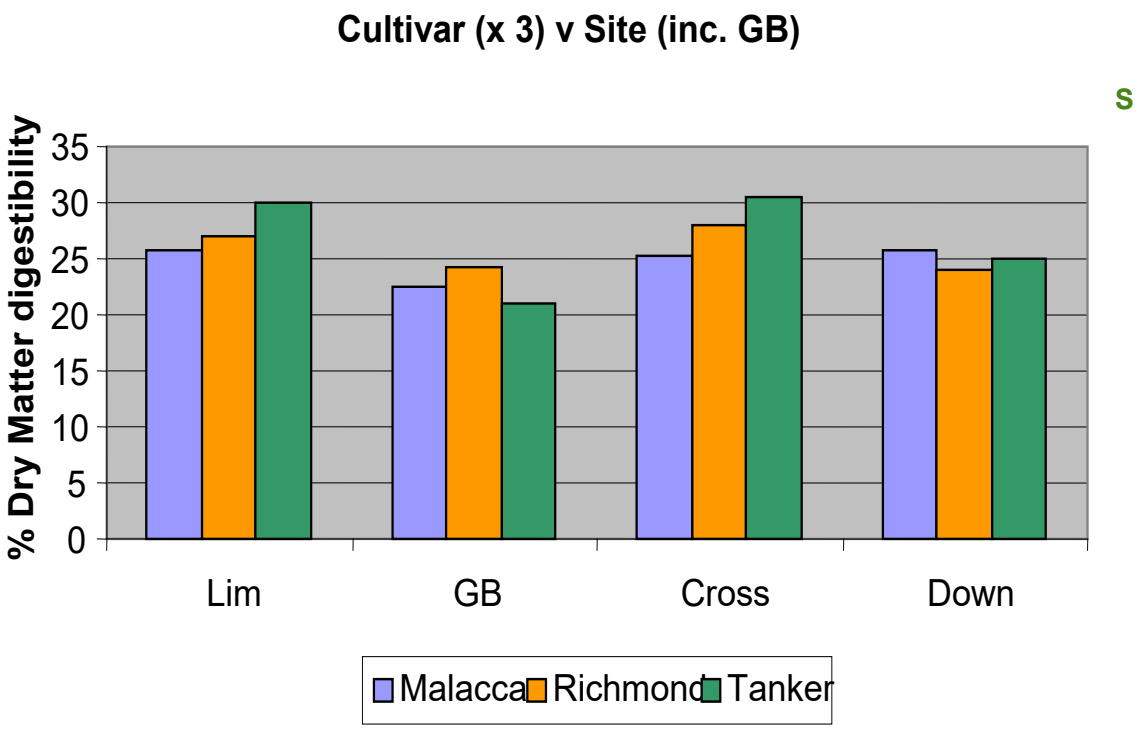


Fig. 3 Dry matter and dry matter digestibility of 3 wheat cultivars – Malacca, Richmond and Tanker - grown on 3 Northern Ireland and 1 GB sites (Year 2 experiment).




S

APS - NIHPBS

Fig. 4 Comparison of changes in % Dry matter, % Ash, % Dry matter digestibility, %NDM, %weight loss 2 (structural hemicellulose & lignin) and %ash residue (DTG) in straw from treated (+ fungicide, + PGR) and untreated 4 wheat cultivars. (Year 2 experiment)

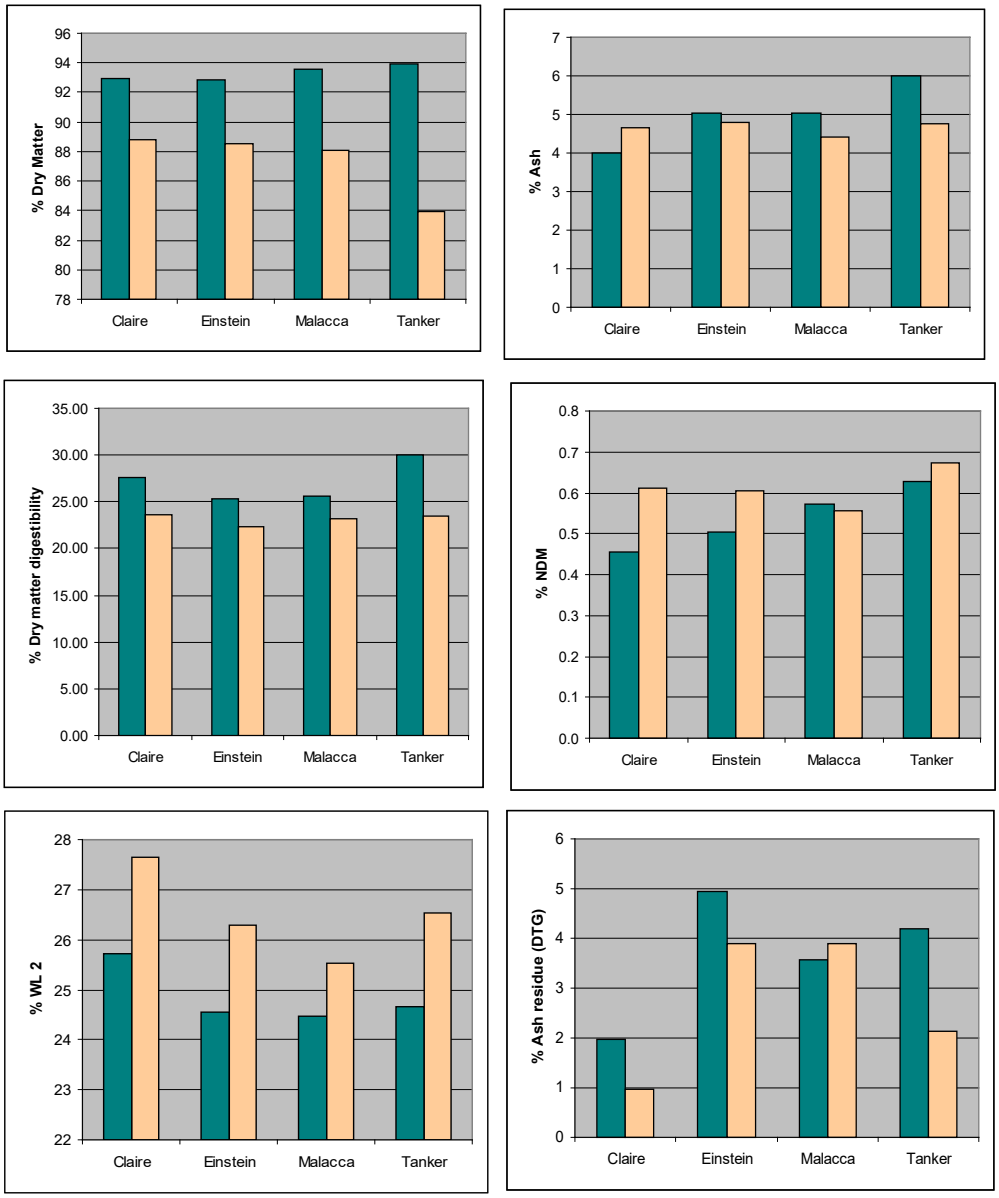



Table 6. F values for %DMD and %HDMD for restricted n=16 data comparisons.

| **Model 1** | d.f | % DMD | %HDMD |
|---|---|---|---|
| Source of variation | | | |
| *CULTIVAR* | 3 | 0.023 | 0.151 |
| **FUNG** | 1 | <0.001 | <0.001 |
| **PCR** | 1 | 0.034 | 0.035 |
| **Residual** | 10 | | |
| Total | 15 | | |

Fig. 5 The effect of Fungicide and PGR application on dry matter, dry matter digestibility, and ash of 4 wheat cultivars – Claire, Einstein, Malacca and Tanker - grown on a single Northern Ireland site at PTS Crossnacreevy (Year 2 experiment)

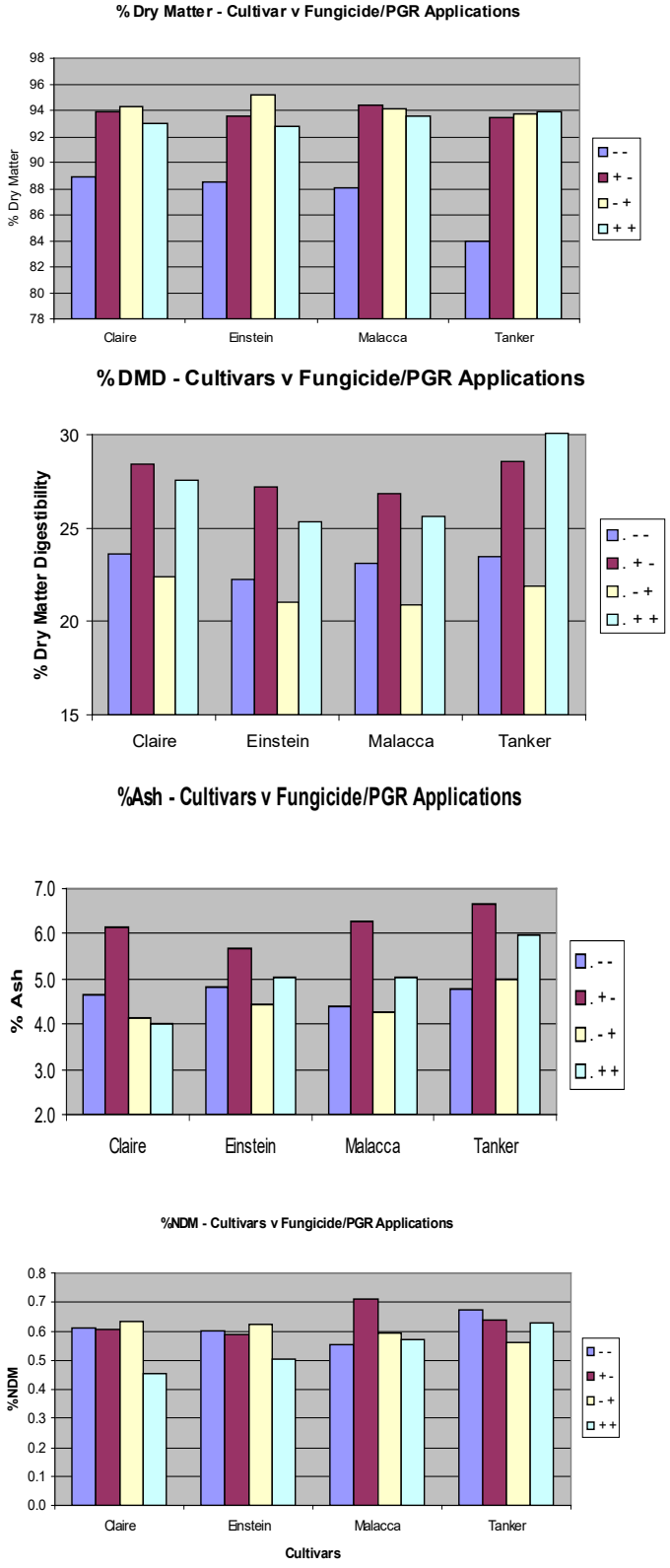
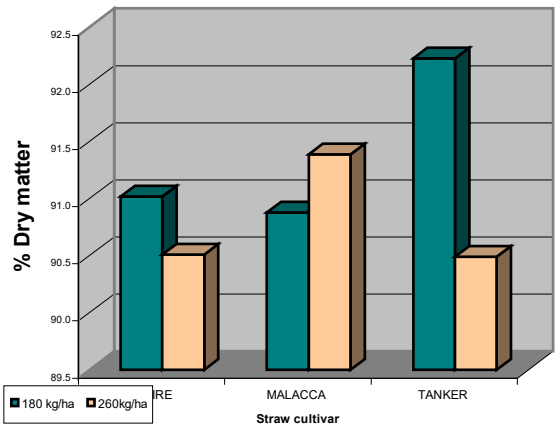
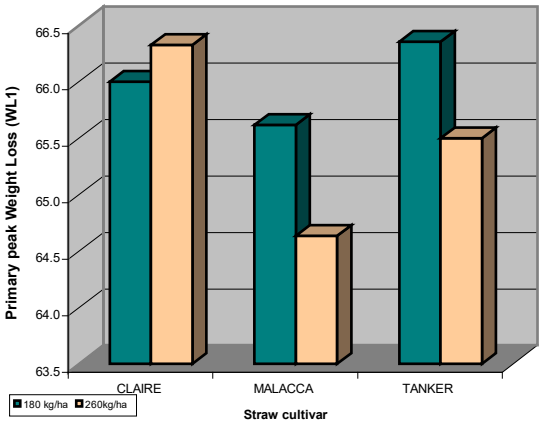
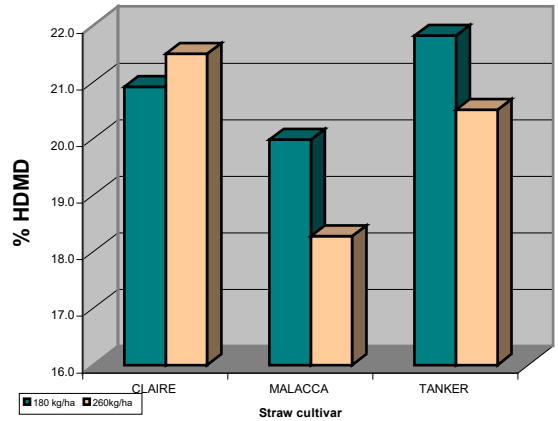
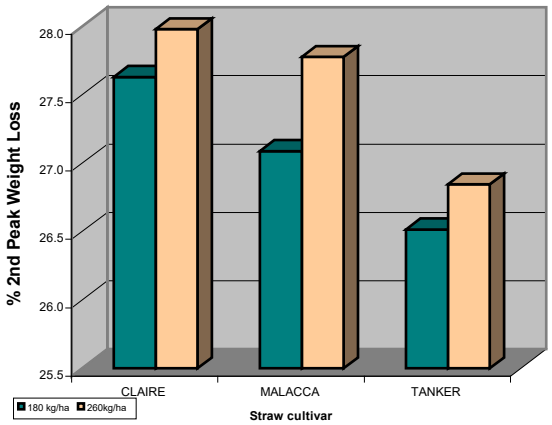


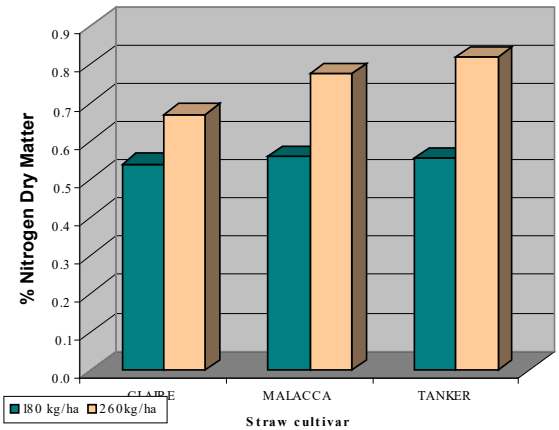
Fig. 6 Effect of nitrogen application rates of 180 kg N/ha and 260 kg N/ha on the analytical parameters in 3 wheat straw cultivars (Year 3 experiment)

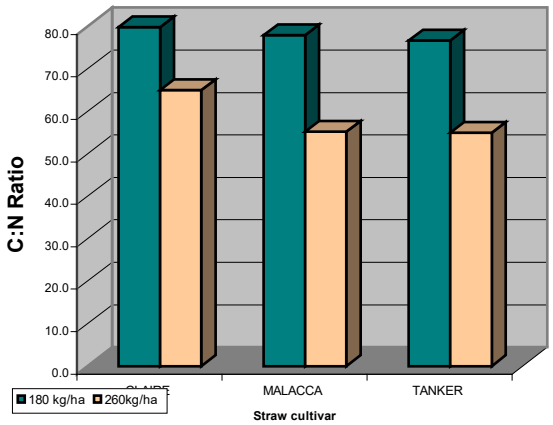


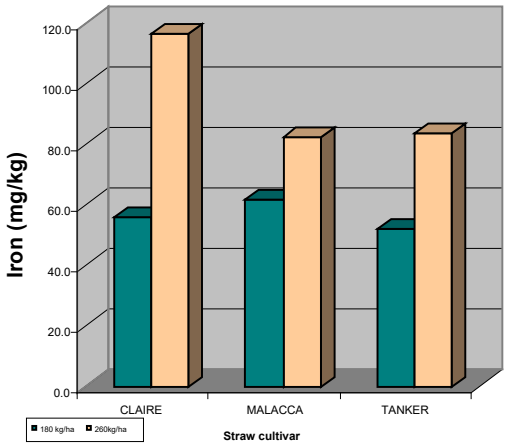



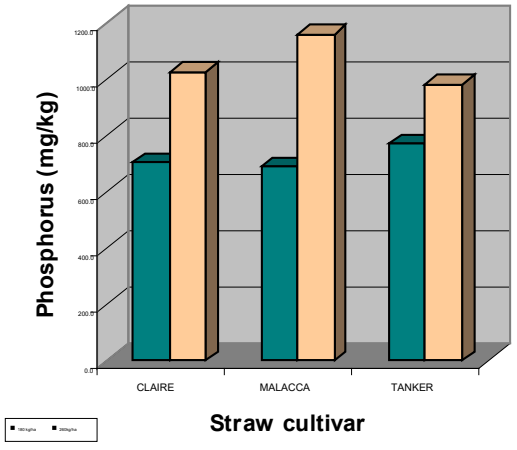



Table 7. Analysis for 10 wheat straw cultivars from NI sites with mean, Standard Error of Means (SEM), Significant differences and LSD

| Cultivar | % DM | Ec (LG) | pH (NFL) | TSC | TSP | Wt Loss 1 | Pk Area 1 | Wt Loss 2 | % Ash | % NDM | P | K | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Access | 83.99 | 938 | 7.24 | 8.26 | 1.730 | 64.69 | 45.63 | 24.45 | 5.38 | 0.5187 | 0.583 | 5.11 | 2.00 |
| Claire | 88.76 | 511 | 7.62 | 6.09 | 1.293 | 66.47 | 47.66 | 25.52 | 4.03 | 0.5063 | 0.400 | 4.28 | 6.00 |
| Deben | 90.29 | 688 | 7.34 | 7.11 | 1.623 | 64.90 | 45.71 | 24.71 | 4.42 | 0.4827 | 0.507 | 3.22 | 2.33 |
| Einstein | 88.16 | 851 | 7.36 | 5.95 | 1.803 | 64.84 | 45.24 | 25.11 | 4.58 | 0.4823 | 0.533 | 6.10 | 3.67 |
| Malacca | 89.10 | 442 | 7.29 | 3.99 | 1.273 | 65.45 | 47.20 | 24.42 | 4.25 | 0.524 | 0.47 | 3.83 | 4.67 |
| Napier | 90.51 | 796 | 7.18 | 5.96 | 1.683 | 65.43 | 46.45 | 24.88 | 4.41 | 0.510 | 0.620 | 4.73 | 3.0 |
| Option | 90.42 | 730 | 7.10 | 4.76 | 1.633 | 65.08 | 45.82 | 25.06 | 4.85 | 0.557 | 0.570 | 5.21 | 4.67 |
| Richmond | 89.80 | 573 | 7.27 | 6.99 | 1.663 | 65.27 | 46.52 | 24.70 | 4.33 | 0.461 | 0.493 | 3.24 | 2.0 |
| Robigus | 90.72 | 933 | 7.48 | 5.88 | 1.553 | 64.56 | 44.96 | 25.47 | 4.64 | 0.455 | 0.470 | 5.44 | 3.0 |
| Tanker | 89.12 | 996 | 7.67 | 12.61 | 2.807 | 66.21 | 46.29 | 24.74 | 5.21 | 0.546 | 0.597 | 7.16 | 7.0 |
| **Mean** | **89.09** | **746** | **7.36** | **6.76** | **1.706** | **65.29** | **46.15** | **24.905** | **4.61** | **0.505** | **0.524** | **4.83** | **3.83** |
| **SEM** | **1.733** | **92.3** | **0.108** | **0.992** | **0.226** | **0.413** | **0.351** | **0.212** | **0.219** | **0.029** | **0.034** | **0.58** | **0.417** |
| **Sign** | **ns** | ** | * | *** | ** | **ns** | *** | * | ** | **ns** | ** | ** | *** |
| **LSD** | **-** | **274.2** | **0.32** | **2.946** | **0.672** | **-** | **1.042** | **0.631** | **0.652** | **0.089** | **0.102** | **1.72** | **1.24** |

Table 8. Significant difference mapping (F pr values) for chemical parameters for n=16 (4 cultivars, +/- Funcicide, +/- PGR) data comparisons.

| | | % DM | pH | Ec | % Ash | % NDM | % DMD | % HDMD | TSC | TSP | WT Loss 1 | PK Ar 1 | WT Loss 2 | *Residue* | Mn | Ca | P | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | d.f. | | | | | | | | | | | | | | | | | |
| *CULTIVAR* | 3 | 0.156 | 0.11 | 0.14 | 0.08 | 0.7 | 0.08 | 0.350 | 0.04 | 0.24 | 0.033 | 0.023 | 0.009 | <.001 | 0.505 | 0.079 | 0.116 | 0.006 |
| **FUNG** | 1 | 0.003 | 0.09 | 0.02 | 0.01 | 0.6 | 0.001 | 0.003 | 0.1 | 0.15 | 0.45 | 0.201 | 0.003 | 0.001 | 0.02 | 0.007 | 0.028 | 0.003 |
| **PGR** | 1 | 0.002 | 0.97 | 0.08 | 0.02 | 0.21 | 0.07 | 0.127 | 0.59 | 0.32 | 0.093 | 0.437 | 0.046 | 0.164 | 0.065 | 0.141 | 0.06 | 0.52 |
| **FUNG.PCR** | 1 | 0.001 | 0.04 | 0.08 | 0.04 | 0.27 | 0.32 | 0.785 | 0.65 | 0.54 | 0.397 | 0.148 | 0.1 | 0.02 | 0.47 | 0.121 | 0.369 | 0.081 |
| **CULTIVAR.PCR** | 3 | 0.14 | 0.97 | 0.77 | 0.22 | 0.99 | 0.53 | 0.726 | 0.54 | 0.45 | 0.517 | 0.383 | 0.761 | 0.093 | 0.376 | 0.658 | 0.957 | 0.579 |
| *CULTIVAR.FUNG* | 3 | 0.089 | 0.82 | 0.28 | 0.31 | 0.43 | 0.33 | 0.512 | 0.14 | 0.32 | 0.227 | 0.338 | 0.469 | 0.075 | 0.485 | 0.497 | 0.687 | 0.098 |
| Total | 15 | | | | | | | | | | | | | | | | | |

Statistical significance measured by ANOVA P <0□5 , P □0.01 , or P <□.001

Fungicide and plant growth regulator residues

*Year 1 experiments*

Pesticides detected in the straw samples were restricted, at most, to single fungicides (Table 11). These reflect the different fungicides used in Northern Ireland (fenpropimorph) and Wellesbourne (tebuconazole). No plant growth regulator residues were detected, even on the treated cultivars Clare and Weston.

*Year 2 experiments*

Analysis of straw for the plant growth regulator (PGR), chlormequat, showed that residues could be detected in treated straw at 0.5 to 7.8 mg/kg (Table 12). Residues were lower at the Downpatrick site and higher at Wellesbourne. This may be due to the lower rainfall at the Wellesbourne site. No residues were detected in any of the untreated straw samples. Fungicide (fenpropimorph and tebuconazole) residues detected in the Northern Ireland straw samples are shown in Table 13. Residue levels were again lower on the Downpatrick site and lower on cultivar Tanker than on the other cultivars. Residues in the GB straw samples were mainly breakdown products (biphenyl and 2-phenyphenol) and the fungicide tebuconazole (Table 14).

*Year 3 experiments*

Chlormequat residues in the treated straw samples were generally higher than in the straw used in the Year 2 experiments and were highest in the cultivar Hereward grown at Wellesbourne (Table 15). The main fungicide detected was tebuconazole, with phenyl breakdown compounds also detectable (Table 16). Tebuconazole residues were similar in the straw samples, except in cultivar Claire grown at Downpatrick, which were higher. No fungicide was detected in the cultivar Tanker grown at Kirton.

Table 9. Significant difference mapping (F pr values) for chemical parameters for n=18 (3 cultivars, 2 nitrogen rates, 3 reps) data comparisons in Year 3 straw samples

| | | **DM %** | **pH** | **EC** | **Ash %** | **NDM %** | **HDMD %** | **TSC** | **TSP** |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | d.f. | | | | | | | | |
| **CULTIVAR** | 2 | 0.015 | 0.008 | 0.099 | 0.006 | 0.43 | 0.210 | 0.30 | 0.19 |
| **NITROGEN** | 1 | 0.002 | 0.64 | 0.128 | 0.61 | 0.003 | 0.440 | 0.96 | 0.69 |
| **CULTIVAR.NIT** | 2 | <0.001 | 0.27 | 0.07 | 0.20 | 0.56 | 0.063 | 0.50 | 0.31 |

| | **PK** | **PK** | **PK** | **PK** | **WT** | **Pk** | **PK** | **Resi** | **P** | **K** | **Fe** | **LT** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

| | Area 1 | HT 1 | Temp 1 | Width 1 | Loss 2 | Area 2 | HT 2 | due | | | | Internode 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | | | | | | | | | | | | |
| **CULTIVAR** | 0.116 | 0.001 | 0.003 | <0.001 | 0.03 | 0.094 | 0.425 | 0.56 | 0.76 | 0.008 | 0.55 | <0.001 |
| **NITROGEN** | 0.002 | 0.165 | 0.78 | 0.95 | 0.15 | 0.03 | 0.01 | 0.02 | <0.001 | 0.01 | 0.02 | <0.001 |
| **CULTIVAR.NIT** | 0.155 | 0.53 | 0.89 | 0.98 | 0.86 | 0.07 | 0.221 | 0.19 | 0.35 | 0.966 | 0.50 | 0.002 |

Statistical significance measured by ANOVA P <0.05 , P <0.01 , or P <0.001

Table 10. Comparison of mean with minimum and maximum values of all parameters analysed for wheat straw samples – 2003 - 2005 harvests combined.

| Parameters | | Units | n | Mean | Min | Max |
|---|---|---|---|---|---|---|
| Dry Matter | | % | 84 | 90.18 | 69.27 | 95.23 |
| pH | | - | 84 | 6.86 | 6.17 | 7.64 |
| Conductivity | | µS/cm | 84 | 751.83 | 154.30 | 2750.00 |
| Nitrogen Dry Matter | | % | 84 | 0.57 | 0.33 | 1.09 |
| Ash | | % | 84 | 5.58 | 2.92 | 9.89 |
| C:N ratio | | - | 84 | 80.13 | 38.74 | 129.93 |
| DMD* | | % | 54 | 25.47 | 18.71 | 30.56 |
| HDMD* | | % | 84 | 22.99 | 10.54 | 29.63 |
| Total soluble carbohydrate | | g/kg DM | 80 | 6.10 | 2.87 | 19.23 |
| Total soluble polyphenol | | g/kg DM | 80 | 1.72 | 1.08 | 3.88 |
| DTG | WT-L1** | % | 80 | 65.12 | 61.17 | 68.77 |
| | Peak Area 1 | % | 80 | 45.90 | 41.21 | 51.07 |
| | Peak Height 1 | mg/min | 80 | 0.51 | 0.40 | 0.77 |
| | Peak Temp 1 | °C | 80 | 315.58 | 294.80 | 339.21 |
| | Peak Width 1 | °C | 80 | 57.63 | 23.09 | 67.13 |
| | WT-L2* | % | 80 | 25.63 | 22.55 | 28.78 |
| | Peak Area 2 | % | 80 | 10.42 | 7.97 | 12.32 |
| | Peak Height 2 | mg/min | 80 | 0.13 | 0.09 | 0.19 |
| | Peak Temp 2 | °C | 80 | 432.26 | 412.56 | 450.60 |
| | Peak Width 2 | °C | 80 | 45.81 | 29.40 | 67.30 |
| | Residue | % | 80 | 3.50 | 0.29 | 8.89 |
| Minerals | Aluminium | mg/kg | 84 | 175.24 | 80.42 | 310.48 |
| | Calcium | mg/kg | 84 | 2826.31 | 1901.20 | 7716.48 |
| | Copper | mg/kg | 84 | 7.99 | 3.11 | 24.60 |
| | Iron | mg/kg | 84 | 66.69 | 15.82 | 424.00 |
| | Potassium | mg/kg | 84 | 4791.34 | 795.77 | 12448.00 |
| | Magnesium | mg/kg | 84 | 826.26 | 323.1 | 1780.04 |
| | Manganese | mg/kg | 84 | 23.75 | 3.99 | 53.85 |
| | Sodium | mg/kg | 84 | 235.75 | 99.57 | 637.49 |
| | Phosphorus | mg/kg | 84 | 650.12 | 254.51 | 1455.82 |
| | Zinc | mg/kg | 84 | 4.32 | 0.16 | 12.62 |
| Internode length (mean) | | cm | 34 | 18.83 | 11.95 | 31.75 |
| Internode diameter (mean) | | cm | 34 | 0.13 | 0.09 | 0.26 |
| Node diameter (mean) | | cm | 34 | 0.13 | 0.10 | 0.22 |
| Elasticity Modulus (mean) | | | 34 | 478.17 | 307.74 | 686.85 |

* DMD – Dry matter Digestibility and HDMD Hemicellulose Dry Matter Digestibility
** Weight loss in primary (1) and secondary (2) peaks as determined by DTG analysis

Table 11. Fungicide residues detected in straw samples for Year 1 experiments

| Cultivar, N rate, Site (NI or GB) | Year | Fungicide detected | Level found, mg/kg |
|---|---|---|---|
| Claire, low N, NI | 2003 | fenpropimorph | 0.02 |
| Claire, high N, NI | 2003 | fenpropimorph | 0.03 |
| Hereward, GB | 2002 | tebucanazole | 0.04 |
| Hereward, GB | 2003 | tebucanazole | 0.10 |
| Weston, GB | 2002 | tebuconazole | 0.15 |
| Malacca, GB | 2003 | tebuconazole | 0.07 |
| Richmond, GB | 2002 | none | - |

Table 12. Plant growth regulator (Chlormequat mg/kg) residues in treated straw samples for Year 2 experiments

| Cultivar | Growing Site (2004 unless stated) | | | | | |
|---|---|---|---|---|---|---|
| | Kirton | Wellesb. (2003) | Wellesbourne | Cross | DownP | Lim |
| Axona | 1.3 | | | | | |
| Hereward | | | 7.8 | | | |
| Tanker | 1.7 | | | 1.3 | 0.7 | 1.0 |
| Richmond | | 6.7 | 4.8 | 2.2 | 0.6 | 0.5 |
| Claire | | | | 1.3 | 0.5 | 1.2 |
| Malacca | | | 1.2 | 1.5 | 0.4 | 1.2 |

Table 13. Fungicides (mg/kg) detected in treated Northern Ireland straw samples for Year 2 experiments

| Cultivar | Site | Fenpropimorph | Tebuconazole |
|---|---|---|---|
| Claire | Cross | 0.09 | 0.09 |
| Claire | DownP | 0.01 | 0.08 |
| Claire | Lim | 0.115 | 0.11 |
| Malacca | Cross | 0.09 | 0.09 |
| Malacca | DownP | 0.01 | 0.08 |
| Malacca | Lim | 0.12 | 0.025 |
| Richmond | Cross | 0.11 | 0.22 |
| Richmond | DownP | 0.01 | 0.09 |
| Richmond | Lim | 0.09 | 0.13 |
| Tanker | Cross | 0 | 0.08 |
| Tanker | DownP | 0 | 0.06 |
| Tanker | Lim | 0.035 | 0.035 |

Table 14. Fungicides detected in GB straw samples from Year 2 experiments

| Cultivar | Site | Year | Tebuconazol | Biphenyl | 2-Phenyphenol |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| | | | e | | |
| Tanker | Kirton | 2004 | 0 | 0.02 | 0.03 |
| Hereward | Welles | 2002 | 0.04 | 0.02 | 0.06 |
| Hereward | Welles | 2003 | 0.55 | 0.02 | 0.05 |
| Hereward | Welles | 2004 | 0 | 0.02 | 0.05 |
| Malacca | Welles | 2003 | 0 | 0 | 0 |
| Malacca | Welles | 2004 | 0 | 0.03 | 0.06 |
| Richmond | Welles | 2004 | 0 | 0.03 | 0.05 |
| Axona | Kirton | 2004 | 0 | 0.03 | 0.05 |

Table 15. Plant growth regulator (chlormequat) detected in straw samples used in Year 3 experiments

| Cultivar | Site, treatment | Chlormequat, mg/kg straw |
|---|---|---|
| Claire | DownPatrick | 5.51 |
| Malacca | DownPatrick | 3.48 |
| Tanker | DownPatrick | 13.90 |
| Claire | Crossnacreevy 180N | 12.06 |
| Malacca | Crossnacreevy 180N | 10.67 |
| Tanker | Crossnacreevy 180N | 15.61 |
| Claire | Crossnacreevy 260N | 13.90 |
| Malacca | Crossnacreevy 260N | 7.47 |
| Tanker | Crossnacreevy 260N | 10.76 |
| Hereward | Wellesbourne, -PGR | 0 |
| Hereward | Wellesbourne, +PGR | 33.0 |
| Tanker | Kirton, -PGR | 0 |
| Tanker | Kirton, +PGR | 13.0 |

Table 16. Fungicides (mg/kg) detected in straw samples used in the Year 3 experiments

| | | | | |
|---|---|---|---|---|
| Malacca | DownPatrick | 0.17 | 0.06 | 0.03 |
| Tanker | DownPatrick | 0.23 | 0.08 | 0.03 |

| | | | | |
|---|---|---|---|---|
| Claire | Crossnacreevy 180N | 0.24 | 0.22 | 0.14 |
| Malacca | Crossnacreevy 180N | 0.12 | 0.20 | 0.11 |
| Tanker | Crossnacreevy 180N | 0.26 | 0.13 | 0.07 |
| Claire | Crossnacreevy 260N | 0.17 | 0.22 | 0.09 |
| Malacca | Crossnacreevy 260N | 0.11 | 0.18 | 0.08 |
| Tanker | Crossnacreevy 260N | 0.20 | 0.17 | 0 |
| Hereward | Wellesbourne, -PGR | 0.16 | 0.02 | 0.23 |
| Hereward | Wellesbourne, +PGR | 0.27 | 0 | 0.08 |
| Tanker | Kirton, -PGR | 0 | 0.03 | 0.28 |
| Tanker | Kirton, +PGR | 0 | 0.03 | 0.16 |

Mechanical Properties of Straw Samples

*Year 1 experiments*

*Tensile strength tests*. Most of the straw samples did not fail at the maximum load applied (10 N). The results in Table 17 show clear differences between the samples. Cultivar Richmond had the greatest Young's modulus (stiffness) and was also the least stretchable. Addition of extra nitrogen fertiliser to cultivar Claire resulted in stiffer straw. Storage of cultivar Hereward resulted in a loss in stiffness.

Table 17. Mechanical properties of wheat straw samples measured with an Instron testing machine at room temperature

| Cultivar, N rate Site (NI or GB) | Instron Tensile Tests | |
|---|---|---|
| | Strain at max. load, (mm/m) | Young's modulus, MPa |
| Claire, low N, NI | 700 | 921 |
| Claire, high N, NI | 960 | 1034 |
| Hereward, GB | 980 | 1065 |
| Hereward, GB | 780 | 846 |
| Weston, GB | 620 | 883 |
| Malacca, GB | 760 | 1046 |
| Richmond, GB | 350 | 1446 |

*Dynamic mechanical analysis of straw.* Results are expressed as Young's modulus (stiffness) and tan delta (a measure of the energy lost by the straw during the test). The results show that temperature and straw moisture had large effects on the mechanical properties, and could alter the relative differences between straw types. Values obtained at 50°C for the different samples are shown in Table 18. The results show that the differences between the samples depended on the test conditions: dry, wet (100% RH) or immersed. Under dry conditions, cultivar Weston was the stiffest whereas under wet or immersed conditions, cultivar Malacca was the stiffest. Differences in energy loss (Tan D) between the samples also depended on the moisture condition of the test samples. Under moist conditions, the order was Weston

(greatest), Malacca, Richmond (smallest). Under dry conditions, the order was Richmond (greatest), Weston, Malacca (smallest).

Table 18. Dynamic mechanical properties of wheat straw samples measured by Triton at 50 C

| Cultivar | Young's modulus, Mpa | | | Energy loss (tan delta) | | |
|---|---|---|---|---|---|---|
| | dry | wet | immersed | dry | wet | immersed |
| Weston, GB | 52 | 17 | 52 | 0.08 | 0.27 | 0.27 |
| Malacca, GB | 38 | 34 | 65 | 0.07 | 0.24 | 0.23 |
| Richmond, GB | 29 | 34 | 30 | 0.13 | 0.19 | 0.22 |

*Year 2 experiments*

*Tensile strength tests*. Straw of the wheat cultivar Malacca was stiffer than that of the cultivar Tanker, with cv. Richmond intermediate (Fig. 7). Straw grown at the Crossnacreevy site was stiffer than that grown at Downpatrick, Limavady and in GB. The use of PGR did not have a clear effect on the stiffness of straw pieces of standard length.

Bench-scale composting experiments

*Year 1 experiments*

At spawning, the average compost moisture was 71.8 %. The average contents of N, ammonium N and ash were 1.99, 0.10 and 20 % of DM. Average compost pH was 7.8. Mushroom mycelial growth and pot experiment yields from compost prepared from the seven straw batches are shown in Table 19. Differences in mycelial growth between the samples were small. The highest mushroom yields were obtained from compost made from wheat cultivars Claire (low N application) and Malacca. Compost made from the stored 2002 Hereward straw produced the lowest yield.

*Year 2 experiments*

At spawning, the average compost moisture content was 72.5%. The average contents of N, ammonium N and ash were 1.97, 0.13 and 14.4% of DM respectively. The average compost pH was 7.60.

Straw grown at the Limavady site produced higher mushroom yields than straw of the same cultivars grown at the other sites (Fig. 8). The site differences were clearer than the differences between wheat cultivars, although straw of the cultivar Malacca produced a higher mushroom yield than cultivars Claire and Tanker. When results from both Northern Ireland and GB sites were considered, straw treated with PGR generally cropped better than untreated straw (Fig.9). There were no clear effects of site or wheat cultivar on mycelial growth rate, but

PGR treated straw generally resulted in slightly faster mycelial growth, when all sites were considered. Differences in yield between composts made from wheat straw of with and without fungicides were not significant.

Table 19. Mushroom mycelial growth and yield in pot tests from composts prepared from different straw samples in Year 1 experiments

| Cultivar, N rate | Crossnacreevy, NI | Mycelial growth mm/day | Mushroom yield, g/kg compost DM |
|---|---|---|---|
| Claire, low N | Crossnacreevy, NI, 2003 | 4.6 | 689 |
| Claire, high N | Wellesbourne, GB, 2003 | 5.1 | 414 |
| Hereward | Wellesbourne, GB, 2003 | 4.7 | 442 |
| Hereward | Wellesbourne, GB, 2002 | 4.3 | 328 |
| Weston | Wellesbourne, GB, 2002 | 5.0 | 371 |
| Malacca | Wellesbourne, GB, 2002 | 4.4 | 635 |
| Richmond | Wellesbourne, GB, 2003 | 4.5 | 354 |

Fig. 7 Effect of site and wheat cultivar on elastic modulus of straw

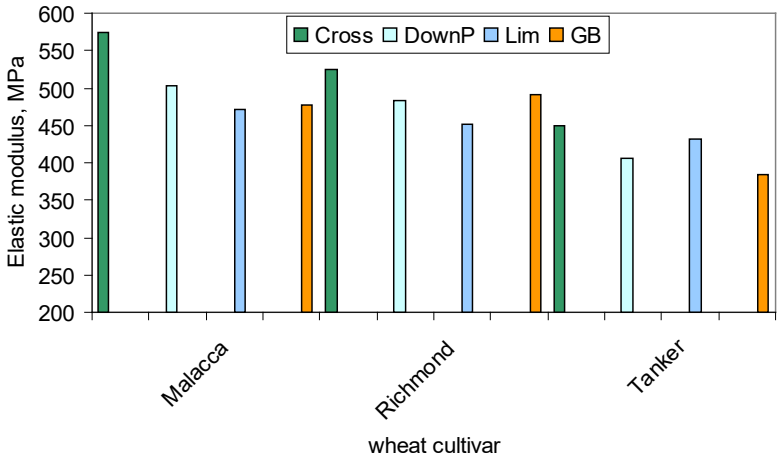


Fig. 8 Effect of wheat cultivar and site on mushroom yield in flask experiment composts

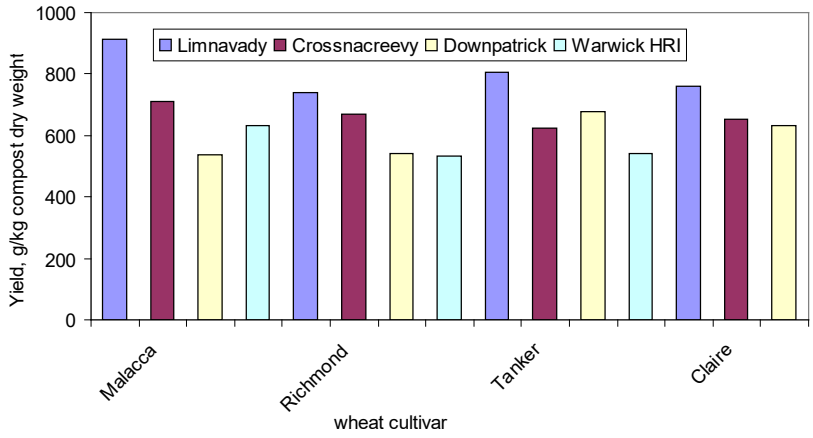
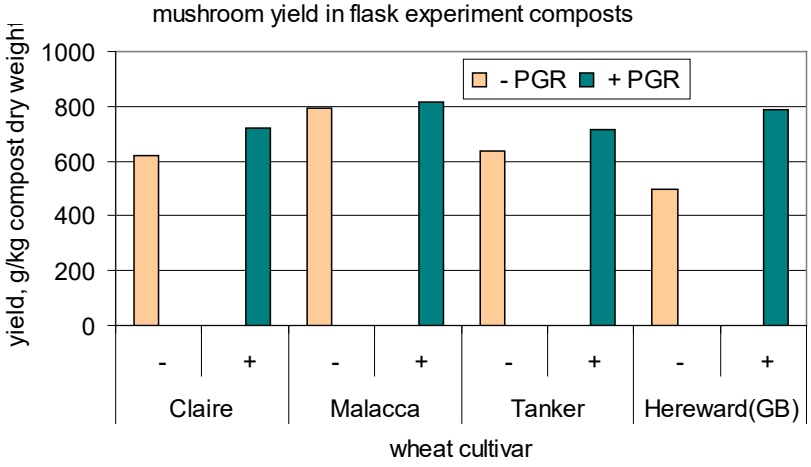



Fig. 9 Effect of plant growth regulator and wheat cultivar on mushroom yield in flask experiment composts




*Year 3 experiments*

The effect of wheat cultivar in flask composting experiments on subsequent mushroom yield is shown in Figs. 10 to 12. The difference in mushroom yield between three wheat cultivars grown at Downpatrick and Crossnacreevy was not significant (Fig. 10). Wheat straw grown at the Crossnacreevy site at the higher rate of nitrogen fertiliser produced a higher mushroom yield than straw grown at the lower rate (Fig. 11). Straw of the cultivar Hereward produced a higher mushroom yield than straw of the cultivar Tanker but plant growth regulator application did not have a consistent effect on subsequent mushroom yield (Fig. 12).

When data from both Years 2 and 3 was considered, there was no significant effect of straw residues of PGR (chlormequat) or fungicide (tebuconazole) and subsequent mushroom yield (Figs. 13 and 14). There was no effect of the residue on mushroom mycelial growth rate in the compost. There was no relationship between the mycelial growth and mushroom yield on composts prepared from different straw samples.

Windrow composting experiments

*Year 2 experiments*

At spawning, composts had an average moisture content of 73.9%. The average N, ammonium N and ash contents were 2.58, 0.055 and 25% of DM respectively. The average compost pH at spawning was 7.77.

Relative mushroom yields from different straw composts differed between two replicate composts and crops (Fig.15). Most of the straw types produced a better yield in the repeat crop, but 2-year old straw from cv Hereward (-PGR) produced a better yield in the first crop. This indicates that the composting method and conditions can influence the relative performance of different straw types.

Straw of the same wheat cultivar and PGR treatment that had been stored dry for 1 year (2003) generally cropped better than fresh straw (2004) (Fig.15). Straw stored for 2 years from 2002 (cv Hereward, -PGR) produced a lower yield than 1 year old straw of the same type. Straw from the different winter wheat cultivars (Hereward, Malacca, Richmond and Tanker) treated with PGR from the 2004 season produced similar mushroom yields. The spring wheat cultivar Axona produced a higher yield than the winter wheat cultivars from the same season (2004) and PGR treatment. Straw of two winter wheat cultivars (Hereward and Malacca) treated with PGR generally produced a slightly higher yield and faster mycelial growth rate than untreated straw (Fig. 16).

Fig. 10 Effect of growing site and wheat cultivar on subsequent mushroom yield, Year 3 flask experiment

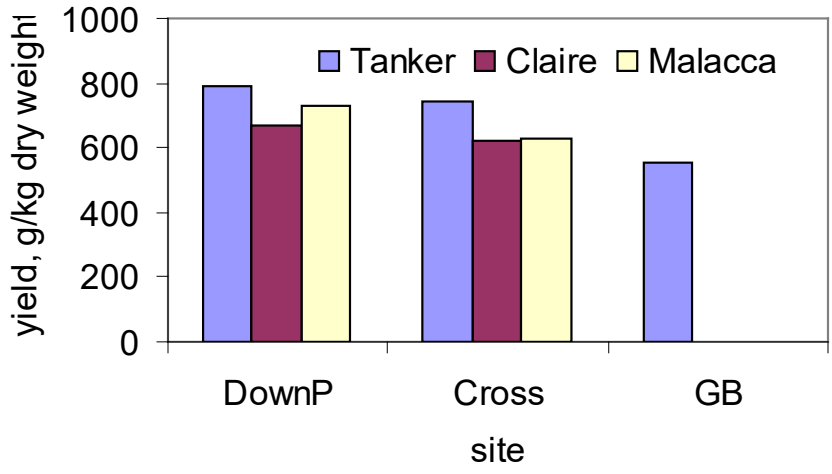



Fig. 11 Effect of wheat cultivar and nitrogen fertiliser on subsequent mushroom yield, Year 3 flask experiment

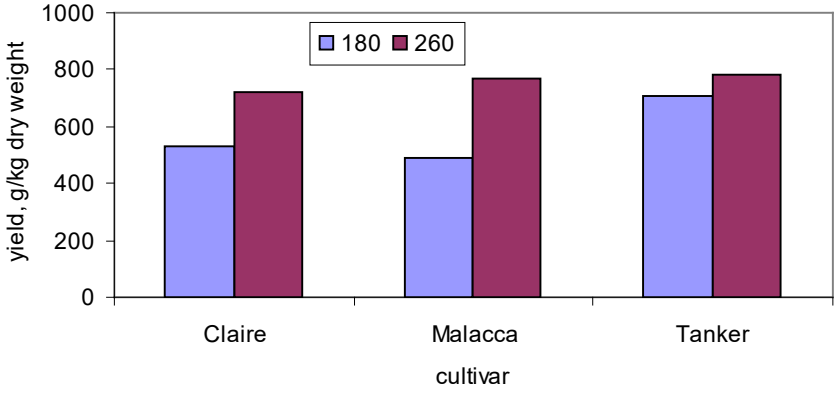


Fig. 12 Effect of wheat cultivar and plant growth regulator application, Year 3 flask experiment

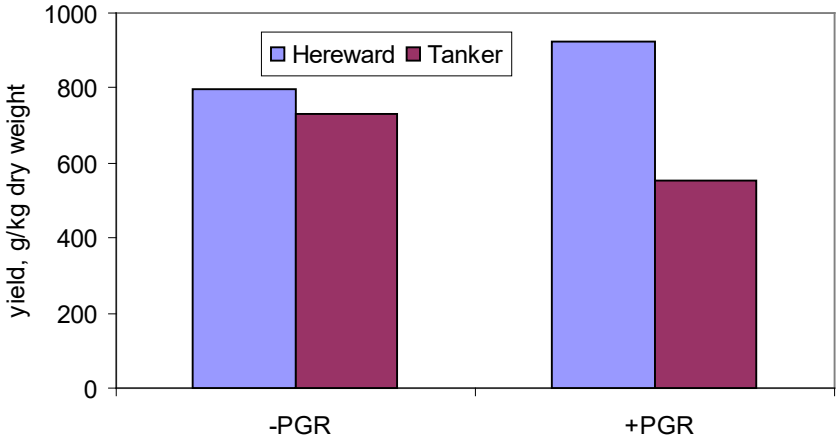



Fig. 13

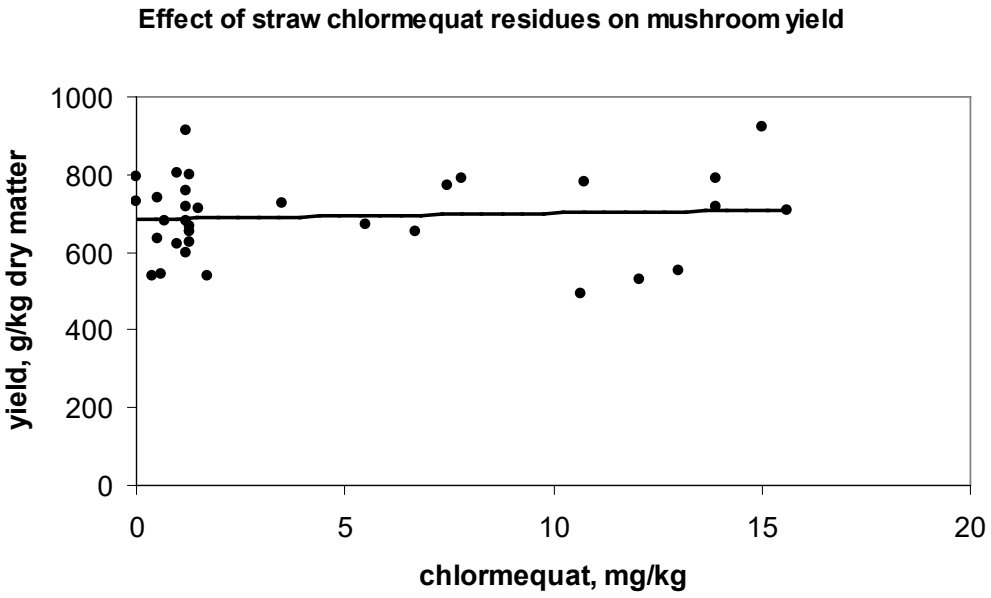



Fig. 14

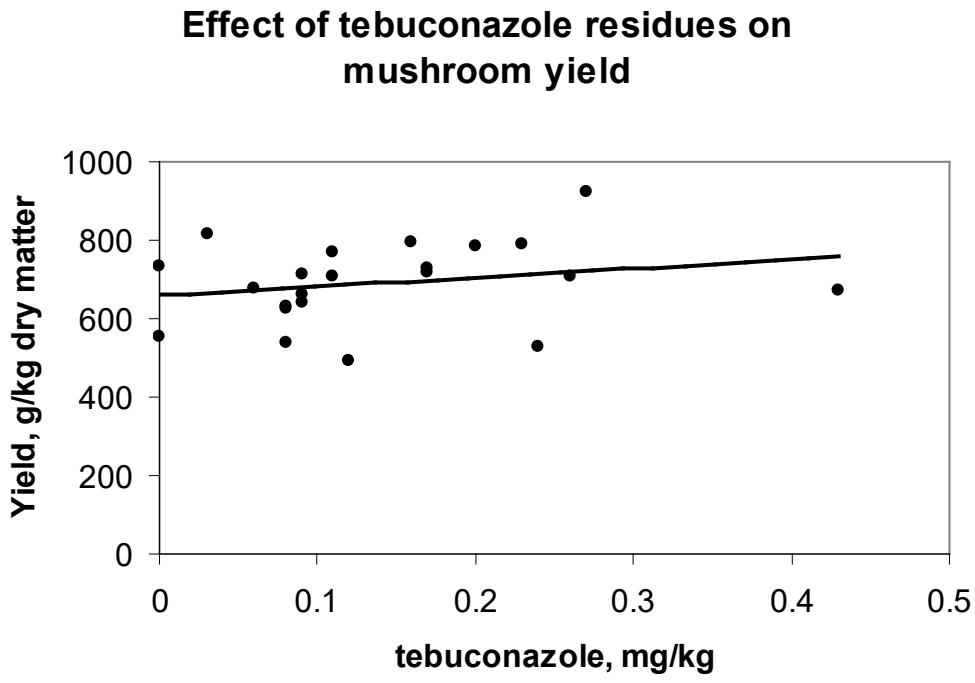


Fig. 15 Effect of wheat straw on mushroom yield in two series of windrow composts

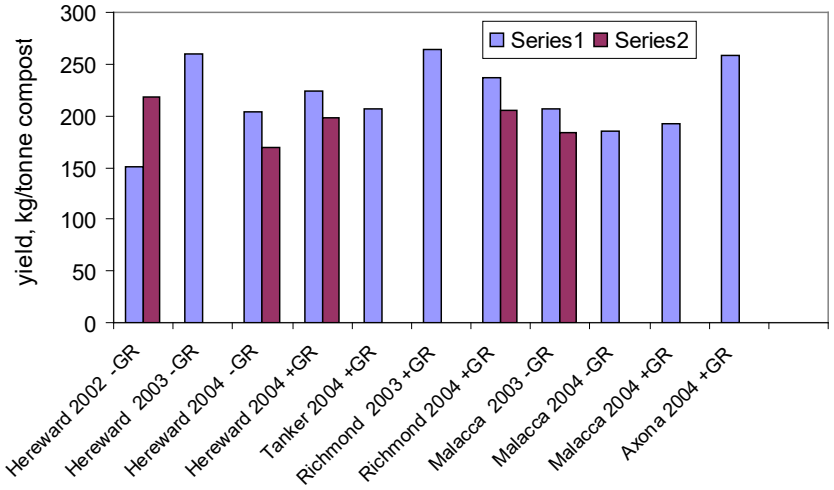



Fig. 16 Effect of plant growth regulator on mushroom yield from windrow composts

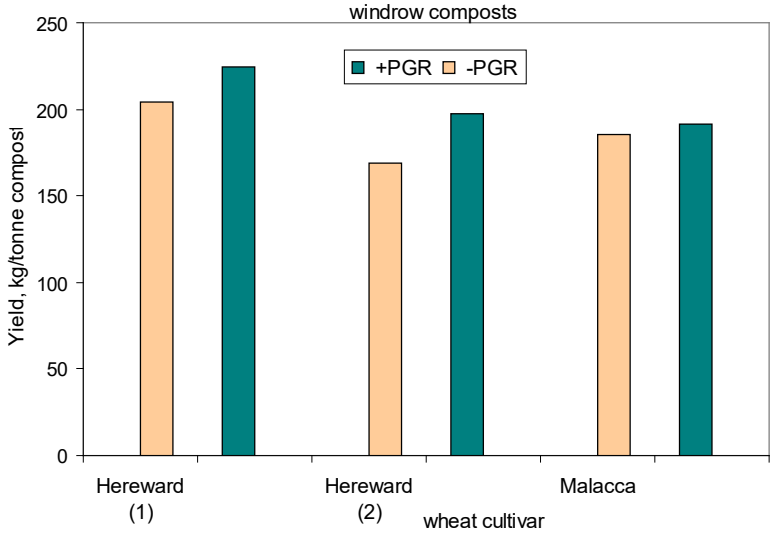
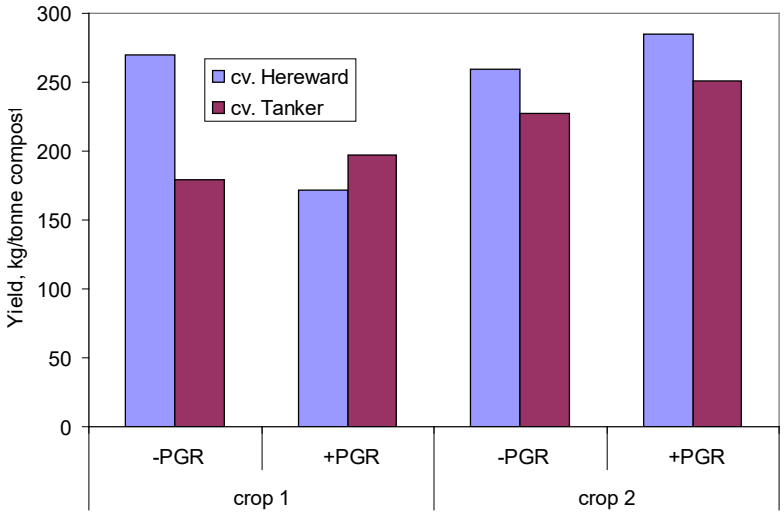



Fig. 17 Effect of wheat cultivar and plant growth regulator on mushroom yield from windrow composts



*Year 3 experiments*

At spawning, the average compost moisture content was 75.1%. The average N, ammonium N and ash contents were 2.57, 0.017 and 24.2% of DM respectively. The average compost pH at spawning was 7.73.

As in the Year 2 experiments, the relative difference in mushroom yield differed between the two replicate composts and crops (Fig. 17). Straw from the cultivar Hereward produced a higher mushroom yield than straw from the cultivar Tanker. Chlormequat application to straw did not significantly affect mushroom yield (Fig. 17).

**Discussion**

The results of this work confirm earlier work which has shown that different winter wheat cultivars grown under the same conditions can produce straw with significantly different chemical characteristics (Knapp et al 1983; Sharma et al 2000). In agreement with previous work, treatment of wheat with the fungicide tebuconazole and the growth regulator chlormequat resulted in straw with high dry matter digestibility and generally lower nitrogen content (Sharma et al 2000). Savoie et al (1992) showed that application of tebuconazole, as well as other fungicides, significantly affected the dry matter content and degradability of wheat straw, but the effects were less important than the effects of nitrogen fertiliser and growth regulator applications. Here, some of the effects of nitrogen fertiliser applications to wheat on the chemical composition of straw varied across different cultivars. However, higher nitrogen application resulted in higher straw contents of nitrogen, potassium, phosphorus and iron. The DGT (weight loss in the secondary peak WL 2 – structural hemicellulose and lignin) data also indicated that straw grown on plots with higher nitrogen applications had greater hemicellulose and cellulose fractions and less lignin, possibly due to the faster growth and longer stem length. The greater availability of mineral and organic nutrients in the straw grown on higher nitrogen plots may therefore explain the higher mushroom yield from compost grown on this straw.

The DGT (weight loss in the secondary peak WL 2 – structural hemicellulose and lignin) analyses of straw from different sites were similar. Here it is possible that the greater dry digestibility of straw from the Limnavady site (which produced the best mushroom yield) was the most important factor.

The present results have shown that the effects of wheat cultivar and applications fungicide and growth regulator on the chemical composition of straw, and subsequent mushroom compost productivity are relatively small compared with effects of growing site and nitrogen fertiliser.

The large-scale experiments show that the difference in yield between composts prepared from the 'best' and 'worst' straw types from the same season was about 50 kg mushrooms/ tonne compost. The factor which had the greatest influence on straw properties and subsequent mushroom cropping was growing site. The increase in yield would therefore justify transporting straw from more distant sources if these can be shown to reliably produce better yields than local but 'poorer' wheat straw sources.

**Conclusions Part 1**

1. Growing site had a greater effect on the chemical analysis of wheat straw and its subsequent mushroom cropping performance than cultivar. However, mushroom yields from the spring wheat cultivar Axona were better than from several winter wheat cultivars.
2. Straw produced at the Limavady site in Northern Ireland produced compost with a higher yield potential than straw from two other sites in Northern Ireland and from GB. This straw had a higher dry matter content and higher dry matter digestibility than straw from the same treatments grown on the other sites.
3. Samples of straw that had either a high dry matter digestibility and/or larger relative amounts of cellulose and hemicellulose to lignin (determined by thermogravimetry) produced better mushroom yields than straw samples with low values of these measurements.
4. The tensile properties of straw did not relate to the subsequent mushroom yield potential or mycelial growth rate of the compost produced.
5. Straw from wheat treated with the plant growth regulator (PGR) chlomequat and fungicide differed in terms of dry matter, ash and dry matter digestibility from untreated straw.
6. PGR or fungicide applications to straw did not significantly affect either mushroom yield or mycelial growth rate from the compost produced.
7. Wide variations in the residues of chlormequat and fungicide on treated straw were found, but these did significantly affect mushroom mycelial growth or yield.
8. Large-scale experiments showed significant differences between straw types; compost made with straw from wheat cultivar Hereward produced a higher mushroom yield than straw from cultivar Tanker. Differences in mushroom yield between the 'best' and 'worst' straw sources were about 50 kg mushrooms/ tonne compost.
9. There was general agreement between the results of flask and windrow composts produced with same straw samples.

10. Straw from plots treated with high rates of nitrogen fertiliser resulted in a higher mushroom yield in flask composting experiments than straw from plots with a lower rate of nitrogen fertiliser. The straw from higher nitrogen plots had higher N, P, K and Fe contents, and DGT analysis also indicated higher contents of hemicellulose and cellulose than straw from lower nitrogen plots.
11. Straw that was stored dry for 1 year produced higher mushroom yields than fresh straw of the same type (wheat cultivar, applications of PGR, fungicide and N fertiliser), whereas two-year old straw produced a lower yield.

## Part 2. Reducing spawn-running time.

### Introduction

Processing time is a major factor influencing the cost of mushroom compost production. In particular, the rate of degradation to produce a suitable compost (in aerated or windrow Phase I), and the subsequent colonisation of the compost with mushroom mycelium in expensive spawn-running facilities (Phase III). Due to the shorter production cycle, there has been move away from using pasteurised (Phase II) compost in cropping rooms to using colonised (spawn-run or Phase III) compost. Producing a sufficient supply of Phase III compost at a competitive price is a key point in the future of the UK mushroom industry.

Previous compost research has focussed on the factors which affect mushroom yield, rather than the rate of mycelial colonisation, and these are not necessarily related (Flegg & Wood 1985, Smith et al 1995, Sharma et al 2000). Factors that have been shown to affect the rate of mycelial colonization of compost include moisture content, pH, ammonium N, salinity, temperature and $CO_2$ (Flegg & Wood 1985). Identification of the factors that influence the mushroom mycelial colonization of compost could lead to a significant reduction in spawn-running time and associated capital and operating costs.

Obtaining colonised (spawn-run) compost clearly depends on incorporating a suitable mushroom inoculum (spawn) into the pasteurised (Phase II) compost. For many years in the UK, the standard amount of spawn added has been 8 litres per tonne of compost, equivalent to 0.5% w/w. This rate was based on the work of Cook & Flegg (1962), but since this time, compost analyses and preparation methods have changed significantly. A number of farms apply spawn to the surface of the compost, a practice known as ‘top-spawning’. However, it is unclear whether this is beneficial. The most commonly used substrate for spawn is rye grain, but other formulations based on millet grain and vermiculite (Speedy spawn) are also available. These latter formulations have smaller particle sizes than rye grain, and therefore have more points of inoculation for the same incorporation rate.

Mushroom nutrients or 'supplements' have been added to compost in order to enhance yield. These are usually protein-based materials and are widely used for adding to colonised (spawn-run) compost. A difficulty in adding them to compost at spawning is that the nutrients can also be available to competitor moulds (Gerrits, 1988). New compost supplements have recently become available that may be suitable for use at spawning. If the nutrients are selectively available to the mushroom mycelium, the materials may the potential to enhance mushroom mycelial growth and yield.

After spawning into trays or blocks, the compost is compressed in order to increase the quantity of compost per unit of cropping area. The level of compression has a significant effect on the porosity of the spawned compost and could have an effect on the subsequent spawn-run. However, the effect of this compression on spawn-running and subsequent mushroom yield has not been previously examined.

**Objectives**

To test the following hypotheses:

- Can extracellular laccase be used as a measure of mycelial growth in different compost samples?
- Is the rate of mycelial growth in compost related to its chemical analysis?
- Can the rate of spawn-running be improved by modifying the compost?

**Materials and Methods**

Measuring spawn-running

*Measurement of mycelial growth using growth tubes*

A growth tube method, adapted from Smith et al (1995) was used for measuring linear mycelial growth in compost. Spawn (8 g, Sylvan A15) was filled in the end of a boiling tube which was then filled with 30 g of compost up to a mark 100 mm from the closed end. The position of the mycelial growth front was measured at 3-daily intervals until the compost in the tube was fully colonised by mycelium. Tubes filled with spawn and wetted fine grade vermiculite (30 g, moisture content 73% w/w) were used as control samples for each batch of tubes. The tubes were placed horizontally in an incubator at 25°C.

*Laccase activity of composts*

The method of Wood & Goodenough (1977), modified by Smith et al (1989) was used for determining the laccase activity of composts. Compost samples (50 g) were agitated for 1 hour at 20°C in 100 ml of 0.01 M phosphate buffer, pH 7.0. The coarse solid debris was removed by filtration using nylon gauze and the filtrate clarified by centrifugation at 12,000 rpm for 15 min

at 20°C. The supernatant extract was then stored at -15 C until use.

The laccase activity of the thawed extract was determined polarographically using an oxygen electrode (Rank BrothersLtd, Cambridge). Samples (1.0 ml) of compost extract were mixed with 1.7 ml of sodium acetate-acetic acid buffer, 0.02 M, pH 5.0 in the electrode chamber. P-phenylenediamine (0.3 ml 0.1 M) was added to the chamber. The amount of oxygen consumed (related to the laccase activity) was recorded on a chart recorder. This was determined from the slope of the graph of time v oxygen concentration in the electrode cell.

Laccase determinations were conducted using 16-days spawn run compost (colonised by mushroom mycelium), unspawned pasteurised compost, and laccase obtained from Sigma (extracted from *Rhus vernificera*) was used as a control (1 mg per ml suspension). Three replicates samples were analysed. The laccase enzyme activity was determined from the calculation of Wood & Goodenough (1977) which showed that the consumption of 1 umol of oxygen consumed in the electrode was equivalent to 1 enzyme unit of activity in 1 ml of the extract.

*pH and temperature measurement*

Compost pH was measured before, during and after spawn-run as described in the previous section. The temperature of compost in trays during spawn-run was measured with multi-point data loggers.

*Compost analysis*

Phase II (pasteurised) composts were analysed for nitrogen, ammonium nitrogen, ash, minerals P and K, TSC and HDMD, and were also analysed using vis-NIR and DTG as described in Part I of the report.

Composts for measuring spawn-running

*Commercial Phase II composts*

Pasteurised compost was obtained from 11 different composting sites of the participating partners on two separate occasions.

*Experimental Phase II composts*

Windrow composts were produced using a standard procedure at Warwick HRI using straw from a single source (cv. Hereward) and standard amounts of poultry manure (500 kg per tonne of straw) and gypsum (30 kg per tonne of compost). Composts with the following formulations were prepared and compared with standard straw + poultry manure above (A):

B. As (A) replacing 30% of the poultry manure with an equivalent amount of nitrogen in ammonium sulphate

C. As (B) but also adding 300 kg of composted green waste.

The cropping performance and rate of mycelial growth using the spawn strain Sylvan A15 in each compost was determined.

Experiments on spawn-running rate

*Effect of compost pressure*

Phase II compost was prepared using the above windrow procedure. Compost was spawned with the strain A15 at 0.5 %w/w. At spawning, trays were filled as follows:

(a) 30 kg, no pressure applied at spawning
(b) 50 kg, no pressure applied at spawning, pressed at casing
(c) 50 kg, no pressure applied at spawning, re-mixed and pressed at casing
(d) 50 kg, pressure 10.3 MPa applied for 4 seconds
(e) 50 kg, pressure 10.3 MPa applied for 12 seconds
(f) 60 kg, pressure 10.3 MPa applied for 12 seconds

Trays of treatment (a) were re-filled at casing to an equivalent weight of 50 kg/tray at spawning (47 kg spawn-run compost). None of the compost was supplemented. Compost temperatures were recorded hourly with multipoint data-loggers.

*Spawn rate and type*

A series of four crops were spawned at 0.5% w/w (equivalent to 8 litres of spawn/tonne compost) and 0.8% w/w, using the strain Sylvan A15. In a further experiment, rye grain spawn (strain Lambert 901 and Sylvan A15) was compared with 'Speedy Spawn' and millet spawn (both strain 901 Lambert Spawn) at the same rates (0.5% w/w). In the fourth crop, half the trays were also top-spawned by sprinkling 100g of spawn over the surface.

*Supplementation and spawn rate*

The following supplement treatments were used at spawning:

(a) no supplement
(b) Spawn Mate ProMycel 480 (Amycel) added 1% of compost weight
(c) Natural Gold (IPP) added at 1% of compost weight.

In a further experiment, the following treatments were used:

(a) no supplement
(b) Lambert Spawn T6 added at 1% of compost weight.
(c) Lambert Spawn Soya Supplement added at 1% of compost weight.

*Effect of straw type*

The effects of straw type on mycelial growth were covered in Part 1. The aim of these tests was determine if there were any relationships between the analysis of the compost prepared from different straw types in flask and windrow experiments and subsequent mushroom mycelial growth rate and yield. 64 samples from the laboratory scale Phase II composts from different straw types were scanned for Vis-NIR spectra. Seven samples from 2006 were not scanned due to inadequate material. 27 samples from the straw windrow compost samples were also analysed. The visible and NIR spectra of the compost samples were assessed for similarities and differences in the various wavelengths.

**Results**

Measurement of spawn-running

Four methods were identified that could be used to measure the rate of spawn-run (mycelial colonisation) of compost:

- measuring the fall in compost pH
- measuring the amount of laccase enzyme in extracts from the compost
- temperature recording
- measuring the mycelial front in a growth tube, using moistened vermiculite as a control.

Both of the latter two methods were found to be highly dependant on the moisture content of the compost: drier composts increased the temperature rise and mycelial extension rate.
The commercial phase II composts differed in pH (7.13- 7.65), EC (3.95 – 6.39 mS/cm), and dry matter (64.4 – 71.8%). However, there was no clear effect of these parameters on yield or spawn running in the pot experiments.

The extension rate of mycelial colonisation was closely correlated with the fall in pH during spawn-running (Fig. 18). Compost pH declined from about day 7 until day 21 after spawning (Fig.19). The amount of laccase enzyme produced by mushroom mycelium continued to increase between days 10 and 16 of spawn-running, and in some composts continued to increase until day 21 of spawn-running (Fig.20). Commercial composts with a higher level of laccase enzyme at the end of spawn-running produced better yields than composts with lower levels (Fig.21). A similar relationship between Phase III compost laccase and subsequent mushroom yield was found for experimental composts produced at Warwick HRI (Fig. 22).

Fig. 18 Relationship between compost pH drop in spawn-run and mushroom mycelial growth rate

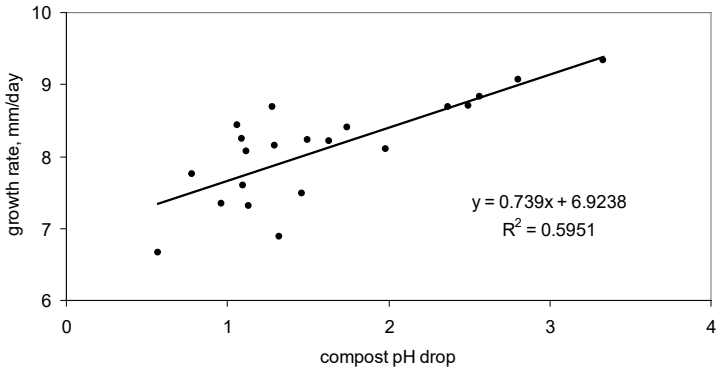



Fig. 19 Compost pH during spawn-run of commercial composts

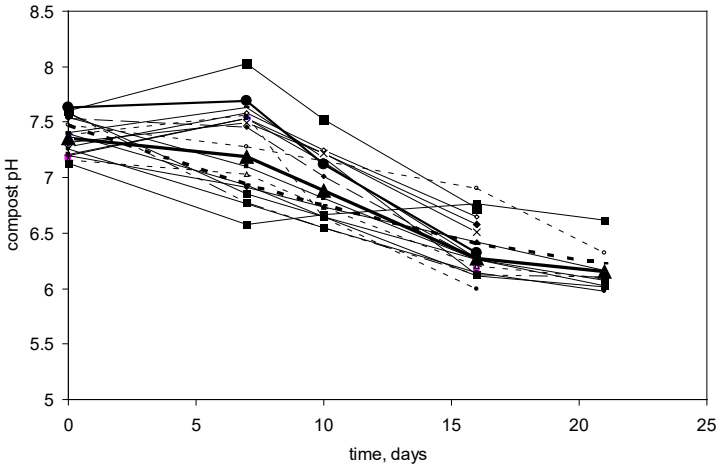



Fig. 20 Laccase in compost during spawn-run of commercial composts

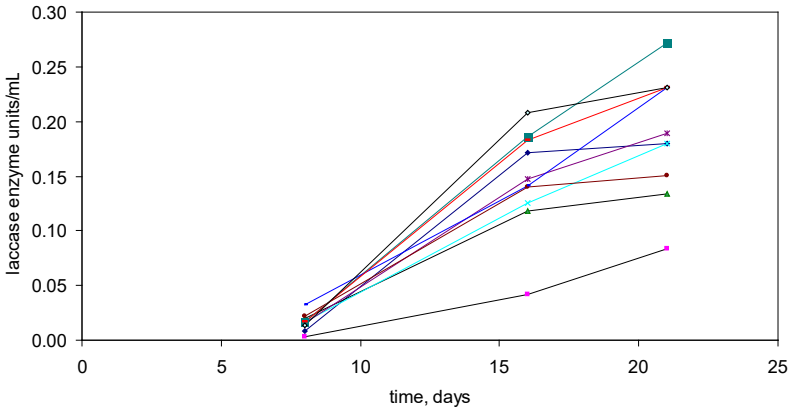


Fig. 21 Relationship between laccase in spawn-run compost extracts and mushroom yield

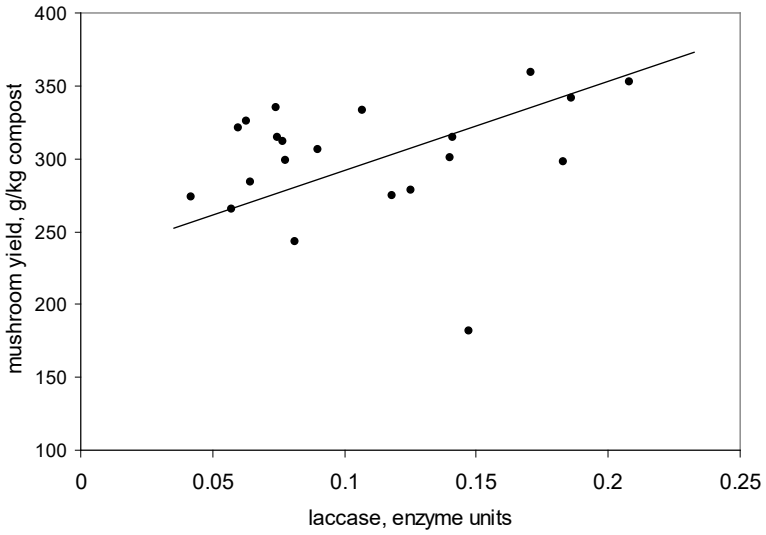



Fig. 22 Relationship between laccase in spawn-run compost extracts and mushroom yield from experimental composts

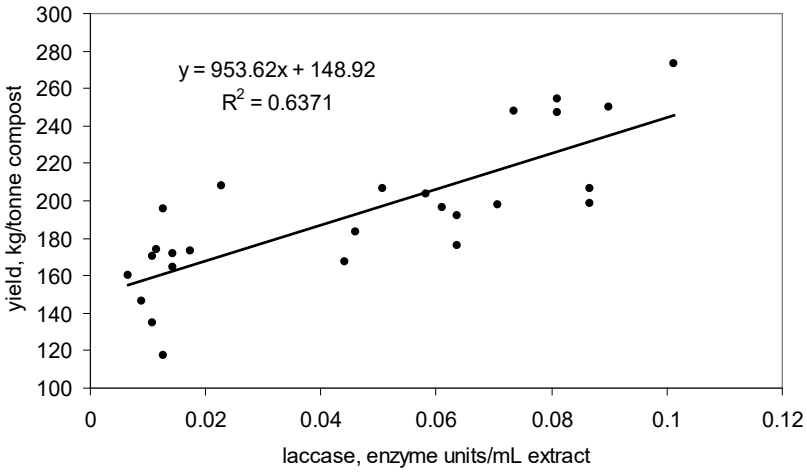


Fig. 23 Relationship between fall in compost pH during spawn-run and mushroom yield

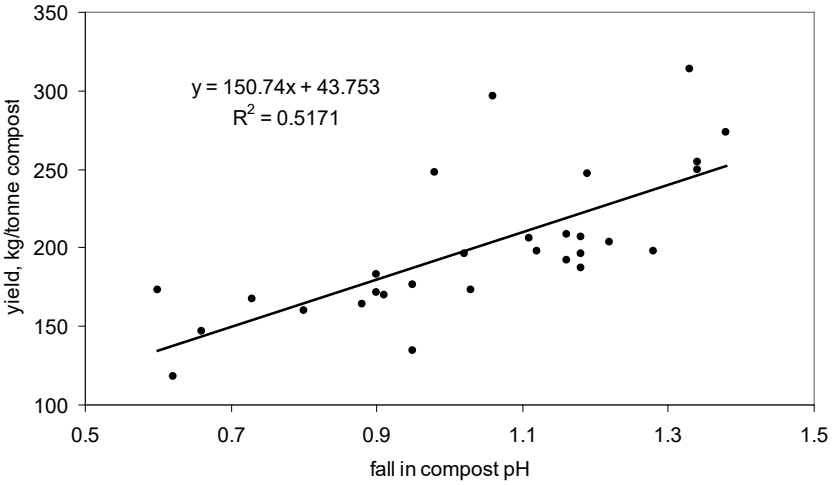



Fig.24 Effect of supplementation and spawning rate on spawn-running temperatures
N no supplement; P ProMycel, G Natural Gold
Spawn rate 0.5, 0.8 % w/w

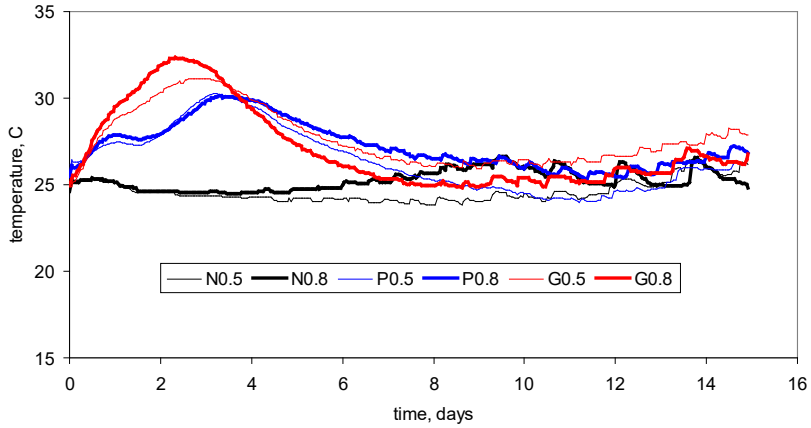



Fig. 25 Effect of spawn rate (w/w) on spawn-running temperatures

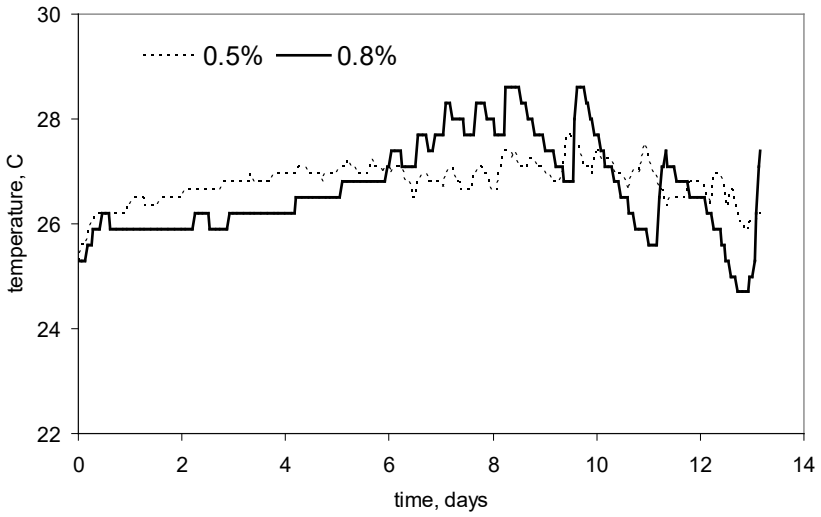


Fig. 26 Effect of supplementation of Phase II compost on mushroom yield

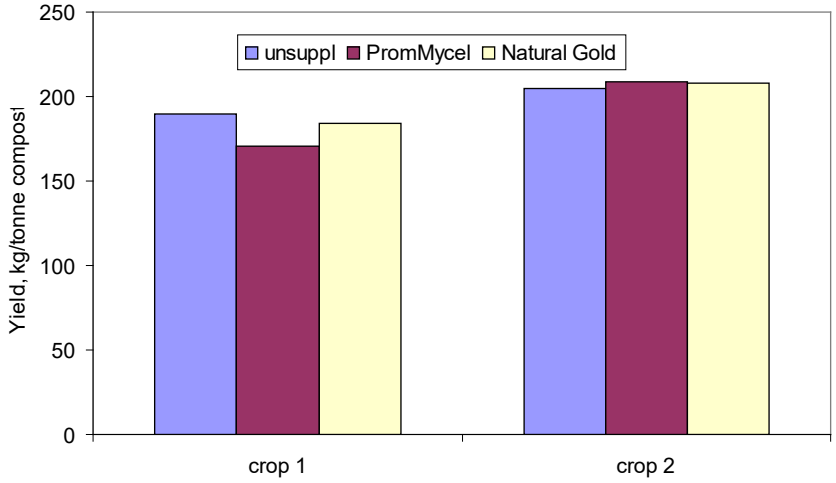



Fig. 27 Effect of supplementation of Phase II compost on mushroom yield

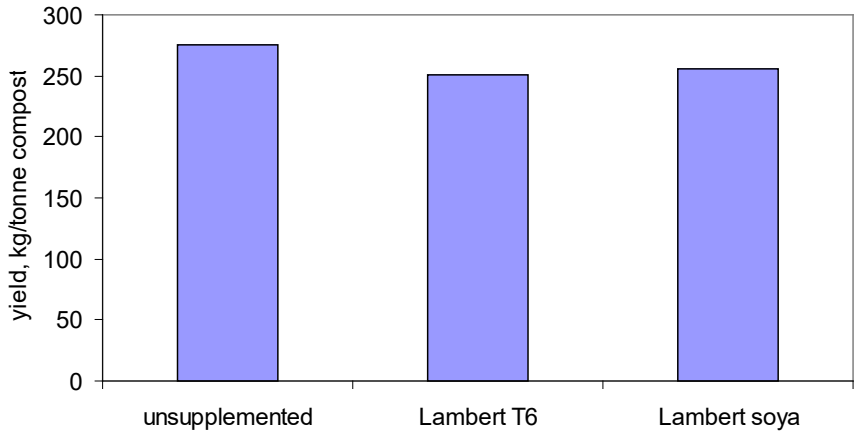



Fig. 28 Effect of spawn rate on mushroom yield

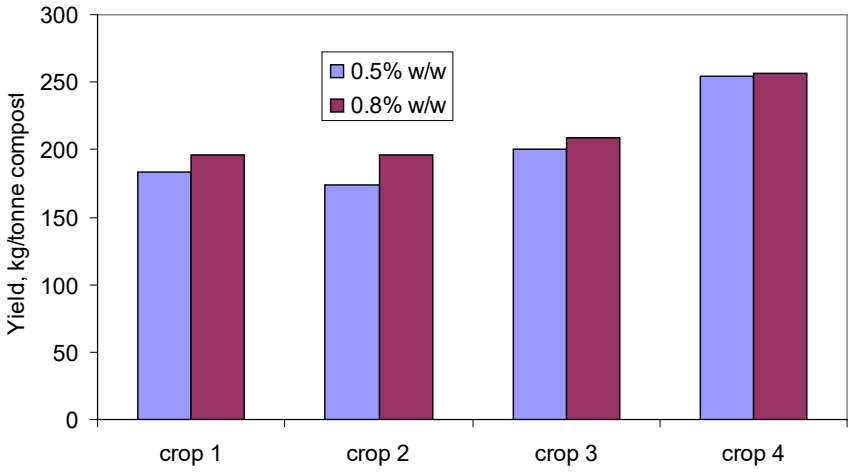


Fig. 29 Effect of spawn type on mushroom yield

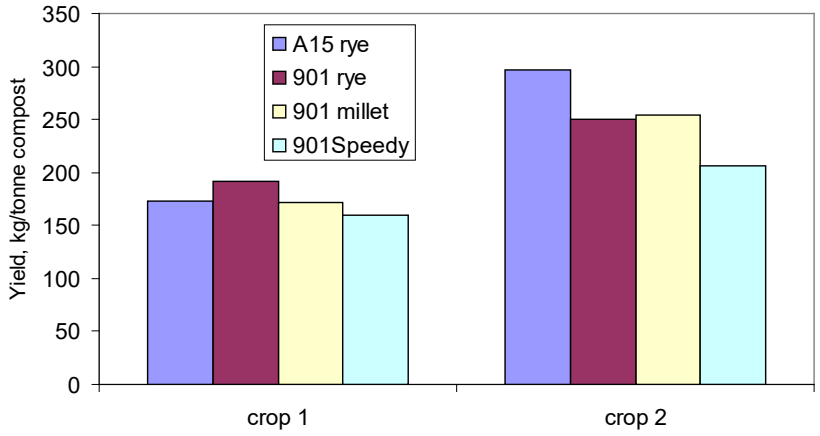



Fig.30 Effect of compost nitrogen source on mushroom yield

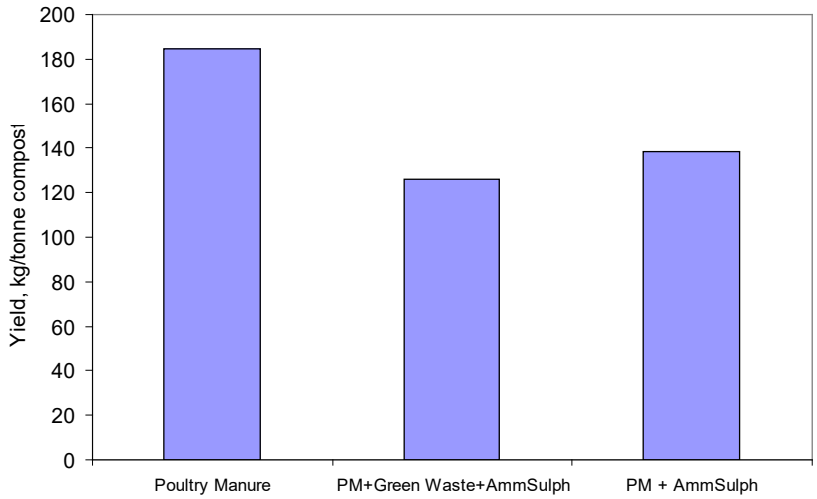



Fig. 31 Effect of composting pressing time on mushroom yield

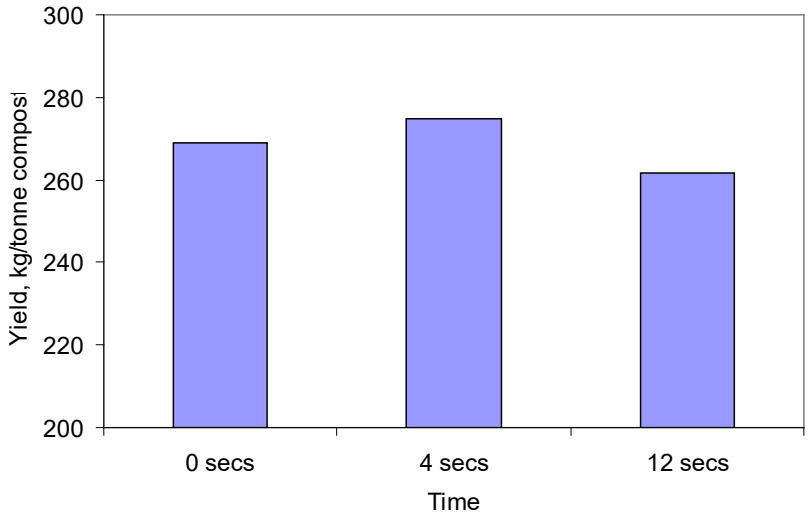


Table 20. Effect of spawn-rate and supplementation on spawn-running

| Spawn rate %/w/w | Supplement (1% w/w) | pH drop | Laccase units/mL extract 10days | 16days |
|---|---|---|---|---|
| 0.5 | none | 0.90 | 0.019 | 0.052 |
| 0.8 | none | 1.18 | 0.012 | 0.069 |
| 0.5 | ProMycel | 0.20 | 0.003 | 0.013 |
| 0.8 | ProMycel | 0.73 | 0.008 | 0.050 |
| 0.5 | Natural Gold | 0.95 | 0.007 | 0.072 |
| 0.8 | Natural Gold | 1.16 | 0.007 | 0.072 |

There was also a relationship between the fall in compost pH during spawn-running and subsequent mushroom yield (Fig. 23).

Batches of commercial Phase III compost were analysed for laccase enzyme. Values ranged between 0.01 and 0.06 laccase enzyme units/mL extract.

Effects of spawn rate and supplementation on spawn-running

Supplementation did not have a significant effect on mycelial growth rate in growth tubes. The effects of compost supplementation on compost temperatures during spawn-running are shown in Fig. 24. Both supplements resulted in an initial rise in compost temperature (before mycelial

growth had started). Increasing the spawn rate from 0.5 to 0.8 % w/w resulted in an earlier rise in compost temperatures during spawn-run (Fig. 25). None of the supplements used at spawning had a significant effect on mushroom yield (Figs. 26 and 27).

The higher spawn rate resulted in an earlier compost temperature peak and greater pH drop and laccase enzyme during spawn-run than the lower rate (Fig. 25, Table 20). This meant that the higher rate reduced spawn-run by 1 -2 days. Compost spawned at the higher spawn rate (0.8% w/w) generally resulted in a higher yield than the lower rate (0.5% w/w) across the four crops (Fig. 28). The effect of different types of spawn (Lambert 901and Sylvan A15 at 0.5% w/w) on yield in two crops is shown in Fig. 29. There was no significant difference in yield between rye and millet grain, but ‘Speedy Spawn’ resulted in a poorer yield and spawn-run.

Surface spawning with 100 g spawn per tray had no significant effect on the speed of spawn-run or on subsequent mushroom yield (average yields were 252 kg/ tonne compost with surface spawning and 262 kg/tonne compost without surface spawning).

Effects of compost analysis, formulation and pressure on spawn-running

Comparing mycelial growth rate in different straw + poultry manure composts showed that the analysis of the compost (other than moisture content) had only a small effect on the rate of mycelial growth rate. The optimum moisture content for mycelial growth rate was around 70%

at spawning. Compost nitrogen content at spawning (within the range 2.3 to 2.65 of DM) had no significant effect. Mycelial growth rate only declined when the compost pH exceeded 7.85.

Addition of ammonium sulphate to the compost formulation resulted in a much higher ammonium nitrogen content at spawning (0.39% of DM) compared with poultry manure compost (0.03% of DM). Addition of green waste compost to the formulation resulted in a higher compost ash content at spawning (35% of DM) compared with poultry manure compost (28% of DM). Ammonia levels in Phase II were lower when 30% of the poultry manure was replaced (600 ppm) compared with 800 ppm with poultry manure. However, composts with ammonium sulphate took 2 days longer to clear of ammonia. Replacing 30% of the nitrogen from poultry manure with green waste compost and/or ammonium sulphate significantly reduced mushroom yield (Fig. 30).The effect of increasing the compaction (pressing time) of compost in trays following spawning is shown in Fig. 31. Increased compaction of compost by pressing for 12 secs retarded spawn-running and reduced mushroom yield compared with pressing the compost in trays for 4 secs.

Effect of straw type and compost analysis

*Laboratory flask straw composts*

Major differences in the compost samples from different straw types were in the visible segment of the spectra Fig. 31A. There were fairly close correlations between the vis-NIR spectral analysis of the compost samples and mycelial growth rate (Fig. 31B) and with fall in compost pH during spawn-run (Fig. 32B). However, the correlation between vis-NIR analysis of compost samples and mushroom yield was relatively poor, even after 10 outlier samples were excluded (Fig. 31C).

*Windrow straw experiment composts*

A relationship between visible and NIR spectra of the compost samples and mushroom yield was not observed. However, a relationship between the derivative thermogravimetric analysis (DTG) of the compost samples and subsequent mushroom yield was identified (Fig. 31D).The results therefore indicate that vis-NIR analysis of composts made from different straw batches may provide useful information on the subsequent mycelial growth in the composts, but not on mushroom yield. Information on potential mushroom yield from composts is more likely to be obtained from DTG analysis.

Figure 31A. An overlay of raw spectra (400-2500 nm) obtained from 64 laboratory scale compost samples showing differences in the absorbance especially in the 400-1300 nm range.

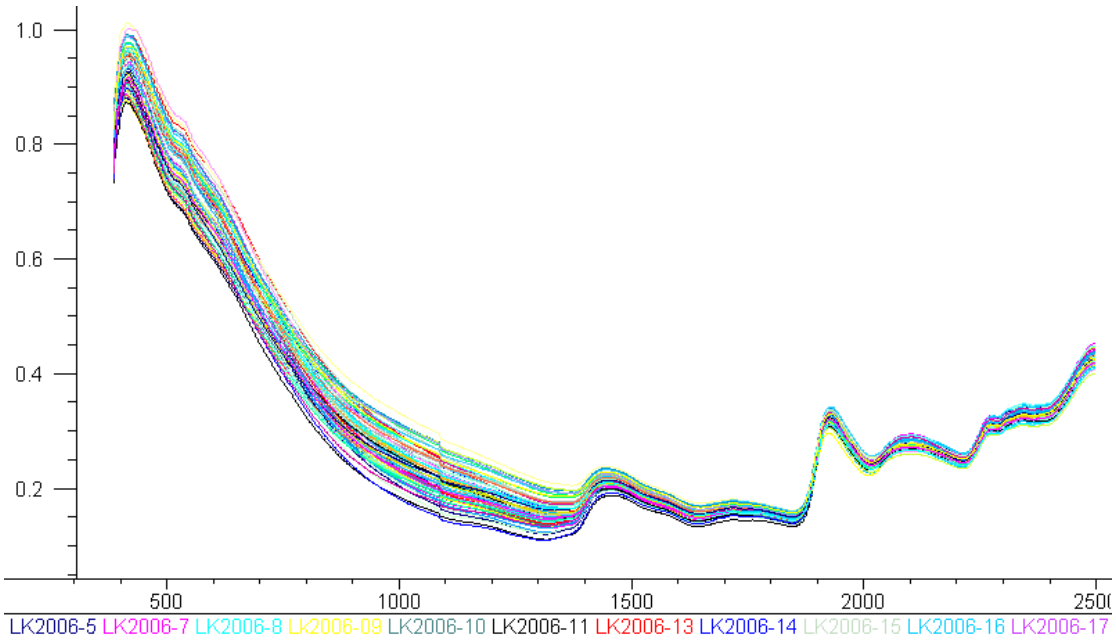



Figure 31B. Relationship between visible and NIR spectra of compost and mushroom mycelial growth rate, showing an $R^2$ of 0.73.

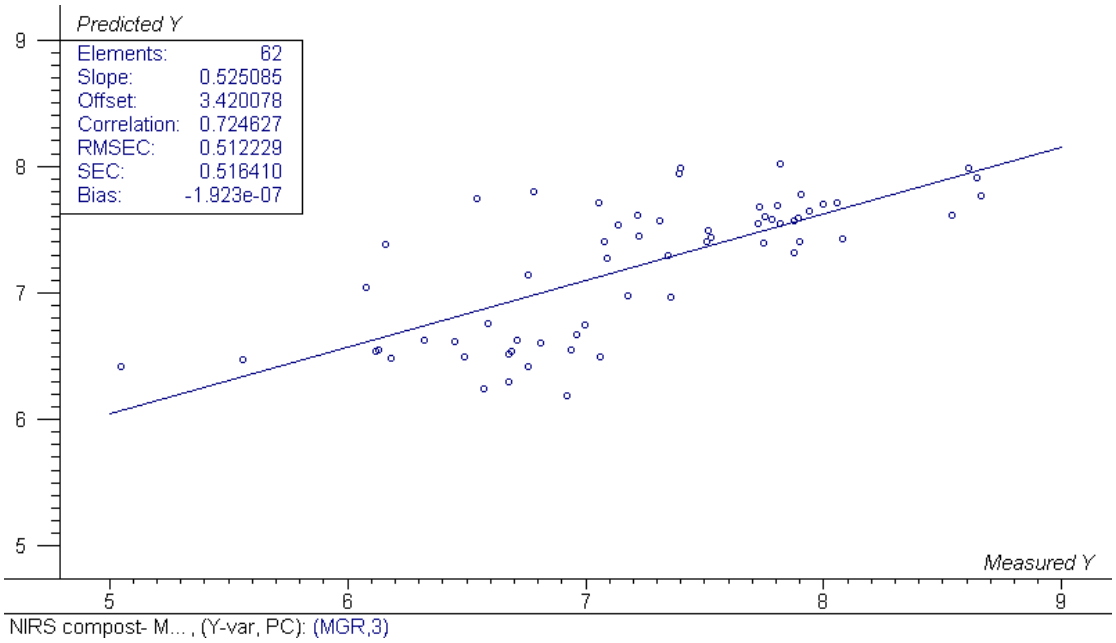


Figure 31C. Relationship between visible and NIR spectra of compost and pH of spawn-run compost showing an $R^2$ of 0.70.

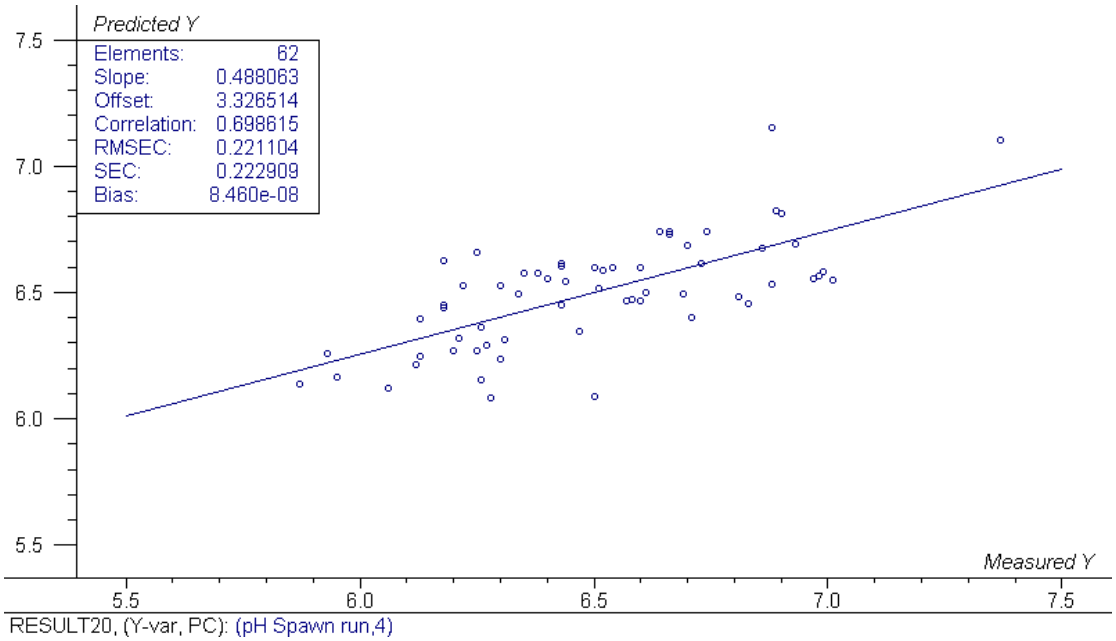
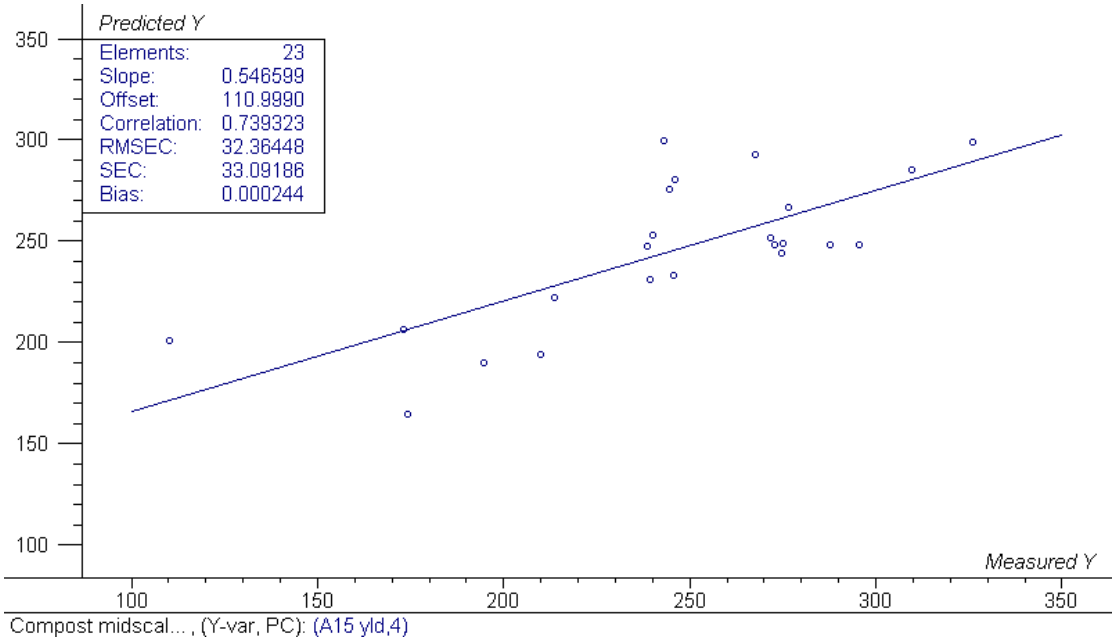



Figure 31D. Relationship between DTG parameters listed and measured yield from the 23 windrow scale compost samples.



## Discussion

The relationship between laccase in compost extracts and mushroom mycelial growth agrees with the results of Matcham et al (1985) who found a similar relationship for mushroom mycelium growing on rye grain spawn. These results further show that laccase enzyme production during spawn-running can be used to assess the quality of colonised Phase III compost. However, similar relationships have been found between the fall in compost pH during spawn-run and mycelial growth rate and mushroom yield. This is due to the oxalic acid produced by mushroom mycelium during spawn-run (Flegg & Wood, 1985). Compost with a greater amount of mushroom mycelium therefore has a greater amount of oxalic acid, which results in a larger fall in compost pH. Since pH is much easier to measure than laccase enzyme in compost extracts, the former method is more practically usable.

Cooke & Flegg (1962) stated that a spawn rate of 0.5% w/w was optimal, while van Gils (1988) recommends a lower rate of 0.3% w/w. The results of this work show that a spawn rate of 0.5% w/w is sub-optimal in terms of spawn-run and mushroom yield. A higher spawn rate of 0.8% w/w increased average mushroom yield by 15 kg / tonne compost and reduced spawn-run time by 1 day. However, addition of spawn by top-spawning or alternatives to rye grain were found to give no benefits.

Altering the nutritional status of the compost by changing the nitrogen source or by addition of supplements did not result in an improved spawn-run. However, the relationship between the results of NIR analysis of composts and mycelial growth rate demonstrates that the compost can have a significant effect on spawn-running time. The Vis-NIR analysis can detect changes in fibre fractions, especially availability of hemicellulose. Amorphous hemicellulose can provide an available nutrient source to the mushroom mycelium. In addition cellulose and nitrogen-lignin fractions are also detected by Vis-NIR spectra.

On commercial sites generally, high levels of compaction are used to increase the quantity of compost that can be filled into a tray, or the amount of compost that can be pressed into a block. The adverse effect of excessive compaction of compost in trays on spawn-run and mushroom yield demonstrated in these experiments means that the level of compaction used commercially should be carefully considered.

## Conclusions Part 2

1. Increases in compost temperature during spawn-running were highly dependent on compost moisture: drier composts resulted in higher temperatures during spawn-running.
2. The extension growth rate of mushroom mycelium on Phase II compost was related to a fall in pH of the compost; however, a faster mycelial colonisation of compost (spawn-run) did not necessarily correspond with a higher mushroom yield.
3. The cropping performance of commercial composts was related to the amount of laccase enzyme at the end of spawn-running and the fall in compost pH during spawn-running.
4. The amount of laccase enzyme produced by mushroom mycelium increased to day 16 of spawn-run, and in some composts increased further to day 21. Compost pH declined in all the composts to day 21.
5. Fall in compost pH may be a good indicator of the rate of spawn-run but the amount of laccase enzyme in compost extracts is a better indicator of the quality of spawn-run.
6. Increasing the time of pressing of Phase II compost from 4 to 12 seconds retarded spawn-running and depressed mushroom yield. However, pressing for 4 seconds had no significant effect on spawn-running or yield compared with unpressed compost.
8. A higher spawn rate (0.8% w/w) resulted in greater laccase content, greater compost pH drop and higher mushroom yield than a lower rate (0.5% w/w).
9. Standard rye and millet grain spawn formulations resulted in a faster spawn-run and higher mushroom yield than a proprietary product 'Speedy Spawn'.
10. None of supplements (ProMycel, Natural Gold and Lambert T6) had a significant effect on spawn-running (except for an early increase in compost temperature) or yield.
11. Replacing 30% of poultry manure nitrogen in compost with ammonium sulphate (with lime or green waste compost) retarded spawn-run and reduced mushroom yield.
12. There were fairly close correlation between the vis-NIR spectral analysis of the compost samples prepared from different straw batches and subsequent mycelial growth rate and with fall in compost pH during spawn-run. This could be due to the availability of compost nutrients to mushroom mycelium, particularly amorphous hemicellulose and cellulose.
13. There were no relationships between the vis-NIR spectral analysis of the composts and subsequent mushroom yield. However, the results from DTG analysis of compost samples did correlate with their subsequent mushroom yield.

**Part 3. Effects of recycled water analysis, treatment and application on odours and compost.**

**Introduction**

The mushroom industry is under environmetal pressure due to its odour emissions. A previous HortLINK project showed that sulphides, produced from sulphur-containing proteins in poultry manure under anaerobic conditions, were the main cause of the odours from mushroom composting (Noble et al 2001). Methods for detecting sulphides on mushroom composting sites were also developed (Noble et al 2001). Substitution of poultry manure with low odour nitrogen sources and an increased use of aeration resulted in significant reductions in odour and sulphide levels from mushroom composting (Noble et al 2001). Research in Canada has shown that recycled water can contribute significantly to site odour levels and increase odours from susbequent composting (Duns et al 1999). If environmentally unacceptable levels of smell are to be eliminated, the use of recycled run-off or 'goody' water and watering management needs to be improved. Determining the relevant chemical and microbial properties of recycled water, and influences of water treatment, could lead to a significant reduction in the odours from mushroom composting and water storage faclities. Recycled water quality and management may also have effects on the efficiency of the composting process and subsequent mushroom growth. Anaerobic run-off liquor can also have a deleterious effect on subsequent mushroom growth (Heineman & Engels 1953).

If the quality of goody water is to be monitored and improved, the industry needs methods that can be used on-site for measuring properties of goody water that relate to its potential for creating nuisance odours. It is well established that aeration of goody water and liquid organic wastes can diminish the production of anaerobic odours. However, there are a variety of aeration systems and configurations that are available and used on composting sites and other applications such sewage treatment works. It is unclear as to which system is best suited for goody water treatment.

**Objectives**

To test the following hypotheses in relation to odours from recycled water:

- Are odours produced by recycled water related to its chemical and microbial composition?
- Does the odour produced by recycled water relate to its composition in terms of chemical compounds or the microbial community structure determined from phospholipid fatty acid (PLFA) analysis?
- Can the chemical and microbial composition of recycled water be modified so that it is less detrimental in terms of odour and sulphide emissions and mushroom growth?

**Materials and Methods**

Collection of goody water samples

Goody water was obtained from the storage pits or tanks of 14 compost yards. Samples were

obtained either by immersing a 9 litre bucket to the full depth of the storage pit, or from the recirculation pipe which drew liquid from the bottom of the pit or tank. Common ingredients for the compost prepared on the sites were wheat straw, poultry manure and gypsum. Horse manure was used on four of the sites and urea was used on six of the sites. The water was stored in 3 litre plastic bottles for up to 2 weeks at 2 C before analysis of the liquid (after returning to room temperature) and of freeze dried material. Samples (1 litre) were freeze-dried for 1 week and the weight of the resulting dry matter was determined. The dried samples were then used for further analysis.

Analysis of goody water

An assessment of goody water colour was made:
0 Clear
1 Very pale
2 Pale brown
3 Brown
4 Dark brown
5 Very dark brown
6 Near black

*Redox potential*

Redox indicator electrodes measure the ratio of oxidised species to reduced species in a solution. A WaterWatch System 2600 (EuaxSys (UK) Ltd, Camelford, Cornwall) probe and data logger were used for measuring redox potential. Goody water (1 litre) was filled in a beaker at room temperature, the electrode was immersed in the water and the redox potential measured after 2 min.

*Dissolved oxygen concentration*

Dissolved oxygen was measured with a polarized probe meter (Hanna Instruments). Goody water (1 litre) at room temperature was filled in a beaker and stirred with a magnetic stirrer. The probe was immersed in the water and the dissolved oxygen was measured when a stable reading was obtained after 2 min.

*Conductivity and pH*

Electrodes were used to measure the conductivity and pH of goody water at room temperature.

*Analysis of sulphate reducing bacteria (SRB)*

The populations of sulphite reducing *Clostridia* spp. (an indicator species of sulphite reducing

bacteria) were determined by counting the colony forming units (cfu) using selective media.

*Analysis of Phospholipid fatty acids*

*Extraction.* Freeze-dried goody water (0.5 g) fresh was placed in a 50 ml tube with a teflon-lined screw cap. Citrate buffer was added to make the water content of sample and buffer 1.5 ml. $CHCl_3$ (1.9 ml), 3.8 ml MeOH and 2 ml Bligh and Dye ($CHCl_3$:MeOH:buffer) were added, vortexed and left to separate for 2 hours. The mixture was then vortexed and centrifuged (ca 2500 rpm for 10 min). The supernatant (using pipette and handler) was transferred into another test tube and washed (vortex, centrifuge and transfer as above) with 2.5 ml Bligh and Dye. The phases were split by adding 3.1 ml $CHCl_3$ and 3.1 ml buffer. The mixture was again vortexed for 1 min and left overnight to separate. 1 ml of the lower organic phase was transferred to a small test-tube and evaporated under a stream of nitrogen gas

*Lipid fractionation.* The lipids were separated into different classes with increasing polarity: neutral lipids (including hydrocarbons, free fatty acids and sterols), glycolipids and polar lipids (phospholipids). Silicic acid was activated at $120^0$C for 1 h. Funnels were washed twice with $CHCl_3$:MeOH, and the column packed by placing a small ball of glass wool (washed) in the 'bottom'. The funnel was washed through with $CHCl_3$ and 0.5g silicic acid 'dissolved' in $CHCl_3$ added. The dry lipid material was dissolved in 3x100µl $CHCl_3$ and transfered to column. Neutral lipids were eluted with 5 ml $CHCl_3$, glycolipids with 20 ml acetone, and polar lipids with 5 ml MeOH. The MeOH eluate was collected in small tubes and evaporated down under nitrogen.

*Mild alkaline methanolysis.* The samples were dissolved in 1 ml MeOH:toluene. 1 ml 0.2 M KOH was added and incubated in water bath at $37^0$C for 15 min. 2 ml hexane:$CHCl_3$ (4:1), 0.3 ml 1M acetic acid and 2 ml water were added. The mixture was vortexed for 1 min and centrifuged for 5 min. The upper organic phase was transferred to a new test tube. The organic phase was evaporated under a stream of nitrogen and the sample was stored in a freezer at –20°C. The mixture was dissolved in 100 ml hexane for analysis in GC-MS using a wax column and the bacterial fatty acids standard for reference retention time and the C19:0 for quantitation.

*Amino acid analysis*

The analyses were conducted by JEOL (UK) Ltd. Freeze-dried goody water samples were hydrolysed to produce amino acids from the protein content. The amino acid composition was then separated by HPLC and the amino acids identified by mass spectrometry.

Analysis of air surrounding goody water

*Odour sampling*

Odour samples were collected in 20 L Teflon bags as follows:

(i) from 1 litre Duran bottles containing 100 mL of goody water

(ii) from goody water pits or tanks on composting sites .

Two replicates were made for each sample. The odour samples were then transported to IGER North Wyke and analysed within 24 hours.

*Olfactometry (Odour panel analysis)*

Odour concentration (OC) was determined by an odour panel using dilution olfactometry. A dynamic dilution olfactometer (type Olfactomat "C", Project Research, Amsterdam) was used according to recommendations in van den Berg (1992), i.e. a forced choice type presentation where six panellists were required to choose between two sniffing ports, one containing odourless air, and the other diluted, odorous air. Threshold values, at which 50% of the panel could just detect an odour, were determined and odour concentration (OC) expressed as Odour Units $m^{-3}$ (OU $m^{-3}$) air. A range of six dilutions was presented to the panellists in steps of ascending concentrations, each differing from the next by a factor of two and each range being presented twice. OC was calculated according to the Dravneiks and Prokop (1975) method. Measurements of the sensitivity of the odour panellists for each set of OC measurements was performed with 198.2 mg $m^{-3}$ (60 ppm) butan-1-ol in nitrogen.

Odour panellists also made an assessment of odour intensity of the samples according to Burton et al (1998). Duran bottles (1 litre) containing 100 mL samples of goody water were presented to the panellists who were required to indicate the subjective strength of the odour, according to the following scale:

0 No odour
1 Very faint odour
2 Faint odour
3 Distinct odour
4 Strong odour
5 Very strong odour
6 Extremely strong odour.

*Gas Chromatography-Mass Spectrometry (GC-MS) analysis*

Volatile compounds were preconcentrated from a 600 ml odour samples by adsorbtion onto silica (Orbo 52, Supelco Inc., Supelco Park, Bellefonte, PA, 16823-0048 USA) and carbon (Orbo 32) based adsorbents. The concentrated odorants were then thermally desorbed from the adsorbents into the GC-MS system for identification and quantification. Chromatographic retention time and mass spectral matching were used to confirm odorant identity. Quantification was performed by desorbing 8 litres of a standard odour identified in the preconcentrated headspace, from the adsorbent.

A Hewlett Packard (hp) (hp Ltd, Heathside Park Road, Cheadle Heath, Stockport, Cheshire, UK) GC-MS system consisting of a 5890 II Series gas chromatograph and a 5972A mass selective detector (MSD II) was used for analysis. A 25 m fused silica (cross linked methyl siloxane) hp-1 column with an internal diameter (id) of 0.2 mm and a 0.34 μm film with a 1 m deactivated fused silica guard column (0.25mm id) were used. The flow rate of the helium the eluting gas was 0.75 ml $min^{-1}$. The Optic temperature programmable injector (Ai Cambridge Ltd, Pampisford, Cambridge, UK) was used to desorb headspace samples from the adsorbents and was initially set at 30˚C and heated to 16˚C $s^{-1}$ for 1 min. An electronic pressure controller was used to offset peak pressure broadening with increasing GC column temperature. The GC oven conditions were an initial temperature of 40˚C, then to 220˚C at 15˚C $min^{-1}$ and remaining at 220˚C for 1 min. The GC-MS interface was at 280˚C. The mass spectrometer scanned from 35 to 250 mass units every 0.2 s to give responses in the ng range.

Volatile organic compounds (VOCs) detected by the mass spectrometer were identified using a probability based matching algorithm and a NIST mass spectral library. Compounds were declared unknown if their matching probability was less than 80 (100 being a perfect match).

*Gas detector tubes*

A Dräger Accuro bellows pump (Drägerwerek, Lübeck, Germany) was used in conjunction with appropriate detector tubes: acetic acid (6722101), carbon disulphide, $CS_2$ (8101891), DMS (6728451), $H_2S$ (8101991 and 8101831), and mercaptan (thiols) (6728981). Detector tubes were used on Teflon bags and for measuring the headspace of Duran bottles containing goody water samples (see above) at the same time as odour measurements. Two replicate measurements were made for each sampling.

*Electronic sulphide analyser*

A method was developed with Casella eti (Monitor Europe) for measuring sulphides in odour samples using a pulsed fluorescence sulphur dioxide analyser. A ML9850B sulphur dioxide analyser, capable of measuring sulphur dioxide in the range 0-500ppb was connected to a thermal oxidiser. The thermal oxidiser converted sulphides into sulphur dioxide which could then be measured.

Effect of gypsum on goody water odour

Gypsum was added to goody water from two sites (6 and 9) at 0, 5, 10 and 20 g /litre and stored at room temperature for 2 months in 2 litre polypropylene bottles. At the end of this

period, the redox potential, pH and volatile sulphides of the liquids were determined, and an assessment of odour intensity made as described above.

Effect of goody water on composting odours and mushroom growth

Bench-scale composting flasks were set up as described in Part 1 on composting tests on straw types. The same compost ingredients were used as previously described (600 g wheat straw, cv Hereward, 200 g sieved poultry manure, 25 g gypsum) with 4 g urea as an additional nitrogen source. Sixteen flask composts were prepared. Two flasks were prepared with fresh water (2L); the other fourteen composts were prepared with 2L of goody water samples collected from 13 composting sites (1 replicate sample). The moisture content at filling of the flasks was 76 – 78% w/w. The oxygen concentration in the flasks was maintained at 3-5% by aeration.

One day after composting in the flask started, the exhaust air from each flask was sampled for hydrogen sulphide, dimethyl sulphide and ammonia using gas detector tubes. The air was also assessed for odour using the same panellists and 0 – 6 odour scale that were used for assessing goody water samples. Composting was completed when the flasks were clear of ammonia, 9 - 11 days after they were filled. Compost from each flask (1.6 kg) was spawned with the strain A15 and cropped in pots as described in Part 1. Mycelial growth rate in samples of the compost was determined using growth tubes as described in Part 2.

**Results**

Goody water storage

Dimensions and other details of goody water pits, and method of sampling are shown in Table 21. Some form of screening of solid matter from the recycled water was conducted on eight of the 14 sites, and nine of the storage pits had some form of aeration. This usually consisted of submerged pipes, submerged propeller with pipes, or recirculation of liquor from the bottom of the pit or storage tank, but on site 13 consisted of a surface aerator.

Effects of aeration

There were wide ranges in pH, electrical conductivity and dry matter content of goody water samples from different sites (Table 22). Sites with aeration had lower dry matter contents than sites without aeration (Table 22). This may have had a greater effect on the quality of the goody water than the oxygenation caused by aeration. Aerated sites had higher dissolved oxygen levels, less negative redox potentials, and lower sulphides, sulphate reducing bacteria and odour levels than unaerated sites (Table 22). Surface aeration (site 13) was ineffective in lowering sulphides and odour levels.

Effects of composting site

There was a reduction in the odour intensity of goody water samples taken from some composting sites 1 and 3 during the 3 years of the project (Fig. 32). However, differences in odour intensity between sites remained fairly consistent. Sites 10 and 11 were usually the most odorous whereas sites 5 and 8 were usually the least odorous.

Indicators of goody water odour

The goody water samples ranged in colour from pale brown to near black, but colour was unrelated to odour intensity.

*Chemical and microbial properties*

There was no effect of goody water pH on odour. There was a relationship between dissolved oxygen concentration and redox potential (Fig.33). However most of the samples had dissolved oxygen concentrations of less than 1 mg/L, although there were differences in redox potential between these samples. There were relationships between the odour intensity of goody water and its redox potential (Fig. 34), dry matter content (Fig. 35) and electrical conductivity (Fig. 36). Dry matter content and electrical conductivity of the samples were highly correlated (Fig.37); the latter measurement can be obtained instantly and does not require freeze-drying of the sample.

Analysis of the dry matter of goody water samples by DGT, showed two distinct weight loss fraction as the temperature was increased. The first weight loss fraction was observed between 150-400°C and probably consisted of holocellulose, carbohydrates, lipids and low molecular weight organic fractions. A second major weight loss fraction was noted between 400-700°C, containing larger molecular weight cross-linked compounds, most likely associated with compost humus fraction and poultry manure. Both of these weight loss fractions accounted for the organic content of samples, typically 45-55%. The dimensions of the second weight loss fraction correlated with odour intensity (Fig. 38). There was no relationship between the residue (ash) content of the samples and their odour intensity.

The predominant microbes in the goody water were gram+ve bacteria, followed by gram-ve bacteria, such as sulphate reducing bacteria e.g. *Clostridia* spp. There was no relationship between the population of sulphate reducing bacteria (SRB) and odour intensity. The average population of SRB was lower in aerated goody water than in aerated samples (Table 23). The total population and percentage of Gram +ve bacteria, determined using PLFA analysis, were higher in non-aerated goody water than in aerated goody water (Figs. 39 and

40). The populations and percentages of different micro-organisms, including sulphate reducing bacteria, were not an indicator of goody water odour.

Samples of goody water with high sulphide levels had high odour intensities (Fig. 41). A large number of chemical compounds were detected in freeze dried samples of goody water. These compounds included fatty acids (e.g. acetic acid), sulphur containing compounds, indole, amines, ketones and aldehydes (Table 20) and amino acids (Table 21). Although sulphides explained most of the odour intensity of goody water odour, a large number of odorous compounds of various types therefore contributed to the odour of goody water.

Fig. 32 Odour intensity of goody water on 12 sites on 4 dates

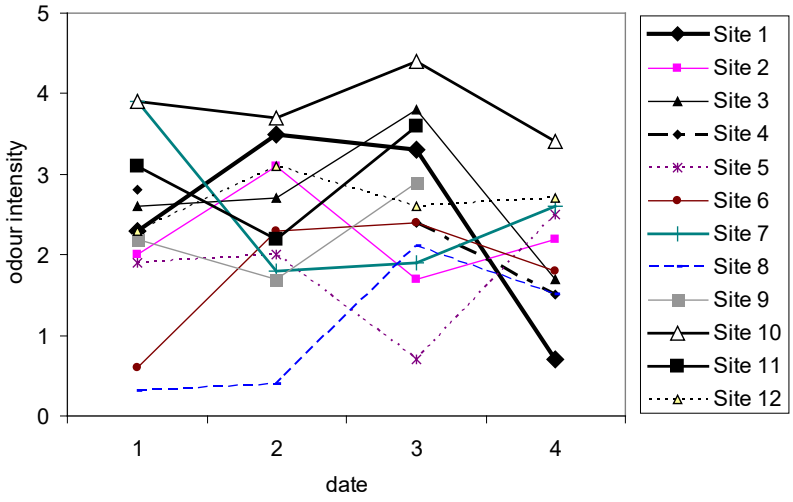



Fig. 33 Relationship between dissolved oxygen and redox potential of goody water samples

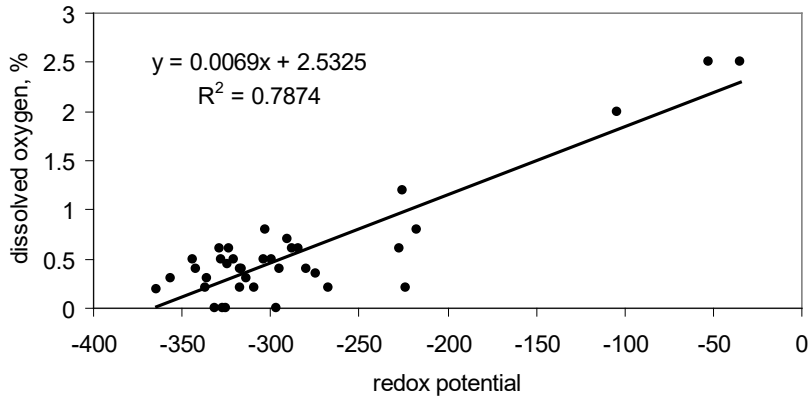


Fig. 34 Goody water redox potential and odour intensity

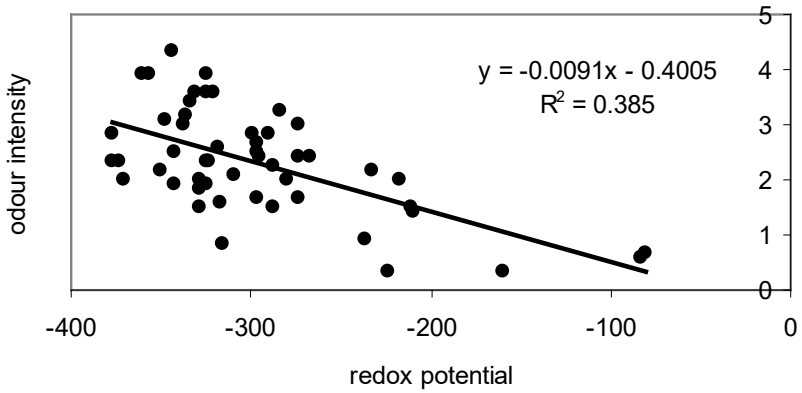



Fig. 35 Goody water dry matter content (log transformed) and odour intensity

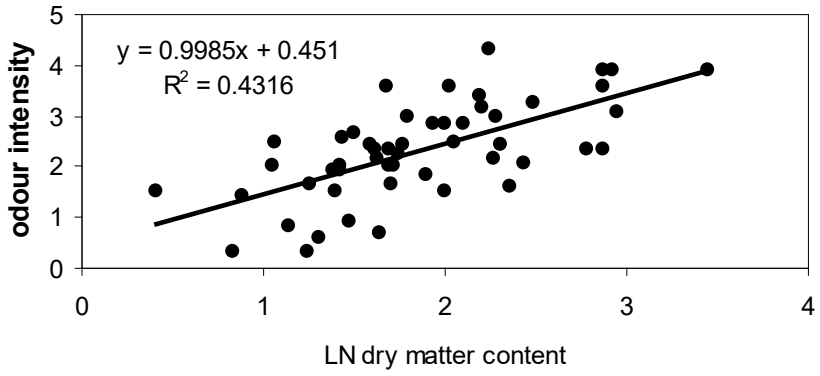


Fig. 36 Goody water electrical conductivity and odour intensity

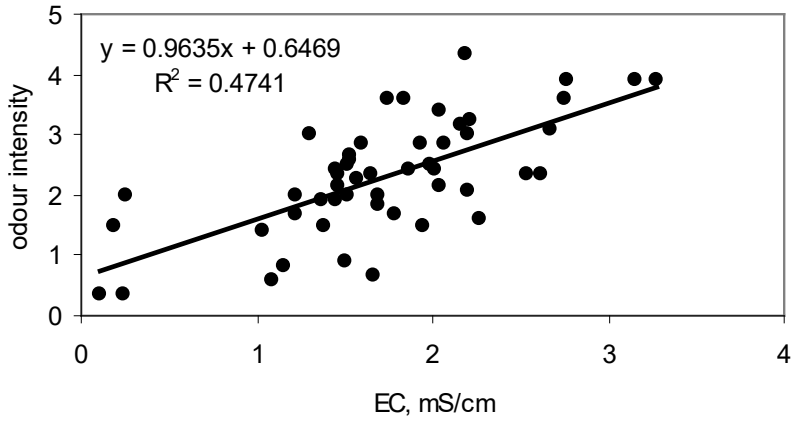
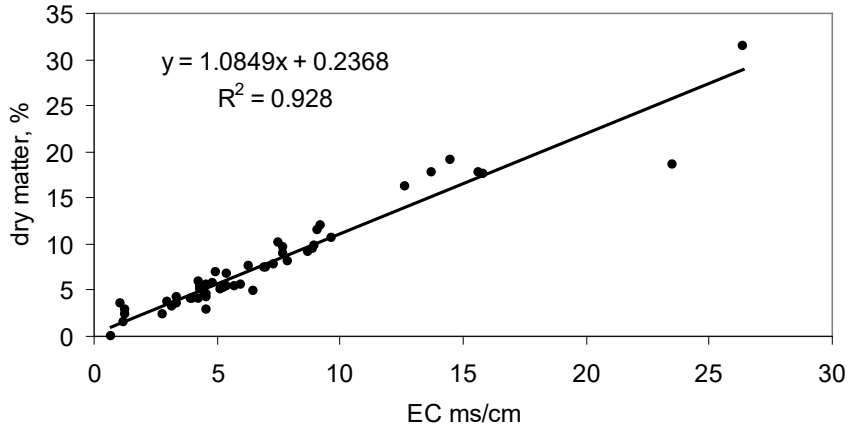



Fig. 37 Relationship between electrical conductivity and dry matter content of goody water samples




Air samples from goody pits

Analysis of air samples from goody water pits showed that this could be a significant source of odour from composting sites. There was a close relationship between the concentration and intensity of goody water odour samples (Fig. 42). Measurements of sulphides in air samples taken from goody water pits, using gas detector tubes (Fig. 43), GC-MS (Fig. 44) or the electronic instrument (Fig. 45) explained some, but not all of the odour concentration.

Hydrogen sulphide and dimethyl sulphide could both be detected with gas detector tubes, but not acetic acid, which could only be detected by GC-MS. Several other volatile odorous sulphur-containing compounds were identified in the air surrounding goody pits using gas chromatography-mass spectrometry (GC-MS) (Table 23). A number of other odorous compounds were identified in these air samples using GC-MS, including alcohols, ketones and

fatty acids (Table 24). Analysis of amino acids demonstrated significant levels of the sulphur containing amino acids, cystiene (Cys) and methionine (Met). These are leached from poultry manure from the composting sites and result in volatile odorous sulphur compounds such as hydrogen sulphide and dimethyl sulphide.

Effect of gypsum on goody water odour

At the end of the storage period (2 months), there was no significant difference in redox potential, odour or sulphides between goody water with and without added gypsum. The sulphate in the gypsum therefore did not appear to be a significant factor in the production of volatile sulphides from goody water. However, it cannot be assumed that this will be the case in large storage tanks over longer periods of time. The prevention of gypsum being washed into the goody water storage tank must therefore be regarded as good practice.

Fig. 38 Height of 2nd peak in DGT analysis and odour intensity of goody water samples

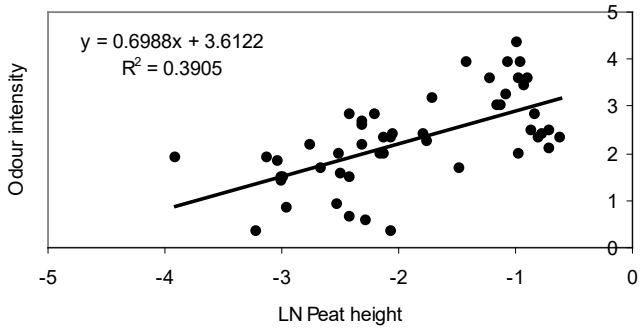



Fig. 39 Effect of goody water aeration on poulation of different microbes using PLFA analsysis

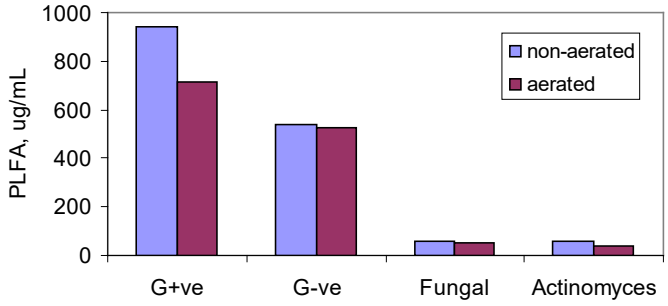


Fig. 40 Effect of goody water aeration on the percenatge of different microbes determined by PLFA analysis

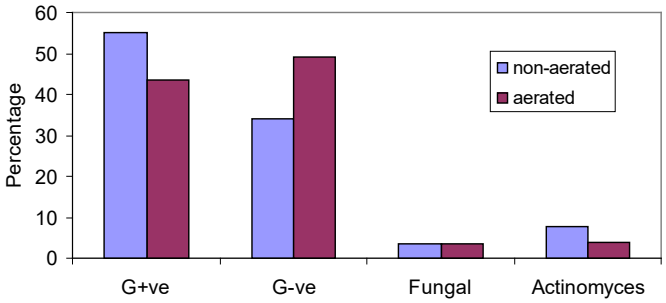



Fig. 41 Sulphides in goody water headspace (log transformed) and odour intensity

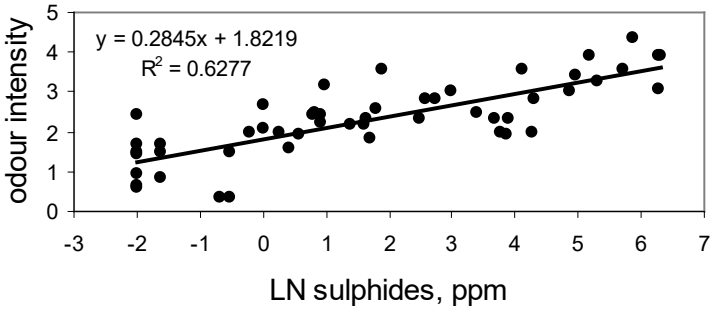



Fig. 42 Relationship between goody water odour intensity and concentration (log transformed)

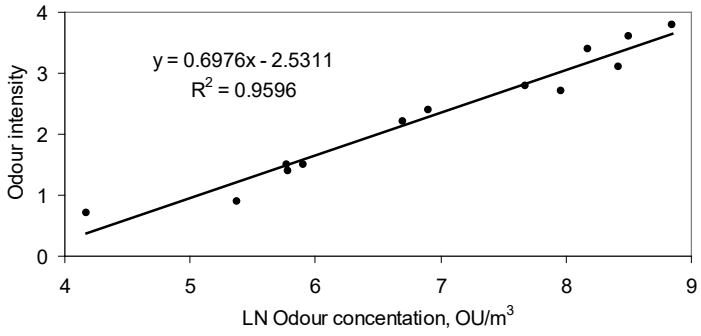



Fig. 43 relationship between sulphides measured with detector tubes and odour concentration

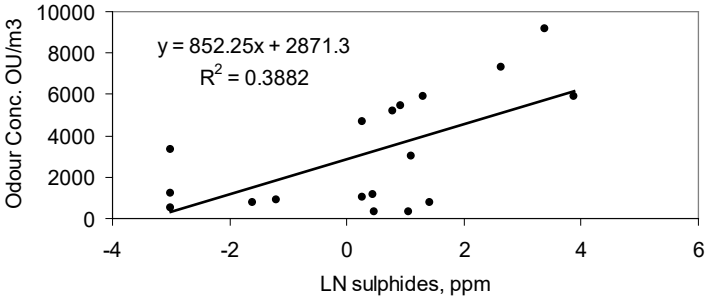


Fig. 44 Relationship between sulphides measured with GC-MS and odour concentration of goody water air

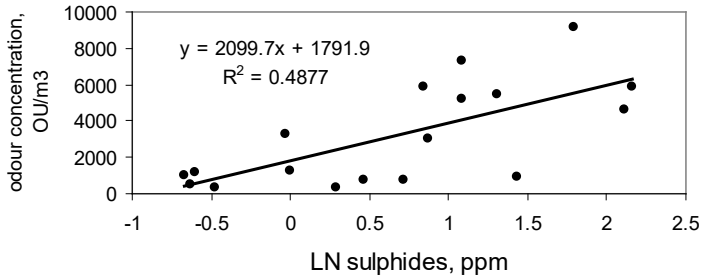



Fig. 45 Relationship between sulphides measured with ME electronic detector and odour concentration

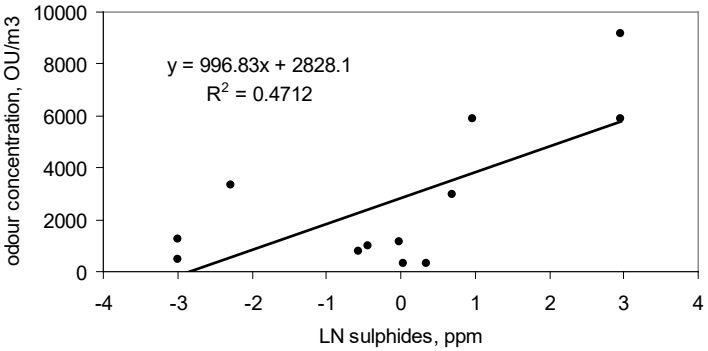



Table 21. Details of goody water pits on different sites

| Site | Depth m | Volume m3 | Screened | Aeration |
|---|---|---|---|---|
| 1 | 2 | 150 | yes | yes |
| 2 | 2 | 80 | no | yes |
| 3 | 4 | 450 | yes | yes |
| 4 | 1.5 | 50 | no | no |
| 5 | 4 | 450 | yes | yes |
| 6 | 4 | 450 | yes | yes |
| 7 | 4 | 200 | no | yes |
| 8 | 2 | 40 | yes | yes |
| 9 | 1.3 | 517 | yes | yes |
| 10 | 4 | 450 | no | no |
| 11 | 2 | 240 | yes | no |
| 12 | 4 | 450 | no | no |
| 13 | 4 | 450 | yes | yes |
| 14 | 4 | 450 | no | no |

Table 22. Average analyses of goody water from aerated and non-aerated storage pits on different sites

| Aeration | pH | Elec Cond mS/cm | Redox | Dissolved oxygen % | DM g/L | SRB cfu/ml | $H_2S$ ppm | DMS ppm | Odour 1-4 scale |
|---|---|---|---|---|---|---|---|---|---|
| non-aerated | 7.27 | 10.11 | -334 | 0.34 | 11.2 | 81 | 127 | 40 | 2.97 |
| aerated | 7.26 | 5.43 | -277 | 0.71 | 6.2 | 26 | 16 | 3 | 1.99 |

Each value is the mean of four replicate visits to 5 non-aerated or 9 aerated sites
SRB: sulphate reducing bacteria, H2S: hydrogen sulphide, DMS: dimethyl sulphide

Table 23. GC-MS analysis of air above goody water pits

| | detected in samples: | min ppb | max ppb |
|---|---|---|---|
| Carbon disulfide | 5 | 0.2 | 1.7 |
| Dimethyl sulphide | 4 | 0.3 | 1.35 |
| Disulfide, dimethyl | 5 | 0.23 | 3.65 |
| dimethyl trisulphide | 4 | 0.1 | 0.45 |
| Ethanol | 4 | 0.93 | 18 |
| Acetone | 4 | 1.15 | 24.93 |
| Isopropylalcohol | 3 | 0.08 | 1.5 |
| Butanal | 4 | 0.27 | 0.87 |
| acetic acid | 4 | 17.5 | 22.64 |
| propanoic acid | 3 | 0.31 | 5 |
| butanoic acid | 3 | 1.26 | 24.4 |
| MEK | 3 | 0.73 | 100.75 |
| pentane, 3-methyl | 2 | 0.32 | 91.8 |
| pentane, 2-methyl | 2 | 0.22 | 19.05 |

Table 24. Compounds detected in 3 or more samples of freeze-dried goody water

| **Compound name** | **Detected in:** |
|---|---|
| Acetaldehyde | 3 |
| Acetamide | 9 |
| Acetamide, N-(aminocarbonyl)- | 6 |
| Acetic acid | 5 |
| Acetic acid, anhydride with formic acid | 4 |
| Acetic acid, hydrazide | 3 |
| Acetic acid, methyl ester | 3 |
| Acetic acid, oxo- | 5 |
| 2-Acetyl-5-methylfuran | 6 |
| l-Alanine ethylamide,(S)- | 6 |
| dl-Alanine ethyl ester | 3 |
| m-Aminophenylacetylene | 10 |
| Ammonium acetate | 6 |
| (2-Aziridinylethyl)amine | 8 |

| | |
|---|---|
| Benzaldehyde, 3-methyl- | 9 |
| Benzaldehyde, 4-methyl- | 5 |
| Benzene, 1-isocyano-2-methyl- | 9 |
| Benzofuran, 2,3-dihydro- | 9 |
| Butanoic acid | 5 |
| Butanoic acid, 2-methyl- | 4 |
| Butanoic acid, 4-chloro- | 4 |
| Carbon dioxide | 7 |
| 1,3,2-Dioxaborolane, 2,4-diethyl- | 3 |
| Ethanimidic acid, ethyl ester | 7 |
| Ethylenediamine | 3 |
| Ethylene oxide | 3 |
| Hexanoic acid | 3 |
| Hexanoic acid, 2-methyl- | 3 |
| Hydrazine, ethyl- | 5 |
| Hydrazine, 1,2-dimethyl- | 6 |
| Hydrazinecarboxamide | 3 |
| Indole | 10 |
| Indolizine | 10 |
| Mequinol | 6 |
| Methanethinol | 4 |
| Monomethyl carbonotrithioate | 3 |
| Pentanoic acid | 4 |
| Pentanoic acid, 3-methyl- | 3 |
| Phenol, 2-methoxy- | 5 |
| 2-Piperidinone | 3 |
| Propanediamide | 5 |
| 1-Pyrrolidinecarboxaldehyde | 3 |
| 2-Pyrrolidinone, 4-methyl- | 3 |
| 5H-1-Pyrindine | 10 |

Table 25. Amino acids detected in goody water samples from different sites

| | Concentration, µg/g | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Compound | 23.6.04 Site 1 | 6.7.04 Site 2 | 6.7.04 Site 3 | 6.7.04 Site 4 | 14.7.04 Site 5 | 14.7.04 Site 6 | 14.7.04 Site 7 | 13.7.04 Site 8 | 27.4.04 Site 1 | 28.4.04 Site 9 |
| P-Ser | 1518.231 | 1885.146 | 1343.960 | 1208.480 | 2351.892 | 1936.903 | 1402.412 | 262.935 | 1951.562 | 1124.577 |
| Tau | | | 37.941 | | 161.265 | 136.612 | 66.410 | 34.628 | | 36.772 |
| PEA | | | | | | 76.542 | 37.652 | 1.670 | 44.686 | 3.773 |
| Urea | 40.389 | 474.818 | | 42.014 | 1466.666 | 68.415 | 49.249 | 15.775 | 64.118 | 49.562 |
| Asp | | 14.614 | 2.081 | 0.582 | | 2.956 | 1.558 | 3.180 | 1.250 | 2.119 |
| Thr | | | 4.182 | 1.523 | | 3.464 | 95.059 | 3.441 | 2.213 | 1.616 |
| Ser | 38.405 | | 80.222 | | | | | 4.062 | 29.596 | |
| Glu | 18.039 | | 45.820 | 4.529 | 3.973 | 90.416 | 31.415 | 63.596 | 14.792 | 23.881 |
| Sar | | | 19.980 | | | | | | | |
| AAA | | | 6.890 | | | | | | | |
| Gly | 5.466 | | 10.075 | 3.180 | | 12.377 | 4.127 | 9.047 | 9.077 | 18.000 |
| Ala | 7.655 | | 14.096 | 8.070 | | 24.852 | 24.705 | 17.066 | 7.412 | 26.446 |
| Cit | 2.498 | | 11.423 | 1.375 | | 4.461 | 5.949 | 2.658 | 12.986 | 13.957 |
| a-ABA | 0.323 | | 2.896 | | | 1.399 | 5.135 | 1.051 | | 5.188 |
| Val | 7.987 | | 36.570 | 5.284 | | 10.330 | 29.180 | 4.086 | 12.453 | 17.793 |
| Cys | | | 39.829 | | | 4.972 | 21.543 | | 2.621 | 6.591 |
| Met | 4.528 | | 51.178 | | | 3.436 | 27.463 | | 5.573 | 5.782 |
| Cysta | | | 4.697 | | | | | | | |
| Ileu | 2.992 | | 3.637 | 1.385 | | 3.219 | 6.957 | | 5.838 | 5.753 |
| Leu | 4.933 | | 18.739 | 2.939 | | 10.092 | 12.841 | 2.477 | 8.005 | 8.458 |
| Tyr | 3.823 | | 13.712 | | | 13.383 | 17.154 | 4.798 | 5.918 | 4.713 |
| Phe | 1.560 | 1.101 | 3.139 | | | 1.949 | | 1.054 | 3.748 | 2.446 |
| b-Ala | | | 33.386 | | | 5.944 | 17.836 | 8.786 | | 2.649 |
| b-ABA | | | 26.078 | | | | 46.603 | | | 8.850 |
| GABA | 3.744 | | 12.875 | 2.481 | 6.329 | 7.040 | 81.103 | 4.162 | 3.182 | 34.396 |
| MEA | 2.973 | | 4.044 | 9.755 | | 3.526 | | | 4.658 | 18.460 |
| NH3 | 1148.794 | 704.914 | 4280.720 | 2603.378 | 8316.941 | 1309.618 | 13969.184 | 4152.484 | 990.017 | 1973.653 |
| Hylys | | | 7.864 | | | | 57.170 | | | 9.477 |
| Orn | 1.667 | | 28.194 | | | 11.603 | 27.563 | 4.141 | 1.467 | 3.239 |
| His | | | 7.761 | | | 2.871 | | | 1.198 | |
| Lys | 9.959 | 3.112 | 88.548 | 4.886 | 16.579 | 51.248 | 93.580 | 24.409 | 7.335 | 15.058 |
| Arg | 4.013 | 4.181 | 32.827 | 3.500 | 6.254 | 27.095 | 15.641 | 6.062 | 8.096 | 7.005 |

Effect of goody water on composting odours and mushroom growth

Analytical and odour details of the 13 samples of goody water and fresh water samples used in the flask composting tests are shown in Table 26. The results show that the water samples from different sites varied widely in terms of pH, EC, dry matter content and odour and sulphide emissions. Sulphide, ammonia and odour measurements from the flask air exhausts are shown in Table 27. These also show a wide range in values. However, the sulphides and odour emissions from composts did not correspond with the sulphide and odour emissions of the goody water that was used in the ingredients. For example, samples 7 and 10, which had high sulphides and strong odours also produced strong compost odours; however, so did sample 12, which did have a strong odour. Conversely, sample 11 which had high sulphides and a fairly strong odour did not produce strong odours or high sulphides during composting. The production of sulphides from the flasks therefore depended on the porosity of the compost, the flow of air through the material, and the development of anaerobic zones in the compost.

The mushroom mycelial growth and mushroom yield on composts prepared from different goody water samples is shown in Table 27. There was no significant relationship between the analysis of the goody water and either mycelial growth or yield from the subsequent compost that was produced.

Table 26. Chemical and odour analysis of water samples collected from 13 composting sites and fresh water used in flask composting tests.

| Site | pH | EC mS/cm | Redox mV | DM % | Odour | Sulphides ppm |
|---|---|---|---|---|---|---|
| 1 | 7.14 | 4.34 | -325 | 6.3 | 2.3 | 12 |
| 2 | 7.30 | 5.41 | -371 | 5.6 | 2.0 | 44 |
| 3 | 6.95 | 4.60 | -318 | 5.5 | 2.6 | 6 |
| 4 | 7.16 | 7.90 | -377 | 8.2 | 2.8 | 74 |
| 5 | 7.58 | 4.28 | -343 | 4.13 | 1.9 | 47 |
| 6 | 7.10 | 2.96 | -83 | 3.7 | 0.6 | 0 |
| 7 | 6.71 | 26.40 | -325 | 31.5 | 3.9 | 180 |
| 8 | 7.27 | 1.11 | -160 | 3.5 | 0.3 | 120 |
| 9 | 7.01 | 7.72 | -350 | 9.7 | 2.2 | 4 |
| 10 | 6.78 | 23.5 | -361 | 18.6 | 3.9 | 550 |
| 11 | 7.06 | 14.52 | -348 | 19.0 | 3.1 | 540 |
| 12 | 7.32 | 12.68 | -373 | 16.2 | 2.3 | 50 |
| 13 | 7.13 | 13.74 | -377 | 17.7 | 2.3 | 40 |
| Fresh water | 7.55 | 0.67 | 20 | 0.0 | 0.0 | 0 |

Table 27. Sulphide and odour analysis of composting emissions and subsequent mushroom mycelial growth and yield from composts prepared from goody water and fresh water used in flask composting tests.

| Site | $H_2S$ ppm | DMS ppm | Ammonia ppm | Odour | Mycelial growth, mm/day | Yield g/kg compost |
|---|---|---|---|---|---|---|
| 1 | 290 | 800 | 50 | 2.5 | 4.8 | 702 |
| 2 | 14 | 6 | 150 | 1.8 | 5.2 | 683 |
| 3 | 10 | 70 | 40 | 2.6 | 4.7 | 749 |
| 4 | 296 | 30 | 40 | 2.9 | 5.0 | 708 |
| 5 | 200 | 60 | 12 | 2.8 | 5.3 | 698 |
| 6 | 35 | 48 | 30 | 3.1 | 4.9 | 737 |
| 7 | 5000 | 5000 | 50 | 4.9 | 5.0 | 726 |
| 8 | 20 | 70 | 20 | 4.3 | 4.8 | 672 |
| 9 | 150 | 180 | 60 | 2.8 | 5.2 | 742 |
| 10 | 1000 | 4000 | 12 | 3.8 | 5.1 | 711 |
| 11 | 1 | 20 | 10 | 2.5 | 4.8 | 678 |
| 12 | 590 | 3200 | 30 | 2.2 | 5.0 | 784 |
| 13 | 20 | 60 | 90 | 3.8 | 5.0 | 663 |
| Fresh water | 4 | 16 | 10 | 1.9 | 4.9 | 731 |

**Discussion**

The results of this work agree with those of Duns et al (1999) which showed that recycled water may be a significant source of odour and sulphide emissions on mushroom composting sites. Odour concentrations of air from goody water storage areas ranged from 300 to 7000 OU/$m^3$ air. This compares with odour concentrations of 1000 to 100,000 OU/$m^3$ air for the pre-wet and Phase I composting areas (Noble et al 2001). Aeration of goody water by means of a submerged air supply was found to reduce the odour intensity, similar to the results found by Burton et al (1998) for piggery slurry. They also found that increasing the duration of aeration reduced the oxygen demand of the water (less negative redox potential) but did not affect pH. Similar to results for odours from pre-wet and Phase I mushroom composting areas (Noble et al 2001), the main cause of the odours were volatile sulphur compounds. Measurement of two of these compounds, hydrogen sulphide and dimethyl sulphide, again explained most of the odour intensity of the samples. However, this correlation was found to be less precise than the same relationship for air samples from composting areas, which indicates that a wider range of compounds (sulphur compounds and others) may be responsible for the odour of goody water.

The measurement of sulphides with gas detector tubes is satisfactory for sulphide levels exceeding 0.5 ppm. Similar to previous results for composting emissions (Noble et al 2001), a pulsed fluorescence $SO_2$ analyser was able to detect sulphides at less than 50 ppb in goody water air samples. This instrument could therefore be used for detecting low levels of sulphides from composting and goody water storage areas, both on and off site.

The dimensions of the second weight loss fraction in the thermogravimetric (DGT) analysis correlated with odour intensity. This may be due to odorous compounds in the samples (such as sulphides) being bound in the larger molecular weight compounds that are indicated by the second weight loss fraction.

The results of this work support the hypothesis that odour intensity of goody water relates to its chemical and biological composition. The odours are caused by the leaching of poultry manure into the recycled water supply, leading to the breakdown of sulphur containing amino acids by sulphide reducing bacteria under anaerobic conditions. This results in the emission of odorous sulphur compounds. The process can be prevented by washing less poultry manure out of the pre-wet area, by applying some of the poultry manure later in the composting process. The solid material in leached poultry manure can also be screened out of the goody water, but dissolved components will be retained in the leachate. Submerged aeration can prevent the anaerobic decomposition of the sulphur-containing proteins. The effects of these treatments on the quality of the goody water can easily be measured on-site by determining the electrical conductivity (which indicates dry matter content) and redox potential (which indicates oxygen demand).

There is no indication from this work that less odorous goody water leads to less odorous compost. Nor is there any indication that the growth of mushroom mycelium or mushroom yield is affected by goody water analytical parameters over a wide range of values. The main benefit from improving the quality of the goody water is therefore a direct effect of reducing odours from the goody water storage area and when the liquid is sprayed on to the compost and yard surfaces.

**Conclusions Part 3**

1. Aeration of goody water by submerged pipes, propeller type aerators, or continuous recirculation reduced odour and sulphide emissions. However, this effect was confounded by aerated goody water pits having a lower dry matter content.
2. Surface aeration of goody water pits was ineffective in reducing odour.

3. Redox potential was found to be better indication of the oxygen demand of goody water samples since a dissolved oxygen meter could not discriminate between several samples which had zero values, but differing redox potentials and odours.
4. Goody water odour was correlated with redox potential and dry matter content (or electrical conductivity). Dry matter content and electrical conductivity of goody water were highly correlated. Analysis of goody water dry matter using DGT also explained some of the odour intensity of the samples.
5. A large number of chemical compounds were detected, both in freeze-dried goody water and in air samples taken from above goody water pits. Sulphur-containing compounds explained most but not all the odour associated with goody water air. Indoles, amines, ketones, aldehydes and alcohols were also detected in variable amounts.
6. Sulphides (hydrogen sulphide and dimethyl sulphide) from goody water could easily be detected with gas detector tubes. Low concentrations of these sulphides could also be detected with an electronic analyser.
7. There was a wide range in the pH of goody water but this did not affect odour.
8. Microbial profiles of goody water samples were determined using phospholipid fatty acid (PLFA) analysis and measurement of *Clostridia* spp. to estimate populations of sulphate reducing bacteria. Aerated goody water had a lower population of sulphate reducing bacteria and overall population of Gram +ve bacteria than non-aerated goody water.
9. The addition of gypsum to goody water samples did not result in an increase in odour or volatile sulphides. However, the prevention of gypsum being washed into the goody water storage tank and forming a source of sulphur for sulphate reducing bacteria, must be regarded as good practice.
10. Goody water samples with wide ranges in pH, EC, redox potentials and dry matter contents produced composts with similar mushroom mycelial growth rates and mushroom yields.
11. The sulphide emissions and odours produced from flask composts were not related to the odour intensities and sulphide emissions of the goody water samples that were used to prepare them.

## TECHNOLOGY TRANSFER

### Exploitation plan

Plans for commercial exploitation of the results are divided into the industry sectors: composters, mushroom growers and instrument manufacturers. The results will also have relevance to industries outside of those participating in the project.

Composters

The results of this work have already been incorporated into the Defra Process Guidance Note on Mushroom Substrate Production PG6/30 (NIPG6/30 in Northern Ireland). In particular the following recommendations are to be implemented on commercial sites to control odours:

- Minimise volume of goody water by separate storage of rainwater
- Aeration of goody water by a submerged system
- Measuring of Redox potential, minimum value of -280 mV (this replaced a previous stipulated minimum oxygen concentration of 2% which was not achievable)
- Specified maximum dry matter content of 8% w/w in goody water
- Screening of solid material and splitting of poultry manure applications to avoid leaching
- Measuring of sulphides (maximum value 2 ppm).

Similar recommendations for goody water treatment have been made by the Irish Environmental Protection Agency in Waste Licences issued to mushroom composters.

The above requirements have been introduced on participating composting sites within the last 12 months. The equipment required for measuring redox potential and electrical conductivity is low cost (less than 3500). The guidance note is due for review in 2008. In the light of the final report, the above figures will be reviewed to prepare new industry guidelines (by mid 2007) – in particular the need to measure EC and the required frequency of sampling.

The consistently improved performance of straw from certain sites and stored straw over fresh material means that composters will review their straw supply and storage regimes (within 9 months). Winter wheat trials are being conducted on different sites with different planting densities and cultivars to test the differences found in this work (within 1 year) and to optimise straw production for composting..

Shorter spawn-runs resulting from increasing the spawn-rate above 0.5% w/w means that the currently used spawn rates will be reviewed (within 6 months). Measurement of compost pH at the beginning and end spawn-run will be a useful indication to the quality of a spawn-run and may anticipate problems with compost quality (of immediate application).

Mushroom growers

The benefits of higher spawn rates also apply mushroom growers. The adverse effect of excess compaction of compost in trays and blocks will be evaluated by growers using these systems, and may lead to lower pressures being applied (with a resultant improvement in spawn-run).

A HDC Factsheet on 'Optimising spawn-running conditions' is to be prepared in the next 3 months.

Instrument manufacturers

The pulsed fluorescence SO2 has been shown to be useful objective tool in measuring low levels of odorous sulphides on and around recycled water storage and other areas of composting sites. The use of instruments for this purpose, as well on waste and sewage sludge composting sites will be a potential market.

Other waste industries

The results of this work on odour minimisation from leachate will be of direct relevance to other waste composting industries. In particular, the methods used for measuring and improving the anaerobic status of the liquid. The use of vis-NIR and DGT analysis to determine the chemical properties of composts could have a potential use in determining the suitability and stability of composted wastes for plant growing media.

Information on cultural factors affecting straw properties will be of use to other users of straw e.g. as biofuel.

**Publications and Presentations resulting from the project**

Publications

Improved efficiency and environmental impact of mushroom composting. HortLINK Leaflet HL 0163.

Noble R, Kilpatrick M, Compost research, HDC News October 2004.

Noble R, Kilpatrick M, Better mushroom composting, The Mushroom People, 3a, No. 174, September 2005, 14-15.

Noble R, Kilpatrick M. Better mushroom composting. HDC News, October 2005.

Noble R, Effects of straw properties on composting and cropping, Australia Mushroom Growers Association Journal, December 2005

Noble R, Mushroom Compost Research, Canadian Mushroom World, March 2006.

Noble R, Kilpatrick M, Solving bad smells from goody water, HDC News December 2006.

Noble R, Kilpatrick M, Solving bad smells from goody water, Mushroom People, December 2006.

Results from this project on the management of goody water on composting sites have been incorporated into the Defra Process Guidance Note (PG6/30).

Presentations

Noble R, Compost Research, Warwick HRI Mushroom Subject Day/ Mushroom Growers Association Conference, October 2004.

Noble R, The effects of wheat straw on mushroom composting. Australian Mushroom Growers' Association Conference, October 2005.

Noble R. Reducing mushroom composting odours. University of Guelph, Horticulture Research Institute Centenary presentation, Canada, April 2006.

Noble R, Research on Mushroom Composting, Presentation to the Canadian Mushroom Growers Association, Ontario, Canada, April 2006.

**References**


Anonymous (2002) Pocket guide to varieties of cereals, oilseeds & pulses. National Institute of Agricultural Botany, Cambridge, 216 pp.

Anonymous (2004) The Agricultural Budgeting & Costing Book No. 54. Agro Business Consultants Ltd. Melton Mowbray, Leics.

Burton CH, Sneath RW, Misselbrook TH, Pain BF (1998) The effect of farm scale aerobic treatment of piggery slurry on odour concentration, intensity and offensiveness. J. agric. Engng. Res. 71: 203-211.

Chalaux N, Olivier JM, Minvielle N (1991) Bench-scale composting and wheat straw degradability. In: Maher MJ (ed) Science and Cultivation of Edible Fungi, Balkema, Rotterdam, pp 207-214.

Cook D & Flegg PB (1962) The effect of rate of spawning on the cropping of the cultivated mushroom. Report of the Glasshouse Crops Research Institute 1961, pages 118 – 121.

Dravnieks A, Prokop Wh (1975) Source emission odour measurement by a dynamic forced triange olfactometer. J. Air Pollution Control Assn. 25:28-35.

Duns GJ, Ripley BD, Rinker DL (1999) Monitoring the production of odorous compounds during Phase I mushroom composting. Mushroom News 47 (11) 12-23.

Flegg PB, Wood DA (1985) Growth and fruiting. In: The Biology and Technology of the Cultivated Mushroom (PB Flegg, DM Spencer, DA Wood eds), John Wiley & Sons, Chichester, 43-62.

Gerrits JPG (1988) Nutrition and compost. In: The Cultivation of Mushrooms (ed) LJLD van Griensven. Darlington Mushroom Laboratories Ltd, Sussex, England, pages 29 – 72.

Heineman P & Engels L (1953) Toxicite des acides organiques sur le mycelium d'Agaricus hortensis et especes voisines en culture pure. Mushroom Science II, 49-51.

Knapp JS, Parton JH, Walton NI (1983) Enzymic saccharification of wheat straw – differences in the degradability of straw derived from different cultivars of wheat straw. Journal of the Science of Food and Agriculture 34, 433 – 439.

Lyons G, McCall RD, HSS Sharma (2000) Physical degradation of wheat straw by the in-vessel and windrow methods of mushroom compost production. Can. J. Microbiol. 46: 817-825.

Matcham SE, Jordan BR, Wood DA (1985) Estimation of fungal biomass in a solid substrate by three independent methods. Applied Microbiology and Biotechnology 21: 108 – 112.

Nix J, Hill P, Edwards A (2005) Farm management Pocketbook. The Andersons Centre. Melton Mowbray, Leics.

Noble R, Fermor TR, Evered CE, Atkey PT (1997) Bench-scale preparation of mushroom substrates in controlled environments. Compost Sci. Utiliz. 5: 32-43.

Noble R, Gaze RH (1998) Composting in aerated tunnels for mushroom cultivation: influences of process temperature and substrate formulation on compost bilk density and productivity. Acta Hortic. 469: 417-427.

Noble R, Hobbs PJ, Dobrovin-Pennington A, Misselbrook TH, Mead A (2001) Olfactory response to mushroom composting emissions as a function of chemical concentration. J. Environ. Qual. 30: 760-767.

Savoie JM, Chalaux N, Olivier JM (1992) Variability in straw quality and mushroom production: Importance of fungicide schedules on chemical composition and potential degradability of wheat straw. Bioresource Technology 41: 161-166.

Sharma HS & Kilpatrick M (2000) Mushroom (Agaricus bisporus) compost quality factors for predicting potential yield of fruiting bodies Can. J. Microbiol 46: 515-519.

Sharma HS, Lyons G, Chambers J (2000) Comparison of the changes in mushroom (Agaricus bisporus) compost during windrow and bunker stages of phase I and II. Ann. Appl. Biol. 136: 59-68.

Sharma HSS, Faughey G, Chambers J, Lyons G, Sturgeon S (2000) Assessment of winter wheat cultivars for changes in straw composition and digestibility to fungicide and growth regulator treatments. Ann appl. Biol. 137: 297-303.

Smith JF, Claydon N, Love ME, Allan M, Wood DA (1989) Effect of substrate depth on extracellular endocellulase and laccase production of *Agaricus bisporus*. Mycol. Res. 93: 292-296.

Smith JF, Wood DA, Thurston CF (1995) Growth measurement of Agaricus bisporus mycelium in composted substrates as an indicator of compost selectivity and mushroom productivity. In : (TJ Elliott, ed) Science and Cultivation of Edible Fungi, Balkema, Rotterdam, pp293-301.

Van den Berg M (1992) Sensory odour measurements using an olfactometer. Nederlands Normilisatie Institut, Kalfjeslaan 2, Postbus 5059, 2600 GB Delft, Netherlands.

Van Gils JJ (1988) Cultivation. In: The Cultivation of Mushrooms (ed) LJLD van Griensven. Darlington Mushroom Laboratories Ltd, Sussex, England. Pages 263 – 308.

Wood DA, Goodeneough PW (1977) Fruiting of *Agaricus bisporus,* Changes in extracellular activities during growth and fruiting. Arch. Microbiol. 114: 161-165.