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:	Annual Report Year 1 October 2003– September 2004
Key Workers:	Ralph Noble, Mairead Kilpatrick, Phil Hobbs, Shekar Sharma Andreja Dobrovin-Pennington, Gary Lyons
Locations of Project:	Warwick HRI, Wellesbourne, Warwick, CV35 9EF DARDNI, Applied Plant Science Division, Northern Ireland Horticultural and Plant Breeding Station, Loughall, Co. Armagh, BT60 8JB Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon, EX20 2SB
Project Co-ordinator:	Mr Martyn Dewhurst, Tunnel Tech Ltd, 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

CONTENTS Page

Consortium members:

Warwick HRI

Department of Agriculture and Rural Development for Northern Ireland

Institute of Grassland and Environmental Research

Horticultural Development Council

Mushroom Growers' Association

Blue Prince Mushrooms Ltd

Carbury Mushrooms Ltd

Custom Compost Ltd

Greenhill Compost Ltd

J. Rothwell & Son Ltd

James A. Gooding Ltd

McGeary's Mushroom Compost

Monaghan Mushrooms Ltd

Shackleford Mushrooms Ltd

Tandragee Compost

Tunnel Tech Ltd

JEOL (UK) Ltd

Casella eti

Triton Technology Ltd

Government Sponsors

Department for the Environment, Food and Rural Affairs

Department of Agriculture and Rural Affairs for Northern Ireland

GROWER SUMMARY

Background and Objectives of the Work

Processing time is a major factor influencing the cost of 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 facilties (Phase III). 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.

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. However, the key physical and chemical properties of straw that relate to suitability for mushroom composting and subsequent cropping have not yet been identified.

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. 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 also have a critical effects on the efficiency of the composting process and subsequent mushroom growth.

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

- Methods have been developed for measuring the chemical and physical properties of straw, its behaviour during composting, and subsequent mushroom growth on the compost. These methods will form the basis for further experimental work in the second and third years of the project, to determine whether the suitability of different types of wheat straw can be related to their properties.
- The chemical properties of straw were determined using Derivative thermogravimetry (DTG), Near Infra-Red Spectroscopy (NIR), as well as analytical procedures for measuring the contents of soluble carbohydrates, ash, and other components. These analyses showed significant differences between cultivars, and within the same cultivar grown at high and low N, and in stored and new season straw.

- The mechanical (tensile) properties of wheat straw were determined using two types of instrumentation. These were based on the Instron Universal testing machine and the Triton Technology dynamic mechanical analysis.
- Significant differences in mechanical properties were detected between wheat straw cultivars. However, temperature and moisture have a large influence on the mechanical properties of straw and the relative difference between samples.
- Different straw samples resulted in different levels of respiration and degradation during composting.
- The resultant composts produced from different straw samples resulted in different rates of colonisation by mushroom mycelium, and different mushroom yields in pot cropping tests.
- For laboratory and intermediate-scale composting tests in Year 2 of the project, straw samples from different wheat cultivars grown with and without fungicide and plant growth regulators have been obtained from six sites in England and Northern Ireland.

Reducing spawn-running time

- Compost temperature was not a reliable indicator of spawn-running since drier composts tended to increase in temperature greater and faster than moister composts.
- Compost pH fell during spawn-running, but was not indicative of the amount of mushroom mycelium due to differences in the initial compost pH.
- Methods were developed for measuring the colonisation rate of mushroom mycelium in compost using growth tubes.
- The optimum moisture content of pasteurised compost for mycelial growth rate was 72-73%.
- Compost pH, and nitrogen, ammonium N and ash did not affect mycelial growth rate within the ranges of values tested.
- A method based on an oxygen electrode was successfully used to detect the presence of laccase produced by mushroom mycelium and the difference between spawn-run (Phase III) and pasteurised compost (Phase II).
- The laccase method will be further examined in Year 2 of the project to determine if it can reliably measure the amount of mushroom mycelium produced on different compost samples during spawn-running.

Effects of recycled water analysis on odours

- Techniques were developed for measuring the chemical, microbial and odour properties of recycled goody water in storage pits.
- 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 had differing Redox potentials and odours.
- Goody water samples with a lower Redox potential tended to produce more volatile sulphides and were more odorous than samples with a less negative Redox potential.
- There were also wide ranges in the dry matter content, pH, conductivity, sulphite reducing bacteria and chemical analysis of goody water samples obtained from different sites.
- Composts prepared from the most anaerobic goody water samples (lowest Redox potentials) also produced the most volatile sulphides and strongest odours during subsequent composting.

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)

Primary milestones 1.1, 2.1 and 3.1 and secondary milestones 1.4, 3.4, 3.5 have been achieved within the specified timescales. Primary milestones 1.2 and 1.5 will take 3 months longer than anticipated due to seasonality of straw production. The timescale for the remaining milestones appears to be reasonable.

SCIENCE SECTION

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. However, the key physical and chemical properties of straw that relate to suitability for mushroom composting and subsequent cropping have not yet been identified.

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). Wheat cultivars are known to differ with respect to the physical stiffness of their straw (Anon 2002). 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 previous HortLINK project on mushroom composting odours showed that up to 30% of rape straw could be used to substitute the standard wheat straw. Rape straw and barely straw (in horse manure) are now used commercially by most mushroom composters in the UK, to augment wheat straw supplies.

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

The following hypotheses will be tested:

- Is straw biodegradability in composting related to its physical and/or chemical properties? (previous work at DARD indicated that the biodegradability of wheat straw samples in composting is related to the enzymatic digestion of their dry matter in a hemicellulase-cellulase bioassay, modified from Sharma et al 2000)
- Is the biodegradability of straw in composting related to its subsequent suitability for mushroom mycelial growth and cropping?
- Can the biodegradability and suitability of straw be related to its cultural source?

Materials and Methods

Chemical analyses of straw samples

Straw samples were analysed for pH, electrical conductivity, dry matter content, total soluble carbohydrates (TSC), total soluble polyphenol (TSP), and ash. Straw samples were also analysed using derivative thermogravimetry (DTG) and vis-Near Infrared Spectroscopy (NIR) according to the methods outlined below.

Thermogravimetric analysis (DTG)

Wheat straw 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 subsamples were taken from each wheat straw sample. The pieces of straw were cut into lengths of between 3-4 cm and 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 bandpath 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 analysed using WINISI software (Foss). Vis-NIR data was analysed using the Partial Least Squares method to determine relationships between samples and chemical data.

Pesticide Analysis (GC-MS)
An acetone (100 ml) extraction of the straw sub-samples (10g) 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 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.

Physical analyses of straw samples

Tensile strength tests
Tensile tests were conducted with an Instron universal testing machine (model 4301, Instron Ltd, High Wycombe, UK) at 20 C and 100% relative humidity. The tensile tests were conducted according to the methods of Andrews et al (2002). 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 equipment

Wheat straw samples (600 g) were mixed with 200 g of sieved poultry manure, 4 g urea, 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 h 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.

Compost analysis

Analyses were conducted on freeze-dried, finely milled samples of the compost ingredients and of the substrates before and after processing in the flask composting equipment. Dry matter (DM), N, ammonium (NH^{+}_{4}) and ash contents and pH were determined as described previously (Noble and Gaze, 1994).

Mushroom cropping procedure

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).

Experiments

Wheat straw samples

The above methods were tested on the straw samples in Table 1. Two of the samples (cv. Claire) were grown at the Northern Ireland Plant Breeding Station at Crossnacreevy; the other five samples were grown at Wellesbourne, Warwicks.

Table 1. Wheat straw samples assessed

Sample	Cultivar	Age, months	Source	Growth regulator	Harvest method	N fertiliser kg / ha
1	Claire	6	Crossnacreevy, NI	CCC	intact	100
2	Claire	6	Crossnacreevy, NI	CCC	intact	200
3	Hereward	6	Wellesbourne	none	chopped	standard
4	Hereward	18	Wellesbourne	none	chopped	standard
5	Weston	18	Wellesbourne	CCC	chopped	standard
6	Malacca	18	Wellesbourne	none	half cut	standard
7	Richmond	6	Wellesbourne	CCC	chopped	standard

Results

Chemical analyses of straw samples

Analyses are shown in Table 2. Variation exists in the chemical parameters assessed based on cultivar type and crop husbandry regimes. Straw from cultivars Claire (high N application) and Weston were the most alkaline. Cultivar Claire also produced straw with the highest conductivity, dry matter content and total soluble carbohydrates. Cultivars Weston and Malacca had the highest ash contents.

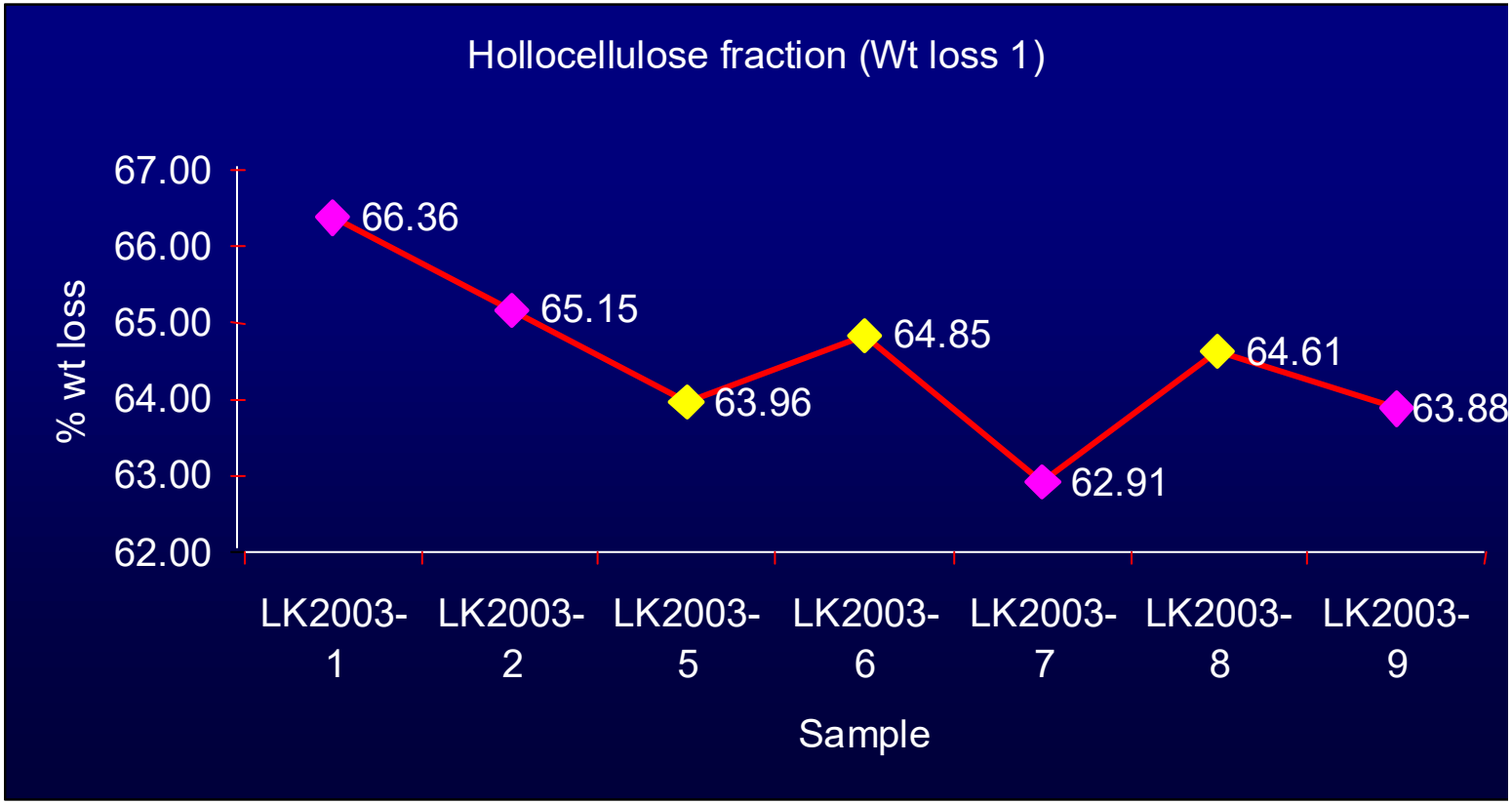
Table 2. Wheat straw samples assessed

Sample	Cultivar, N	pH	EC uS/cm	Dry matter, %	TSC g/kg DM	TSP g/kg DM	Ash % of DM
1	Claire, low	7.87	598	95.3	12.4	3.5	4.4
2	Claire, high	8.97	627	95.6	11.8	3.6	4.6
3	Hereward	7.50	314	94.4	9.3	2.6	5.3
4	Hereward	7.87	318	93.2	9.5	2.4	5.4
5	Weston	8.96	469	94.0	7.9	2.7	7.6
6	Malacca	8.45	237	94.7	6.2	2.4	7.2
7	Richmond	8.11	337	94.3	7.8	2.4	6.3

Derivative thermogravimetry analyses results are shown in Figures 1-3. Figure 1 depicting the holocellulose fraction indicates significant differences in the fibre fraction of all samples analyses by DTG. Highest values were quantified in the cultivar Claire grown in Northern Ireland. Similarly in Figure 2 significant differences in the structural hemicellulose and lignin fractions are evident. Figure 3 shows the derivative thermograms of three of the wheat straw cultivars – Claire, Hereward and Richmond. Weight loss 1 profiles indicate the two UK cultivars to be similar but distinct from the cultivar Claire. Additional analyses from the 2004 samples will confirm if this is a consistent difference. The shape of the thermogram profiles provides additional information on the proportional balance between the structural hemicellulose and lignin fractions of the samples. The influence of PGR on cultivars Claire and Richmond are exhibited as greater degree of lignification (WL2) on the thermal profile. The NIR raw and transformed spectra of all 7 samples are shown in Figures 4a and 4b respectively. NIR analysis indicates chemical differences between samples with GB samples differing significantly from those of NI origin. Good correlation has been shown between the NIR spectral characteristics and the fibre fractions as measured by DTG (Figure 5).

Figure 1 Hollocellulose fraction weight loss at peak 1 for all 7 straw samples.

DTG



***P* <0.001**

◆ **- Indicates PGR used**

Figure 2 Structural hemicellulose and lignin fractions weight loss at peak 2 for all 7 straw samples.

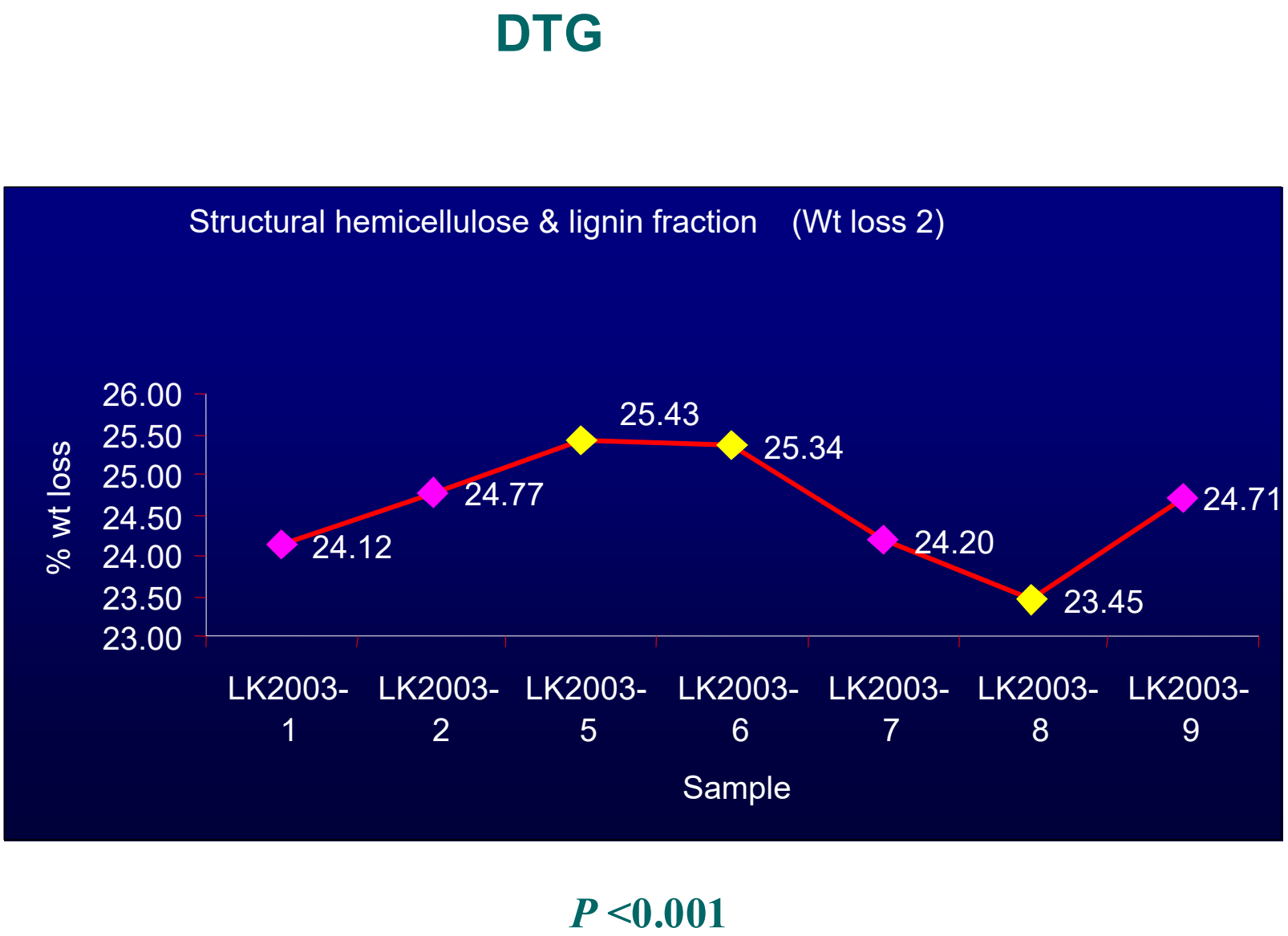
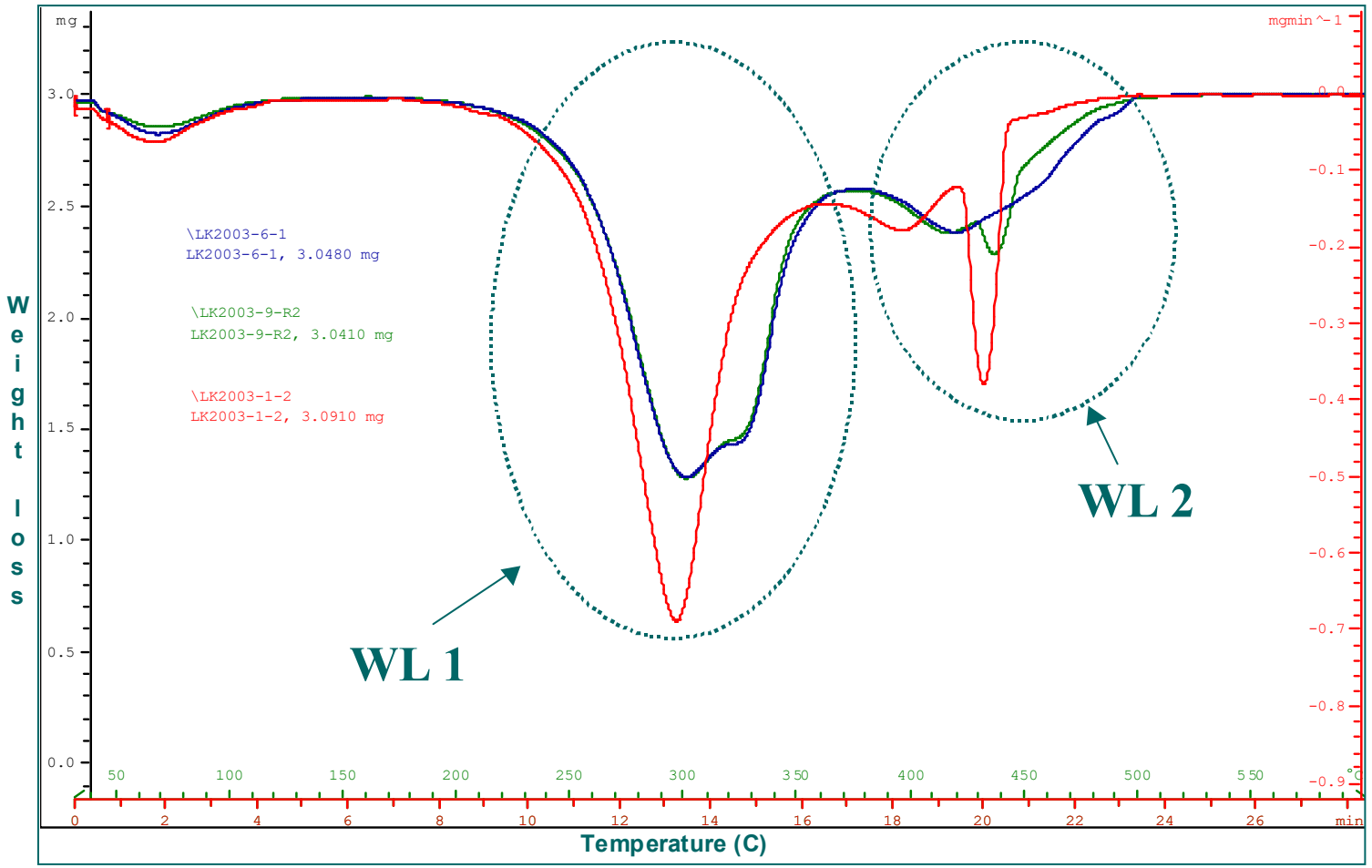


Figure 3

Derivative thermograms of 3 of the straw cultivars



◆ - Indicates PGR used

Hereward 2003 *Richmond + CCC*

Figure 4a - Raw Spectra

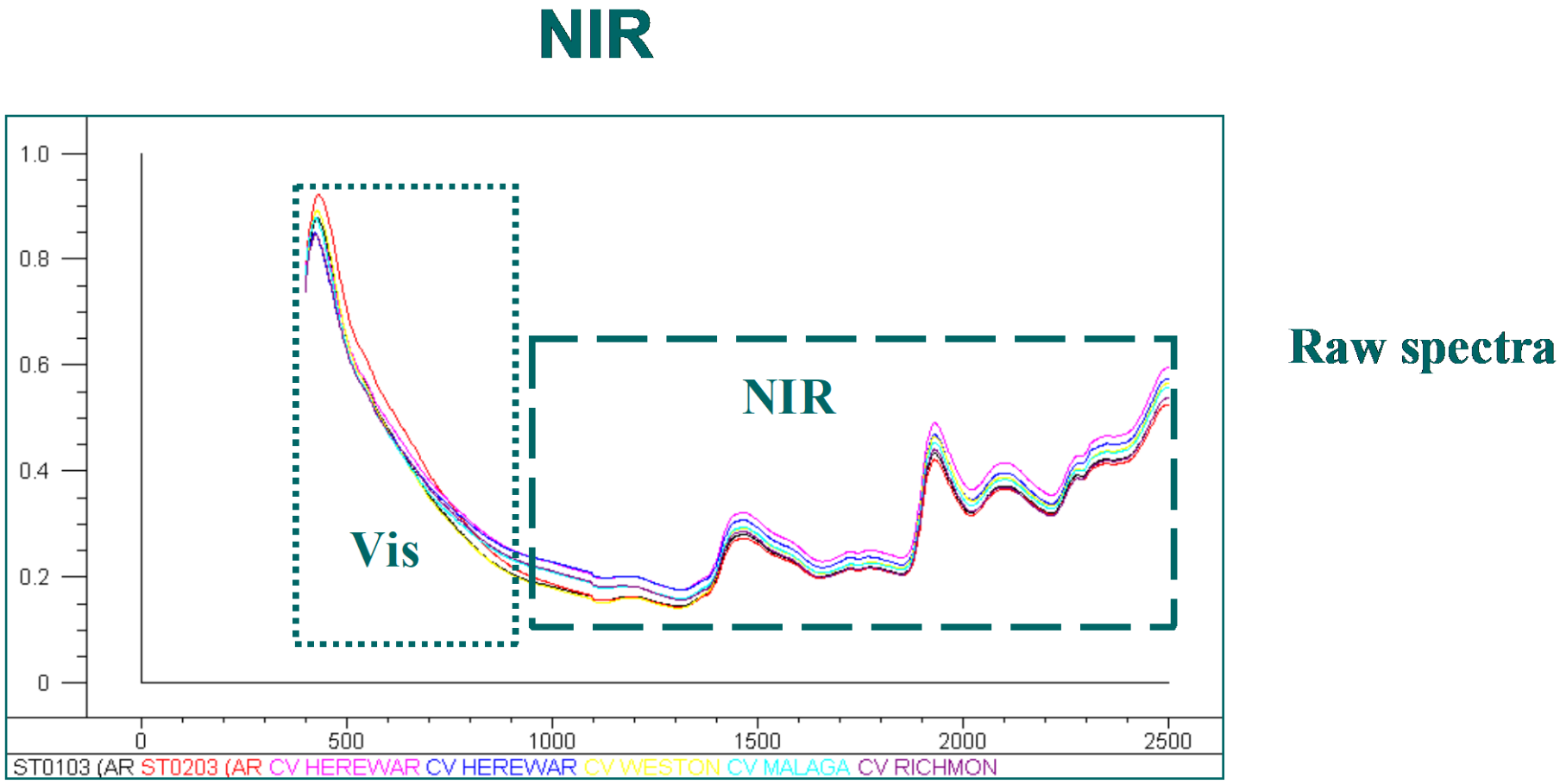
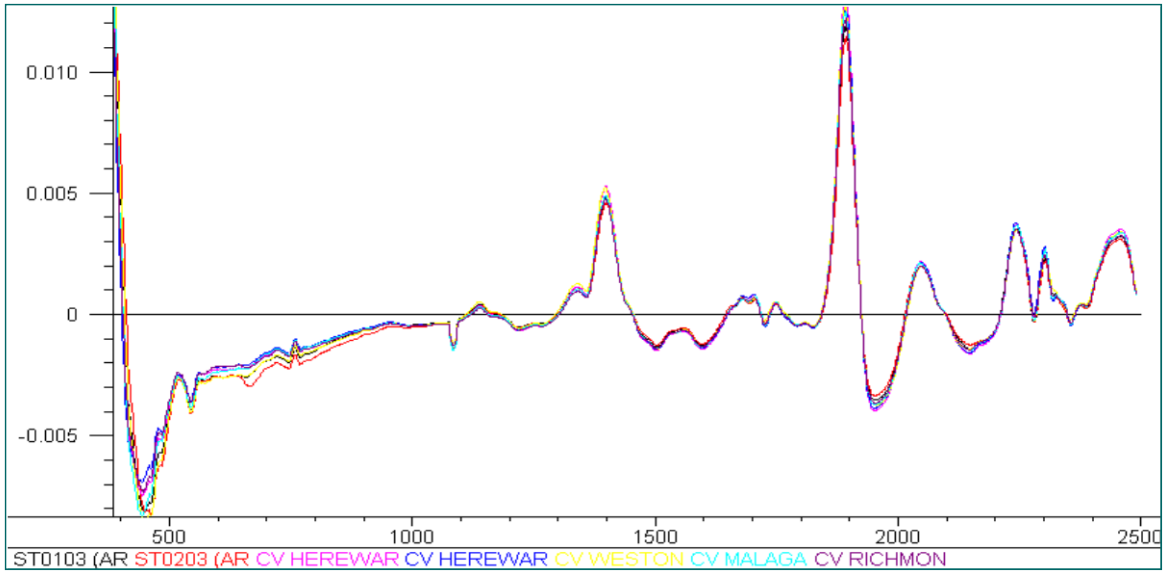


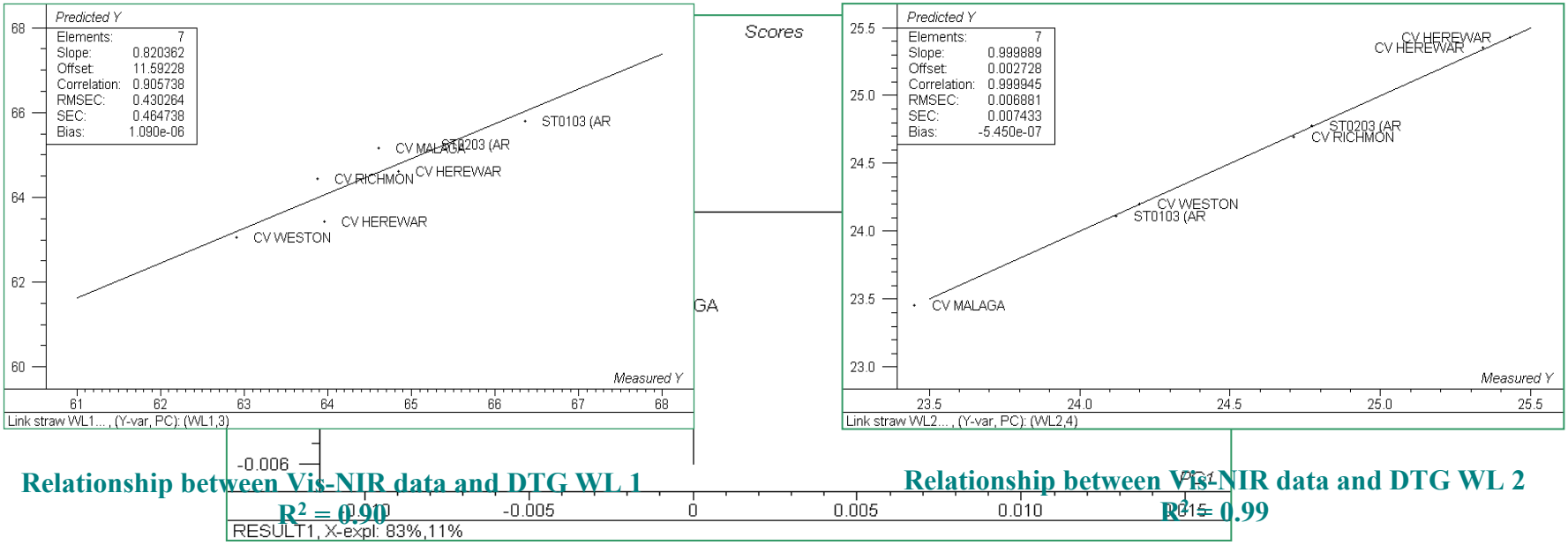
Figure 4b – Transformed spectra



Transformed spectra

Figure 5 -

NIR Score plot for 7 straw



Relationship between Vis-NIR data and DTG WL 1

R^2 = 0.99

Relationship between Vis-NIR data and DTG WL 2

R^2 = 0.99

Figure 6. Load – displacement graphs of tensile tests on straw samples using an Instron Universal testing machine.

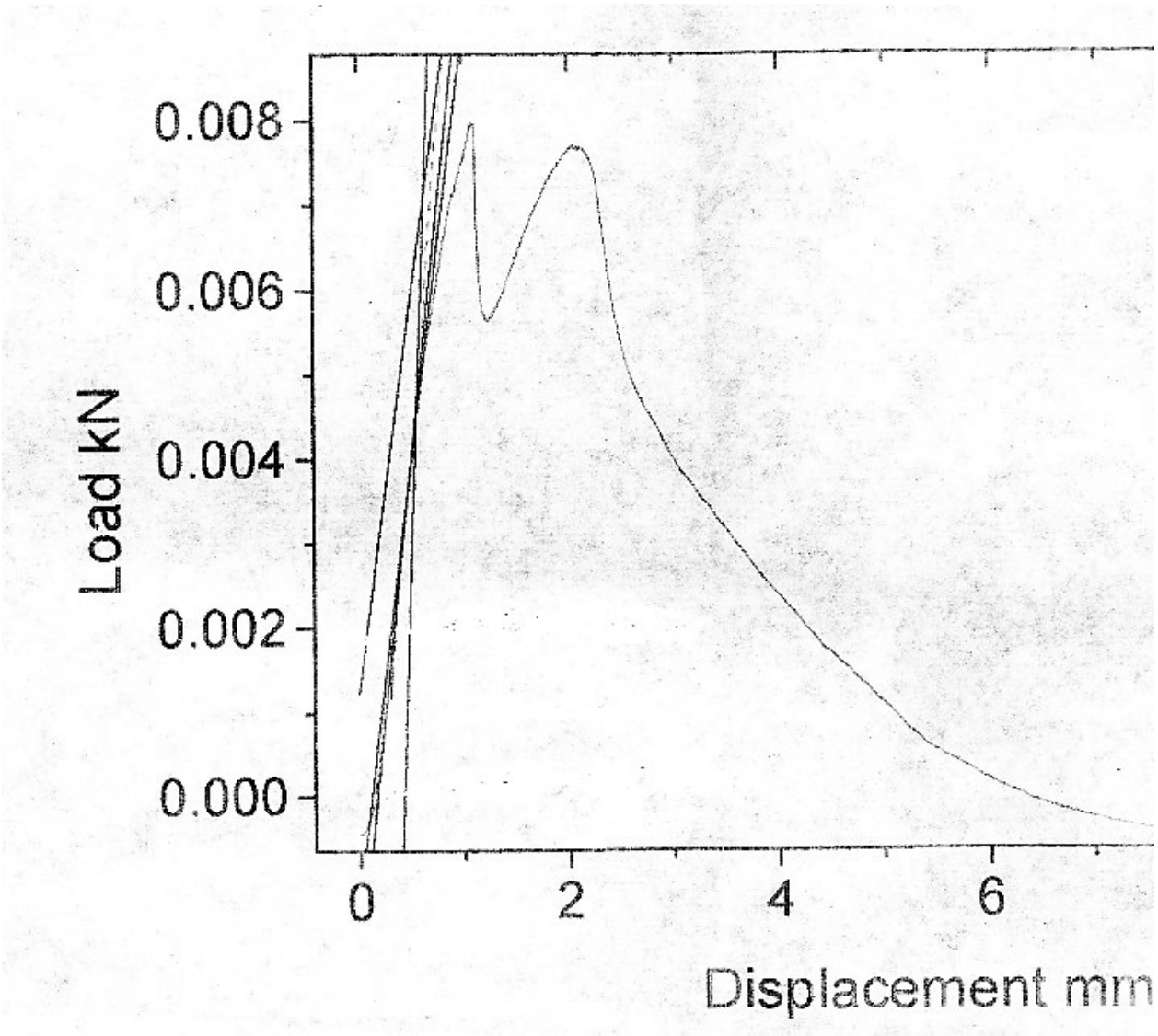


Figure 7a Dynamic mechanical properties of straw cv Malacca, TanD

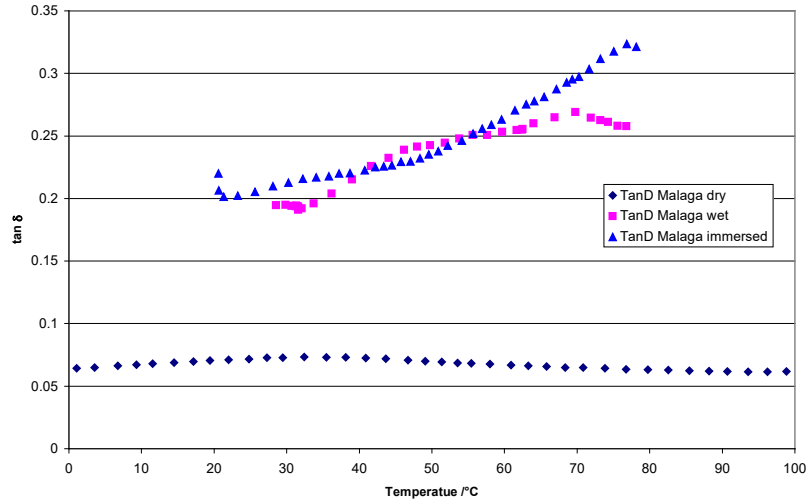


Figure 7b cv Richmond TanD

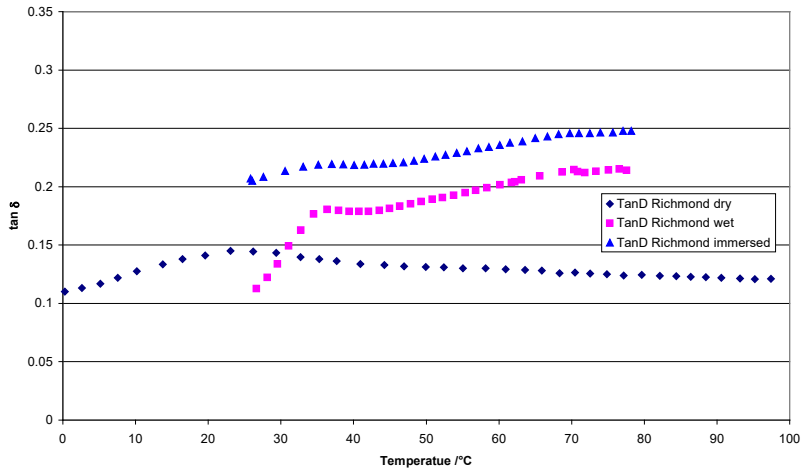


Figure 7c cv. Western TanD

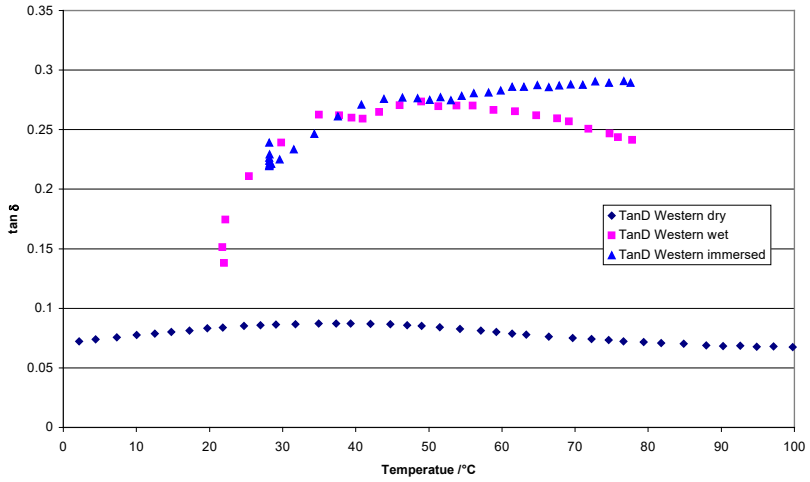


Figure 8 a Dynamic mechanical properties of straw- Youngs Modulus of Elasticity cultivars Weston, Richmond and Malacca Samples tested standard air oven

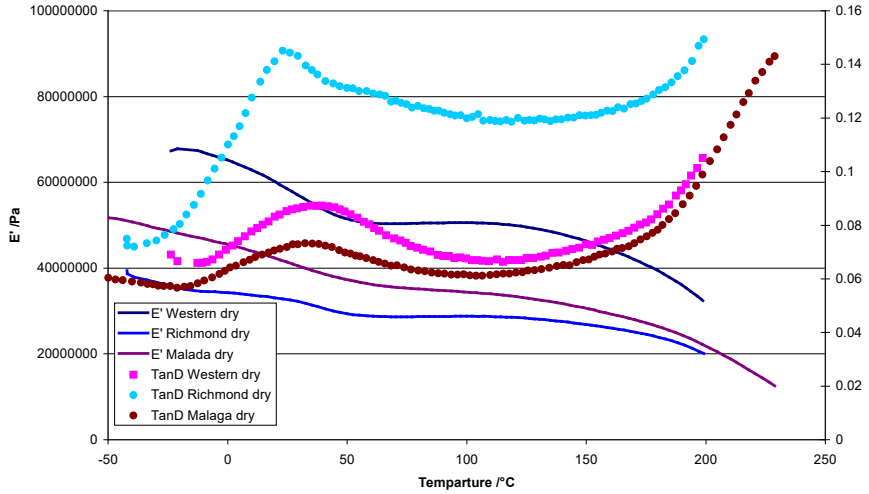


Figure 8 a Dynamic mechanical properties of straw- Youngs Modulus of Elasticity cultivars Weston, Richmond and Malacca Samples tested standard air oven

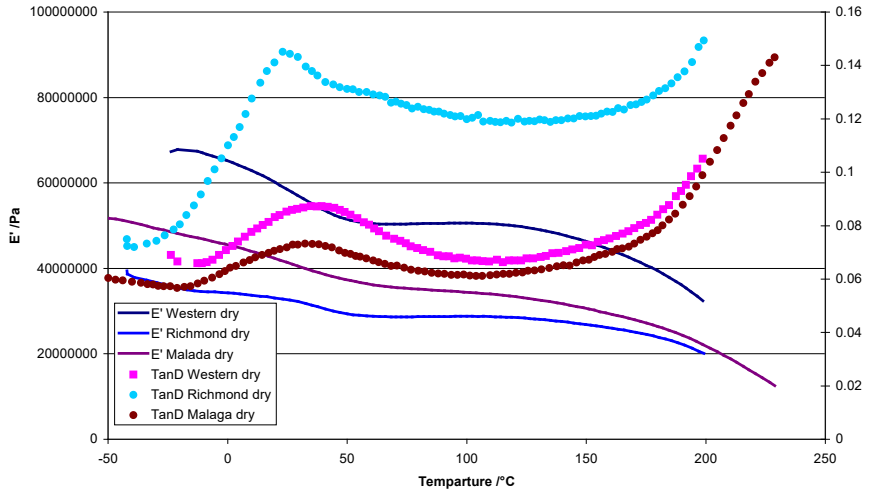


Figure 8c Dynamic mechanical properties of straw - Youngs Modulus of Elasticity cultivars Weston, Richmond and Malacca, samples tested immersed

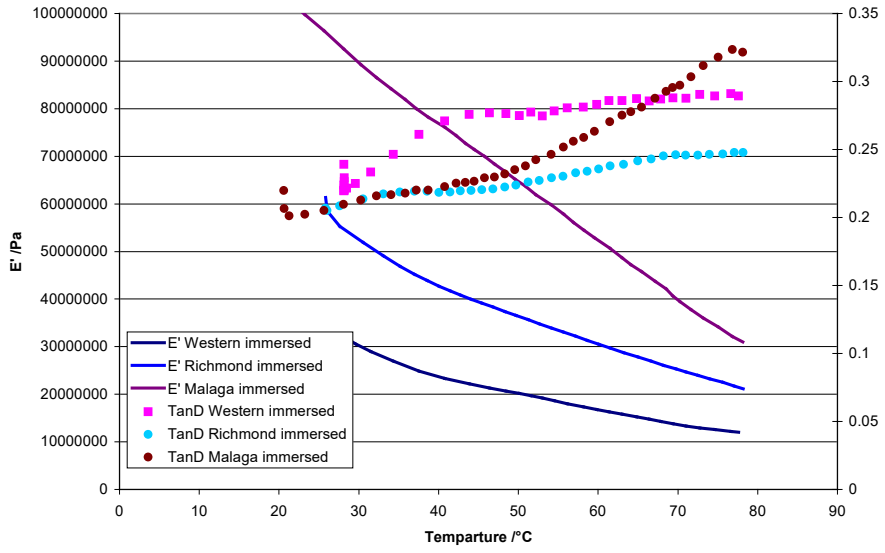
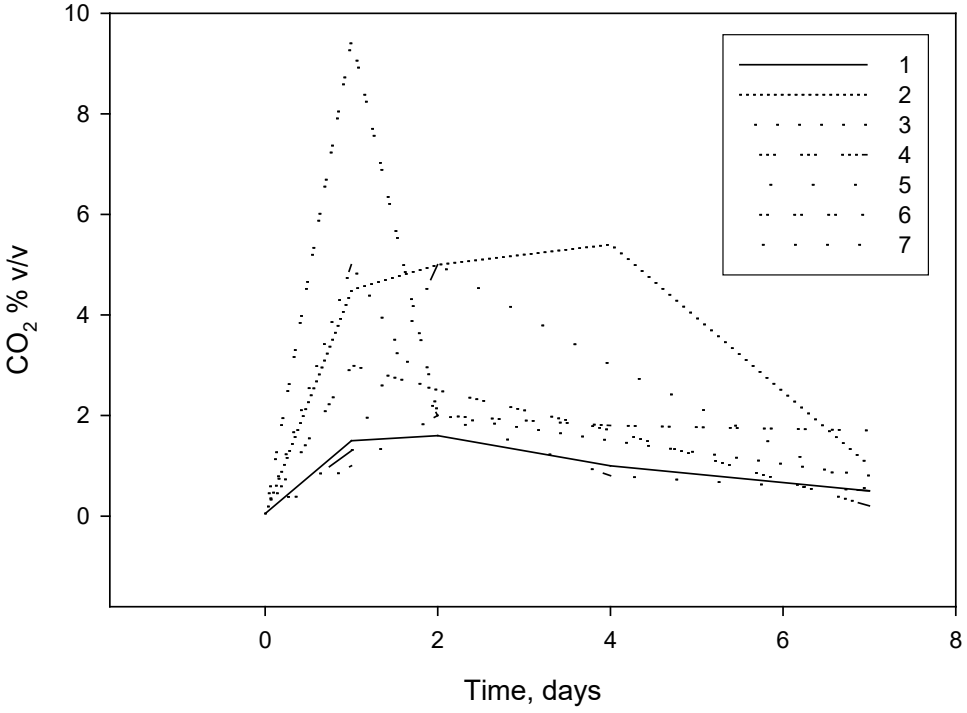
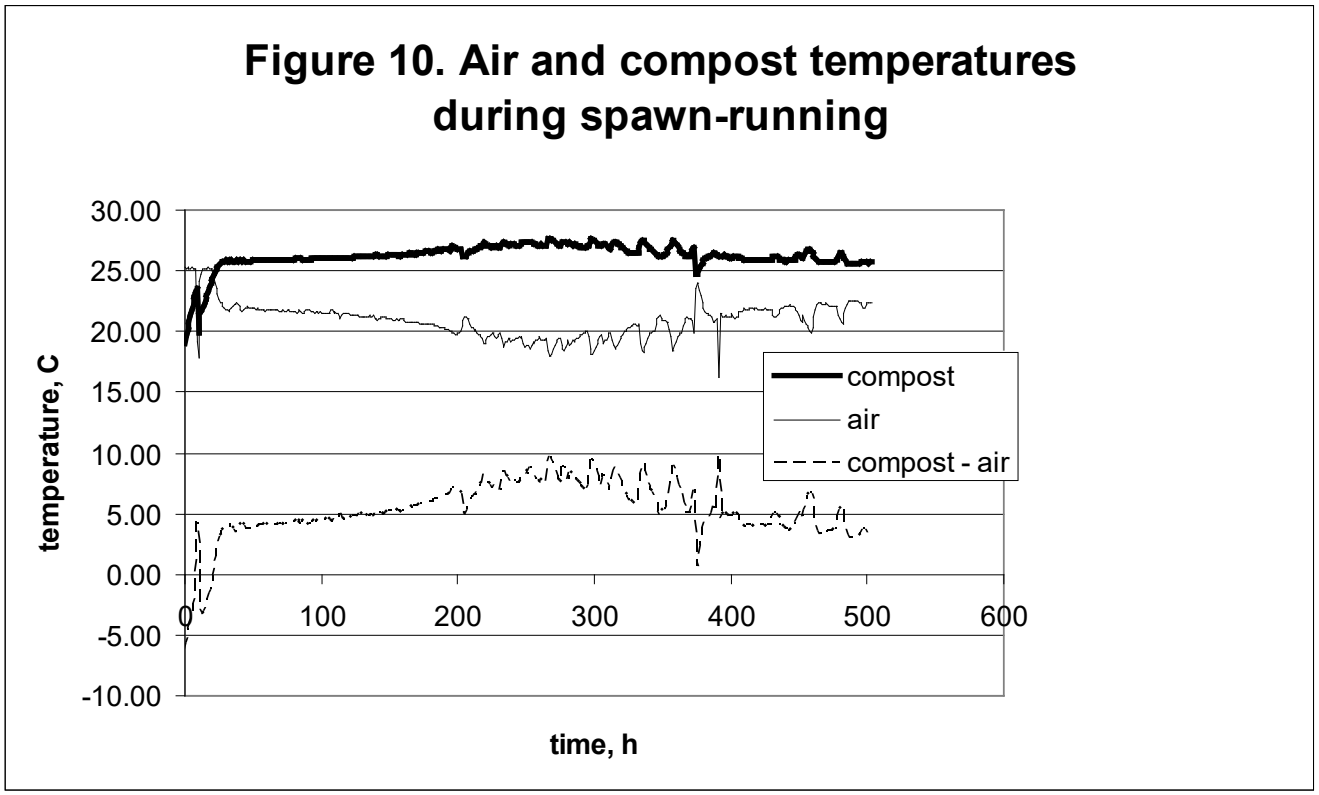


Figure 9. Carbon dioxide concentration during composting of straw samples 1 to 7





Pesticides detected in the straw samples were restricted, at most, to single fungicides (Table 3). These reflect the different fungicides used in NI (fenpropimorph) and Warwicks. (tebuconazole). No plant growth regulator residues were detected.

Table 3. Fungicide residues detected in straw samples

Sample	Cultivar	Pesticide detected	Level found, mg/kg
1	Claire, low N	fenpropimorph	0.02
2	Claire, high N	fenpropimorph	0.03
3	Hereward	tebucanazole	0.04
4	Hereward	tebucanazole	0.10
5	Weston	tebuconazole	0.15
6	Malacca	tebuconazole	0.07
7	Richmond	none	-

Physical analyses of straw samples

Tensile strength tests

Typical force displacement tests results from the Instron testing machine are shown in Figure 6. The slope is a measure of stiffness (Young's modulus) of the straw, with a steeper slope indicating greater stiffness. The strain at the maximum load (about 10N) is an indication of the 'strechability' of the straw sample. Most of the straw samples did not fail at the maximum load applied (10N). An example of tissue failure is shown in Figure 6.

The results in Table 4 show clear differences between the samples. Cultivar Richmond had the greatest Young's modulus (stiffness) and was also the least stretchable. Addition of extra N fertiliser to cultivar Claire resulted in stiffer straw. Storage of cultivar Hereward resulted in a loss in stiffness.

Table 4. Mechanical properties of wheat straw samples measured with an Instron at room temperature

Sample	Cultivar	Instron Tensile Tests	
		Strain at max. load, (mm/m)	Young's modulus, MPa
1	Claire, low N	700	921
2	Claire, high N	960	1034
3	Hereward	980	1065
4	Hereward	780	846
5	Weston	620	883
6	Malacca	760	1046
7	Richmond	350	1446

Dynamic mechanical analysis of straw

Results obtained at a range of temperatures for three straw samples are shown in Figures 7 and 8. These 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 have large effects on the mechanical properties, and can alter the relative differences between straw types. Values obtained at 50 C for the different samples are shown in Table 5. 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 5. Mechanical properties of wheat straw samples measured by Triton at 50 C

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

Composting tests on straw types

Carbon dioxide measurements during flask composting are shown in Figure 9 and Table 6. Cultivar Claire (straw samples 1 and 2) resulted in a higher carbon dioxide evolution than cultivar Weston (straw sample 7). Carbon losses during composting, measured by initial and final ash content, were highest in cultivars Claire and stored Hereward, and lowest in cultivar Malacca (Table 6).

Table 6. Carbon and carbon dioxide losses during composting of different straw samples

Sample	Cultivar	Carbon dioxide loss, g/kg compost	Carbon loss, g/kg compost
1	Claire, low N	31.0	38.8
2	Claire, high N	43.5	31.5
3	Hereward	24.6	25.4
4	Hereward	27.2	39.6
5	Weston	23.2	26.0
6	Malacca	26.0	14.5
7	Richmond	31.7	26.2

Mushroom mycelial growth and cropping

Mushroom mycelial growth and pot experiments yields from compost prepared from the seven straw batches are shown in Table 7. 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 hereward straw produced the lowest yield.

Table 7. Mushroom mycelial growth and cropping in pot tests from composts prepared from different straw samples

Sample	Cultivar	Mycelial growth mm/day	Mushroom yield, g/kg compost DM
1	Claire, low N	4.6	689
2	Claire, high N	5.1	414
3	Hereward	4.7	442
4	Hereward	4.3	328
5	Weston	5.0	371
6	Malacca	4.4	635
7	Richmond	4.5	354

Straw samples for 2004 Composting Experiments

Straw from Wellesbourne and Kirton

Wheat straw cultivars, plant growth regulator (CCC) application and growing locality and year of straw for 2004 composting and analytical tests are shown in Table 8. These show a total of six wheat cultivars from the Wellesbourne and Kirton sites. Two cultivars were grown with and without PGR. New season and stored straw was obtained for three cultivars. All the samples were grown with fungicide (tebuconazole).

Table 8. Wheat straw samples from Wellesbourne and Kirton for 2004 composting tests

Sample	Cultivar	Age, months	Source	Growth regulator
1	Hereward	3	Wellesbourne	CCC
2	Hereward	3	Wellesbourne	none
3	Hereward	15	Wellesbourne	none
4	Hereward	27	Wellesbourne	none
5	Weston	15	Wellesbourne	CCC
6	Malacca	3	Wellesbourne	none
7	Malacca	3	Wellesbourne	CCC
8	Malacca	15	Wellesbourne	none
9	Richmond	3	Wellesbourne	CCC
10	Richmond	15	Wellesbourne	CCC
11	Tanker	3	Kirton	CCC
12	Axona	3	Kirton	none
13	Axona	3	Kirton	CCC

Wheat straw samples, plant growth regulator, and nitrogen application treatments for Northern Ireland straw samples are shown in Table 9. Ten wheat cultivars were grown on three sites. Some of the cultivars were grown with and without PGR and fungicide, and two (Richmond and Tanker) are common to those grown in England. The wheat cultivars cover a range in straw length and strength characteristics.

Table 9. Wheat straw samples from Northern Ireland for 2004 composting tests

#	Variety	Year	F	PGR	SITE		Var #	Trial ID
11	Claire	2004	+	+	DownP	DARD	101	WW04NI12T
13	Malacca	2004	+	+	DownP	DARD	103	WW04NI12T
32	Option	2004	+	+	DownP	DARD	104	WW04NI12T
9	Tanker	2004	+	+	DownP	DARD	105	WW04NI12T
29	Napier	2004	+	+	DownP	DARD	106	WW04NI12T
23	Access	2004	+	+	DownP	DARD	111	WW04NI12T
20	Deben	2004	+	+	DownP	DARD	112	WW04NI12T
17	Richmond	2004	+	+	DownP	DARD	115	WW04NI12T
15	Einstein	2004	+	+	DownP	DARD	117	WW04NI12T
26	Robigus	2004	+	+	DownP	DARD	118	WW04NI12T
10	Claire	2004	+	+	Cross	DARD	101	WW04NIO9T
12	Malacca	2004	+	+	Cross	DARD	103	WW04NIO9T
31	Option	2004	+	+	Cross	DARD	104	WW04NIO9T
8	Tanker	2004	+	+	Cross	DARD	105	WW04NIO9T
28	Napier	2004	+	+	Cross	DARD	106	WW04NIO9T
22	Access	2004	+	+	Cross	DARD	111	WW04NIO9T
19	Deben	2004	+	+	Cross	DARD	112	WW04NIO9T
16	Richmond	2004	+	+	Cross	DARD	115	WW04NIO9T
14	Einstein	2004	+	+	Cross	DARD	117	WW04NIO9T
25	Robigus	2004	+	+	Cross	DARD	118	WW04NIO9T

#	Variety	Year	F	PGR	SITE		Var #	Trial ID
38	Claire	2004	+	+	LIM	CEL	101	WW04NI35T
42	Malacca	2004	+	+	LIM	CEL	103	WW04NI35T
33	Option	2004	+	+	LIM	CEL	104	WW04NI35T
34	Tanker	2004	+	+	LIM	CEL	105	WW04NI35T
30	Napier	2004	+	+	LIM	CEL	106	WW04NI35T
24	Access	2004	+	+	LIM	CEL	111	WW04NI35T
21	Deben	2004	+	+	LIM	CEL	112	WW04NI35T
18	Richmond	2004	+	+	LIM	CEL	115	WW04NI35T
46	Einstein	2004	+	+	LIM	CEL	117	WW04NI35T
27	Robigus	2004	+	+	LIM	CEL	118	WW04NI35T
41	Claire	2004	-	-	LIM	DARD	101	WW04NI10U
45	Malacca	2004	-	-	LIM	DARD	103	WW04NI10U
37	Tanker	2004	-	-	LIM	DARD	105	WW04NI10U
49	Einstein	2004	-	-	LIM	DARD	117	WW04NI10U
40	Claire	2004	-	+	LIM	CEL	101	WW04NI35U
44	Malacca	2004	-	+	LIM	CEL	103	WW04NI35U
36	Tanker	2004	-	+	LIM	CEL	105	WW04NI35U
48	Einstein	2004	-	+	LIM	CEL	117	WW04NI35U
39	Claire	2004	+	-	LIM	CEL	101	WW04NI35L
43	Malacca	2004	+	-	LIM	CEL	103	WW04NI35L
35	Tanker	2004	+	-	LIM	CEL	105	WW04NI35L
47	Einstein	2004	+	-	LIM	CEL	117	WW04NI35L

Conclusions Part 1

1. Methods have been developed for measuring the chemical and physical properties of straw, its behaviour during composting, and subsequent mushroom growth on the compost. These methods will form the basis for further experimental work in the second and third years of the project, to determine whether the suitability of different types of wheat straw can be related to their properties.
2. The chemical properties of straw were determined using Derivative thermogravimetry (DTG), Near Infra-Red Spectroscopy (NIR), as well as analytical procedures for measuring the contents of soluble carbohydrates, ash, and other components. These analyses showed significant differences between cultivars, and within the same cultivar grown at high and low N, and in stored and new season straw.
3. The mechanical (tensile) properties of wheat straw were determined using two types of instrumentation. These are based on the Instron Universal testing machine and the Triton Technology dynamic mechanical analysis.
4. Significant differences in mechanical properties were detected between wheat straw cultivars. However, results at Triton Technology showed that the test conditions (temperature and moisture) have a large influence on the mechanical properties of straw.
5. Different straw samples resulted in different levels of respiration and degradation during composting.
6. The resultant composts produced from different straw samples resulted in different rates of colonisation by mushroom mycelium, and different mushroom yields in pot cropping tests.
7. For laboratory and intermediate-scale composting tests in Year 2 of the project, straw samples from different wheat cultivars grown with and without fungicide and PGR have been obtained from six sites in England and Northern Ireland.

Part 2. Reducing spawn-running time.

Processing time is a major factor influencing the cost of 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 facilties (Phase III). 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.
The following hypotheses will be tested:

- 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

Composting procedure
Compost was prepared according to the procedure in Noble et al (1998). Straw bales were formed into windrows and wetted on day 0 without a pre-wetting procedure. Poultry manure was added on days 2 and 7, and water applications and windrow turns were made on alternate

days during a 16 day period. Water applications after day 10 were varied according to compost moisture content. Composts were prepared with moisture contents at filling of bulk pasteurisation tunnels of 70-80%.

Composts were pasteurised at 58 C for 6 hours and conditioned at 45-48 C. For the Phase II pasteurisation regime, the tunnels were filled with 2.5 t of material from the Phase I stage to a height of 0.9 – 1.1 depending on the ingredients. Following a 20 h equalisation of compost temperature at 45 - 48°C, the composts were pasteurised at 58 - 60°C for 6 h. 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. Details of temperature and airflow control during Phase II are given in Noble & Gaze (1998).

Measurement of mycelial growth

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.

Compost analysis

Analyses were conducted on freeze-dried, finely milled samples of the compost ingredients and of the substrates before and after processing in the Phase II tunnels. Dry matter (DM), N, ammonium (NH^+_4) and ash contents and pH were determined as described previously (Noble and Gaze, 1994). Derivative thermogravimetric analyses were conducted on the compost samples, similar to those conducted on the wheat straw samples (previously described).

Measurement of cellulose and lignin

The Ritter method (Rahn et al, 2002) was used for determining the cellulose and lignin contents of dried samples of the composts. Cellulose was determined by acid digestion and then determining the concentration of soluble carbohydrates that were formed.

Oven dried and milled compost samples (1 g) were refluxed with 10 ml of 72% sulphuric acid and 320 ml of distilled water for 2h. The samples were then vacuum filtered with a Buchner funnel and the residue collected in a GF/A filter paper. The filtrate was then analysed for soluble carbohydrates by addition of phenol and determining the resultant yellow coloration with a spectrophotometer (Rahn et al, 2002).

Lignin and ash were determined by first drying the filter papers and the burning off the lignin in a furnace at 400 C. The ash content was determined by subtraction of the ash of the filter paper.

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 h 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 (fully 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

Spawn-running Temperatures and Mushroom Cropping Procedure

The cropping procedure is outlined in Noble & Gaze (1998). The composts were spawned using the Hauser A15 (Sylvan Spawn Ltd, Peterborough, UK) and 2100 (Amycel-UK Ltd, Burton-on-Trent, UK) strains. Spawned trays were stacked four high in cropping sheds. Air and compost temperatures during spawn-running were measured with electronic probes and data logger. Mushrooms were picked as large buttons (diameter 30-40mm) over a 24-days period (three flushes of mushrooms).

Results

Temperatures during spawn-running
A typical profile of air and compost temperatures during spawn-running is shown in Figure 10. The following parameters were recorded:
(a) the maximum difference between air and compost temperatures
(b) the time taken to reach the maximum compost temperature.
The maximum difference between air and compost temperatures ranged from 5 to 18 C. This difference was generally greater in drier composts (Figure 11). There was no correlation between (a) and (b) above, or with any compost analysis factors (N, ammonium or ash content, pH) or mushroom yield.

Compost analysis
Moisture, nitrogen and pH
Compost moisture, nitrogen, ammonium nitrogen, ash and pH are shown in Table 11. Compost Ph fell during spawn-running (Fig.12), but this depended on the initial compost pH, and the final pH was not indicative of the amount of mycelium in the compost.

Measurement of hollocellulose, cellulose and lignin.
Compost hollocellulose (DGT), cellulose and lignin are shown in Table 11. Analyses of the composts is continuing and correlations between the different compost factors and mycelial growth will be tested once the data is complete.

Mycelial growth rate
There was no relationship between mycelial growth rate in compost and mushroom yield (Figure 13). There was no effect of compost pH, ammonium nitrogen content, nitrogen content or ash content on mycelial growth rate (Figures 14 and 15). However, compost moisture

content had a significant effect on mycelial growth rate, with an optimum of about 73% at spawning (Figure 15).

Laccase activity of composts
Chart recorder printouts showing the consumption of oxygen resulting from the presence of laccase in the extracts are shown in Figures 17-19. The addition of P-phenylenediamine to the compost extract in the oxygen electrode resulted in decrease in the oxygen concentration when laccase was present. This is shown by a slope in the chart recorder printout (Figures 17 and 18). Results for spawned compost and laccase suspension were consistent. Extracts from unspawned compost did not show a significant consumption of oxygen i.e. slope in the chart recorder printout (Figure 19; Table 10). The mean value obtained for spawn-run compost was similar to that obtained by Wood & Goodenough (1977) of 0.39 laccase enzyme units / ml extract.

Table 10. Laccase activity of compost extracts and *Rhus* laccase suspension

Sample	Laccase enzyme units / ml extract
Spawn-run compost	0.4709
Pasteurised unspawned compost	0.0092
Laccase suspension	0.1591

Conclusions Part 2

1. Compost temperature was not a reliable indicator of spawn-running since drier composts tended to increase in temperature greater and faster than moister composts.
2. Methods were developed for measuring the colonisation rate of mushroom mycelium in compost using growth tubes.
3. Optimum moisture content of pasteurised compost for mycelial growth rate was 72-73%.
4. Compost pH, and nitrogen, ammonium N and ash did not affect mycelial growth rate within the range of values tested.
5. Compost pH declined during spawn-running, but final pH was not indicative of the amount of mycelium in the spawn-run compost.
6. A method based on an oxygen electrode was successfully used to detect laccase and the difference between spawn-run (Phase III) and pasteurised compost (Phase II).

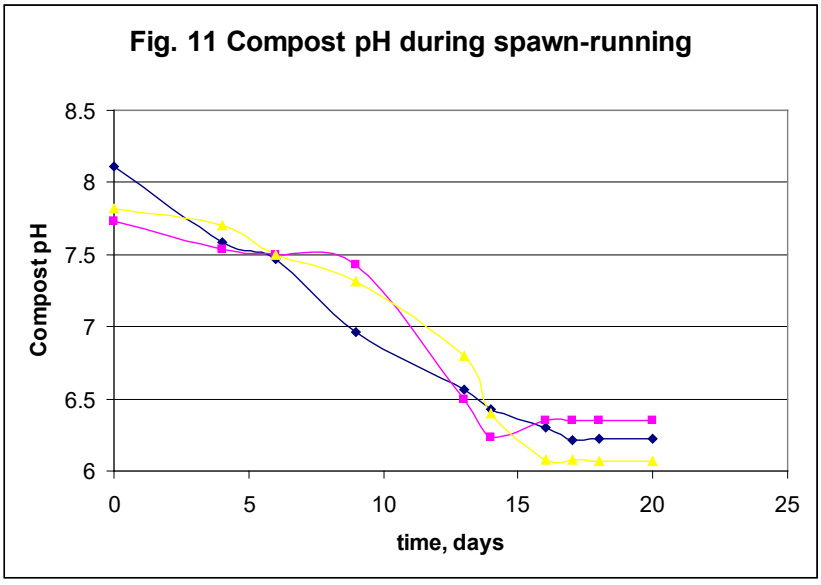


Fig. 12 Relationship between compost moisture and spawn running temperature

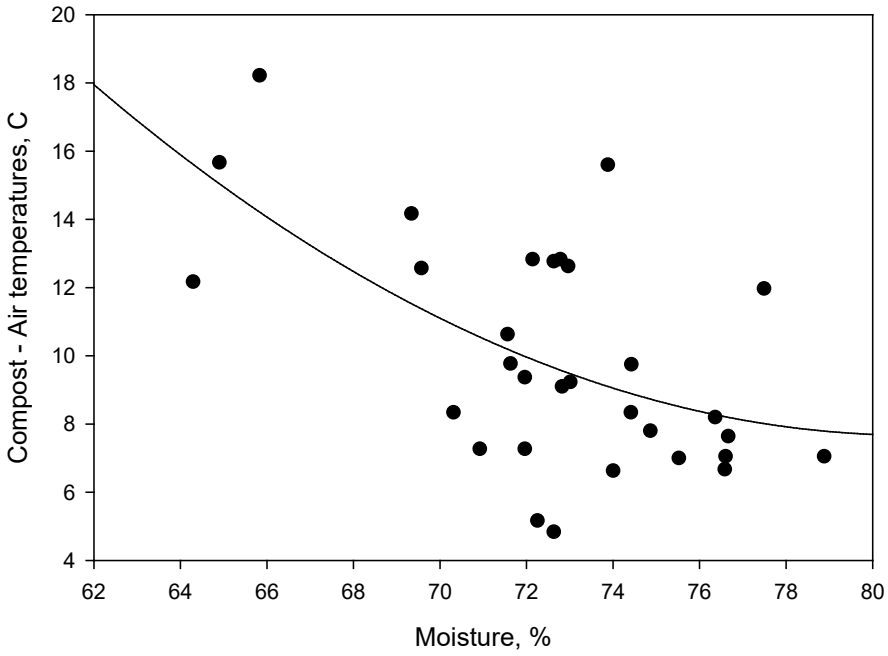


Fig. 13 Mushroom mycelial growth rate and yield

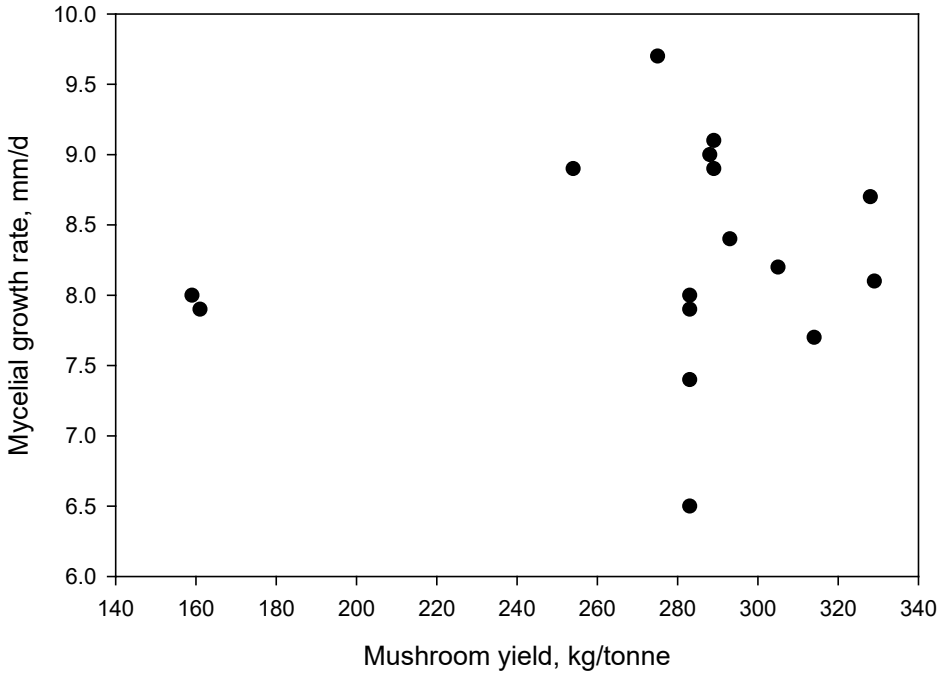


Fig. 14 Relationship between compost pH and mycelial growth rate

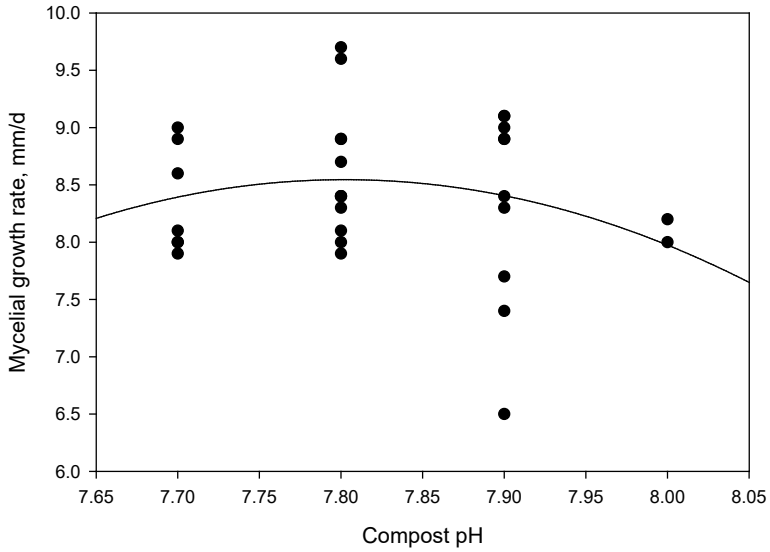


Fig. 15 Relationship between total compost N and mycelial growth rate

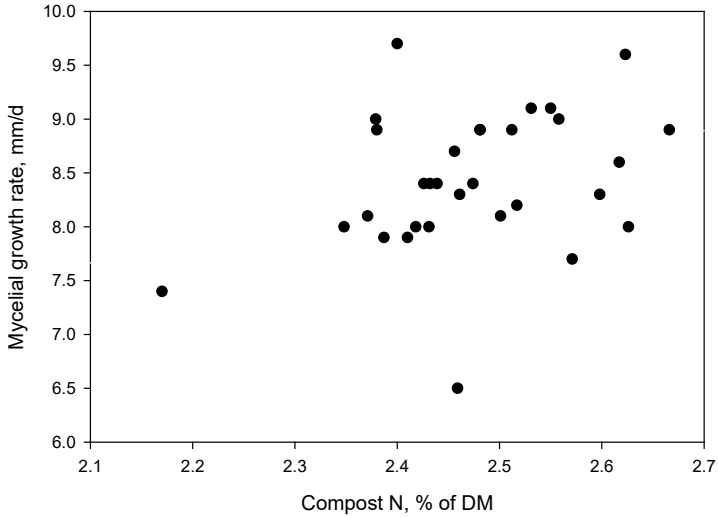


Fig.16 Relationship between compost moisture and mycelial growth rate

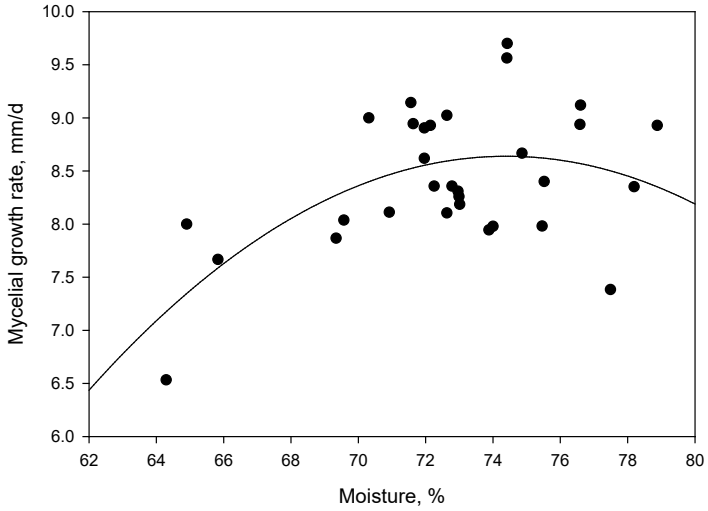


Figure 17. Oxygen consumption in oxygen electrode following addition of laccase enzyme. The slope indicates the rate of oxygen consumption and hence laccase concentration.

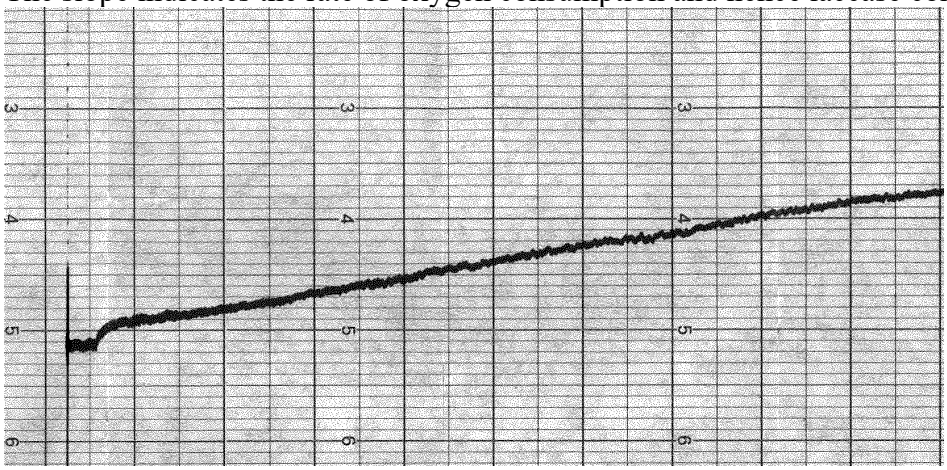


Figure 18. Oxygen consumption in pasteurised compost extract .
The shallow slope indicates a low concentration of laccase.

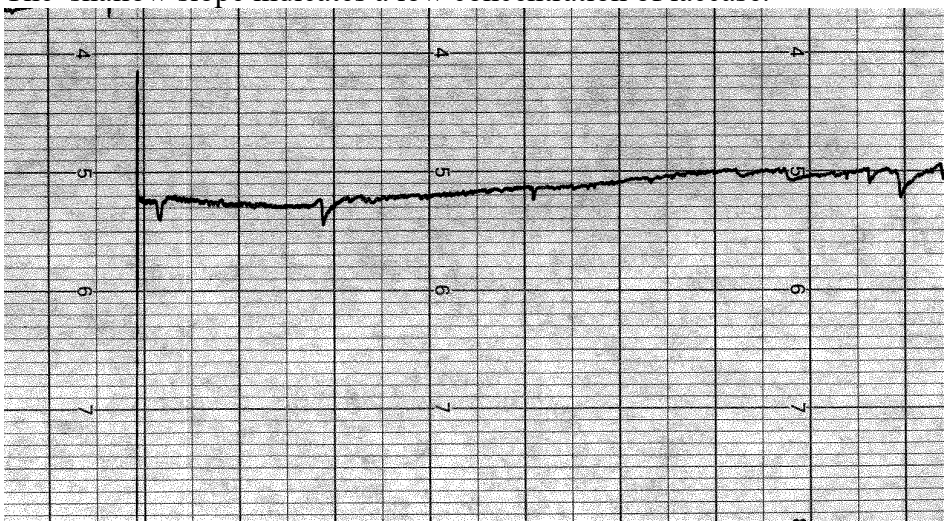


Figure 19. Oxygen consumption in spawn-run compost extract .
The slope indicates a high concentration of laccase.

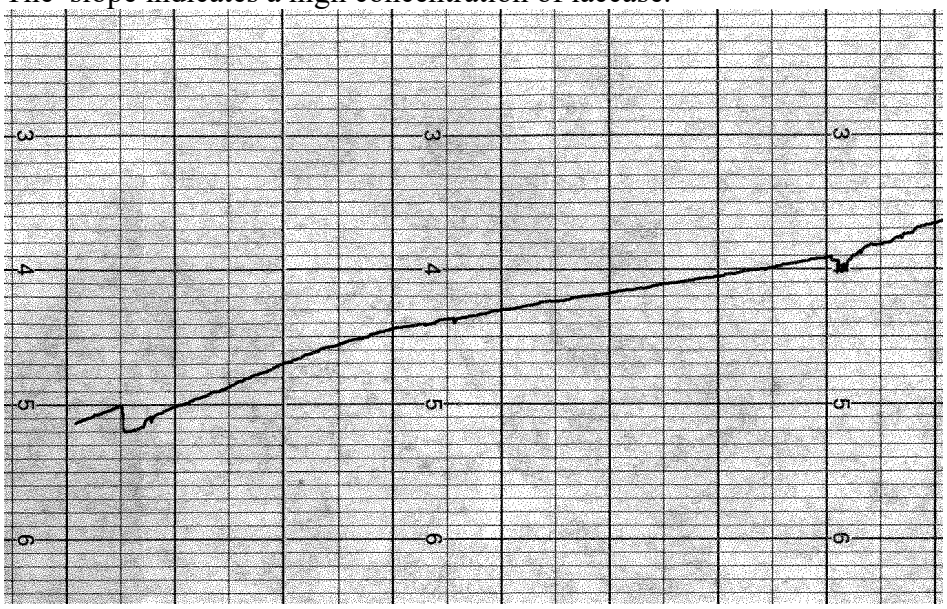


Table 11. Analysis of Phase II composts, mushroom mycelial growth rate and mushroom yield

	Growth	Yield	Compost analysis								DTG analysis	
Compost Ref	rate mm/day	kg/t	% of dry matter N	NH4	Ash	pH	moisture %	cellulose mg/g	lignin mg/g	debris ash mg/g	hollocellulose wt loss %	humus fraction % wt loss
16/02A	8.9		2.646	0.020	24	7.8	72.0	341	258	35	51.2	20.5
16/02B	8.6		2.576	0.041	25	7.7	72.0	404	280	46	56.4	17.0
16/02C	9.0		2.310	0.069	24	7.7	72.6	464	290	45	55.0	17.9
16/02D	8.4		2.352	0.080	23	7.9	72.2	292	563	39	52.6	20.9
01/03A	9.1	268	2.520	0.030	22	7.9	71.6					
01/03B	8.9	268	2.436	0.045	24	7.7	72.1					
01/03C	8.3	268	2.562	0.036	22	7.8	73.0	380	298	42	56.5	17.6
01/03D	8.4	268	2.380	0.046	25	7.8	72.8					
02/03	8.9	279	2.478	0.034	25	7.9	71.6	265	308	51	50.2	19.4
03/03	8.7	268	2.380	0.076	22	7.8	74.9	294	308	48	50.2	18.6
05/03	9.0	263	2.520	0.038	25	7.9	70.3	282	379	50	54.2	17.8
06/03N	9.1	218	2.478	0.053	22	7.9	76.6	275	344	50		
06/03W	8.9	170	2.310	0.070	24	7.8	78.9	297	329	73	50.0	18.1
07/03	8.1	220	2.466	0.035	26	7.7	70.9	311	287	39	55.1	18.6
08/03N	8.3	210	2.422	0.039	22	7.9	73.0	275	344	75		
08/03W	8.4	110	2.380	0.059	25	7.8	78.2	269	401	71		
10/03	8.2	257	2.478	0.039	24	8	73.0	313	326	67		
11/03ED	6.5	220	2.422	0.037	27	7.9	64.3	269	357	62		
11/03D	8.0	220	2.380	0.038	28	8	64.9	282	377	59		
11/03N	7.9	220	2.352	0.035	25	7.8	69.3	271	347	61		
11/03W	7.4	220	2.100	0.070	22	7.9	77.5	271	346	54		

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. 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 also have a critical effects on the efficiency of the composting process and subsequent mushroom growth.

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). Substitution of poultry manure with low odour nitrogen sources and an increased use of aeration resulted in significant reductions in odour 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). However, the cause of odours from recycled water in terms in microbial populations (sulphide producers and other anaerobes) and chemical compounds such as sulphides or fatty acids has not been determined. This would enable effective methods of recycled water treatment to be formulated. Methods for detecting low concentrations sulphides on mushroom composting sites have been developed using pulsed fluoresence analysers (Noble et al 2001). Anaerobic run-off liquor can also have a deleterious effect on subsequent mushroom growth (Heineman & Engels 1953).

Here, the following hypotheses will be tested in relation odours from recycled water:

- Are odours (sulphides) produced by recycled water related to its chemical and microbial composition?
- Does the odour produced by composting relate to the composition of recycled water, in terms of chemical compounds or the microbial community structure determined from PCR and PLFA analysis?
- Are the presence of particular compounds in recycled water (e.g. organic acids) detrimental to mushroom growth and cropping?
- 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 of 13 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. 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.

Analysis of goody water

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 when a stable reading was obtained after 10 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 5 min.

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

Analysis of sulphite 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 teflon-lined screw cap. Citrate buffer was added to make the water content of sample and buffer 1.5 $CHCl_3$ (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 now 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 120^0C for 1 h. Funnels were washed twice with $CHCl_3$:MeOH, and the column packed by placing small ball of glass wool (washed) in '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 3x100□1 $CHCl_3$ 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 37^0C 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 store sample in 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.

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 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 h.

Olfactometry (Odour panel analysis)
Odour concentration (OC) was determined by an odour panel using dilution olfactometry. A dynamic dilution olfactometer (type DTM, 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 Prokov (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.

Samples were also tested with a newer Olfactomat "C" Project Research, Amsterdam instrument, which operated on the same principles as the DTM instrument.

Odour panellists also made an assessment of odour intensity of the samples according to Burton et al (1997). 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 l 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 1m 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 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-site in the same way as sampling odours for collection in Teflon bags, 24h after on-site sampling. Dräger tubes were also used for measuring the headspace of Duran bottles containing goody water samples (see above) and the air inside composting flasks (see below). 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 an 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 sulphide into sulphur dioxide which could then be measured.

Preparation of composts from goody water samples

The same bench-scale composting procedure used for composting wheat straw samples was used. Goody water (2 litres) was used in place of fresh water, except for the control treatment. Odours were assessed after 24 h in the flasks using 6 panellists and the above 0-6 odour intensity scale.

Results

Dimensions and other details of goody water pits, and method of sampling are shown in Table 12. Some form of screening of solid matter from the recycled water was conducted on eight of the 13 sites, and nine of the storage pits had some form of aeration. This consisted of submerged pipes, submerged propeller with pipes, a surface aerator and/or recirculation of liquor from the bottom of the pit or storage tank.

Chemical and microbial analysis of goody water samples

Analysis of all the samples collected is still incomplete, but results obtained to date are shown in Table 13. The results show large differences between samples in terms of pH, conductivity, Redox potential, dry matter content, sulphite reducing bacteria (SRB, *Clostridia* spp.). Hydrogen sulphide and odour of the different samples also showed a wide range (Table 13). When all the data has been collected (November 2004), analyses will be conducted to determine which factors are related to each other, and which are the important factors in terms of odour emissions from goody water. A general trend is that the samples with the most

negative Redox potential also resulted in the greatest hydrogen sulphide and odour emissions. The subsequent composts prepared from these samples also resulted in the greatest sulphide and odour emissions (Table 14).

Figure 20 shows the relationship between dissolved oxygen concentration at Redox potential of goody water samples. Although several of the samples had a zero value of dissolved oxygen, they had different Redox potentials, which demonstrates their oxygen demand. This demonstrates the problem of using dissolved oxygen meters which do not provide information on the oxygen demand of samples with a zero oxygen concentration.

Phospholipid fatty acid analysis of the goody water samples is currently being conducted.

Analysis of air samples from goody water pits

Analysis of air samples from goody water pits showed that this could be significant source of odour from composting sites (Table 15). Hydrogen sulphide and dimethyl sulphide could both be detected with gas detector tubes, but not acetic acid (Table 15). Several other volatile odorous sulphur-containing compounds were identified in the air surrounding goody pits using gas chromatography-mass spectrometry (GC-MS) (Table 16). A number of other odorous compounds were identified in these air samples using GC-MS, including alcohols, ketones and fatty acids (Table 17). Examples of GC-MS spectra obtained at IGER and JEOL are shown in Figures 21 and 22.

Conclusions Part 3

1. Techniques were developed for measuring the chemical, microbial and odour properties of recycled goody water in storage pits.
2. 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.
3. Goody water samples with a lower Redox potential tended to produce more volatile sulphides and were more odorous than samples with a less negative Redox potential.
4. There also wide ranges in the dry matter content, pH, conductivity, sulphite reducing bacteria and chemical analysis of goody water samples obtained from different sites.
5. Composts prepared from the most anaerobic goody water samples (lowest Redox potentials) also produced the most volatile sulphides and strongest odours during subsequent composting.

Table 12. Details of goody water pits on different sites

Site	Depth sample, m	Depth m	Volume m3	Screened	Aeration
1	1	2	150	yes	yes
2	2	2	80	no	yes
3	4	4	450	yes	yes
4	1			no	no
5	1	4	450	yes	yes
6	4	4	450	yes	yes
7	0.5	4	200	no	yes
8	1.5	2	10	yes	yes
9	1	1.3	517	yes	yes
10	4	4	450	no	no
11	1.4	2	240	yes	no
12	4	4	450	no	no
13	4	4	450	yes	yes

Table 13. Chemical and microbial (sulphite reducing bacteria, SRB) analysis of goody water samples from different sites

Site	pH	Conduct uS/cm	Redox	DM g/L	SRB cfu/ml	H_2S ppm	Odour
1	6.93	484.6	-146				
1	7.34	456	-329	5.57	0		
1	7.53	405	-303	5.43	20	12	2.3
2	7.3	541	-371	5.42	0	20	2
3	6.95	460	-318	4.23	0	6	2.6
4	7.16	790	-377	8.18	43	30	2.8
5	7.58	428	-343	4.13	49	11	1.9
6	7.1	296	-83	3.69	0	0	0.6
7	6.71	264	-325	31.46	76	180	3.9
8	6.88	186.6	-106				
8	5.49	173.5	-96				
8	7.27	111	-160	3.47	0	0	0.3
9	7.54	481	-287	5.75			
9	7.01	772	-350		0	4	2.2
10	7.77	539.6	-175				
10	6.78	2350	-361			540	3.9
11	7.06	1452	-348		86	540	3.1
12	8.51	455.1	-204				
12	7.32	1268	-373			50	2.3
13	6.57	457.1	-171				
13	7.13	1374	-377		5	40	2.3
water	7.55	67			0		

Table 14. Analysis of emissions from flask composts prepared from goody water and fresh water

Site	H_2S ppm	DMS ppm	Odour
1	290	800	2.5
2	14	6	1.8
3	10	70	2.6
4	396	30	2.85
5	200	60	2.8
6	35	48	3.1
7	5000	5000	4.9
8	20	70	4.3
9	150	180	2.8
10	1000	4000	3.8
11	1	20	2.5
12	590	3200	2.2
13	20	60	3.8
water	3.5	13	1.85

Table 15. Analysis of odour emissions from goody water pits using gas detector tubes

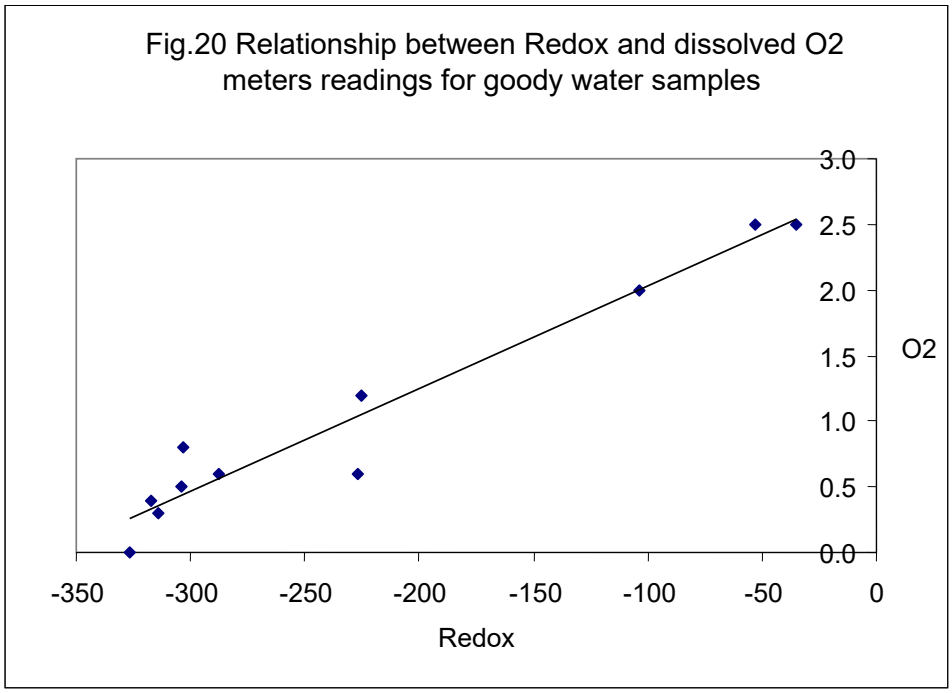
Sample	Odour OU/m3	H2S ppm	DMS ppm	Acetic ppm
1	8474			
2	7968			
3	929	0.3	0	0
4	4662	0.3	1	0
5	14766	0.2	2	0
6	20086			
7	5435	0.5	2	0

Table 16. Volatile sulphur containing compounds identified in emissions from goody water pits by GC-MS

Sample	**methanethiol**	**Carbon disulphide**	**Dimethyl sulphide**	**Dimethyl disulphide**	**dimethyl trisulphide**
1	0.32	3.07	0.34	4.86	0.14
2	0.35	18.18	2.31	7.10	0.28
3	57.40	2950.50	595.20	1813.27	489.35
4	0	0.12	0.53	3.18	0.13
5	0	0.14	0.24	1.09	0.14

Table 17. Other volatile compounds identified in emissions from goody water pits by GC-MS

Compound	**Concentration, mg/m3**	
	min	max
Acetic acid	0.05	44.78
Acetone	10.1	10.6
Butanal	0.12	0.24
Butanoic acid	0.13	1.54
1-Butanol	35.09	52.08
Ethanol	2.60	3.41
Ethyl benzene	2.52	34.64
Furan	0.17	0.24
Heptane	0.49	0.50
Hexane	0.23	0.32
Methyl Ethyl Ketone	0.05	0.91
3-(methylthio)-propanal	3.22	8.46
Phenol	3.45	4.42
1,2,4-trimethyl benzene	0.85	21.48
p-Xylene	1.24	35.22



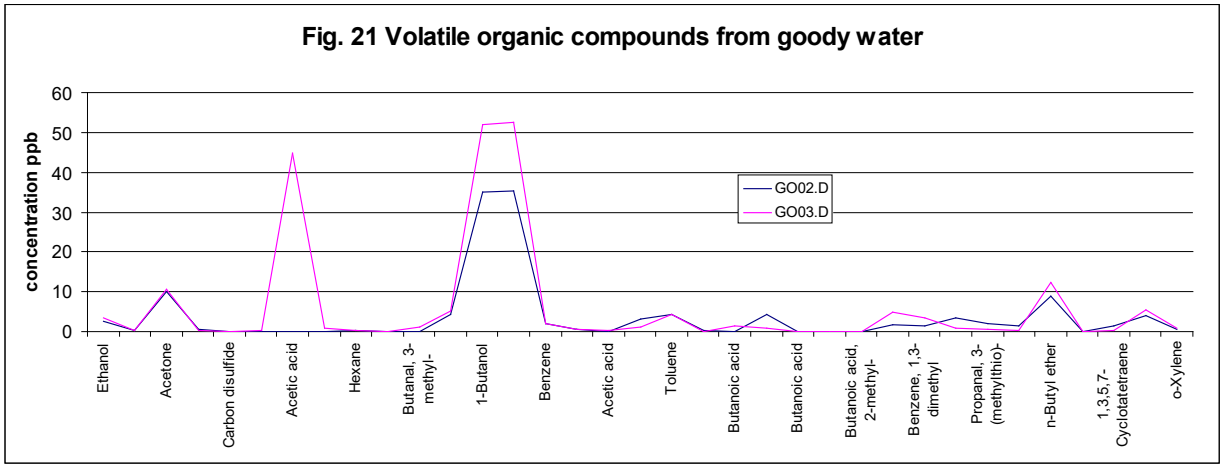
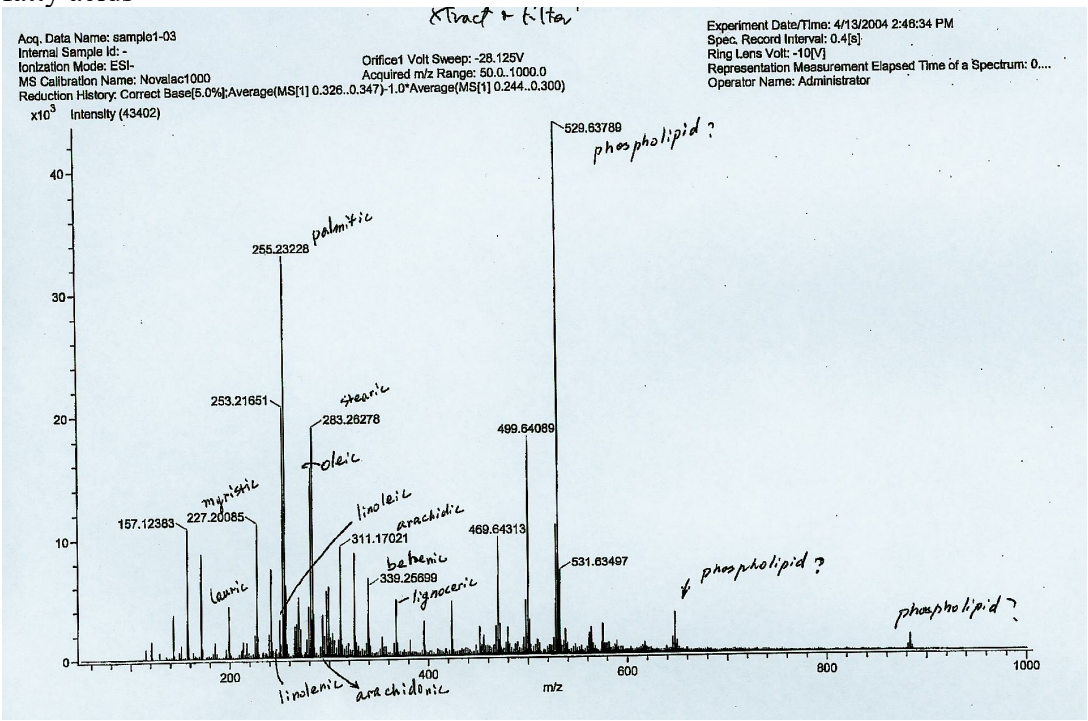


Figure 22. GC-MS analysis of goody water at JEOL indicating presence of phosopholipids and fatty acids



TECHNOLOGY TRANSFER

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.

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

References

Andrews J, Adams SR, Burton KS, Edmondson RN. 2002 Partial purification of tomato fruit peroxidase and its effect on the mechanical properties of tomato fruit skin. J. Exp. Botany 53, 2393-2399.

Anonymous. 2002. NIAB Guide to varieties of cereals, oilseeds & pulses. Autumn 2002. National Institute of Agricultural Botany, Cambridge.

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. p 207-214.

Dravneiks A, Prokop, W H 1975 Source emission odour measurement by a dynamic forced-choice triangle olfactometer. J Air Pollut Cont Assn 25: 1.

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

Flegg PB, Wood DA 1985. Growth and fruiting. In: The Biology and Cultivation of the Edible Mushroom. Flegg PB, Spencer DM, Wood DA (eds). John Wiley & Sons Ltd. Chichester, UK 141-177.

Heinemann P, Engels S. 1953. Toxicitie des acides organiques sur le mycelium d'Agaricus Hortensis et especes voisines, en culture pure. Mushroom Science 2: 49-51.

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

Noble, R. and R.H. Gaze. 1994. Controlled environment composting for mushroom cultivation: substrates based on wheat and barley straw and deep litter poultry manure. J. Agric. Sci. Camb. 123: 71-79.

Noble R. & Gaze R.H. (1998) Composting in aerated tunnels for mushroom cultivation: Influences of process temperature and substrate formulation on compost bulk density and productivity. Acta Horticulturae 469: 417-427.

Noble, R., T.R. Fermor, C.E. Evered, and P.T. Atkey. 1997. Bench-scale preparation of mushroom substrates in controlled environments. Compost Sci. Utiliz. 5 (3): 32- 43.

Noble R., Gaze R.H. and Willoughby N. (1998) A high yielding substrate for mushroom experiments: Formula 3. Mushroom Journal 587: 27,28.

Noble, R. P. J. Hobbs, A. Dobrovin-Pennington, T.H. Misselbrook and A.Mead. 2001. Olfactory Response to Mushroom Composting Emissions as a Function of Chemical Concentration. Journal of Environmental Quality 30: 760 - 767.

Rahn CR, Bending GD, Lillywhite RD, Turner MK 2002. Chemical characterisation of vegetable and arable crop residue materials: a comparison of methods. J. Science Food Agriculture 79:1715-1721.

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

Sharma HSS, Kilpatrick M. 2000. Mushroom (*Agaricus bisporus*) compost quality factors for predicting potential yield of fruiting bodies. Canadian Journal Microbiology 46:515-519.
Smith JF, Claydon N, Love ME, Allan M, Wood DA. 1989. Effect of substrate depth on extracellular endocellulose and laccase production of *Agaricus bisporus*. Mycol. Res. 93:292-296.
Smith J.F., Wood, D.A. and Thurston, C.F. (1995) Growth measurement of *Agaricus* mycelium in composted substrates as an indicator of compost selectivity and mushroom productivity. In: *Science and Cultivation of Edible Fungi* (ed. T.J. Elliott), 293-301.
Van den Berg M, 1992 Sensory odour measurements using an olfactometer. Nederlands Normalisatie Instituut, Kalfjeslaan 2, Postbus 5059, 2600 GB Delft, Netherlands.
Wood DA & Goodenough PW, 1977. Fruiting of Agaricus bisporus Changes in Extracellular enzyme activities during growth and fruiting. Archives of Microbiology 114:161-165.